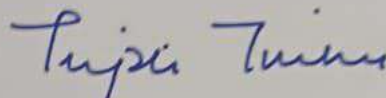


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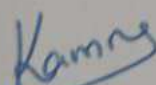
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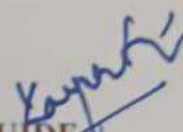

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**TO EVALUATE THE EFFECT OF VIBRATORY STIMULUS ON
LEVELS OF BIOMARKERS DURING ORTHODONTIC TOOTH
MOVEMENT**

DISSERTATION

Submitted to

**BABU BANARASI DAS UNIVERSITY, LUCKNOW, UTTAR
PRADESH**

*In the partial fulfillment of the requirements for the degree
of*

MASTER OF DENTAL SURGERY

**In
ORTHODONTICS AND DENTOFACIAL ORTHOPAEDICS**

By

Dr. Ashima verma

Under the guidance of

Dr. Tripti Tikku

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**BABU BANARASI DAS COLLEGE OF DENTAL SCIENCES,
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LIST OF ABBREVIATIONS

S.NO	ABBREVIATED FORM	FULL FORM
1.	RAP	Regional Acceleratory Phenomenon
2.	IL	Interleukin
3.	RANKL	Receptor Activator of Nuclear Factor-kappa B
4.	TNF	Tumor necrosis factor
5.	MOPS	Micro-osteoperforation
6.	PAOO	Periodontontally accelerated osteoperforations
7.	LLLT	Low Level Laser Therapy
8.	et al	et alia (and others)
9.	MMP	Matrix Metalloproteinases
10.	IgM	Immunoglobulin M
11.	PDGF	Platelet derived growth factor
12.	OTM	Orthodontic tooth movement
13.	PDL	Periodontal ligament
14.	MAPK	Mitogen activated protein kinase
15.	GCF	Gingival Crevicular Fluid

LIST OF ABBREVIATIONS

16.	NiTi	Nickel Titanium
17.	O.P.G	Orthopentamogram
18.	MBT	Mclaughlin Bennett Trevisi
19.	SS	Stainless Steel
20.	GIC	Glass Ionomer Cement
21.	ELISA	Enzyme linked immunosorbent Assay
22.	HRP	Horseradish peroxidase
23.	Pg/ml	Pico gram per milli liter
24.	µg/mL	Micro gram per milli liter
25.	P value	Probability value
26.	t	Value of t test
27.	df	Degree of freedom
28.	Hz	Hertz
29.	OPG	Osteoprotegerin
30.	LIES	Low level laser therapy

ABSTRACT

Aim: To evaluate and compare the levels of biomarkers during enmasse retraction of anterior teeth (using sliding mechanics) using vibratory stimulus (with electric powered tooth brushes) in a split mouth study design at different time intervals.

Material and Method: Samples for the study included 15 patients (age range of 18-25 yrs) who were undergoing fixed orthodontic treatment after extraction of all first premolars. Enmasse retraction of anterior teeth was initiated on .019x.025SS wire using Niti closed coil spring with a force of 250 gms. Split mouth study design included two Groups, Group I was (control, n=15, canine of left side where no vibratory stimulus was provided) and Group II (n=15, canine of right side where vibratory stimulus was given using electric powered tooth brush). GCF was collected at different time intervals -T0 (baseline) immediately after applying the retraction force, T1- (after 24 hrs), T2- (20 days), T3- (2 months) from gingival sulcus using absorbent points. Mean levels of IL-6 and RANKL were measured following standard protocol of GCF storage, transfer and analysis using ELISA analyzer, Data was tabulated and adequate statistical comparisons were made using SPSS software.

Result: IL-6 had a trend of (T0>T2>T3>T1) in Group I, and (T3>T2>T1>T0) in Group II whereas RANKL had a trend of (T2>T3>T0>T1) in Group I and (T1>T2>T3>T0) in group II. On intra group comparison statistical significant difference was seen between T0- T1, T1-T2 for IL-6 in group I, and between T0-T1, T0-T2, T0-T3 in Group II both for IL-6 and RANKL. Level of IL-6 and RANKL were significantly higher in Group II then in Group I at T1, T2, T3.

Conclusion: Vibratory stimulus significantly elevated level of biomarker, hence, electric powered tooth brushes can be preferred in adults subjects for increasing biomarker levels thereby accelerating tooth movement.

KEYWORDS: Biomarkers, Electric powered tooth brushes, IL-6, RANKL, ELISA.

Orthodontic treatment includes correction of dental and skeletal mal relationship to achieve good esthetics with functional harmony. However, long duration of treatment makes it difficult for adult subjects to seek comprehensive orthodontic care. Also increased orthodontic treatment time can result in a multitude of damaging effects like: white spot lesions, dental caries, root desorption, gingival inflammation and decreased patient compliance¹. Uribe et al.¹ found that about 85% of adult patients and 95% of adolescent patients wished that orthodontic treatment lasted 18 months or less; this would be a 25% reduction in the overall treatment duration than the most reported average duration of treatment in their study¹. This increased duration of orthodontic treatment led to need for accelerating the rate of tooth movement during fixed orthodontic treatment. The acceleration of tooth movement has focused on three main approaches: Pharmacologic, surgical methods and physical / mechanical stimulation method¹. The use of drugs like vitamin D, Thyroid hormones, parathyroid hormone, corticosteroids, prostaglandins, interleukins, misoprostol accelerates tooth movement. However, these drugs had unwanted side effects that had to be considered before prescribing them¹. Surgical methods like Corticotomy, Osteotomy also accelerates tooth movement based on Regional Acceleratory Phenomenon (RAP). As the bone is irritated surgically, RAP starts with initiation of inflammation cascade, release of various inflammatory mediators like IL- 1, IL-6, IL-8, RANKL, TNF alpha etc that causes increased Osteoclastogenesis, hence faster tooth movement¹. These conventional surgical methods were extremely invasive and not well accepted by patient at times. Hence, newer surgical methods like piezosurgery, fiberotomy, micro osteoperforations (MOPS), Periodontontally accelerated osteoperforations (PAOO), wilkodontics evolved that were comparatively less invasive, but still considered as minor surgical procedures¹. Mechanical or physical stimulation methods are non-invasive and non-pharmacological procedures and include vibration methods², Low level laser therapy, electric currents, therapeutic ultrasound, pulsed electromagnetic field etc., Low level laser therapy (LLLT)³ stimulates cells through photobiological action, thereby accelerates bone remodeling, reduce pain and is well tolerated and does not have any traumatic effects. Besides this Low intensity direct electric currents (LIES) and Pulsed electromagnetic field were also found to speed up orthodontic tooth movement³. However all these methods not only need expensive equipments but also need multiple visits of patients to clinical set up. Various

methods have been used to induce vibratory stimulus like pulse vibration device (AcceleDent)⁴, Ultrasonic or powered tooth brushes, the tooth massager etc. that could be used by patients at home⁴. Amongst these vibratory stimulating devices, AcceleDent and tooth massager are patented devices and are expensive⁴. Ultrasonic brushes emit waves of higher frequency that is 20,000 Hz which is undesirable⁴. Powered tooth brushes are available in the frequency range of 60 Hz - 333Hz which is required and are comparatively cheaper, easily available and have been used in previous studies to reduce pain due to orthodontic treatment or to accelerate the tooth movement⁴.

The results of studies evaluating efficacy of various vibratory devices showed mixed results regarding accelerated tooth movement³⁻⁴, where few studies showed significant accelerated tooth movement, other studies demonstrated non-significant difference between both control and experimental side³⁻⁴.

A biomarker is defined as a biological molecule found in blood, tissues or other body fluids that indicate a normal or abnormal process, condition or disease. Various biological molecules like TNF alpha, IL-1, IL-6, IL-8, IgM, alpha-glucosidase, acid phosphatase, alkaline phosphatase, MMP-13, MMP-8, MMP-9, PDGF, RANKL etc are released on application of mechanical stresses during OTM. The assessment of these molecules as biomarkers would provide better understanding of OTM at molecular level⁵.

Histologically, vibratory stimulus had shown to accelerate cell proliferation, collagen synthesis, RANKL expression in animal studies⁶. Leethanakul et al.² explained the mechanism of action and biological effects at molecular level on application of vibratory stimulus to accelerate tooth movement². On application of orthodontic forces bioelectric signals are produced that are reinforced by discontinuous forces produced by vibratory devices. Thus, there is increase in the number of osteoclasts on the compression/convex side with enhanced expression of RANKL in osteoblasts, PDL, fibroblasts and osteoclasts and there is induction of signaling molecules like MAPK (mitogen activated protein kinase), c-fos and nitric acid to induce osteoclastogenesis. Various studies demonstrated that biomarkers released during OTM were reinforced because of vibratory stimulus.⁷⁻¹⁴ Hence it could be anticipated that biomarker level would be raised on application of vibratory stimulus. As powered tooth brushes are cheaper and can be used by patient at home it was decided to use

them for providing vibratory stimulus in present study.⁷⁻¹⁰

IL-6 is an earliest bone resorption marker, and is released during early phase of orthodontic tooth movement. Another potent biomarker is RANKL which binds to RANK and initiates osteoclastogenesis. Therefore the levels of IL-6 and RANKL would be evaluated in the present study.

Biomarkers can be detected in blood/serum¹⁵ or saliva^{16,17} or GCF¹⁸ etc. The collection of serum/blood is an invasive procedure that may be painful and may cause anxiety or discomfort for some individuals. Also, there is risk of infection or contamination and levels fluctuate due to various factors, such as diet, exercise or diurnal variation or presence of inflammatory conditions in other parts of body. Moreover biomarkers are present in minimal quantities in serum. Salivary biomarkers had been used to assess pubertal growth spurt, bone turnover or understanding OTM at molecular levels¹⁶. However similar to serum, salivary biomarkers are present in very low concentration, requiring highly sensitive tests to detect them. The composition of saliva is influenced by its origin and various environmental and psychological factors. Thus, qualitative analysis of salivary marker can be reliably performed however; detecting levels of these biomarkers in saliva remains a significant challenge.

To overcome these limitations of serum or saliva, biomarkers could be evaluated in GCF. GCF is a transudate or exudate that arises at gingival margin. GCF biomarkers can indicate the cellular response of periodontium. Advantages of GCF collection are that it is minimal invasive, cost effective, ease of sample collection and not influenced by systemic conditions of body. Therefore, in the present study it was decided to collect GCF for assessing the biomarkers released during orthodontic tooth movement¹⁷.

GCF can be collected using pre-weighed twisted threads, microcapillary pipetting, washing method, absorbent paper points, paper strips etc¹⁷. Pre-weighted twisted threads are placed in gingival crevice and then weighted to assess the GCF collected however weighing them is a difficult task¹⁷⁻¹⁸. For micro capillary pipetting, pipette is kept in fluid for 10-20 mins so it is time consuming and can be traumatic for patient; also it is difficult to remove the entire sample from the tubing¹⁹. Absorbent paper points are placed in gingival crevice for 30 seconds only, to assess biomarker levels in

GCF²⁰. It is less traumatic and easiest way and most commonly used method to collect GCF²¹. Hence it was decided to use absorbent paper point to collect GCF in the present study.

The efficacy of vibratory stimulus on accelerating tooth movement, on application of orthodontic force, by measuring rate of movement of adjacent teeth in extraction space had been assessed in previous studies but none of the studies have evaluated biomarker level during space closure in orthodontic tooth movement using sliding mechanics. As IL-6 and RANKL bone resorptive biomarker at molecular level are released when tooth movement is initiated, it was decided that their levels can be evaluated on control and experimental side during space closure. The levels would be assessed at (baseline, 24 hrs, 48 hrs, 20 days) of starting retraction using NiTi closed coil spring after placement of 0.19X 0.25 SS with crimpable hooks between lateral incisor and canine².

For this split mouth study design was selected where maxillary canine of one side served as experimental side where patient was asked to use vibratory brush and canine of other side served as control. The attractiveness of the split mouth design is that it eliminated the inter-participant variation associated with differences in initial extraction space sizes, malocclusion types and variations in inter-appointment interval, as each participant acted as his/her own control.

Considering this aim of this study was to evaluate and compare the levels of biomarkers (IL-6 and RANKL) during space closure (using sliding mechanics) using vibratory stimulus (with electric powered tooth brushes) in a split mouth study design at different time intervals.

AIM

To evaluate and compare the levels of biomarkers during enmasse retraction of anterior teeth (using sliding mechanics) using vibratory stimulus (with electric powered tooth brushes) in a split mouth study design at different time intervals.

OBJECTIVES

1. To evaluate the level of IL6 in GCF on distal side of maxillary canine on control and experimental side (vibratory stimulus given with electric powered tooth brush) during enmasse retraction of anterior teeth at different time intervals (baseline, 24 hours, 20 days and two months).
2. To evaluate the level of RANKL in GCF on distal side of maxillary canine on control and experimental side (vibratory stimulus given with electric powered tooth brushes) during enmasse retraction at different time intervals (baseline, 24 hours, 20 days and 2 months).
3. To make intra-group comparison for both biomarkers levels between different time intervals.
4. To make inter-group comparison, for levels of each biomarkers at different time intervals.

Stark TM, Sinclair PM (1987)²² determined whether the application of a simple surgically noninvasive, pulsed electromagnetic field could increase both the rate and amount of orthodontic tooth movement observed in guinea pigs and evaluated the electromagnetic field's effects on bony physiology and metabolism and searched for possible systemic side effects. Laterally directed orthodontic force was applied to the maxillary central incisors of a sample of 40 young male, Hartley guinea pigs (20 experimental, 20 control) by means of a standardized intraoral coil spring inserted under constricting pressure into holes drilled in the guinea pigs' two maxillary central incisors. The application of a pulsed electromagnetic field to the experimental animals significantly increased both the rate and final amount of orthodontic tooth movement observed over the 10-day experimental period. The experimental animals also demonstrated histologic evidence of significantly greater amounts of bone and matrix deposited in the area of tension between the orthodontically moved maxillary incisors.

Brudvik P, Rygh P. (1991)²³ The purpose of this study was to investigate the occurrence of orthodontic root resorption in connection with local injection of prostaglandin E2 (PGE2). The material consisted of 25 male Wistar rats. The control group comprised six animals where no force was applied. In five animals 0.1 ml of 0.1 µg/ml PGE2 was injected in the gingival area of the upper right first molar. In one animal no PGE2 was injected. The animals were killed after 3 days. The experimental tooth movement groups consisted of 19 animals. Duration of experiments was 3 days, 7 days, and 10 days. The maxillary first molars on both sides were each moved mesially by means of a coil spring. On the right side 0.1 ml of PGE2 0.1 µg/ml was injected in the gingiva on the buccal side of the upper first molar on days 0, 3, 5, and 7. On the left side no injection of PGE2 was performed. In three animals in the 7-day group the vehicle (Waymouth medium) was injected. There was no significant difference in root resorption between the experimentally moved teeth with and without local injection of PGE2, but a trend towards more root resorption was registered on the teeth where such injections had been performed.

Sandy JR, Farndale RW, Meikle MC. (1993)²⁴ This review highlights recent developments in bone cell biology, evaluates previous research, and offers future direction toward improving our understanding of events that mediate orthodontic tooth movement. The in vivo and in vitro models that have been developed to examine the responses of connective tissues and how they have contributed to our understanding of

the mechanisms involved in mechanically induced bone remodeling are discussed in detail. Osteoblasts are now recognized as the cells that control both the resorptive and the formative phases of the remodeling cycle, and receptor studies have shown them to be the target cells for resorptive agents in bone. The osteoblast is perceived as a pivotal cell, controlling many of the responses of bone to stimulation with hormones and mechanical forces. It is apparent that not all the cellular responses induced by mechanically deformed tissues can be explained by the current paradigm emphasizing the importance of prostaglandin production and cAMP elevation; the mobilization of membrane phospholipids giving rise to inositol phosphates offers an alternative second messenger pathway. It is also argued from circumstantial evidence that changes in cell shape produce a range of effects mediated by membrane integral proteins (integrins) and the cytoskeleton, which may be important in transducing mechanical deformation into a meaningful biologic response.

Grieve WG 3rd, Johnson GK, Moore RN, Reinhardt RA, DuBois LM. Prostaglandin E (1994)²⁵ examined GCF levels of two potent bone resorbing mediators, Prostaglandin E(PGE) and interleukin - 1 β (I L-1 β), during human orthodontic tooth movement. 10 patients, each having one treatment tooth undergoing orthodontic tooth movement and contralateral control tooth were examined. GCF sampled at control sites and treatment sites before activation and at 1, 24, 48 and 168 hours. PGE and IL-1 β levels were determined with radioimmunoassay. At 1 and 24 hours mean GCF IL-1 β levels were significantly elevated at treatment teeth compared with control teeth. GCF levels of PGE for treatment teeth were significantly higher at 24 and 48 hours than the control teeth.

Leiker BJ, Nanda RS, Currier GF, Sinha PK, Howes RI. (1995)²⁶: The long-term effects of varying concentrations and frequencies of injectable, exogenous prostaglandin E2 (PGE2) on the rate of tooth movement and the amount of root resorption were evaluated. There were 132 male Sprague-Dawley rats 8 weeks old that initially weighed 225 to 250 gm. Five animals were in a pilot study, while seven were baseline controls and eight were appliance controls. The remaining 112 animals were divided into two experimental time periods of 2 and 4 weeks. Then, each experimental time period was divided into four subgroups of 14 animals based on concentration levels of PGE2 injections, i.e., 0.1, 1.0, 5.0 and 10.0 μ g. Half of these animals in the dosage subgroup received a single injection at appliance placement and the other half received weekly injections. A fixed orthodontic appliance consisting of closed-coil nickel-titanium springs

were ligated between the maxillary incisors and maxillary first molars. The initial activating force was 60 gm. The results showed that injections of exogenous PGE 2 over an extended period of time in rats did enhance the amount of orthodontic tooth movement. However, there was no statistically significant difference in tooth movement between the single and multiple injection groups or among the four concentration levels of PGE 2 used in either the 2- or 4-week time periods. The amount of root resorption as seen from scanning electron micrographs did increase with the use of prostaglandin injections, specifically with increased numbers of injections and with increased concentrations of PGE 2.

Yavuzylmaz E, Yamalik N, Bulut S, Saatci U. (1995)²⁷ determined levels of two important cytokines, interleukin 1-p (IL-1p) and tumour necrosis factor-a (TNF-a) in GCF and, in addition, serum IL-1p levels, were determined in patients with severe and rapid periodontal breakdown by use of ELISA. While IL-1p was detected in all of the GCF samples studied, TNF-a could only be detected in about half the samples. The mean GCF IL-1p level was 38.45 f 13.99 pg/mL, and the mean TNF-a level was 3.20 f 1.39 pg/mL, respectively. The GCF 11-18 levels also presented a strong positive correlation with the mean pocket depths. Although weak, both of the cytokines also presented correlations with the presence of bleeding on probing. Additionally GCF samples contained increased IL-1 p levels when compared with the serum samples suggesting local production mechanisms. So the study suggest that these cytokines may be involved in the pathogenesis of periodontal diseases (IL-1p being more significant), and also may help in defining the active phase of periodontal breakdown.

Yonemori K, Matsunaga S, Ishidou Y, Maeda S, Yoshida H (1996)²⁸ The mechanism by which electrical stimulation causes osteogenesis is unknown. Bone marrow of the rabbit was stimulated by direct electrical current or electromagnetic fields to clarify the mechanism of osteogenesis by electrical stimulation. A total of 105 rabbits were separated into five groups: a direct current stimulation by Kirshner wire insertion group; a pulsed electromagnetic fields stimulation (PEMF) group; a PEMF with Kirshner wire insertion group; a Kirshner wire insertion group; and an intramedullary drilling control group. Measurement of intramedullary new bone formation and determination of alkaline phosphatase activity within the bone marrow were performed. Argylophilic nuclear organizer region (AgNOR) staining was done to evaluate the change in proliferative activity of the osteoblasts during electrical stimulation. In the direct current stimulation

group and the PEMF accompanied by the insertion of the Kirshner wire group, alkaline phosphatase activity in the bone marrow and AgNOR staining increased at 7 days after surgery. At 14 days after surgery, alkaline phosphatase activity and proliferative activity of osteohlast were significantly higher in these two groups than in the other groups (PEMF group, Kirshner wire insertion alone group, intramedullary drilling group). Intramedullary new bone formation was most active in the direct current stimulation group. Electromagnetic stimulation of the inserted Kirshner wire also promoted bone formation significantly. The Kirshner wire insertion alone group and the intramedullary drilling group showed bone formation, but it was significantly less. Electromagnetic stimulation without the insertion of the Kirshner wire showed little bone formation. These findings revealed that the degree of osteogenesis induced by electrical stimulation is influenced by the tissue environment, and that osteogenesis is promoted markedly when electrical stimulation is provided in the environment of inflammation and reactive cells.

Uematsu S, Mogi M, Deguchi T. (1996)²⁹ identified and quantified the 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 was collected before activation, and at 1, 24, and 168 hr after the initiation of tooth movement. Cytokine levels were determined by ELISAs. The concentrations of interleukin (IL)-1B, IL-6, tumor necrosis factor- α , epidermal growth factor, and B₂-microglobulin were significantly higher in the experimental group than in the controls at 24 hr after the experiment was initiated. In contrast to cytokine alteration, the amount of total protein in the GCF exhibited a gradual increase, but no significant difference was observed between the control and experimental groups. Since all cytokines in GCF play an important role in the bone remodeling processes .

Mogi M, Otogoto J, Ota N, Inagaki H, Minami M, Kojima K. (1999)³⁰ identified and quantified the various growth factors, apoptosis-related modifiers [soluble form of Fas (sFas) and bcl-2] and cytokines in the gingival crevicularfluid (GCF) of patients with different severities of periodontitis as compared with those of controls. GCF samples were taken from patients with periodontal disease and from controls. The concentrations of epidermal growth factor, transforming growth factor (TGF)- α , interleukin (IL)-1b, IL-6, interferon-g, b2-microglobulin (b2-MG), and apoptosis-related modi@ers sFas and bcl-

2 in the samples were determined by enzyme-linked immunosorbent assay. TGF- α was significantly lower in patients with periodontal disease than in the controls. In contrast, the concentrations of IL-1 β , IL-6, and b2-MG were significantly higher in the group with severe periodontal disease than in the controls. The amount of total protein in the GCF was considerably higher in the disease group than the controls ($p < 0.05$). TGF- α , IL-1 β , and b2-MG concentrations were associated (Spearman rank correlation, $r < 0.05$ for all) with clinical measures of disease severity (pocket depth) and inflammation (bleeding when probed). Apoptosis-related modiers (sFas and bcl-2) could not be detected in any samples. These results suggest that the growth factor TGF- α and certain cytokines are associated with the presence of periodontal disease.

Alhashimi N, Frithiof L, Brudvik P, Bakhiat M. (2001)³¹ performed situ hybridization to measure the messenger RNA expression of IL-1 β , 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-1 β 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. So they concluded that these proinflammatory cytokines may play important roles in bone resorption after the application of orthodontic force.

Lee KJ, Park YC, Yu HS, Choi SH, Yoo YJ. (2003)³² evaluated the effects of a light continuous force and an interrupted force with weekly reactivation on interleukin-1 (IL-1) and prostaglandin E2 (PGE2); among 10 adults with 4 premolars extracted. 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-1 and PGE2 levels were measured. For E1, the IL-1 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-1 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-1 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-1 secretion, but there might be limitations in increasing the mediator levels, because of the feedback mechanisms in vivo. In addition, the analysis of crevicular fluid is a useful method for assessing cellular response to orthodontic force in vivo.

Mogi M, Ootogoto J, Ota N, Togari A. (2004)³³ the receptor activator for NF- B ligand (RANKL) plays an important role in osteoclast formation. A recent study with animal models suggests the involvement of RANKL in the pathogenesis of this periodontal disease. However, no one has examined the level of RANKL in the body fluid of human subjects. This communication reports on the in vivo concentrations of RANKL and the RANKL decoy receptor osteoprotegerin (OPG) in the gingival crevicular fluid (GCF) of periodontal subjects with severe, moderate, and mild forms of the disease. An increased concentration of RANKL and a decreased concentration of OPG were detected in GCF from patients with periodontitis (*p < 0.05 vs. control subjects). The ratio of the concentration of RANKL to that of OPG in the GCF was significantly higher for periodontal disease patients than for healthy subjects (*p < 0.01). Taken together, these data suggest that RANKL and OPG contribute to osteoclastic bone destruction in periodontal disease.

Güvenç Bas, aran, Törün Özer, Filiz Acun Kaya, and Orhan Hamamci (2006)³⁴ determined levels of interleukins 2, 6, and 8 during tooth movement, and test whether they differ from each other with leveling and distalization forces used in various treatment stages of standard orthodontic therapy in fifteen patients. The patients were seen at baseline, 7 days later, and 21 days later, and as the teeth were leveled. In first 6 months of treatment, only leveling forces were applied. After 6 months before distalization starts 4th GCF sample (serves as baseline score for distalization). Scores of days 7 and 21 after 6 months of the distalization treatment were also recorded. Results showed no statistical differences in IL-2 levels except that it increased at the 7th and

21st days of leveling and distalization. IL-6 showed no significant fluctuations either statistically or mathematically. IL-8 showed a statistically significant decrease on the 7th day of leveling. Again on the 7th and 21st days of the distalization, it increased to some extent. GCF volumes were greater on the 7th and 21st days of leveling and distalization, and returned to baseline levels after leveling.

Krishnan V, Davidovitch Z (2006)³⁵ studied all major findings from the beginning of research in biology of tooth movement to recent development that provide detailed insight into molecular, cellular and tissue level reactions and various deleterious effects of orthodontic forces. He discussed about various signalling molecules, their release and effect on bone deposition and resorption during Orthodontic tooth movement.

Darendeliler MA, Zea A, Shen G, Zoellner H. (2007)³⁶ studied whether or not high-frequency and low-magnitude vibration affects orthodontic tooth movement caused by magnetic or/and mechanical forces. In forty-four 7-week-old Wistar rats, neodymium-Iron-Boron (Nd-Fe-B) magnets and Sentalloy closed coil springs were placed between maxillary or mandibular first molars and incisors. Under vibrations induced by pulsed electromagnetic field (PEMF), the coil spring moved significantly more amount of tooth movement than that of coil-magnet combination. Under a non-PEMF scenario, there was no significant difference in tooth movement between coil spring and coil-magnets combination.

Makoto Nishimura ,Mirei Chiba, Toshiro ohashi, Masaaki sato, Yoshiyuki shimizu, Kaoru Igarashi and Hideo Mitani (2008)¹³ evaluated the effects of mechanical stimulation by resonance vibration on tooth movement of maxillary first molars of 6-week-old male Wistar rats were moved to the buccal side by using an expansive spring for 21 days and the time interval selected were 0,7,14 days of orthodontic tooth movement. Tooth movement in the experimental group was significantly greater than in the control group ($P < .05$). Enhanced RANKL expression was observed at fibroblasts and osteoclasts in the periodontal ligament of the experimental group on day 3. The number of osteoclasts in the experimental group was significantly increased over the control group on day 8 ($P = .05$). They concluded that application of resonance vibration might accelerate orthodontic tooth movement via enhanced RANKL expression in the periodontal ligament without additional damage to periodontal tissues such as root resorption.

M Yamaguchi (2009)³⁷ in a review article highlighted the role of the receptor activator of nuclear factor-kappa (RANK), receptor activator of nuclear factor-kappa ligand (RANKL), and osteoprotegerin (OPG) in periodontal tissue reactions, in response to orthodontic forces. They found that concentrations of RANKL in GCF increased during orthodontic tooth movement, and the ratio of concentration of RANKL to that of OPG in the GCF was significantly higher than in control sites. In vivo studies have shown the presence of RANKL and RANK in periodontal tissues during experimental tooth movement of rat molars, and that PDL cells under mechanical stress may induce osteoclastogenesis through up regulation of RANKL expression during orthodontic tooth movement.

Kau CH, Nguyen JT, English JD (2010)³⁸ The purpose of this study was to determine the clinical effects of a cyclical force generating device on tooth movement and overall orthodontic treatment time. In addition, the levels of patient compliance and patient satisfaction were evaluated. Patients who were undergoing active orthodontic treatment with fixed appliances, with no previous history of orthodontic treatment, were invited to participate in this study. The subjects were instructed to use the device for 20 minutes daily for a period of 6 consecutive months. Rates of tooth movement, patient compliance, and patient perception data were evaluated. Fourteen patients completed the study. The total rate of movement for the mandibular arch was 0.526 mm per week or 2.1 mm per 28-day month; the total rate of movement for the maxillary arch was 0.759 mm per week or 3.0 mm per 28-day month. There was a statistically significant difference between the rates of movement in the maxillary arch compared with the mandibular arch ($p > 0.05$). The patient compliance rate as measured by data recorded from the devices by the study engineer indicated a 67% compliance rate. Overall patient satisfaction with the device increased over the course of treatment time for most variables as indicated by the mean scores. Paired t-test value indicated that there was a statistically significant change in overall satisfaction for all variables measured with the exception of drooling and noise. It was concluded that the rates of movement of teeth during orthodontic treatment were increased with the use of the device; patient compliance with use of device was 67%; and patient acceptance of and compliance with the device was clinically significant.

Teixeira CC, Khoo E, Tran J, et al. (2010)³⁹ hypothesized that stimulating the expression of inflammatory cytokines, through small perforations of cortical bone, increases the rate of bone remodeling and tooth movement. Forty-eight rats were divided

into 4 groups: 50-N force applied to the maxillary first molar (O), force application plus soft tissue flap (OF), force application plus flap plus 3 small perforations of the cortical plate (OFP), and a control group (C). From the 92 cytokines studied, the expression of 37 cytokines increased significantly in all experimental groups, with 21 cytokines showing the highest levels in the OFP group. After 28 days, microcomputed tomography, light and fluorescent microscopy, and immunohistochemistry demonstrated higher numbers of osteoclasts and bone remodeling activity in the OFP group, accompanied by generalized osteoporosity and increased rate of tooth movement.

Suamphan et al (2010)⁸ found significantly higher rate of canine movement on experimental side where 60g of orthodontic force was applied in combination to mechanical vibratory stimulus from electrical tooth brush of frequency 125 Hz at T1(1 month of canine retraction) and T2(3 months of canine retraction) . They found trend of IL-1 beta for control group as $T3(132.73 \pm 205.01) > T2(107.98 \pm 208.04) > T0(81.85 \pm 156.26) > T1(56.62 \pm 87.21)$ and trend for Accedent group was $T0(41.72 \pm 33.32) > T2(29.57 \pm 27.72) > T3(26.98 \pm 22.92) > T1(26.97 \pm 10.84)$. However, intergroup comparision at each time showed statistically non significant difference.

Showkatbakhsh R, Jamilian A, Showkatbakhsh M. (2010)⁴⁰ found that that with exposure to pulsed electromagnetic field, canine retraction was significantly more than the control group ($P < .001$) after 5.0 ± 0.6 months, suggestive of favorable affect of physical stimulation method on acceleration of tooth movement.

Gkantidis N, Christou P, Topouzelis N. (2010)⁴¹ assessed showed acceleration of canine retraction rate with corticotomy during the first month of-therapy ($WMD = 0.73; p < 0.01$) and with low- intensity laser ($WMD = 0.42$ mm/month; $p < 0.001$) in a period longer than 3 months in comparision to control group.

Madureira DF, Telles CS, Da Silva JS, et al. (2012)⁴² found Interleukin (IL)-6 (IL-6) production was significantly elevated in the PDL on day 1 after force application. Significantly strong positive correlations between GCF and PDL in experimental group were seen on days 3 (interferon-gamma), 7 (IL-10), 14 (IL-17A), and 28 (IL-17A, tumor necrosis factor- alpha), and significantly strong negative correlation were seen on days 14 (interferon- gamma) and 21 (IL-2, IL-10).

Hamamci O, Başaran G, Tözüm TF. (2012)⁴³ assessed and found insignificant difference in the level of IL-6 from GCF collected around implants and distobuccal site

of maxillary canines i.e. treatment group and mesial to second premolar(control) during canine distalization using NiTi closed coil spring.They also found statistically nonsignificant difference on intragroup comparison of T1 with other time interval ,however they did not make intragroup comparison between all time intervals.

Zhang C, Li J, Zhang L, Zhou Y, Hou W, Quan H, et al. (2012)⁴⁴ isolated human periodontal ligament stem cells(PDLSC) from premolar teeth and randomised into vibration and static cultures. The effect of vibration on PDLSC proliferation ,differentiation and osteogenic potential was assessed at genetic and protein level.Alkaline phosphatase activity was gradually increased with the frequency of vibration, to a peak at 50 Hz, and the level of osteocalcin was significantly higher than control following vibration at 40 Hz, 50 Hz, 60 Hz, 90 Hz and 120 Hz. Levels of Col-I, Runx2 and Osterix were significantly increased by LMHF mechanical vibration at frequencies of 40 Hz and 50 Hz.The author suggested beneficial effect of vibration of cell turnover.

Tuncer BB (2013)⁴⁵ evaluated and found non -significant difference in levels of RANKL with time ,on retracting the maxillary canines by sentalloy closed coil springs with a continuous force of 200gm on the segmental .016x.022SS wire, four weeks after 1st premolar extraction .The mean value was highest at 7 days ($1.29 \pm 1.0 \text{ pmol/l}$)>at baseline(pre-treatment- $1.19 \pm .71 \text{ pmol/l}$) > at 1st month($1.04 \pm .50 \text{ pmol/l}$)>then at 24hrs($.96 \pm .55 \text{ pmol/l}$)>least at 1 hr ($.83 \pm .30 \text{ pmol/l}$).

Meeran NA (2013)⁴⁶ the various neurotransmitters, growth factors, interleukins, leptins and enzymes like aspartate aminotransferase, cathepsin K and matrix metalloproteinases have the potential to serve as biological markers in order to monitor and validate orthodontic tooth movement.

Gkantidis N, Mistakidis I, Kouskoura T, Pandis N (2014)⁴⁷ assessed the available evidence on the effectiveness of accelerated orthodontic tooth movement through surgical and non-surgical approaches in orthodontic patients in a meta analysis.Two studies on corticotomy and two on low-intensity laser, which had low or unclear risk of bias, were mathematically combined using the random effects model. Higher canine retraction rate was evident with corticotomy during the first month of-therapy (WMD = 0.73;p < 0.01) and with low-intensity laser (WMD = 0.42 mm/month; p < 0.001) in a period longer than 3 months. The quality of evidence supporting the interventions is moderate for laser

therapy and low for corticotomy intervention. They concluded that there is some evidence that low laser therapy and corticotomy are effective, whereas the evidence is weak for interseptal bone reduction and very weak for photobiomodulation and pulsed electromagnetic fields.

Bowman SJ. (2014)⁴⁸ conducted a Randomized con-trolled trial for assessing accelerated tooth movement with subject using AcceleDent. They reported 2.71mm per month of initial maxillary dental alignment in 23 premolar-extraction patients—a 51% reduction in treatment time compared to the control group, with no adverse findings such as root resorption or pain. The differences in align-ment speed may be attributed to the typically faster tooth movement in the maxillary arch.

Kumar AA, Saravanan K, Kohila K, Kumar SS. (2015)⁴⁹ described all the biomarkers seen in GCF during orthodontic tooth movement such as prostaglandin E2 , substance P, Epidermal growth factor, Rankl, osteoprotegerin , granulocyte macrophage colony stimulating factor, alpha 2 microglobulin, Interleukin 1beta, 2, 8 (bone remodelling), myeloperoxidase, hyaluronic acid, Chondroitin sulfate, Pentaxrin, Osteocalcin, Insulin growth factors, Pyridinoline, deoxypyridinoline, N - telopeptine, Dentine matrix protein, acid phosphatase, Alkaline phosphate(bone forming biomarker), Aspartase amino transferase, Cathepsin B, Matrix metalloproteins, beta glucuronidase, Lactate dehydrogenase etc. The roles of these biomarkers gives a better understanding of ongoing cellular process and clinical usefulness leading to proper choice of mechanical loading, to improve n shorten the period of treatment time, avoiding adverse consequences.

Woodhouse NR, DiBiase AT, Johnson N, Slipper C, Grant J, Alsaleh M, et al. (2015)⁵⁰ conducted a randomized clinical trial and investigated the effect of supplemental vibrational force (Aceledent) on rate of orthodontic tooth alignment with fixed appliances. In eighty-one subjects they found no evidence that supplemental vibrational force with an AcceleDent device can increase the rate of initial tooth alignment or reduce the time required to achieve complete alignment when used in conjunction with a preadjusted edgewise fixed appliance.

Pavlin D, Anthony R, Raj V, Gakunga PT (2015)²¹ conducted a RCT to assess the effect of vibratory (AcceleDent) on the rate of orthodontic tooth movement. The mean rate

of maxillary canine retraction was significantly higher for the AcceleDent® group with 1.16 mm/month compared to 0.79 mm/month in the control group.

Ahmed et al (2015)⁴ found elevated levels of IL-1 beta and TNF -alpha in saliva in subjects using AcceleDent® device (30Hz) in comparison to control group. The mean value of both salivary IL-beta (pg/ml) and TNF-alpha (pg/ml) among study group were higher than control group at T1, followed by T2, then T3 with statistically significant difference ($p < 0.05$).

Leethanakul C, suamphan S, Jitpukdeebodintra S, Thongudomporn U and Charoemratrote C (2015)² investigated the effects of application of vibratory stimuli on IL-1beta secretion in GCF during maxillary canine distalization in split mouth study design in 15 subjects. Light force of 60g was applied to canine for 3 months on both sides, however vibratory stimuli was provided using an electric toothbrush 15 minutes a day, on experimental side only. GCF was collected from mesial and distal sides of each canine at each monthly appointment. Both, at pressure and tension site, levels of IL-1beta was increased at baseline to soon after bracketing (T0) to 1 month after canine retraction and decreased thereafter at each monthly interval (T2, T3), but difference was significant at pressure side only for each group. On comparing groups significant difference was noted for levels of IL-1beta only at T3 on pressure side with high value on experimental side. This was similar to significantly high rate of tooth movement on experimental side than control side at T2 and T3. Results showed that difference was significant for pressure site only and the levels of IL-1beta decreased at each monthly intervals (T2 and T3).

Chouinard et al (2016)⁶ found no statistically significant difference in levels of biomarkers (IL-1-beta, IL-8 and TNF-alpha in saliva) during orthodontic tooth movement with fixed appliances and vibration device (AcceleDent=30Hz) in a randomized control trial. The biomarker secretion was assessed at T0 (before bonding), T1 (5-6 weeks after bonding), T2 (10-12 weeks), T3 (15-18 weeks). Trend for IL-1-beta level for AcceleDent group ($T0 = 41.72 \pm 33.32$ pg/ml $> T2 = 29.57 \pm 27.75$ pg/ml $> T3 = 26.98 \pm 22.92$ pg/ml $> T1 = 26.97 \pm 10.84$ pg/ml) and for control group $T3 = 132.73 \pm 205.01$ pg/ml $> T2 = 107.98 \pm 208.04$ pg/ml $> T0 = 81.85 \pm$

156.26pg/ml> T1= 56.62±87.21pg/ml.

BOWMAN J. (2016)⁵¹ have studied effect of vibration on molar distalization. Sixty-five adolescent Class II patients, were planned for upper-molar distalization, divided into vibration (V using acceleDent A) and control (C) groups. Non-significant differences were noted between the V and C groups in terms of gender, age, canine eruption, and third-molar status. There was a significant difference, however, with respect to second-molar eruption status. There were no statistically significant differences between the two groups with regard to upper first-molar tipping, intrusion, or crown distalization, or in the number of days required to achieve a super-Class I molar relationship. The patients in the V group demonstrated a 27% greater rate of crown movement (1.1mm per month) compared to the C group (.9mm per month; $p = .053$). They also showed 71% more movement of the molar root apex (2.9mm vs. 1.7mm per month; $p = .03$).

Castroflorio T, Gambero EF, Caviglia GP, Deregibus A. (2017)⁵² found significantly elevated levels of RANKL on experimental side i.e. where isolated 2nd molar was distalised using aliners whereas contralateral side 2nd molar was used as control at T1. The trend for RANKL on test side was $T3(2.5 \pm 2.1 > T2(1.3 \pm 0.9) > T1(1.2 \pm 0.8) > T0(0.5 \pm 0.4)$ with statistical difference at T1 ($P=.004$).

Wu Q, Zhou X, Huang D, Ji Yingchen, Kang F (2017)²⁰ investigated the inflammatory factors in serum 10 days- after orthognathic surgery . They treated the osteocyte-like cell line MLO-Y4 with recombinant mouse IL-6 and IL-6 receptor (IL-6R), and used quantitative RT-PCR and Western blotting to explore Receptor activator of nuclear factor- κ B ligand (RANKL) expression at both the mRNA and protein level. They found that IL-6 and RANKL were stimulated in serum 3-7days after orthognathic surgery. Therefore, IL-6 and IL-6 receptor enhanced the expression of RANKL at both the mRNA and protein level in MLO-Y4 cell line.

Miles P, Fisher E, Pandis N. (2018)⁵³ conducted a RCT to assess the effect of the AcceleDent A appliance on the patient who had undergone maxillary premolar extractions during space closure in forty class II adolescents. The space was measured parallel to the occlusal plane from the cusp tips of the teeth mesial and distal to the extraction spaces. Results showed no clinically or statistically significant difference in the rate of space closure both in control and AccleDent groups.

DiBiase AT, Woodhouse NR, Papageorgiou SN, Johnson N, Slipper C, Grant J, et al. (2018)⁵⁴ did not found statistically significant increase in rate of space closure both in acceledent and control group at baseline, T1(start of space closure), T2(first visit after T1), T3(end of space closure), T4(completion of treatment).

Jain S, Raghav P, Khera A, Arya A. (2018)⁵⁵ found no significant difference in rate of orthodontic tooth movement after first, second and third month consecutively on comparison between control and experimental site using powered tooth brush(60Hz frequency) , whereas fourth month showed less tooth movement of experimental side as compared to control side.

Azeem et al (2019)⁵⁶ found the amount of tooth movement was similar for canines on the vibration side and on the non-vibration side (mean 0.81 ± 0.10 mm and 0.82 ± 0.11 mm, respectively, $p > 0.05$). Acceleration of rate of tooth movement had variable results in above mentioned studies. Though we did not measure the rate of tooth movement, in present study, however levels of IL-6 were highest at T3 in Group II, suggestive of fact that change at molecular level on applying mechanical stresses were reinforced by vibratory stimulus. This was also stated by Leethankal² that there is increase in number of osteoclast on compression side with enhanced expression of biomarkers. Thus, it could be anticipated that higher levels of IL-6 at T3 will also contribute to increased rate of tooth movement on application of vibratory stimulus. However similar trend was not seen in levels of RANKL that could be due to the fact that release of RANKL is dependent, not on IL-6 alone rather other biomarkers also influnce its release. Additional factors like age, sex, bone molecular density, variation in cellular activity in each individual would also control release of biomarker as tooth movement progresses.

Studies that recorded level of biomarkers on application of orthodontic force are discussed below.

Jayaprakash PK, Basavanna JM, Grewal H, Modi P, Sapawat P, Bohara PD. (2019)⁵⁷ found significantly elevated levels of IL-6 in GCF on moving canine distally by elastic chain exerting an initial force of 250 g on experimental side only in subject with age range $\pm$ of 15.9 years at 24 hours concentration of IL-6 was higher (0.065 ± 0.005 * pg / μ g) than at baseline(0.030 ± 0.008 pg/ μ g), At 168 hr (7days) mean level of IL-6 (0.044 ± 0.007 pg / μ g) were higher than baseline but difference was not significant. On other side where no forces was

applied, there was decrease in level of IL-6 at 24 hr (0.016 ± 0.006 pg / μ * g) than at baseline (0.034 ± 0.0091 pg / μ * g) at 168 hr (0.036 ± 0.0091 pg / μ * g) and the difference was statistically non- significant between 168hr and baseline.

Mohammed A, K S, Mohindroo A, Shetty A, Shetty V, Rao S, Shetty P. (2019)⁵⁸ evaluated IL-6 in PDL of extracted 1 premolar each tooth was assigned a time point for its extraction at 2 hours, 15th day or the 30th day after application of force. Brackets were bonded to the experimental teeth at 5 degrees to the long axis of the tooth, 200-250 g of force was exerted on placing 0.014 Ni-Ti wire. They found a significant increase on experimental teeth in the level of IL-6 on 15th day (14.91) than on 30th day (11.1) and least was at 2hrs (3.4) which was non- significant in comparison to control

Siriphan N, Leethanakul C, Thongudomporn U. (2019)³ used electric powered tooth brush at 30Hz and 60Hz and RANKL and OPG levels were analysed immediately before canine distalization (T1) , at 24 hours (T2), 48 hours (T3) and 7 days (T4) and 3 months (T5). They found rate of canine distalization was not significantly different between experimental groups and control group. No within- or between-group differences in RANKL and OPG were observed ($P > 0.05$), for 30 Hz and 60 Hz group , however RANKL on the control side showed statistically significant difference on intragroup comparison with highest value at T2(450.34 pg/ml) > T4(428.63 pg/ml) and T3(425.34) > T1(189.48) ($P < 0.001$).

Ahmed N, Bhat KR, Joseph R, Younus A. (2020)⁵⁹ reported many biomarkers in the GCF during the course of orthodontic tooth movement. These biomarkers provide vital information about the micro-environment. These include inflammatory mediators like Prostaglandin E-2, Substance P, Epidermal growth factor, Transforming growth factor, Rankl, Osteoprotegerin, Granulocyte macrophage colony stimulating factor, Alpha-2 microglobulin , Interleukin 1 beta, 2,6,8, Myeloperoxidase enzyme in PMN and Metabolic products of paradental remodeling like Hyaluronic acid, Chondroitin, Pentaxrin-3, Osteocalcin, Insulin growth factor, Pyridinoline, deoxypyridinoline, N-telopeptide, Dentin matrix protein. Besides this they also contain Enzymes like Acid phosphatase, Alkaline phosphatase, Aspartate amino transferase, Cathepsin B, Matrix metalloproteins (1,2,8), Beta glucuronidase, Lactate dehydrogenase. The biomarkers in the GCF reflect the changes occurring during orthodontic treatment.

Reiss S, Chouinard MC, Landa DF, Nanda R, Chandhoke T, Sobue T et al. (2020)⁶⁰ compared the effect of supplemental vibratory force Accedent on biomarkers of bone remodelling during mandibular anterior alignment (MAA) on forty patients (aged 15–35yrs) with that of control group . Salivary multiplex assay was completed to analyse the concentration of various biomarkers (TNFa,IL-6,MMP9,IL-3, IL-8, MMP1, MMP8, IL-1b, Dkk1, MMP13, SOST, RANKL, OPN, IL-11, IL18, OPG,TGFb1, RANK/OPG) of bone remodelling before treatment (T0) and at three following time points (T1, T2, T3), 4–6 weeks apart.The results showed that no significant difference in the changes in salivary biomarkers of bone remodelling between Accedent® and control group at any time point over the trial duration . So they concluded that supplemental vibratory force during orthodontic treatment with fixed appliances did not affect any biomarkers of bone remodelling .

Garg A.K, Tikku T, Srivastava K, Khanna R, Maurya RP, Verma SL (2021)⁶¹ in a clinical study on 24 subjects evaluated Intermittent vibratory stimulation to accelerate tooth movement using electric powered tooth brush to accelerate individual canine retraction using 150 gm of force with NiTi coil spring.In a split mouth study design, rate of tooth movement was measured on study models at time interval, T0(start of canine retraction), T1 (after 1st month), T2 (after 2nd month) and T3 (after 3rd month).There was an increased rate of canine movement on the side of vibratory stimulation (right side) in comparison to the control side (left side) for all time intervals and can be used to decrease treatment time in adults.

Agrawal A and TM Chouribe (2021)⁹ assessed the effect of vibrational force on biomarkers for orthodontic tooth movements in a systematic review .Only one study showed highly statistically significant levels of IL-1 beta with <.001.The outcomes of studies were found to be mixed due to methodological heterogeneity due to different devices and frequencies of vibration. Rate of tooth movement was correlated with levels of released biomarkers under the influence of vibrational force in these studies, but it was found to be significant only in few studies. Results of all RCTs advocated that various kinds of biomarkers change due to changes in movement of teeth.

Shaikh A. (2021)¹⁸ found elevated levels of IL-6 and TNF-alpha in GCF using Low-Intensity laser therapy (LILT) in comparison to control at day 2, day 9, day 29, day 49

and day 60. The trend for IL-6 in experimental group was {Day 02=0.959 > Day 29=0.909> Day 09= 0.894> Day 60= 0.847} and in control group trend was Day 02=0.651=Day 09=0.651< Day 29=0.586< Day 60=0.477}.

Zheng J, Yang K. (2021)⁶² found trend for the level of RANKL on study side where low level laser therapy (LLLT) during orthodontic tooth movement was used for canine retraction as Day 21=2.48 ± 0.47 >Day 14 = 2.20 ± 0.17 >Day 7= 2.17 ± 0.58> Day 29= 1.97 ± 0.17 >Day 0= 1.91 ± 0.19 and on control side trend was as Day 21= 2.15 ± 0.29 >Day 0=2.07 ± 0.48 > Day 14= 1.98 ± 0.34 > Day 28= 1.88 ± 0.36 > Day 7= 1.80 ± 0.21. However the levels of RANKL showed statistically non significant difference compared to baseline in control group at all time intervals, however, in study group the level was significant only at Day 21, when compared to baseline.

Akbar A, Ur Rehman A, Fatima M. (2022)⁶³ found there was no statistically significant difference in the amount of tooth movement between the experimental and control side (p= 0.22) on using electric tooth brush.

Gujar AN, Shivamurthy PG. (2023)⁷ found significantly high rate of tooth movement in both groups that used electric toothbrushes with 125 Hz or 150Hz in comparison to non vibratory group at each time interval I.e. T0-T1,T1-T2,T2-T3. They analysed a different biomarker PGE2 where levels elevated significantly on using electric powered tooth brush in comparison to control and levels increased more with higher frequency brush at T1,T2,T3. Intragroup comparison revealed statistically significant difference in mean levels between different time intervals in each group, however they did not compare each group separately with one another.

Shaadounh RI, Hajeer MY, Al-Sabbagh R, Alam MK, Mahmoud G, Idris G. (2023)⁶⁴ conducted a clinical study at the Department of with six patients in whom extraction of upper first premolars followed by en-masse retraction was done followed by electrical stimulation on the maxillary anterior region . The results showed that mean treatment duration to complete the en-masse retraction was 5.66±0.81 months, concluding that low-intensity direct electrical current could be an effective method to accelerate orthodontic movement.

Pascoal S, Oliveira S, Ascione M, Pereira J, Carvalho Ó, Pinho T. (2024)¹⁰ in a systematic review assessed the impact of high (>30 Hz) and low (≤30 Hz) frequency vibrations on orthodontic tooth movement (OTM). Low-frequency vibrations did not

seem to accelerate OTM with aligners or fixed appliances, despite some positive outcomes in certain studies. Conversely, high frequency vibrations were linked to increased aligned tooth movement with or during space closure with fixed appliances. In vivo studies reported favourable results with high-frequency vibrations (60 Hz to 120 Hz), which stimulate bone biomarkers, facilitating alveolar bone remodelling and suggested larger-scale research is needed to strengthen its potential in orthodontics.

This in vivo study was conducted in Department of Orthodontics and Dentofacial Orthopaedics, Babu Banarasi Das College of Dental Sciences, BBD university in collaboration with Department of Biochemistry KGMU, Lucknow with the aim to evaluate and compare the level of biomarkers (IL-6 and RANKL) in GCF during space closure (using sliding mechanics) using vibratory stimulus (with electric powered tooth brushes) at different time intervals. The sample for the study consisted of 15 patients with a clinical presentation of class I type 2 malocclusion (Bi-maxillary protrusion) and class II Div I malocclusion (18-25yrs) undergoing fixed Orthodontic treatment who required first premolar extraction as a part of treatment. The subjects were divided into two groups based on use of vibratory stimulus. Maxillary canine of left side-of all subjects were grouped as Group I(control) where no vibratory stimulus was provided. Group II (experimental side) included maxillary canine of right side where vibratory stimulus was provided using electric powered brush. GCF collection was done from distal surface of maxillary canine at different time (baseline, 24 Hours, 20 Days, 2 Months).

Criteria for sample collection:

After careful evaluation of all diagnostic records (Lateral cephalograms, O.P.G, Study models) samples for the study were selected based on following criteria-

Inclusion criteria:

1. Adult patients with range of 18-25 years.
2. Patients with Angle's class I Bi-mallixary protrusion or Class 2 div 1 malocclusion in whom extraction of all first premolars is planned as a part of Fixed orthodontic treatment.
3. Patient having full complement of teeth till second molar.
4. All subjects in good general health with healthy periodontal tissues.
5. No radiographic evidence of bone loss in any tooth.

EXCLUSION CRITERIA:

1. Patients having history of chronic diseases like diabetes, hypertension.
2. History of taking drugs changing the hormonal balance such as contraceptives, antihistamines or non-steroidal anti-inflammatory drugs, Bisphosphonate in the past four months or during the fixed orthodontic treatment.

3. Patient not willing to participate in the study.
4. A total of 15 subjects based on above mentioned inclusion and exclusion criteria were selected.

Ethical committee Approval:

Approval from the Ethical and Research committee of Babu Banarasi Das College of Dental Sciences; BBD University was taken prior to conducting the study. Written informed consent was obtained from the selected subjects participating in the study before beginning of the treatment as per the guidelines of university by explaining them the purpose, procedure and consequences of the study.

Materials and Equipments used in the study:**A. Material used for diagnosis and making impression:(Fig.1)**

1. Gloves
2. Mouth-mask
3. Mouth -mirror
4. Tweezer
5. Explorer
6. Kidney tray
7. Cheek retractor
8. Photographic Mirror
9. Impression tray
10. Bowl
11. Spatula
12. Alginate impression material (NEOALGIN)
13. Orthokal



FIG.1- Materials used for Diagnosis and Impression Making:

- | | | | |
|--|---------------|-------------------|-----------------------|
| 1. Gloves | 2.Mouth-mask | 3.Mouth-mirror | 4.Tweezer |
| 5. Explorer | 6.Kidney tray | 7.Cheek retractor | 8.Photographic Mirror |
| 9. Impression trays | 10.Bowl | 11.Spatula | |
| 12. Alginate Impression material(NEOALGIN) | 13.Orthokal | | |

B. Materials used for Cephalometric Analysis(Fig 2)

1. Lateral cephalogram
2. Tracing sheet
3. Pencil
4. Scale
5. Protractor
6. Set square

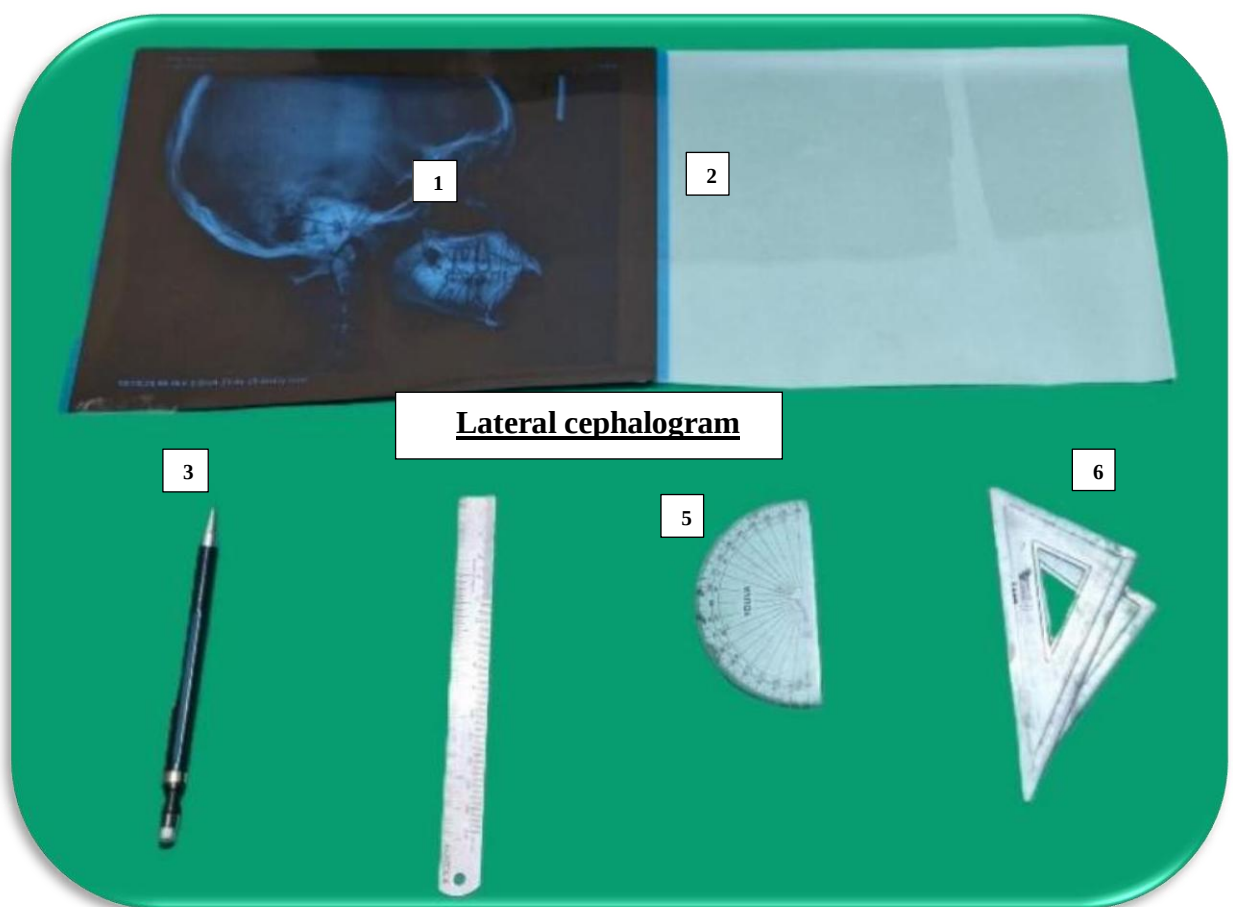


Fig.2: Materials used for cephalometric analysis:

1. Lateral cephalogram
3. Pencil
5. Protractor

2. Tracing Paper
4. scale
6. Set Square

C. Materials used for fixed orthodontic treatment(Fig.3)

1. Cheek retractor
2. Separators
3. Separator placing plier
4. Cotton Rolls
5. Preformed bands with buccal tube on molar as per size with (3M,Unitek)
6. Glass Ionomer cement(luting)
7. MBT Brackets(0.022)
8. MBT Gauge
9. Bracket Holding tweezer
10. Bonding materials:
 - A. Etchant(D-tech)
 - B. Primer(D-tech)
 - C. Bonding adhesive(D-tech)
 11. Light cure gun
 12. Ligature wire
 13. Ligature wire cutter
 14. Arch wire NiTi(0.012,0.014,0.016,0.018,0.020,0.16x0.22,0.17x0.25,0.19x0.25)
and SS(0.12,0.014,0.016,0.018,0.20,0.16x0.22,0.17x0.25,0.19x0.25(Round and Rectangular)
 15. Modules
 16. Mathieu Haeden plier
 17. Distal end cutter

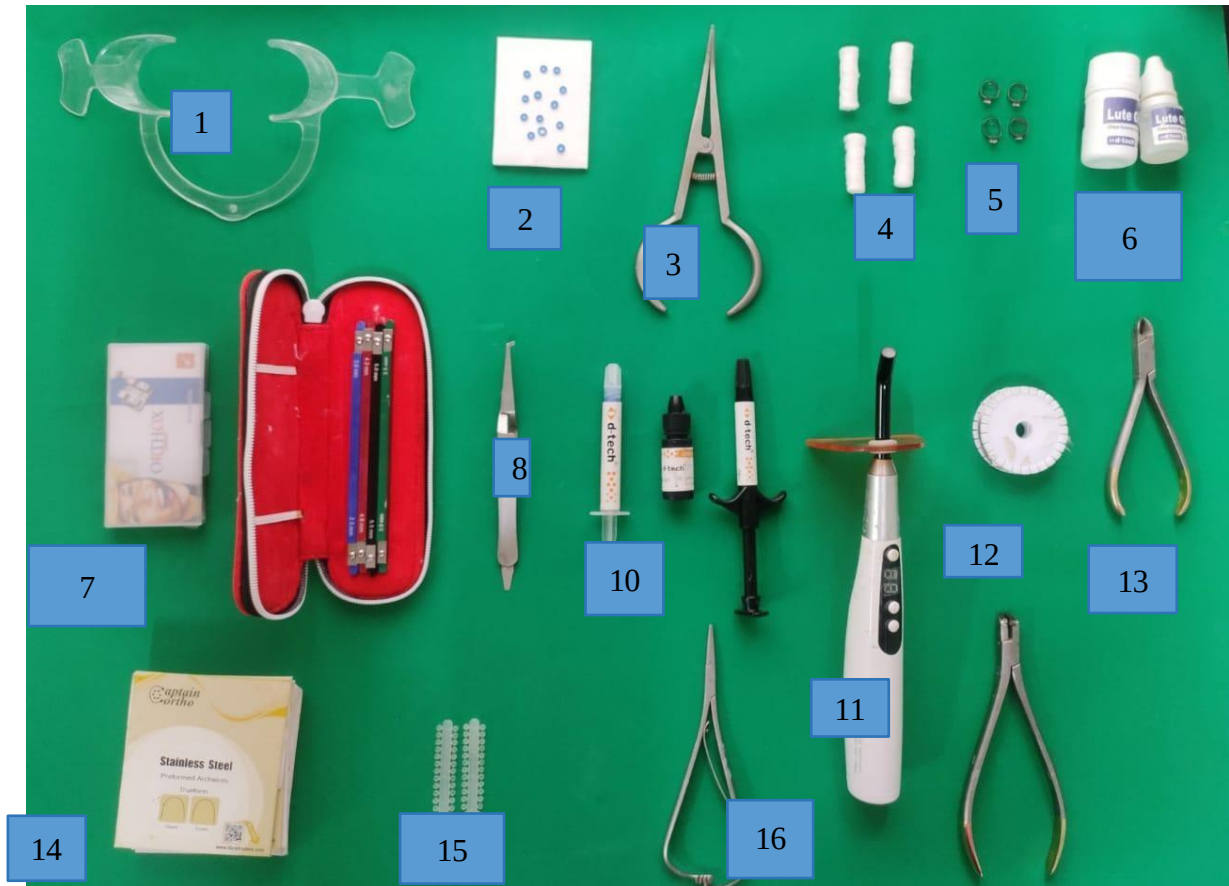


Fig 3: Materials used for fixed orthodontic treatment:

- | | | |
|---|--------------------------|----------------------------|
| 1. Cheek retractor | 2. separators | 3. Separator placing plier |
| 4. Cotton rolls | 5. Pre-formed bands | 6. GIC (luting) |
| 7. MBT bracket kit (0.022) | 8. MBT gauge | 9. Bracket placing plier |
| 10. Bonding materials (etchant, primer, bonding adhesive) | 11. Light cure gun | |
| 12. Ligature wire | 13. Ligature wire cutter | 14. Arch wires |
| 15. Modules | 16. Mathieu plier | 17. Distal end cutter |

D. Material used for space closure(Fig.4):

1. 019"x.025" SS arch wire
2. Crimpable hook
3. Crimpable Plier
4. Niti closed coil spring
5. Dontrix gauge

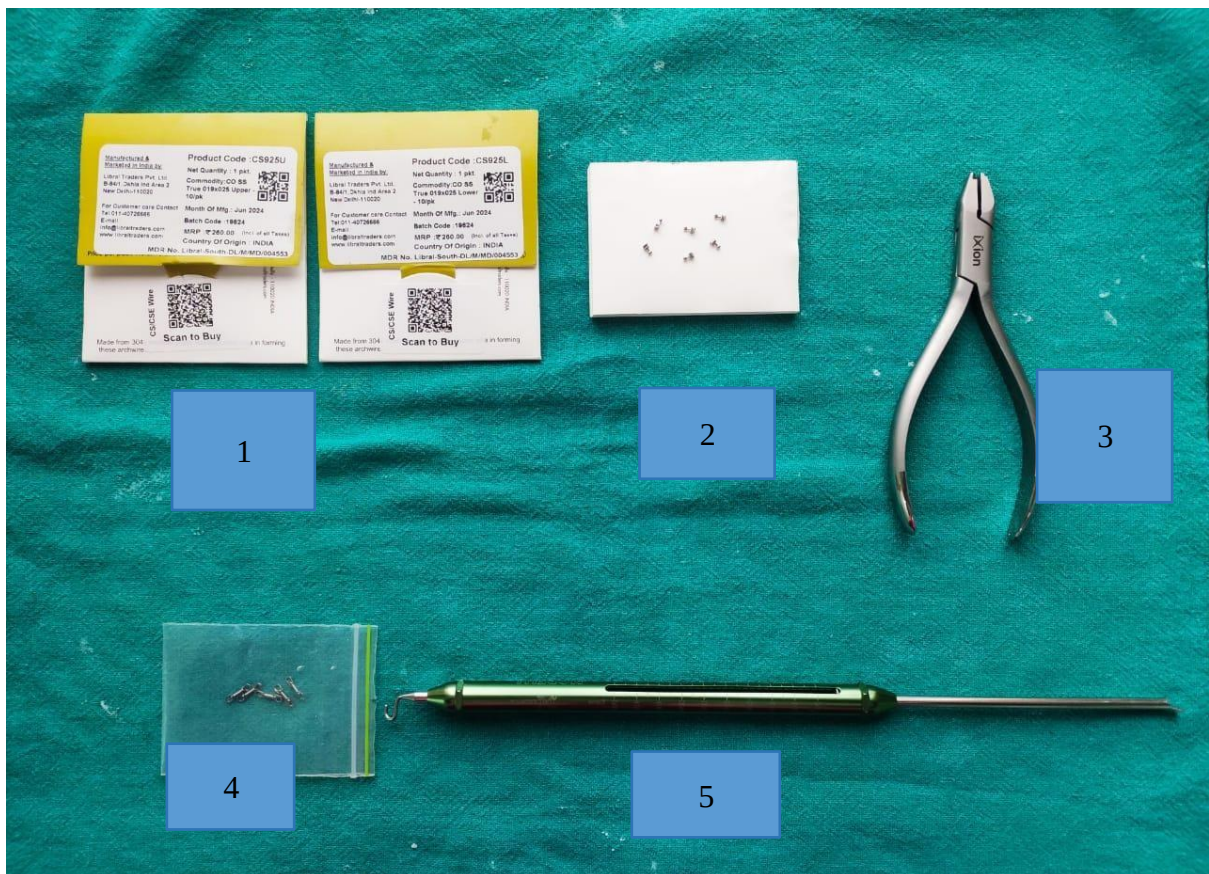


Fig.4-Material used For Space closure:

- | | |
|----------------------------|---------------------------|
| 1. 019"x.025" SS arch wire | 2.Crimpable hooks |
| 3. Crimpable plier | 4.Niti closed coil spring |
| 5. Dontrix gauge | |

E. Material used for vibratory stimulus :

Electric powered tooth brush(Perfora)

Specification: {Medium mode =18000strokes/min= 300 HZ}



Fig 5: Electric powered tooth brush used for vibratory stimulus in study.

F. Materials used for GCF Collection (Fig.6)

1. Mouth wash
2. Gloves
3. Mask
4. Cotton rolls
5. Cheek retractor
6. Absorbent points
7. Suction tips

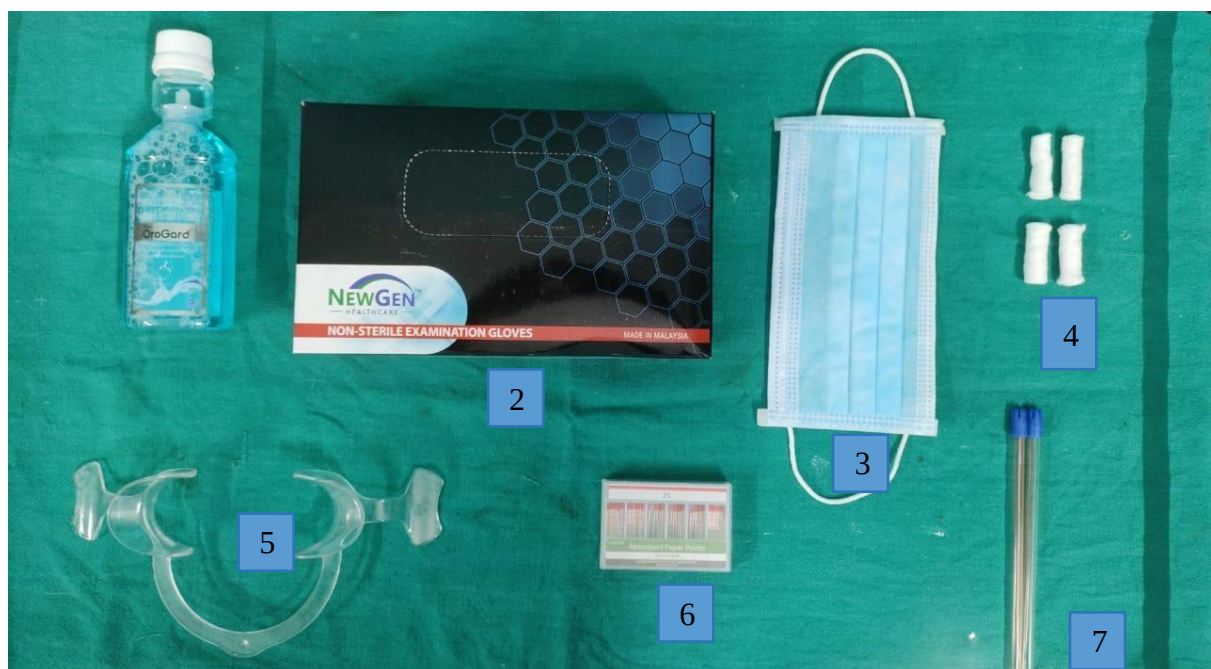


Fig.6:Materials for GCF collection

- | | | |
|-----------------|--------------------|---------------------|
| 1. Mouth wash | 2. Gloves | 3. Masks |
| 4. Cotton Rolls | 5. Cheek retractor | 6. Absorbent points |
| 7. Suction tips | | |

G. Materials used for storage and transfer of GCF(fig.7):

1. Eppendrof tube
2. Phosphate buffer solution(SRL)
3. Ice box for interim storage of sample at 4 degree C.
4. Deep freezer for storing the sample till ELISA test (-80 degree C)



Fig.7:Materials used for storage and transfer of GCF:

- | | |
|--|------------------------------|
| 1. Eppendroff tube | 2. Phosphate buffer solution |
| 3. Ice storage box for interim storage | 4. Deep freezer |

H. Materials used for assessment of biomarker(fig.8):

1. Centrifugation machine
2. Incubator
3. ELISA kit for RANKL (Elabsciences)
4. ELISA kit for IL-6(Elabsciences)
5. .ELISA analyser system(Thermoscientific)



Fig 8: Materials used for assessment of biomarker levels:

1.Centrifugation machine
3.ELISA kit for RANKL
5.ELISA analyser system

2.Incubator
4.ELISA kit for IL-6

METHODOLOGY:

A. For sample selection:

The subjects who came to the Department of Orthodontics and Dentofacial Orthopaedics, BBDCODS,BBDU,Lucknow, with Angle's class I Bi-maxillary protrusion or Class II Div 1 malocclusion , and who fulfilled inclusion and exclusion criteria of study were selected based on clinical examination and study materials.Lateral cephalograms were traced and analysed along with analysis of study models to decide the need of extractions as well as the tooth to be extracted.The subjects in whom extraction of all premolar was planned were selected.

B. For Fixed Orthodontic treatment:

- 1.Brackets of conventional MBT prescription(0.022 slot) was bonded on selected cases following standard protocol.Bands were cemented on molars.
2. Arch wire sequencing was followed for alignment and levelling as per case requirement .(Fig.9)

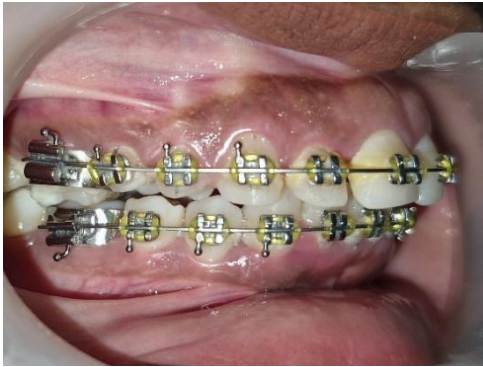


Fig.9: Intraoral photograph of banding and bonding done for one representative subject of study.

First Premolars were extracted in Department of Oral And Maxillofacial surgery under local anaesthesia for all subjects for study.The time of extraction was decided as per individual case requirement.(Fig10)

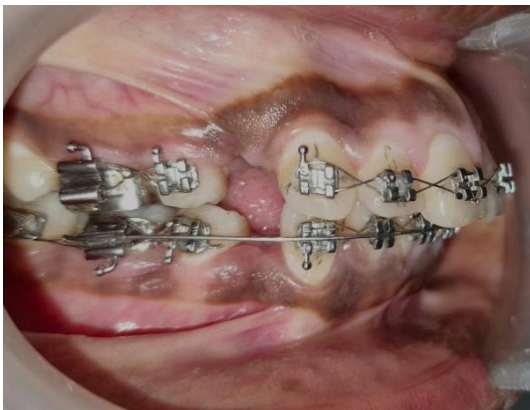


Fig.10: First premolar extractions done for one selected subject of the study.

E.For space closure:(Fig.11,12)

Space closure was initiated when Alignment and leveling was completed and 0.19"x0.25" SS could be placed passively. For space closure crimpable hooks (4mm length) were crimped on .019"x.025" stainless steel wire between lateral incisor and canine.(Fig.11)

2. Space closure was initiated using Niti closed coil spring extended from crimpable hook ,to the hook of buccal tube on maxillary 1st Molar band.

3. Force applied by NiTi closed coil spring using Dontrix gauge and , measured on both sides using Dontrix gauge for stadardization force of 200g was applied in all subjects. .(Fig.12)

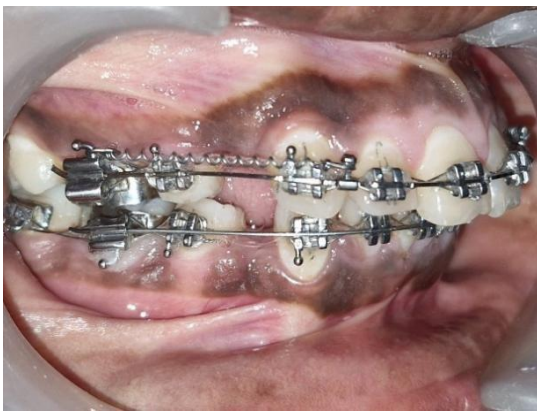


Fig.11:Space closure with NiTi coil spring.



Fig.12:Measurement of optimal force using Dontrix Gauge

F.For Vibratory stimulus:

1. Patient was briefed about control side(left maxillary canine) and experimental side(Right maxillary canine) .
2. All the patients were asked to continue their regular tooth brushing for 2 to 3 mins a day with conventional toothbrush.
3. Patients were instructed not to clean their teeth with electric powered tooth brush rather they were told to hold the toothbrush at desired site to apply mechanical vibration only on the experimental side.
4. On the experimental side patients were instructed to apply the vibratory stimulus through electric powered tooth brush on distal part of maxillary canine of Right side on medium mode(300Hz frequency) for 5 minutes 3 times daily (at regular intervals) for a period of 2 Months (Fig.15).
5. Chart was given to each patient(Table:1)where they could tick mark the number of times they used powered toothbrush.The patient who were not compliant and did not fill the chart properly were excluded from the study.



Fig. 13: Representative patient applying vibratory stimulus on experimental (right side) distal to maxillary canine

No. Of days	Morning (7am-8am)	Evening (3pm-4pm)	Night (9pm-10pm)
Day 1			
Day 2			
Day 3			
Day 4			
Day 5			
Day 6			
Day 7			
Day 8			
Day 9			
Day 10			
Day 11			
Day 12			
Day 13			
Day 14			
Day 15			
Day 16			
Day 17			
Day 18			
Day 19			
Day 20			
For 2 Month			

Table 1: Chart to record timing of using Powered toothbrush
--

G.Pre collection procedure:

The participants were seated comfortably in a dental chair and the procedure for GCF collection was explained to them. The subjects were asked to rinse their mouths with 0.012% chlorhexidine mouthwash. Before collection of GCF, any supragingival soft deposits were removed without causing trauma to the gingival crevice. If any haemorrhage was evident during this procedure, no fluid was collected that day. The target site was then thoroughly irrigated with distilled water, isolated by cotton rolls, cheek retractors and dried by an air syringe.(Fig.14).

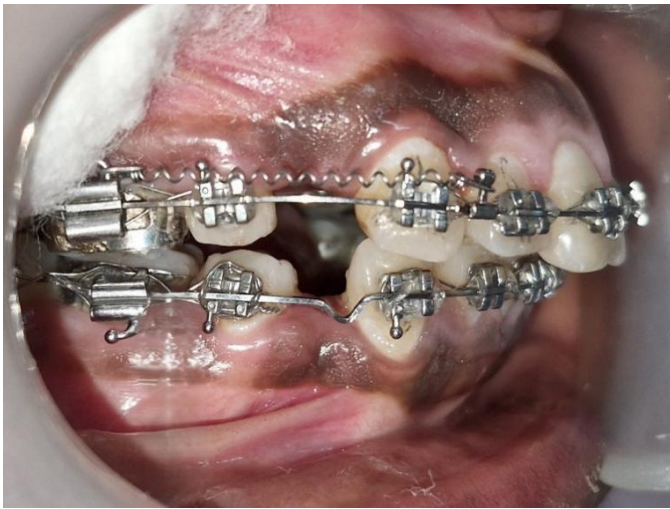


Fig.14. Preparation of site for GCF collection

H. GCF SAMPLE COLLECTION:

1. GCF sample at baseline (T0) was collected soon after placement of 0.019X0.025SS with crimpable hook and before starting retraction using NiTi closed coil spring with a force of 200gms.
2. The absorbent / paper points were gently inserted into the gingival crevice of the selected site (distal to maxillary canine of right and left side) at a depth of 1-2mm for approximately 40 sec. Thereafter absorbent points were removed gently from the sulcus avoiding saliva/ blood contamination. If contamination occurred with blood or saliva, absorbent points were discarded, GCF collection was done later. **(Fig.17 a ,b)**



**Fig.15(a): Absorbent point
distal to maxillary left
Canine(control)**

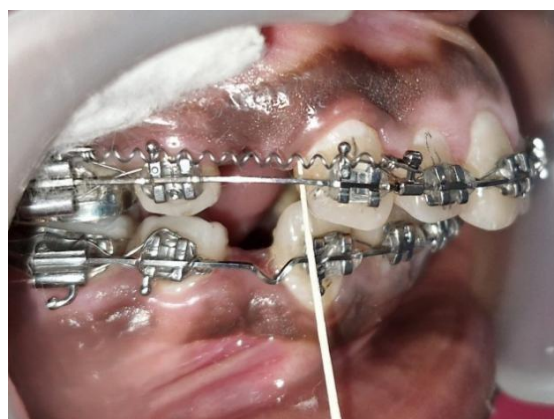


**Fig.15(b): Absorbent point
distal to Right maxillary
canine (experimental)**

4. The same protocol for GCF collection was followed at different time (Table 2):

Different Time of GCF collection				
Groups	T0	T1	T2	T3
Group I (N=15) (Left side) (Control Side)	Baseline	24 hrs	20 Days	2 Months
Group II (Right Side) (Experimental Side) (N=15)	Baseline	24 hrs	20 Days	2 Months

Table 2: Different time at which GCF samples were collected

I. Storage and Transportation of GCF (Fig.16)

Eppendorf tube with collected GCF were then kept in a ice box and placed in a standard freezer for interim storage until they could be transported to Biochemistry lab on the same day for final storage at -80 degree until further analysis.



Fig 16. Material for sample storage and transportation

a. Samples placed in icebox for interim storage.

b. Sample box with eppendorf tube for final storage in deep freezer

J. For Processing of sample: (Fig.17)

1. After collection of all samples of study, samples were processed at laboratory.
2. All frozen samples were thawed at room temperature (18-25° C) .All samples were centrifuged and mixed fully at time of testing for 30 seconds to ensure the complete elution of GCF from paper points. (Fig. 19 a)
3. After being centrifuged, the paper points were removed and supernatants were transferred into a new Eppendorf tube and kept in incubator.(Fig. 19 b & c)



Fig 17. Sample processing before ELISA analysis:
a. Centrifuge machine.
b. Supernatants were transferred into new Eppendorf tubes.
c. Incubator

K.For Biomarker Analysis (ELISA):

After completion of elution of GCF for all the sample, analysis for the assessment of IL-6 and RANKL level in the GCF was done. The method for reagent preparation was as follows-

1. The kit for either of the biomarker (IL6 or RANKL) to be tested was taken from the refrigerator 20 minutes in advance to bring all reagents to room temperature (18-25°C) before use.(Fig.20)
2. Dilute the 25x Concentrated Wash Buffer to 1x working solution.
3. Dilute the Reference Standard to different concentrations
4. Dilute the 100x Biotinylated Detection Ab/Ag to 1x working solution 15 minutes earlier before.
5. Dilute the 100x Concentrated HRP Conjugate to 1x working solution 15 minutes earlier before.



Fig.18:Reagent obtained from ELISA KIT for RANKL and IL-6

After the preliminary steps of reagent preparation microplate reader of ELISA analyser system was turned on and Assay procedure for ELISA was done as shown in following flow chart.

Assay procedure summary (Fig.19)

Add 100 µl of standard or sample to each well. Incubate for 90 minutes at 37°C
(Biomarker molecule present in the sample is bound to the wells by the immobilized antibody)



Remove the liquid. Add 100µl of Biotinylated Detection Ab/Ag Incubate for 1 hour at 37°C



Aspirate and wash for 3 times
(to remove unbound biotinylated antibody)



Add 100 µL of HRP Conjugate, Incubate for 30 minutes at 37°C
(positive well turn bluish in color)



Aspirate and wash for 5 times



Add 90 µl of Substrate Reagent. Incubate for 15 minutes at 37°C
(color of solution changes from blue to yellow)



Add 50 µl of Stop Solution. Determine the OD value at 450 nm immediately.



Absorbance of each well was read immediately on an ELISA reader
using 450nm as the primary wavelength.

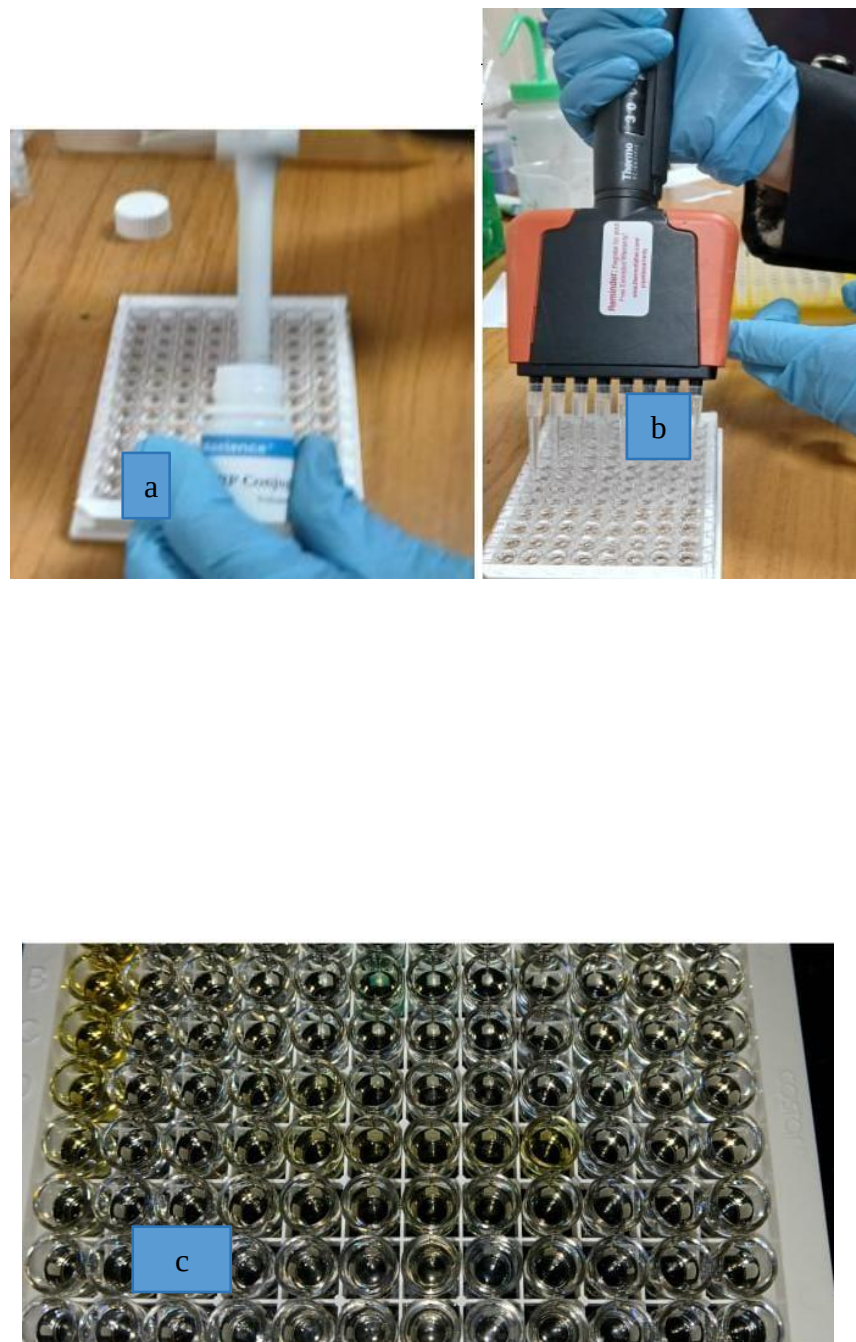


Fig 19(a,b,c)ELISA assay procedure.

a. washing of wells.

b. unbound biotinylated antibody, HRP-conjugated streptavidin is pipetted to wells.

c. Colour changes of positive solution from blue to yellow.

This in vivo study was conducted in Department of Orthodontics and Dentofacial Orthopaedics , Babu Banarasi Das College of Dental Sciences, BBD University in collaboration with Department of Biochemistry KGMU, Lucknow with the aim to evaluate and compare the levels of biomarkers (IL-6 and RANKL) during space closure (using sliding mechanics) using vibratory stimulus (with electric powered tooth brushes) in a split mouth study design at different time intervals. The sample for the study consisted of 15 patients in the age group of 18-25 yrs with a clinical presentation of class I type 2 malocclusion (Bi-maxillary protrusion)and class II Div I malocclusion (18-25yrs) undergoing fixed Orthodontic treatment and who required first premolar extraction as a part of treatment . Based on use of vibratory stimulus the samples was divided into two groups.Group I served as the control group and included canine of left side where no vibratory stimulus was provided. Group II included canine of right side where vibratory stimulus was provided using electric powered brush.GCF was collected from distal surface of maxillary canine at different time intervals (T0=baseline,T1=24hs,T2=20days,T3=2 months).

The result of present study are summarized as follows:

Table 1:Mean value of IL -6 and RANKL for Group I and Group II at different time intervals.

Table 2:Intragroup comparison of IL-6 for Group I at different time intervals.

Table 3: Intragroup comparison of mean values of IL-6 for Group II at different time intervals.

Table 4:Intergroup comparison of mean values of IL-6 between Group I and Group II at T0,T1,T2 and T3.

Table 5:Intragroup comparison of RANLK for Group I.

Table 6:Intragroup comparison of RANKL for Group II .

Table 7:Intergroup comparison of mean values of RANKL for Group I and Group II at each time interval.

Table 8: Overall comparison of Group I versus Group II

Table 1: Mean value of IL-6 and RANKL for Group I and Group II at different time :

				IL-6			RANKL
		Max. (pg/ml)	Min. (pg/ml)	Mean $\pm$ Std. Deviation (pg/ml)	Max. (pg/ml)	Min. (pg/ml)	Mean $\pm$ Std.Deviation (pg/ml)
Group I	T0(n=15)	3.46	2.29	2.8293 $\pm$ 0.290 19.39	27.62	19.39	22.94 $\pm$ 1.838
	T1(n=15)	3.23	2.14	2.518 $\pm$.333 18.99	25.52	18.99	21.995 $\pm$ 1.780
	T2(n=15)	3.20	2.15	2.724 $\pm$.238 20.11	30.15	20.11	23.207 $\pm$ 2.454
	T3(n=15)	3.44	2.29	2.669 $\pm$.344 20.17	27.06	20.17	23.202 $\pm$ 2.367
Group II	T0	5.99	2.43	3.0 238 $\pm$ 0.866	33.79	18.68	25.08 $\pm$ 4.60
	T1	6.87	3.90	4.418 $\pm$ 0.711	40.78	23.84	30.215 $\pm$ 5.471
	T2	8.21	3.88	4.499 $\pm$ 1.095	35.14	23.23	29.168 $\pm$ 2.800
	T3	7.54	3.92	4.568 $\pm$.933	39.10	22.62	28.327 $\pm$.741

The mean value of IL-6 for Group I was highest at T0(2.8293 $\pm$.290 pg/ml), followed by T2(2.7245 $\pm$.23841 pg/ml), then T3(2.669 $\pm$.34490pg/ml), and least was at T1(2.5187 $\pm$ 0.334 pg/ml).(T0>T2>T3>T1).

The mean value for IL-6 for group II was highest at T3 (4.568 $\pm$.933pg/ml), followed by T2 (4.499 $\pm$ 1.095 pg/ml), then T1(4.418 $\pm$ 0.711 pg/ml), and least was at T0 (3.0 238 $\pm$ 0.866pg/ml) .(T3>T2>T1>T0).

The mean value of RANKL for Group I was highest at T2(23.207 $\pm$ 2.454pg/ml), followed by T3 (23.202 $\pm$ 2.367pg/ml), then T0 (22.947 $\pm$ 1.84 pg/ml), and least was at T1(21.99 $\pm$ 1.780 pg/ml).(T2>T3>T0>T1).

The mean value of RANKL for Group II was highest at T1(30.215 ± 5.472 pg/ml) ,followed by T2(29.168 ± 3.800 pg/ml) , then T3 (28.327 ± 4.74 pg/ml) ,and least was at T0(25.086 ± 4.602 pg/ml). (T1>T2>T3>T0).

Table 2: Intra group comparison of IL-6 for Group I at different time interval:

Paired Differences						df	P VALUE
t							
Time interval	Mean ± Std Deviation (pg/ml)	Std. Error Mean	95% Confidence Interval of the Difference				
			Lower	Upper			
T0 - T1	1.189 ± .324	.084	1.1369	1.009	14.183	14	.000***
T0 - T2	1.395 ± .282	.073	1.55	1.238	19.106	14	.278
T0 - T3	1.339 ±.331	.085	1.523	1.156	15.655	14	.424
T1 - T2	.205 ±.274	.070	.357	.054	2.908	14	.011**
T1 - T3	.150 ±.352	.090	.345	.044	1.656	14	.120
T2 - T3	.055 ± .419	.108	.176	.287	.510	14	.618

P > 0.05(Non-significant,NS), P<0.05(Just significant*), P<0.01(Moderate significant**),P<0.001(Highly significant***)

On comparing mean vales of IL-6 for Group I between T0 and T1,higher values were seen at T0 2.829 ± 0.200 pg/ml than T1 2.518 ± 0.333 pg/ml , with a mean difference of 1.189 ± 0.324 pg/ml and this difference was satistically significant (p=.000).

On comparing mean values of IL-6 for Group I between T0 and T2,higher values were seen for T0 $2.8293 \pm .29002$ pg/ml than T2 2.724 ± 0.238 pg/ml , with a mean difference of 1.395 ± 0.282 pg/ml , however the difference was statistically non significant (p= .278)

.On comparing mean values of IL-6 for Group I betweenT0 and T3, higher values were seen for T0 $2.829 \pm .290$ pg/ml than T3 2.66920 ± 0.344905 pg/ml , with a mean difference 1.33987 ± 0.33149 pg/ml, however the difference was statisticallynon significant (p=.424).

On comparing mean values of IL-6 for Group I between T1 and T2,higher values

were seen for T2 2.724 ± 0.238 pg/ml than T1 2.518 ± 0.333 pg/ml ,with a mean

difference -1.21 ± 5.77 pg/ml and this difference was statistically significant ($p=.011$).

On comparing mean values of IL-6 for Group I between T1 and T3, higher values were seen for T3 $2.669 \pm .3449$ pg/ml than T1 2.518 ± 0.333 pg/ml, with a mean difference of -0.150 ± 0.352 pg/ml, however this difference was statistically non-significant ($p=.120$) On comparing mean values of IL-6 for Group I between T2 and T3, higher values were seen in T2 2.724 ± 0.238 pg/ml than T3 $2.669 \pm .344$ pg/ml, with a mean difference of 0.055 ± 0.419 pg/ml, however this difference was statistically non-significant ($p=.618$).

TABLE 3: Intragroup comparison of mean values of IL-6 for GROUP II at different time intervals.

Paired Differences					t	df	P Value
Time intervals	Mean	± Std. Deviation (pg/ml)	Std. Error Mean	95% Confidence Interval of the Difference			
				Lower Upper			
T0 - T1	1.395± 1.158		.29921	2.03674 .75326	4.662	14	.000***
T0 - T2	1.475±1.482		.382520	2.29576 .65491	3.857	14	.002***
T0 - T3	1.544 ± .416		.10746	1.77542 1.31445	14.376	14	.000***
T1 - T2	.080±1.222		.315571	.75717 .596500	.255	14	.803
T1 - T3	.149±1.143		.295154	.782976 .483110	.508	14	.619
T2 - T3	.069±1.502		.387938	.901644 .762444	.179	14	.860

P > 0.05(Non-significant,NS), P<0.05(Just significant*), P<0.01(Moderate significant**),P<0.001(Highly significant***)

On comparing mean values IL-6 for Group II between T0 and T1, higher values were seen for T0 3.0238 ± 0.866 pg/ml than T1 4.418 ± 0.711 pg/ml , with a mean difference of -1.39 ± 1.158 pg/ml, and this difference was statistically significant (p=.000).

On comparing mean values of IL-6 Group II between T0 and T2, higher values were seen for T1 4.4188 ± 0.7115 pg/ml than T0 3.0238 ± 0.86619 pg/ml , with a mean difference of -1.47533 ± 1.481 pg/ml , and the difference was statistically significant(p=.002).

On comparing mean values of IL-6 Group II between T0 and T3, higher difference were seen for T3 4.568 ± 0.933 pg/ml than T0 3.0238 ± 0.866 pg/ml , with a mean difference of -1.544 ± 0.41620 pg/ml , and the difference was statistically significant (p=.000).

On comparing mean values of IL-6 Group II between T1 and T2, higher values were seen for T2 4.499 ± 1.095 pg/ml than T1 4.418 ± 0.711 pg/ml , with a mean difference of 0.803 ± 1.222 pg/ml , however the difference was statistically non-significant ($p=.808$).

On comparing mean values of IL-6 Group II between T1 and T3, higher values were seen for T3 4.568 ± 0.933 pg/ml than T1 4.418 ± 0.7115 pg/ml , with a mean difference of 0.149 ± 1.143 pg/ml , however the difference was statistically non-significant ($p=.619$) .

On comparing mean values of IL-6 Group II between T2 and T3, higher values were seen for T3 4.568 ± 0.933 pg/ml than T2 4.499 ± 1.095 pg/ml with a mean difference of 0.606 ± 1.502 pg/ml , however the difference was statistically non-significant ($p=.860$) .

TABLE 4: Intergroup comparison of Mean values of IL6 both for Group I and Group II at T0,T1,T2,T3.

Time intervals		N	Mean ± Std. Deviation (pg/ml)	Std. Error Mean	P VALUE
T0	Group I	15	2.829 ±.290	.074882	0.417
	Group II	15	3.028 ±.866	.223650	
T1	Group I	15	2.518 ± .333	.086074	0.001***
	Group II	15	4.418 ± .711	.183711	
T2	Group I	15	2.724 ±.238	.061558	0.001***
	Group II	15	4.499 ±1.095	.282961	
T3	Group I	15	2.669 ± .344	.089054	0.001***
	Group II	15	4.568 ± .933	.241030	

P > 0.05(Non-significant,NS), P<0.05(Just significant*), P<0.01(Moderate significant**),P<0.001(Highly significant***)

On comparing mean values of IL-6 between the two groups at T0 ,Group II had higher values of 3.028±0.866 pg/ml than Group I (2.829±.290 pg/ml)and the difference was statistically non significant (p=.4171).

On comparing mean values of IL-6 between the two groups at T1 , GROUP II had higher values of 4.418± 0.711pg/ml than Group I (2.528± .333 pg/ml) and the difference was statistically significant (p=.001).

On comparing mean values of IL-6 between the two groups at T2, Group II had higher values of 4.499 ± 1.096 pg/ml than Group I (2.724 ±.238 pg/ml) and difference was statistically significant (p=.001).

On comparing mean values of IL-6 between the two groups at T3,Group II had higher values of 4.568± .933pg/ml than Group I (2.669 ± .345 pg/ml) and difference was statistically significant (p=.001).

TABLE 5: Intragroup comparison of mean values of RANKL for Group I at different time intervals:

Paired Differences					t	df	P Value	
Time intervals	Mean±	Std. Deviation (pg/ml)	Std. Error Mean	95% Confidence Interval of the Difference				
				Lower				Upper
T0 - T1	.548 ±8.26609		2.13430	5.36561	3.78961	.369	14	.717
T0 - T2	.1.76±5.808		1.49978	2.95737	3.47604	.173	14	.865
T0 - T3	.1.75±6.85831		1.77081	2.67334	4.24812	.704	14	.536
T1 - T2	1.21±5.77988		1.49236	2.15346	4.24812	.702	14	.494
T1 - T3	1.206±7.39549		1.90951	2.18282	6.00815	1.002	14	.334
T2 - T3	.005±5.50518		1.42143	2.18333	3.91400	.609	14	.552

P > 0.05(Non-significant,NS), P<0.05(Just significant*), P<0.01(Moderate significant**),P<0.001(Highly significant***)

On comparing mean values of RANKL for Group I between T0 and T1, higher values were seen for T1 21.99 ± 1.780 pg/ml than T0 22.9473 ± 1.838 pg/ml , with a mean difference of -0.548 ± 8.26609 pg/ml , however the difference was statistically non-significant (p=.717).

On comparing mean values of RANKL for Group I between T0 and T2, higher values were seen for T2 23.207 ± 2.454 pg/ml than T0 22.947 ± 1.838 pg/ml , with a-mean difference of -1.76 ± 5.808 pg/ml ,however the difference was statistically non significant (p=.865).

On comparing mean values of RANKL for Group I between T0 and T3, higher difference were seen for T3 23.202 ± 2.367 pg/ml than T0 22.947 ± 1.838 pg/ml , with a mean difference of -1.75467 ± 6.85831 pg/ml , however the difference was statistically non significant (p=.536).

On comparing mean values of RANKL for Group I between T1 and T2, higher values

were seen for T2 23.207 ± 2.455 pg/ml than T1 21.995 ± 1.780 pg/ml ,with a mean difference of 1.212 ± 5.779 pg/ml , however the difference was statistically non-significant ($p=.494$).

On comparing mean values of RANKL for Group I between T1 and T3,higher values were seen for T3 23.202 ± 2.367 pg/ml than T1 21.995 ± 1.780 pg/ml ,with a mean difference of 0.005 ± 5.505 pg/ml , however the difference was statistically non-significant ($p=.334$) .

On comparing mean values of RANKL for Group I between T2 and T3,higher values were seen for T2 23.207 ± 2.455 pg/ml than T3 23.202 ± 2.367 pg/ml with a mean difference of 0.053 ± 5.505 pg/ml , however the difference was statistically non-significant ($p=.552$) .

TABLE 6: Intragroup comparison of mean values of RANKL for Group II at different time intervals:

Paired Differences					t	df	P VALUE
Time intervals	Mean ± Std. Deviation (pg/ml)	Std. Error Mean	95% Confidence Interval of the Difference				
			Lower	Upper			
T0 - T1	2.28±8.266	2.13430	6.86561	2.28961	1.072	14	.002***
T0 - T2	1.24±5.80861	1.49978	4.45737	1.97604	.827	14	.004***
T0 - T3	.377±6.85831	1.77081	4.17334	3.42267	.212	14	.000***
T1 - T2	1.05±5.779	1.49236	2.15346	4.24812	.702	14	.494
T1 - T3	1.91±7.395	1.90951	2.18282	6.00815	1.002	14	.334
T2 - T3	.865±5.505	1.42143	2.18333	3.91400	.609	14	.552

P > 0.05(Non-significant,NS), P<0.05(Just significant*), P<0.01(Moderate significant**),P<0.001(Highly significant***)

On comparing mean values RANKL for Group II between T0 and T1, higher values were seen for T1 30.215 ± 5.472 pg/ml than T0 25.086 ± 4.602 pg/ml , with a mean difference of -2.288 ± 8.266 pg/ml , and the difference was statistically significant (p=.002).

On comparing mean values of RANKL for Group II between T0 and T2, higher values were seen for T0 25.0867 ± 4.602 pg/ml than T2 29.168 ± 3.800 pg/ml , with a mean difference of -1.24 ± 5.808 pg/ml, and the difference was statistically significant (p=.004).

On comparing mean values of RANKL for Group II between T0 and T3, higher difference were seen for T0 25.086 ± 4.602 pg/ml than T3 28.302 ± 4.741 pg/ml , with a mean difference of $-.37533 \pm 6.85$ pg/ml, and)) the difference was statistically significant (p=.000).

On comparing mean values of RANKL for Group II between T1 and T2, higher values were seen for T1 30.215 ± 5.472 pg/ml than T2 29.168 ± 3.800 pg/ml , with a

mean difference of 1.047 ± 5.779 pg/ml, however the difference was statistically non-significant ($p=.494$).

On comparing mean values of RANKL for Group II between T1 and T3, higher values were seen for T1 30.215 ± 5.471 pg/ml than T3 28.302 ± 4.741 pg/ml, with a mean difference of 1.913 ± 7.395 pg/ml, however the difference was statistically non-significant (1.91267 ± 7.39549) ($p=.334$).

On comparing mean values of RANKL for Group II between T2 and T3, higher values were seen for T2 29.168 ± 3.800 pg/ml than T3 28.302 ± 4.741 pg/ml with a mean difference of 0.865 ± 5.505 pg/ml, however the difference was statistically non-significant ($p=.552$).

TABLE 7: Intergroup comparison of Mean values of RANKL between for Group I and Group II at T0,T1,T2,T3.

Time intervals		N	Mean± Std. Deviation (pg/ml)	Std. Error Mean	P value
T0	Group I	15	22.947±1.838	.47470	0.106
	Group II	15	25.086±4.602	1.18837	
T1	Group I	15	21.995±1.780	.45975	0.001***
	Group II	15	30.215±5.471	1.41285	
T2	Group I	15	23.207±2.454	.63386	0.001***
	Group II	15	29.168±3.800	.98120	
T3	Group I	15	23.202±2.367	.61127	0.001***
	Group II	15	28.3027±4.741	1.22418	

P > 0.05(Non-significant,NS), P<0.05(Just significant*), P<0.01(Moderate significant**),P<0.001(Highly significant***)

On comparing mean values of RANKL at T0 between Group I and Group II ,Group II had higher values of 25.086± 4.602pg/ml than Group I 22.947± 1.838 pg/ml and the difference was statistically non significant (p=.106).

On comparing mean values of RANKL between groups at T1 , GROUP II had higher values of 30.215± 5.472pg/ml than Group I 21.995± 1.780 pg/ml and the difference was statistically significant (p=.001).

On comparing mean values of RANKL between groups at T2, Group II had higher values of 29.168± 3.800 pg/ml than Group I 23.207± 2.455 pg/ml and difference was statistically significant (p=.001).

On comparing mean values of RANKL between groups at T3,Group II had higher values of 28.302 ± 4.741 than Group I 23.202± 2.367 pg/ml and difference was statistically significant (p=.001).

Table 8: Overall comparison of Group I and Group II :

		N	Mean $\pm$ Std. Deviation (pg/ml)	Std. Error Mean	P value
IL-6	Group I	15	2.3104 $\pm$.22554	0.5824	0.001***
	Group II	15	4.1276 $\pm$.53309	.13764	
RANKL	Group I	15	22.4630 $\pm$ 1.42823	.36877	0.001***
	Group II	15	28.9033 $\pm$ 2.71433	.70084	

P > 0.05(Non-significant,NS), P<0.05(Just significant*), P<0.01(Moderate significant**),P<0.001(Highly significant***)

Overall comparison of IL-6 between groups showed higher values in Group II (4.1276 $\pm$.53309 pg/ml) than in Group I (2.3104 $\pm$ 0.22554pg/ml) and this difference was statistically significant (p= 0.001).

Overall comparison of RANKL between groups showed higher values in Group II (28.903 $\pm$ 2.71433 pg/ml) than in Group I (22.4630 $\pm$ 1.42823 pg/ml) and this difference was statistically significant (p= 0.001).

Orthodontic tooth movement is mediated by application of mechanical forces on the teeth through the brackets that move the teeth within the bone by initiating modeling process in the periodontal ligament (PDL). The long duration of treatment with fixed mechanotherapy is one of the greatest challenges in Orthodontics, especially in adult patients as this acts as a major constraint for them in pursuing their treatment¹.

Acceleration of Orthodontic tooth movement in such cases would be beneficial in terms of reducing treatment time and decreasing potential side effects of Orthodontic treatment². Many techniques have been introduced to accelerate the tooth movement like use of drugs, surgical methods and physical / mechanical stimulation methods³. Among mechanical and physical stimulation methods vibratory stimulus is non-pharmacologic, non-invasive method that could be achieved using expensive patent devices like Accedent, the tooth masseuse⁵³ or by ultrasonic brushes that operates at physiologically unacceptable frequency or by powered toothbrushes that are comparatively cheaper, easily available in two frequencies (high and low). Powered tooth brushes had been used in previous studies to reduce pain due to orthodontic treatment and also for accelerating tooth movement, both in animal and human studies. Hence these were selected in present study. Various biological molecules like TNF alpha, IL-1, IL-6, IL-8, RANKL etc are released on application of mechanical stresses during Orthodontic tooth movement. The assessment of these molecules as biomarkers would provide better understanding of OTM at molecular level.

A biomarker is defined as a biological molecule found in blood, tissues or other body fluids that indicate a normal or abnormal process, condition or disease.

According to Leethanakul², on application of orthodontic forces bioelectric signals are produced that are reinforced by discontinuous forces produced by vibratory devices. Hence it could be anticipated that biomarker level would be raised on application of vibratory stimulus using powered brushes, hence it was decided to use them in present study.

IL-6 is an earliest bone resorption marker, and is released during early phase of orthodontic tooth movement¹⁴. Another potent biomarker is RANKL which binds to RANK and initiates osteoclastogenesis⁶⁵. Therefore the levels of IL-6 and RANKL would be evaluated in the present study.

Biomarkers can be detected in blood/serum or saliva or Gingival crevicular fluid etc. The collection of serum /blood is an invasive procedure and levels fluctuate due to various other factors. Salivary biomarkers are also present in very low concentration, requiring highly sensitive tests to detect them and are influenced by various environmental and psychological factors.¹⁵⁻¹⁶

To overcome these limitations of serum or saliva, biomarkers could be evaluated in GCF as it is minimal invasive procedure, cost effective and not influenced by systemic conditions of body. Therefore, in the present study it was decided to collect GCF for assessing the biomarkers released during orthodontic tooth movement.

Most of the previous studies have evaluated efficacy of accelerating tooth movement, by measuring its rate on application of vibratory stimulus³⁻⁴. However, none of the studies have evaluated biomarker level on application of vibratory stimulus during space closure with sliding mechanics. Hence the levels of IL-6, RANKL would be assessed at (baseline, 24 hrs, 20 days and 2 months) during retraction of anterior teeth using sliding mechanics. For this split mouth study design was selected where maxillary canine of one side served as experimental side where patient was asked to use vibratory brush and canine of other side served as control.

Considering this aim of this study was to evaluate and compare the levels of biomarkers (IL-6 and RANKL) during enmasse space closure (using sliding mechanics) using vibratory stimulus (with electric powered tooth brushes) in a split mouth study design at different time intervals.

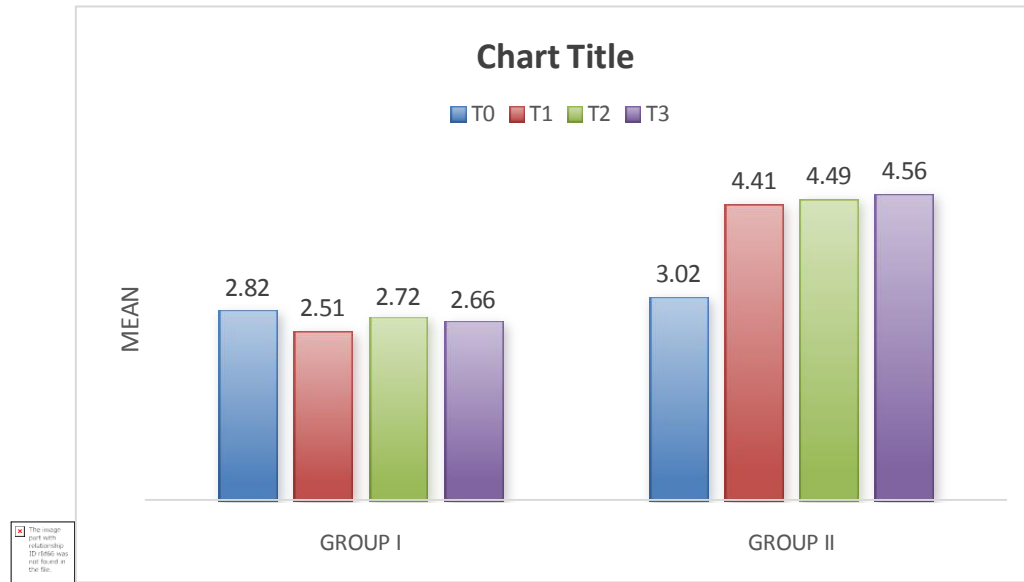
The sample was selected from the patients undergoing fixed Orthodontic treatment in Department of Orthodontics and Dentofacial Orthopaedics, BBDCODS, BBD University, Lucknow and who required space closure by retraction of anterior teeth after extraction of all 1st premolars. The approval from the Ethical and Research Committee of Babu Banarasi Das College of Dental Sciences was taken. Written informed consent was obtained from all selected subjects participating in this study.

After taking prerequisite Orthodontic records and formulation of treatment plan, fixed orthodontic treatment was started following standard protocol. Extractions were done as atraumatic as possible. Alignment and leveling was done following sequential change of

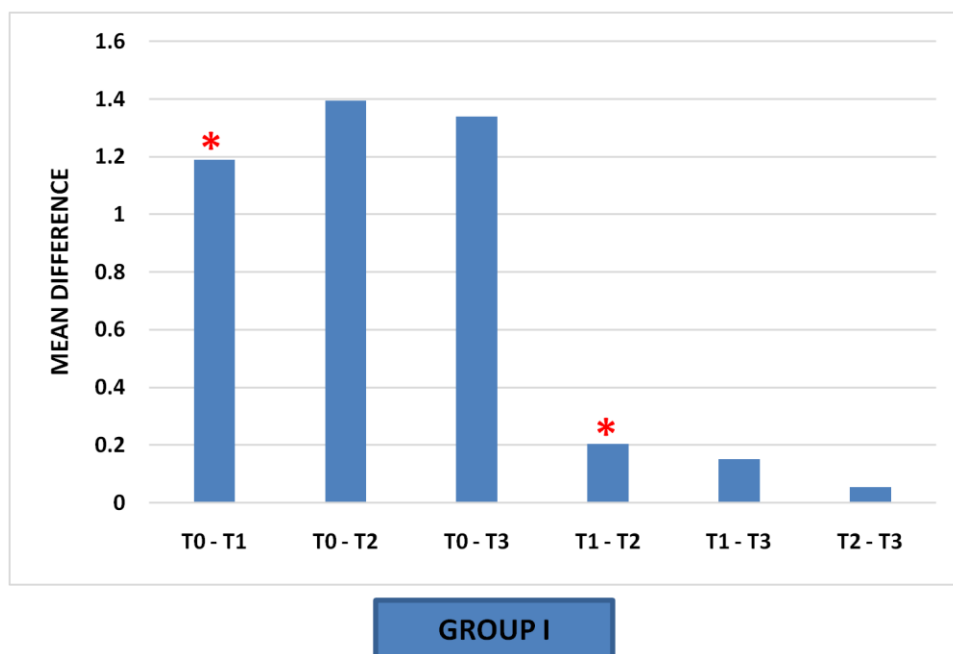
arch wires as per the case. Enmasse retraction of anterior teeth was started after placing 0.019X0.025 S.S. archwire, using NiTi closed coil spring of 9 mm length, stretched from first molar hook to crimpable hook. Force was calibrated at 200-250 grams using Dontrix gauge. Based on use of vibratory stimulus, canine of left side were grouped as Group I and served as control where no vibratory stimulus was provided; Group II included canine of right side where vibratory stimulus was provided using electric powered brush. Patient were instructed not to clean their teeth with vibratory tooth brush rather they were told to hold the toothbrush at desired site to apply mechanical vibration only. GCF was collected from distal surface of maxillary canine at different time (T0=baseline, T1=24hs, T2=20days, T3=2months) using absorbent points. These Absorbent points were placed in Eppendorf tube containing 250ul phosphate buffered saline. These were transported to Department of Biochemistry, KGMU, for storage till analysis is done. All samples were thawed and centrifuged at time of testing, for 30 seconds for elution of GCF from paper points, and supernatants were transferred into a new Eppendorf tube. ELISA kit was opened, reagent were prepared as per instructions and were sequentially added as per protocol. The absorbance of each sample was read at 450 nm using ELISA analyser. Mean values were obtained in pg/ml after comparing values to standard curve as obtained with kit. Data was tabulated and statistical comparisons were done.

DISCUSSION

In the present study trend of level of IL-6 for Group I was highest at T0 ($2.8293 \pm .290$ pg/ml) > T2 ($2.7245 \pm .23841$ pg/ml) > T3 ($2.669 \pm .34490$ pg/ml) > T1 (2.5187 ± 0.334 pg/ml). (T0>T2>T3>T1), with statistically significant difference between T0-T1 and T1-T2.

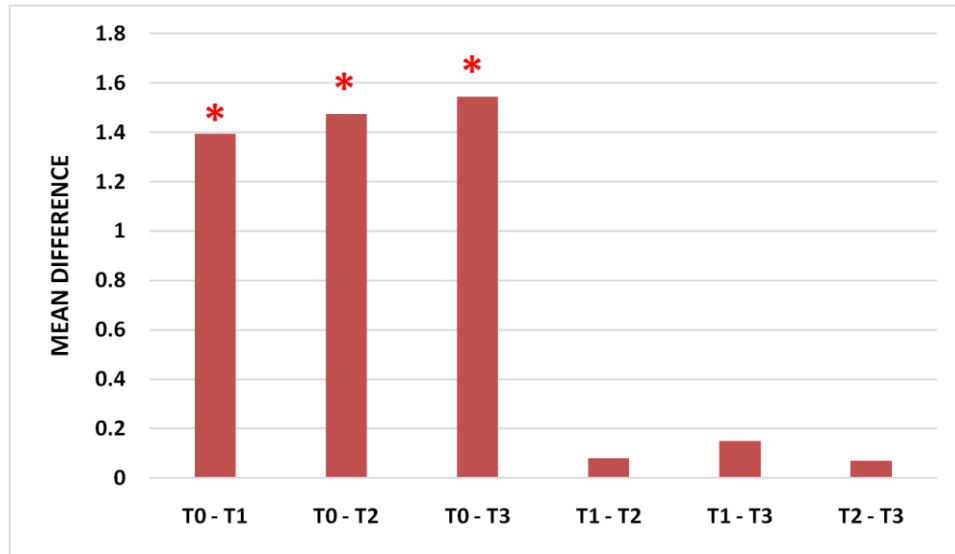


Bardigram 1: Mean values of IL6 for Group I & Group II



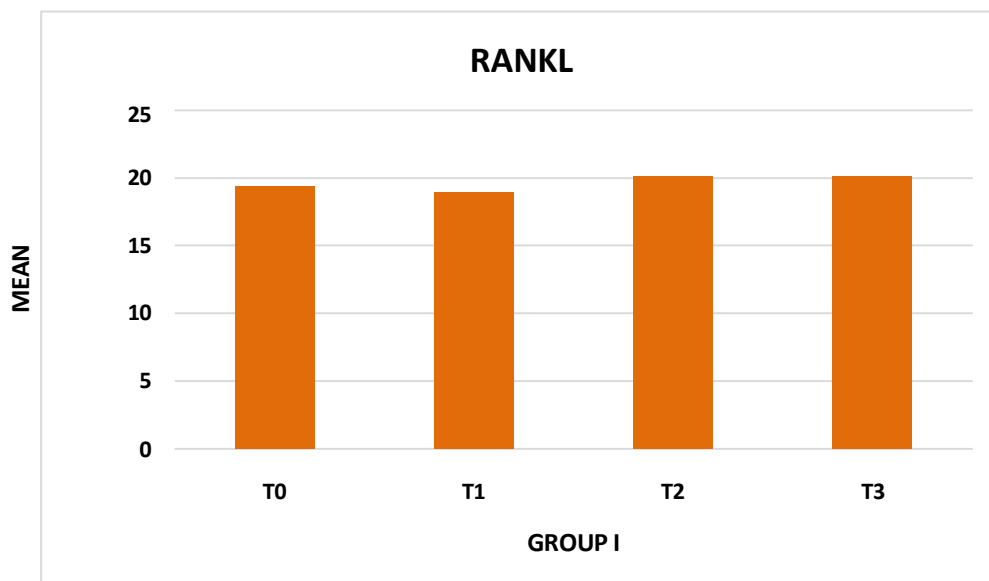
Bar diagram 2: Intragroup comparison for mean levels of IL-6 in Group I

The trend of IL-6 for group II was highest at T3 ($4.568 \pm .933\text{pg/ml}$) >T2 ($4.499 \pm 1.095\text{pg/ml}$) >T1 ($4.418 \pm 0.711\text{pg/ml}$) > T0 ($3.0238 \pm 0.866\text{pg/ml}$) .(T3>T2>T1>T0), having statistically significant difference between T0-T1,T0-T2 and T0-T3 .

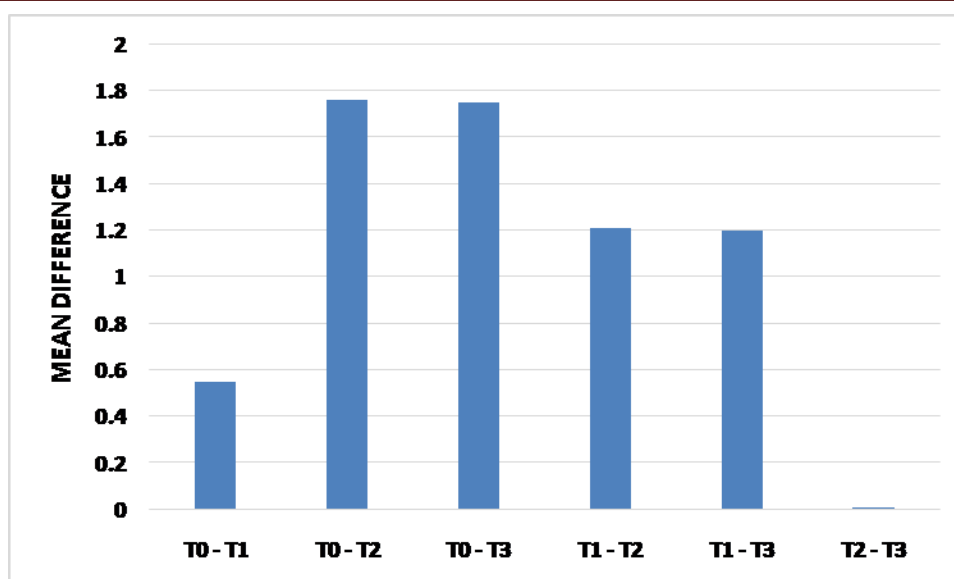


Bar-diagrams 3: Intra-group comparison for mean levels of IL-6 in Group II

The trend of RANKL in present study of Group I was highest at T2 ($23.207 \pm 2.454\text{pg/ml}$) > T3 ($23.202 \pm 2.367\text{pg/ml}$) > T0 ($22.947 \pm 1.84\text{pg/ml}$) > T1 ($21.99 \pm 1.780\text{pg/ml}$). (T2>T3>T0>T1), with statistically non significant difference between time intervals.

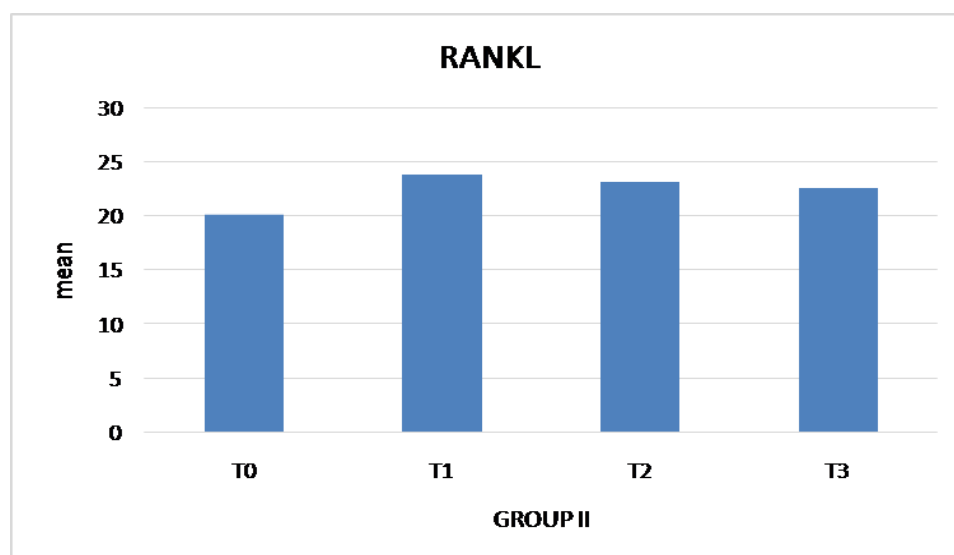


Bar-diagrams 4: Mean levels of RANKL for Group I

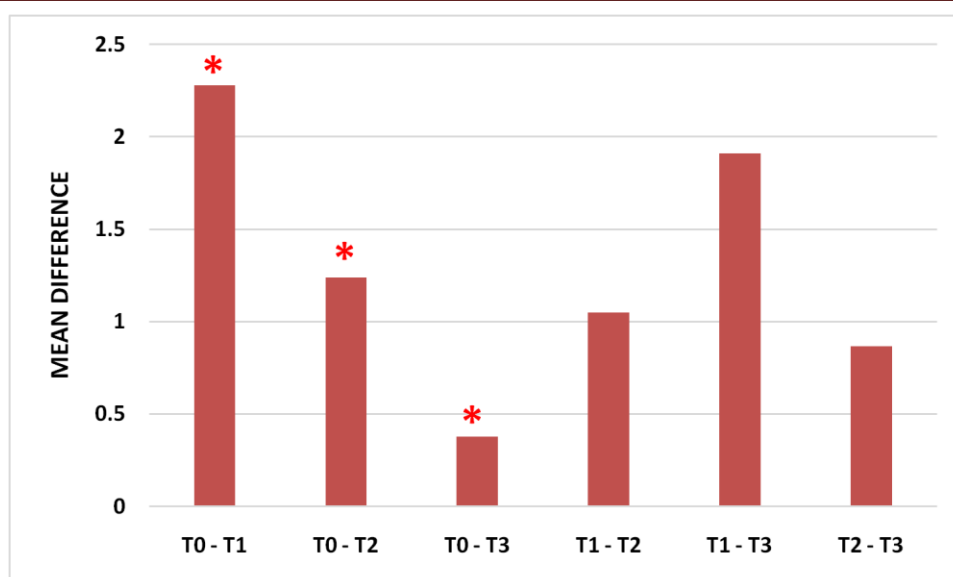


Bar-diagrams 5: Intra group comparison for mean levels of RANKL in Group I

For Group II the trend of level of RANKL was T1(30.215 ± 5.472 pg/ml) ,followed by T2(29.168 ± 2.800 pg/ml) , then T3 ($28.327 \pm .741$ pg/ml) ,and least was at T0(27.93 ± 5.430 pg/ml). (T1>T2>T3>T0), having statistically significant difference between T0-T1,T0-T2 and T0-T3 only

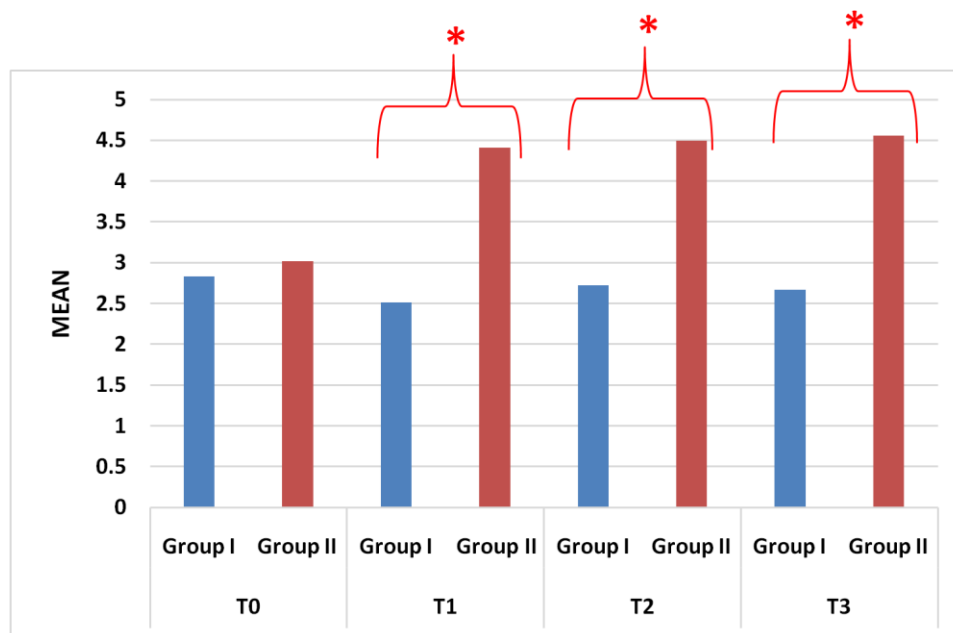


Bar-diagrams 6: Mean levels of RANKL for Group II

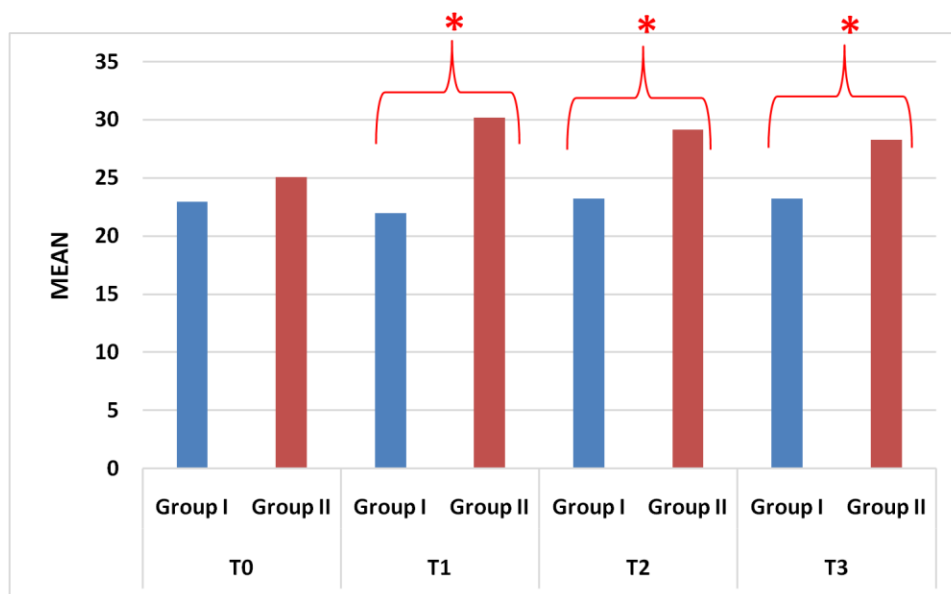


Bar diagram 7: Intragroup comparison for mean value levels of RANKL in GROUP II

On Intergroup comparison mean level of IL-6 as well as RANKL showed significantly higher values in Group II than Group I at T1,T2,T3 whereas at T0 , difference was statistically non significant.



Bar diagrams 8: Intergroup comparison of IL-6 between Group I and Group II



Bar diagrams 9: Intergroup comparison of RANKL between Group I and Group II

The results of the present study showed fluctuations in levels of biomarkers, both on non vibratory (Group I) and vibratory (Group II) side at different time intervals (T0, T1, T2, T3), suggestive of the fact that fluctuation of these biomarkers are a part of intricate process of Orthodontic tooth movement at molecular level. Also, application of vibratory stimulus significantly increased level of both the markers IL-6 and RANKL on experimental site in comparison to control side, suggestive of reinforcement of bone remodeling on application of vibratory stimulus. This also suggests that effect of vibratory stimulus on canine of right side was not transferred to contralateral side as levels of Biomarkers are higher in Group II than Group I. Intragroup comparison of mean values of both biomarkers of Group II amongst time intervals after applying vibratory stimulus did not show statistically significant difference, suggestive of the fact that sustained vibratory stimulus did not increase levels of biomarkers in continuous manner, rather increase was only to certain extent only.

As many studies have not assessed level of similar biomarkers on application of vibratory stimulus, hence results would be compared to studies that had evaluated level of similar or different biomarkers on applying vibratory stimulus using powered brush or any other physical method of accelerating tooth movement by **Leethanakul et al²**, **Siriphan et al⁹**, **Ahmed et al¹⁰**, **Kalemaj et al⁵**, **Chouinard et al⁶**, **Gujar et al⁷**, **Suamphan et al⁸**, **Agrawal et al³**, **Pascoal et al⁴**, **Reiss et al⁶⁰**, **Shaikh et al¹⁸**, **Zheng et al⁶²**, **Zhang et al⁴⁴**, **Yamaguchi et al³⁷** or to studies that had evaluated acceleration of rate of tooth movement on applying vibratory stimulus by **Miles et al⁵³**, **Pavlin et al²¹**, **Bowman et al⁴⁸**, **Woodhouse et al⁵⁰**, **Garg et al⁶¹**, **DiBiase et al⁵⁴**, **Showkatbakhsh et al⁴⁰**, **Gkantidis et al⁴¹**, **Stark et al²²**, **Akbar et al⁶³**, **Jain et al⁵⁵**, **Azeem et al⁵⁶**. Also, the result of control group would be compared to previous studies that recorded level of biomarkers on application of orthodontic force by **Jayaprakash et al⁵⁷**, **Mohammed et al⁵⁸**, **Uematsu et al²⁹**, **Basaran et al³⁴**, **Castroflorio et al⁵²**, **Madureira et al¹¹**, **Tuncer et al⁴⁵**, **Hamamci et al⁴³**.

Leethanakul et al (2015)² found elevated levels of IL-1 β from baseline to soon after bracketing(T0) to 1 month (T1) after canine retraction using 60g of force and using electric powered tooth brush of frequency of 125 Hz . The levels decreased thereafter at each monthly intervals (T2,T3),however the difference was significant at pressure side only at T3. The mean level of IL-1 β showed a trend of T3(0.64 ± 0.33 pg/uL)>T2(0.18 ± 0.09 pg/uL) >T1(0.14 ± 0.04 pg/uL)>T0 (0.04 ± 0.04 pg/uL) >Baseline(0.04 ± 0.02 pg/uL)on experimental side and T1(0.17 ± 0.09 pg/uL)>T3 (0.10 ± 0.11 pg/uL) >T2(0.08 ± 0.06 pg/uL) >T0 (0.06 ± 0.06 pg/uL)>Baseline (0.03 ± 0.02 pg/uL)on control side.

Siriphan et al (2019)⁹ used electric powered tooth brush at 30Hz and 60Hz and RANKL and OPG levels were analysed immediately before canine distalization (T1) , at 24 hours (T2), 48 hours (T3) and 7 days (T4) and 3 months (T5). They found rate of canine distalization was not significantly different between experimental groups and control group.No within- or between-group differences in RANKL and OPG were observed ($P > 0.05$), for 30 Hz and 60 Hz group , however RANKL on the control side showed statistically significant difference on intragroup comparison with highest value at T2(450.34 pg/ml)> T4(428.63 pg/ml) and T3(425.34) > T1(189.48) ($P < 0.001$).

Ahmed et al (2015)¹⁰ found elevated levels of IL-1 β and TNF - α in saliva in subjects using AcceleDent® device (30Hz) in comparison to control group. The mean value

of both salivary IL-beta (pg/ml) and TNF-alpha(pg/ml) among study group were higher than control group at T1, followed by T2, then T3 with statistically significant difference ($p < 0.05$).

Kalemaj et al (2017)⁵ found addition of vibratory stimulus(Acceleration=30) with self ligating brackets , significantly increased levels of IL-1 beta in comparison to conventional-ligating appliance(CLA) as well as self ligating brackets(SLA) in a randomized clinical trial. On comparing effect of vibration , high levels were seen on adding vibration to SLA, at different time intervals t0(at time of appliance placement), t1(after one hour),t2(after one week),t3(after 3 weeks) in comparison to conventional Self ligating brackets,however statistically significant difference were noted at t1(1hr) and t2(1week) only. Similar to this study where addition of vibratory stimulus showed higher level of IL-1beta for selfligating brackets, we found higher levels of IL-6 and RANKL for conventional brackets on addition of vibratory stimulus.

Chouinard et al (2016)⁶ found no statistically significant difference in levels of biomarkers (IL-1-beta , IL- 8 and TNF-alpha in saliva) during orthodontic tooth movement with fixed appliances and vibration device(Acceleration=30Hz) in a randomized control trial. The biomarker secretion was assessed at T0(before bonding),T1(5-6weeks after bonding),T2(10-12 weeks).T3(15-18 weeks).Trend for IL-1-beta level for Acceleration group($T0 = 41.72 \pm 33.32 \text{ pg/ml} > T2 = 29.57 \pm 27.75 \text{ pg/ml} > T3 = 26.98 \pm 22.92 \text{ pg/ml} > T1 = 26.97 \pm 10.84 \text{ pg/ml}$) and for control group $T3 = 132.73 \pm 205.01 \text{ pg/ml} > T2 = 107.98 \pm 208.04 \text{ pg/ml} > T0 = 81.85 \pm 156.26 \text{ pg/ml} > T1 = 56.62 \pm 87.21 \text{ pg/ml}$.

Gujar et al (2023)⁷ found significantly high rate of tooth movement in both groups that used electric toothbrushes with 125 Hz or 150Hz in comparison to non vibratory group at each time interval I.e. T0-T1,T1-T2,T2-T3. They analysed a different biomarker PGE2 where levels elevated significantly on using electric powered tooth brush in comparison to control and levels increased more with higher frequency brush at T1,T2,T3. Intragroup comparison revealed statistically significant difference in mean levels between different time intervals in each group, however they did not compare each group separately with one another.

Suamphan et al (2010)⁸ found significantly higher rate of canine movement on experimental side where 60g of orthodontic force was applied in combination to

mechanical vibratory stimulus from electrical tooth brush of frequency 125 Hz at T1(1 month of canine retraction) and T2(3 months of canine retraction) . They found trend of IL-1 beta for control group as $T3(132.73 \pm 205.01) > T2(107.98 \pm 208.04) > T0(81.85 \pm 156.26) > T1(56.62 \pm 87.21)$ and trend for Accedent group was $T0(41.72 \pm 33.32) > T2(29.57 \pm 27.72) > T3(26.98 \pm 22.92) > T1(26.97 \pm 10.84)$. However, intergroup comparison at each time showed statistically non significant difference.

Agrawal et al (2021)³ in a systematic review assessed the effect of vibrational force on biomarkers for orthodontic tooth movements and found statistically significant levels of IL-1beta in a single study. The outcomes of studies were found to be mixed due to methodological heterogeneity due to different devices and frequencies of vibration.

Pascoal et al (2024)⁴ in a systematic review , assessed the impact of high (>30 Hz) and low (≤ 30 Hz) frequency vibrations on orthodontic tooth movement (OTM) and suggested use of high frequency vibration in future studies for accelerating tooth movements.

Reiss et al (2020)⁶⁰ found that the levels of IL-6 and RANKL in saliva were higher in Accedent group than control group during mandibular anetrior alignment (MAA) at different time intervals i.e. before treatment (T0) , and at three time points 4-6weeks apart(T1,T2,T3). The levels of IL-6 and RANKL were higher in Accedent group than control group however difference was statistically non significant. The level of IL-6 in Accedent group was $T0 = 7.34 \text{ ug/ml} > T1 = 6.99 \text{ ug/ml} > T2 = 6.06 \text{ ug/ml} > T3 = 5.54 \text{ ug/ml}$ and in control group trend was $T3 = 9.21 \text{ ug/ml} > T2 = 5.06 \text{ ug/ml} > T0 = 5.05 \text{ ug/ml} > 4.43 \text{ ug/ml}$. The levels of RANKL in Accedent group $T2 = 29.2 \text{ ug/ml} > T3 = 25.77 \text{ ug/ml} > T0 = 24.32 \text{ ug/ml} > T1 = 19.86 \text{ ug/ml}$ and trend in control group was $T3(25.44 \text{ ug/ml} > T0 = 23.11 \text{ ug/ml} > T2 = 22.75 \text{ ug/ml} > T1 = 13.85 \text{ ug/ml}$.

Shaikh et al (2021)¹⁸ found elevated levels of IL-6 and TNF-alpha in GCF using Low-Intensity laser therapy (LILT) in comparison to control at day 2, day 9, day 29, day 49 and day 60. The trend for IL-6 in experimental group was {Day 02=0.959 > Day 29=0.909 > Day 09= 0.894 > Day 60= 0.847} and in control group trend was Day 02= 0.651=Day 09=0.651 < Day 29=0.586 < Day 60=0.477}.

Zheng et al (2021)⁶² found trend for the level of RANKL on study side where low level laser therapy (LLLT) during orthodontic tooth movement was used for canine retraction as Day 21=2.48 $\pm$ 0.47 > Day 14 = 2.20 $\pm$ 0.17 > Day 7= 2.17 $\pm$ 0.58 > Day 29= 1.97

± 0.17 > Day 0 = 1.91 ± 0.19 and on control side trend was as Day 21 = 2.15 ± 0.29 > Day 0 = 2.07 ± 0.48 > Day 14 = 1.98 ± 0.34 > Day 28 = 1.88 ± 0.36 > Day 7 = 1.80 ± 0.21 . However the levels of RANKL showed statistically non significant difference compared to baseline in control group at all time intervals, however, in study group the level was significant only at Day 21, when compared to baseline.

Zhang et al (2012)⁴⁴ found that Alkaline phosphatase activity was gradually increased with the frequency of vibration, to a peak at 50 Hz, and the level of osteocalcin was significantly higher than control following vibration at 40 Hz, 50 Hz, 60 Hz, 90 Hz and 120 Hz, found in isolated human periodontal ligament stem cells (PDLSC).

Yamaguchi et al (2009)³⁷ found presence of RANKL and RANK in periodontal tissues during experimental tooth movement of rat molars, and that PDL cells under mechanical stress may induce osteoclastogenesis through up regulation of RANKL expression during orthodontic tooth movement.

The overall conclusion drawn after corroborating results of present study to above mentioned studies suggested that addition of vibratory stimulus significantly elevated levels of biomarkers in most of the studies as seen in present study as well for intragroup and intergroup comparison. However the difference in results could be attributed to variation in methodologies, selection of different time interval or variation in biomechanics used with variable force direction and magnitude applied.

Studies that evaluated acceleration of rate of tooth movement on applying vibratory stimulus are discussed below^{53,21,48,50,61,54,40,41,22,63,55,56}.

Miles et al (2018)⁵³ in a RCT found no clinically or statistically significant difference in the rate of space closure both in control and Accedent groups in patients who had undergone space closure following from premolar extraction.

Pavlin et al (2015)²¹ found significantly higher rate of maxillary canine retraction for the AcceleDent® group (1.16 mm/month) compared to the control group (0.79 mm/month).

Bowman et al (2014)⁴⁸ found that average time period needed to attain alignment of the mandibular dentition was significantly shorter in the AcceleDent group (93 days) than in the Study control group (120 days).

Woodhouse et al (2015)⁵⁰ in a randomized clinical trial found that AcceleDent did not significantly increase the rate of initial tooth movement or reduced the amount of time

required to achieve final alignment when used in conjunction with a preadjusted edgewise fixed appliance.

Garg et al (2021)⁶¹ found an increased rate of canine movement on the side of vibratory stimulation (right side) in comparison to the control side (left side) for all time intervals T0(start of canine retraction), T1 (after 1st month), T2 (after 2nd month) and T3 (after 3rd month) and can be used to decrease treatment time in adults.

DiBiase et al (2018)⁵⁴ did not found statistically significant increase in rate of space closure both in accedent and control group at baseline, T1(start of space closure), T2(first visit after T1), T3(end of space closure), T4(completion of treatment).

Showkatbakhsh et al (2010)⁴⁰ found that that with exposure to pulsed electromagnetic field, canine retraction was significantly more than the control group ($P < .001$) after 5.0 ± 0.6 months, suggestive of favorable affect of physical stimulation method on acceleration of tooth movement.

Gkantidis et al (2010)⁴¹ assessed showed acceleration of canine retraction rate with corticotomy during the first month of-therapy (WMD = 0.73; $p < 0.01$) and with low-intensity laser (WMD = 0.42 mm/month; $p < 0.001$) in a period longer than 3 months in comparison to control group.

Stark et al (1987)²² in animal studies showed efficacy of resonance vibration and pulsed electromagnetic field respectively in acceleration of tooth movement.

Akbar et al (2022)⁶³ found there was no statistically significant difference in the amount of tooth movement between the experimental and control side ($p = 0.22$) on using electric tooth brush.

Jain et al (2018)⁵⁵ found no significant difference in rate of orthodontic tooth movement after first, second and third month consecutively on comparison between control and experimental site using powered tooth brush(60Hz frequency) , whereas fourth month showed less tooth movement of experimental side as compared to control side.

Azeem et al (2019)⁵⁶ found the amount of tooth movement was similar for canines on the vibration side and on the non-vibration side (mean 0.81 ± 0.10 mm and 0.82 ± 0.11 mm, respectively, $p > 0.05$). Acceleration of rate of tooth movement had variable results

in above mentioned studies. Though we did not measure the rate of tooth movement, in present study, however levels of IL-6 were highest at T3 in Group II, suggestive of fact that change at molecular level on applying mechanical stresses were reinforced by vibratory stimulus. This was also stated by Leethankal² that there is increase in number of osteoclast on compression side with enhanced expression of biomarkers. Thus, it could be anticipated that higher levels of IL-6 at T3 will also contribute to increased rate of tooth movement on application of vibratory stimulus. However similar trend was not seen in levels of RANKL that could be due to the fact that release of RANKL is dependent, not on IL-6 alone rather other biomarkers also influence its release. Additional factors like age, sex, bone molecular density, variation in cellular activity in each individual would also control release of biomarker as tooth movement progresses.

Studies that recorded level of biomarkers on application of orthodontic force are discussed below^{57,58,29, 34, 52, 11,45,43}.

Jayaprakash et al (2019)⁵⁷ found significantly elevated levels of IL-6 in GCF on moving canine distally by elastic chain exerting an initial force of 250 g on experimental side only in subject with age range $\pm$ of 15.9 years at 24 hours concentration of IL-6 was higher ($0.065 \pm 0.005 \text{ pg} / \mu\text{g}$) than at baseline ($0.030 \pm 0.008 \text{ pg}/\mu\text{g}$). At 168 hr (7days) mean level of IL-6 ($0.044 \pm 0.007 \text{ pg} / \mu\text{g}$) were higher than baseline but difference was not significant. On other side where no forces was applied, there was decrease in level of IL-6 at 24 hr ($0.016 \pm 0.006 \text{ pg} / \mu\text{g}$) than at baseline ($0.034 \pm 0.009 \text{ pg} / \mu\text{g}$) at 168 hr ($0.036 \pm 0.009 \text{ pg} / \mu\text{g}$) and the difference was statistically non-significant between 168 hr and baseline.

Mohammed et al (2019)⁵⁸ evaluated IL-6 in PDL of extracted 1 premolar each tooth was assigned a time point for its extraction at 2 hours, 15th day or the 30th day after application of force. Brackets were bonded to the experimental teeth at 5 degrees to the long axis of the tooth, 200-250 g of force was exerted on placing 0.014 Ni-Ti wire. They found a significant increase on experimental teeth in the level of IL-6 on 15th day (14.91) than on 30th day (11.1) and least was at 2hrs (3.4) which was non-significant in comparison to control.

Uematsu et al (1996)²⁹ found significantly elevated levels of IL-6 in GCF during separate canine retraction in subjects with mean age of 14.4 ± 0.9 years by elastic chain

exerting an initial force of 250 g. The mean levels of IL-6 was significantly higher at 24 hr (0.063 ± 0.004 pg/ μ g) than at baseline (0.020 ± 0.007 pg/ μ g) $p = 0.0441$. At 168 hr (7days) mean level of IL-6 was (0.009 ± 0.006 pg/ μ g.) $p = 0.0147$ was significantly lesser than baseline values.

Basaran et al (2006)³⁴ did not found significant difference in mean value of IL-6 from baseline to 7 days later, and 21 days later, and as the teeth were leveled. The trend observed was baseline=2.447>6 Month +21 days =2.433> 6months +7 days =2.387 >7day=2.373> 21 days=2.327> 6 months=2.320.

Castroflorio et al (2017)⁵² found significantly elevated levels of RANKL on experimental side i.e. where isolated 2nd molar was distalised using aliners whereas contralateral side 2nd molar was used as control at T1. The trend for RANKL on test side was T3(2.5 ± 2.1)>T2(1.3 ± 0.9)>T1(1.2 ± 0.8)>T0(0.5 ± 0.4)with statistical difference at T1($P=.004$)

Madureira et al (2012)¹¹ found Interleukin (IL)-6 (IL-6) production was significantly elevated in the PDL on day 1 after force application. Significantly strong positive correlations between GCF and PDL in experimental group were seen on days 3 (interferon-gamma), 7 (IL-10), 14 (IL-17A), and 28 (IL-17A, tumor necrosis factor-alpha), and significantly strong negative correlation were seen on days 14 (interferon-gamma) and 21 (IL-2, IL-10).

Tuncer et al (2013)⁴⁵ evaluated and found non -significant difference in levels of RANKL with time ,on retracting the maxillary canines by sentalloy closed coil springs with a continuous force of 200gm on the segmental .016x.022SS wire, four weeks after 1st premolar extraction .The mean value was highest at 7 days (1.29 ± 1.0 pmol/l)>at baseline(pre-treatment- $1.19 \pm .71$ pmol/l) > at 1st month ($1.04 \pm .50$ pmol/l)>then at 24hrs($.96 \pm .55$ pmol/l)>least at 1 hr ($.83 \pm .30$ pmol/l).

Hamamci et al (2012)⁴³ assessed and found insignificant difference in the level of IL-6 from GCF collected around implants and distobuccal site of maxillary canines i.e. treatment group and mesial to second premolar(control) during canine distalization using NiTi closed coil spring.They also found statistically nonsignificant difference on intragroup comparison of T1 with other time interval ,however they did not make intragroup comparison between all time intervals.

Similar to present study, all the above mentioned studies showed increase in levels of biomarkers on application of mechanical stimulus during orthodontic treatment but levels fluctuated between different time intervals. As these studies could be compared to control group of our study, variation in intragroup comparison of present with above mentioned studies could be attributed to variation in type of tooth movement, force levels or variation in time intervals selected⁴⁶.

Application of Orthodontic force is considered as mechanical loading which stimulates the early mechanoresponsive cells in alveolar bone i.e. osteocytes. Application of vibratory stimulus augments internal mechanical loading of osteocytes resulting in higher levels of IL-6 thereby increase number of osteoclasts on compression site as suggested by Leethankaul². RANKL is receptor activator of nuclear factor kappa B ligand and is seen on surface of osteoblasts which binds with RANK seen on the surface of osteoclasts for osteoclastogenesis and binds with OPG (osteoprotegrin) during osteoblastogenesis. Mechanical vibration directly increases RANKL level resulting in production of cytokines like VEGF (vascular endothelial growth factor), TNF- α , interleukins, interleukin-B, matrix metalloproteinases and others⁶⁵.

The major limitation of the present study may be linked to differences in the composition of the gingival crevicular fluid (GCF) or the biological response of an individual. Since GCF is sensitive to contamination from plaque and saliva, potential biases should be considered when interpreting the results. Other sources of cytokines, such as those from bone, gingivae, blood vessels, and external factors like plaque accumulation, may also influence the GCF and PDL microenvironments.

Despite of these limitations, the results of present study suggested positive effect of application of vibratory stimulus on levels of biomarkers during enmasse retraction of anterior teeth using sliding mechanics.

According to blood pressure theory, on applying force there is increase in blood flow, decrease in oxygen levels on compression site with corresponding release of biomarkers like IL-6 resulting in bone remodelling. Also initial proinflammatory cytokine like IL-6, subsequently modulates release of RANKL that along with other biomarkers would sustain process of osteoclastogenesis. Future scope of present study would include validating results of present study on large sample size, or co-relation can be established between levels of biomarkers and rate of tooth movement at different time intervals, or

biomarkers can be assessed at other time intervals and in subjects who had been subjected to other methods of space closure. Also, future studies could be done in assessing levels of other biomarkers, both at tension and compression site.

Following conclusions are drawn from the present study with the aim to evaluate and compare the levels of biomarkers during maxillary canine retraction(using sliding mechanics) using vibratory stimulus(with electric powered tooth brushes) in a split mouth study design at different time intervals:-

1. Application of mechanical stress during orthodontic treatment altered the level of biomarkers (IL-6 and RANKL) both in vibratory and non-vibratory groups at different time intervals.
2. The trend for IL-6 for Group I was $T_0 > T_2 > T_3 > T_1$, with statistically significant difference between T_0 - T_1 and T_1 - T_2 .
3. The trend for RANKL for Group I was $T_2 > T_3 > T_0 > T_1$, with no statistically significant difference on intragroup comparison.
4. The trend for IL-6 for group II was $T_3 > T_2 > T_1 > T_0$ and the trend RANKL for group II was $T_1 > T_2 > T_3 > T_0$.
5. Intragroup comparison for each biomarker revealed significant elevation of their levels after application of vibratory stimulus to baseline values. Also, intragroup comparison for each biomarker, for time interval after applying vibratory stimulus did not show significant difference, suggestive of the fact that vibratory stimulus increased levels of biomarkers to certain extent only despite of continuous application during that period.
6. Vibratory stimulus using electric powered tooth brush significantly elevated levels of both the biomarkers at T_1 , T_2 and T_3 .

The most important clinical implication of present study would be that as vibratory stimulus significantly elevated level of biomarker, hence, electric powered tooth brushes can be preferred in adults subjects for increasing biomarker levels thereby accelerating tooth movement. Electric powered tooth brushes being cheap, non-invasive and can be used by patient at home could be good alternative for accelerating tooth movement and reducing treatment time in comparison to other expensive, non-surgical methods or traumatic surgical methods.

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Orthodontic tooth movement is mediated by application of mechanical forces on the teeth through the brackets that move the teeth within the bone by initiating modeling process in the periodontal ligament (PDL) by release of Biomarkers. The long duration of treatment with fixed mechanotherapy is one of the greatest challenges in Orthodontics, especially in adult patients.

Acceleration of Orthodontic tooth movement in such cases would be beneficial and can be achieved by many ways like use of drugs, surgical methods and physical / mechanical stimulation methods. Among these Electric powered tooth brush which is a cheaper and non invasive method of physical stimulation was selected in present study. A biomarker is defined as a biological molecule found in blood, tissues or other body fluids that indicates a normal or abnormal process, condition or disease. Various biological molecules like TNF alpha, IL-1, IL-6, IL-8, RANKL etc termed as biomarkers are released on application of mechanical stresses during Orthodontic tooth movement.

The bioelectric signals produced on applying orthodontic tooth forces are reinforced by vibratory stimulus hence it was anticipated that levels of biomarkers are increased on application of vibratory stimulus.

IL-6 is an earliest bone resorption marker, and is released during early phase of orthodontic tooth movement that initiates release of another potent biomarker RANKL which binds to RANK and sustains osteoclastogenesis. Therefore the levels of IL-6 and RANKL would be evaluated in the present study.

Biomarkers can be detected in blood/serum, saliva or Gingival crevicular fluid etc. The collection of serum /blood is an invasive procedure and levels fluctuate due to various other factors. Salivary biomarkers are also present in very low concentration, requiring highly sensitive tests to detect them. To overcome these limitations of serum or saliva, biomarkers were evaluated in GCF in present study.

Most of the previous studies have evaluated level of biomarkers during orthodontic tooth movement on application of vibratory stimulus. However, none of the studies have evaluated biomarker level on application of vibratory stimulus during enmasse space closure (using sliding mechanics).

Considering this aim of this study was to evaluate and compare the levels of biomarkers (IL-6 and RANKL) during enmasse space closure (using sliding mechanics) using vibratory stimulus (with electric powered tooth brushes) in a split mouth study design at

different time intervals baseline, 24 hours, 20 days, 2 months.

The sample of 15 patients with mean age of 18-25 years was selected from the patients undergoing fixed Orthodontic treatment in Department of Orthodontics and Dentofacial Orthopaedics, BBDCODS, BBD University, Lucknow who required space closure by retraction of anterior teeth after extraction of all 1st premolars. The approval from the Ethical and Research Committee of Babu Banarasi Das College of Dental Sciences was taken. Written informed consent was obtained from all selected subjects participating in this study.

Fixed Orthodontic treatment was initiated following standard protocol. Enmasse retraction of anterior teeth was started on 0.019X0.025 S.S. archwire, using NiTi closed coil spring with a force of 250 grams. Based on use of vibratory stimulus, canine of left side were grouped as Group I and served as control where no vibratory stimulus was provided; Group II included canine of right side where vibratory stimulus was provided using electric powered brush. GCF was collected from distal surface of maxillary canines at selected time intervals using absorbent points. Mean levels of IL-6 and RANKL were obtained following standard protocol of GCF storage, transportation and analysis using ELISA analyser. The absorbance of each sample was read at 450 nm using ELISA analyser. Mean values were obtained in pg/ml after comparing values to standard curve as obtained with kit. Data was tabulated and statistical comparison were done. Following conclusions are drawn from the present study with the aim to evaluate and compare the levels of biomarkers during enmasse retraction on anterior teeth (using sliding mechanics) using vibratory stimulus (with electric powered tooth brushes) in a split mouth study design at different time intervals:-

1. Application of mechanical stresses during orthodontic treatment altered the level of biomarkers (IL-6 and RANKL) ,both in vibratory and non-vibratory groups at different time intervals.
2. Vibratory stimulus using electric powered tooth brush significantly elevated levels in Group II for both the biomarkers at T1, T2 and T3 in comparison to Group I.
3. Intragroup comparison for Group II for each biomarker, revealed significantly higher level of IL-6 and RANKL, after application of vibratory stimulus, to baseline values. Among intragroup comparison for Group II, between time intervals after application of vibratory stimulus, no significant difference was seen suggestive of the fact that vibratory

stimulus increased levels of biomarkers to certain extent only despite of continuous application during that period.

4. On intragroup comparison for Group I, mean levels of IL-6 were significantly higher in T0 than T1 and was higher in T2 than T1.

The most important clinical implication of present study would be that as vibratory stimulus significantly elevated level of biomarker, hence, electric powered tooth brushes can be preferred in adults subjects for increasing biomarker levels, thereby accelerating tooth movement . Also reinforcement of biomarker levels by vibratory stimulus would enable Orthodontic tooth movement in adults at lighter forces that would be optimum and comfortable for the patient. Electric powered tooth brushes being cheap, non-invasive and can be used by patient at home could be a good alternative for accelerating tooth movement and reducing treatment time in comparison to other expensive non surgical methods or traumatic surgical methods.

ANNEXURE-I



BABU BANARASI DAS COLLEGE OF DENTAL SCIENCES

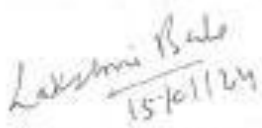
(Faculty of BBD University, Lucknow)

INSTITUTIONAL RESEARCH COMMITTEE APPROVAL

The project titled "To Evaluate The Effect Of Vibratory Stimulus On Levels Of Biomarkers During Orthodontic Tooth Movement." submitted by **Dr. ASHIMA VERMA** Postgraduate student in the **Department of Orthodontics and Dentofacial Orthopaedics** for the Thesis Dissertation as part of MDS Curriculum for the academic year 2022-2025 with the accompanying proforma was reviewed by the Institutional Research Committee in its meeting held on **16th October, 2023** at BBDCODS.

The Committee has granted approval on the scientific content of the project. The proposal may now be reviewed by the Institutional Ethics Committee for granting ethical approval.


(Prof. Dr. Puneet Ahuja)
 Chairperson (IRC)
 Principal
 BBD College of Dental Sciences
 BBD University, Lucknow


(Dr. Lakshmi Bala)
 Co-Chairperson (IRC)
 HOD Biochemistry
 BBD College of Dental Sciences
 BBD University, Lucknow.

ANNEXURE-II



BBD UNIVERSITY

Dr. Lakshmi Bala

Professor and Head, Deptt of Biochemistry and

Member-Secretary, Institutional Ethics Committee (IEC) of BBD University, Lucknow

Communication of the Decision of the VIIIth Institutional Ethics Committee Meeting

IEC Code: 28

BBDU/MDS/28/2024

Date: 07/03/2024

Title of the Project: To Evaluate The Effect Of Vibratory Stimulus On Levels Of Biomarkers During Orthodontic Tooth Movement.

Principal Investigator: Dr. ASHIMA VERMA**Department:** Department of Orthodontics and
Dentofacial Orthopaedics**Name and Address of the Institution:** BBD University, Lucknow**Type of Submission:** NEW, MDS Dissertation proposal

Dear Dr. Ashima Verma

The meeting of the Institutional Ethics Committee (IEC) was held on 06-02-2024 in Conference room, First Floor, BBDCODS, BBD University, Lucknow. Following members were present:

1	Dr. Chandishwar Nath Rtd. Chief Scientist, CDRI, Lucknow.	Chairman
2	Dr. JS Selvastava, Rtd. Chief Scientist, CDRI, Lucknow.	Member
3	Dr. Manodeep Sen, Professor, Department of Microbiology, RMLIMS, Lucknow	Member
4	Dr. Shaleen Chandra, Professor & Dean, Dental Sciences, Atal Bihari Vajpai Medical University (ABVM University), Lucknow	Member
5	Dr. Manuka Khanna, Professor, Deptt. of Political Science, Lucknow University, Lucknow	Member
6	Mr Abhishek Chaudhary, Advocate, Lucknow	Member
7	Dr. Puneet Ahuja, Professor of Oral Pathology and Principal, BBD College of Dental Sciences, BBD University, Lucknow	Member
8	Dr. Lakshmi Bala, Professor & Head, Department of Biochemistry, BBD College of Dental Sciences, BBD University, Lucknow	Member- Secretary

The committee reviewed and discussed your submitted documents of the research proposal in the meeting.

Following comments were suggested and communicated.

Comments:

1. The specific method of obtaining GCF at different time intervals along with the time interval of visits of patients should be mentioned in PID.

Thereafter, the proposal was revised by Principal Investigator and duly approved by the Supervisor and Head of the Department.

Decisions: The committee approved the above proposal from ethics point of view.

Lakshmi Bala
07/03/24

(Dr. Lakshmi Bala)

Member-Secretary IEC

BBD University

Ethics Cell (Vth Floor, BBDCODS)

ethics@bbdu.ac.in

Dr. LAKSHMI BALA
Member-Secretary
Institutional Ethics Committee
BBD University, Lucknow-38

ANNEXURE-III

Babu Banarasi Das College of Dental Sciences

(Babu Banarasi Das University)

BBD City, Faizabad Road, Lucknow – 227105 (INDIA)

Guidelines for Devising a Participant / Legally Acceptable Representative Information Document (PID) in English

Guideline for preparation of the participant information document	
While submitting your project report to the Institutional Ethics Committee, ensure that you have included participant information document and an informed consent form that is prepared as per the guidelines for Good Clinical Practice-Centre for Drug Candidate Optimization (GCP-CDCO2001), International Conference on Harmonization-Good Clinical Practice (ICH – GCP), ICMR ethical guidelines 2006, and the Declaration of Helsinki. The document is important because it enables the participants to make an informed choice. It also has got to be unique because no two research projects are identical. The participant information document (PID should include only those headings listed below which are relevant to that study. Any further information you wish to add, is your choice.	
1.	Participant information document and an consent form in English and Hindi (other languages if required)
2.	Font: Arial spacing of lines with 1.5
3.	Size: 12
4	All the consent forms must have Version No, Date, Page no in the footer
5.	In the case of participants with age ≥ 18 yrs, PID and consent form should be attached while in the case of participant's age ≤ 18 yrs and ≥ 8 yrs the above along with information document and assent form for children (minor) should be attached. In the case of ≤ 8 it will be signed by the guardian.

Potential recruits to your research/trial study must be given sufficient information to allow them to decide whether or not they want to take part. The Information Document should contain

information under the headings given below, and preferably in the order specified. It should be written in simple, non-technical terms and be easily understood by a lay person. Use short words, sentences and paragraphs.

1. Study Title

“TO EVALUATE THE EFFECT OF VIBRATORY STIMULUS ON LEVELS OF BIOMARKERS DURING ORTHODONTIC TOOTH MOVEMENT”.

2. Invitation Paragraph

—You are being invited to take part in a research/trial study. Before you decide it is important for you to understand why the research/study is being done and what it will involve. Please take time to read the following information carefully and discuss it with friends, relatives and your treating physician/family doctor if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

3. What is the purpose of the study?

TO EVALUATE THE EFFECT OF VIBRATORY STIMULUS ON LEVELS OF BIOMARKERS DURING ORTHODONTIC TOOTH MOVEMENT.

4. Why have I been chosen?

As you fulfill the criteria needed for this study .

5. Do I have to take part?

Taking part in the research is entirely voluntary. States:

—It is up to you to decide whether or not to take part. If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part you are still free to withdraw at any time and without giving a reason.

6. What will happen to me if I take part?

You will not be harmed. For fixed orthodontic treatment you have to come for every month during that period the study will be completed.

7. What do I have to do?

You have to come to the given appointment and follow your course of orthodontic treatment.

8. What is the procedure that is being tested?

GCF Sample collected from you will be analysed in lab for level of biomarker during orthodontic tooth movement

9. What are the interventions for the study?

Not an intervention study.

10. What are the side effects of taking part?

No side effects of the research procedure, as we are following normal orthodontic treatment procedure.

11. What are the possible disadvantages and risks of taking part?

No Risk .

12. What are the possible benefits of taking part?

There is no intended clinical benefit to the patient/volunteer from taking part in the study, this will be stated clearly.

13. What if new information becomes available?

—Sometimes during the course of a research project, new information becomes available about the research being studied. If this happens, your researcher will tell you about it and discuss with you whether you want to continue in the study. If you decide to withdraw, your researcher/investigator will make arrangements for your withdrawal. If you decide to continue in the study, you may be asked to sign an updated consent form.

14. What happens when the research study stops?

If the research study finishes/stops nothing will happen to your course of orthodontic treatment.

15. What if something goes wrong?

As it is non invasive procedure where GCF is only collected from you ,nothing can go wrong, however in case of any issue Contact institution or IEC.

16. Will my taking part in this study be kept confidential?

—Yes

17. What will happen to the results of the research study?

The results will be used to compare the levels of biomarkers on vibratory and non vibratory site.

18. Who is organizing the research?

This research study is organized by the academic institution (BBDCODS)

19. Will the results of the study be made available after study is over?

Yes

20. Who has reviewed the study?

You may/should mention that HOD /IRC/IEC of the institution has reviewed and approved the study (you should not however list the members of the Committee).

21. Contact for further information

You should give the patient a contact address for further information. This can be your name or that of another scientist/investigator involved in the study.

Dr Ashima verma

Department of Orthodontics and Dentofacial Orthopaedics

Babu Banarasi College of Dental Sciences.

Lucknow-227105

Mob- 9634646929

Dr. Tripti Tikku (Professor)

Department of Orthodontics and Dentofacial Orthopaedics

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Dr. Kamna srivastava(Reader)

Department of Orthodontics and Dentofacial Orthopaedics

Babu Banarasi College of Dental Sciences.

Lucknow-227105

Mob-9956099502

Name of the PI, Address, e-mail address, Telephone Numbers and name ,
Member Secretary of Ethics Committee of the institution (Dr . Lakshmi
Bala, Member Secretary, bbdcods.iec@gmail.com, ethics@bbdu.ac.in)
with address, e-mail address with telephone numbers 05226191091 (ext.
no. 1291).

*Remember to thank your patient for
taking part in the study!*

The patient information sheet should be dated
The Participant Information document should state that the participant will
be given a copy of the information sheet and the signed consent form.

Signature of PI.....

Name.....

Date.....

ANNEXURE – IV**Babu Banarasi Das College of Dental Sciences****(Babu Banarasi Das University, Lucknow)****BBD City, Faizabad Road, Lucknow – 227105 (INDIA)**

प्रतिभागी के लिए सूचना पत्र

1. अध्ययन शीर्षक

ऑर्थोडॉंटिक दांत आंदोलन के दौरान बायोमार्कर के स्तर पर कंपन सिटमुलस के प्रभाव का मूल्यांकन करना।

2. निमंत्रण अनुच्छेद

आपको एक शोध अध्ययन में भाग लेने के लिए आमंत्रित किया जा रहा है। निर्णय लेने से पहले आपके लिए यह समझना महत्वपूर्ण है कि शोध /अध्ययन क्यों किया जा रहा है और इसमें क्या शामिल होगा। कृपया निम्नलिखित जानकारी को ध्यान से पढ़ने के लिए समय निकालें और यदि आप चाहें तो दोस्तों, रिश्तेदारों और अपने इलाज करने वाले चिकित्सक /पारिवारिक चिकित्सक के साथ इस पर चर्चा करें। हमसे पूछें कि क्या कुछ ऐसा है जो स्पष्ट नहीं है या यदि आप अधिक जानकारी चाहते हैं। यह तय करने के लिए समय निकालें कि आप भाग लेना चाहते हैं या नहीं।

3. अध्ययन का उद्देश्य क्या है ?

विभिन्न समय अंतरालों पर विभाजित मुख अध्ययन डिजाइन में कंपन उत्तेजना (विद्युत चालित ट्यूब्रेशों के साथ) का उपयोग करके (स्लाइडिंग यांत्रिकी का उपयोग करके) अग्र दांतों के सामूहिक निष्कासन के दौरान बायोमार्करों के स्तर का मूल्यांकन और तुलना करना।

4. मुझे इस अध्ययन के लिए क्यों चुना गया है ?

जैसा कि आप इस अध्ययन के लिए आवश्यक मानदंडों को पूरा करते हैं ।

5. क्या इसमें मुझे भाग लेना चाहिए ?

अध्ययन का विवरण जानने के बाद यह आपका निर्णय होगा।

इसमें भाग लेना है या नहीं यह निर्णय लेना आपके ऊपर है। यदि आप भाग लेने का निर्णय लेते हैं तो आपको यह सूचना पत्रक रखने के लिए दिया जाएगा और एक सहमति प्रपत्र पर हस्ताक्षर करने के लिए कहा जाएगा। यदि आप भाग लेने का निर्णय लेते हैं तो आप किसी भी समय बिना कोई कारण बताए वापस लेने के लिए स्वतंत्र हैं।

6. मुझे क्या होगा यदि मैं इस अध्ययन में भाग लेता हूँ।

आपको नुकसान नहीं होगा। निश्चित ऑर्थोडोंटिक उपचार के लिए आपको हर महीने आना होगा उस अवधि के दौरान अध्ययन पूरा हो जाएगा।

7. मुझे क्या करना है ?

आपको दी गई नियुक्ति पर आना होगा और ऑर्थोडॉन्टिक उपचार के अपने पाठ्यक्रम का पालन करना होगा।

8. किस प्रक्रिया का अध्ययन किया जा रहा है ?

ऑर्थोडॉन्टिक क दांत मूवमेंट के दौरान बायोमार्कर के स्तर के लिए आपसे एकत्र किए गए जीसीएफ नमूने का प्रयोगशाला में विश्लेषण किया जाएगा।

9. इस शोध में कौन से हस्तक्षेप दिए जाएंगे ?

हस्तक्षेप अध्ययन नहीं .

10. इस अध्ययन में भाग लेने के क्या दुष्प्रभाव हैं ?

शोध प्रक्रिया का कोई दुष्प्रभाव नहीं है , क्योंकि हम सामान्य ऑर्थोडॉन्टिक उपचार प्रक्रिया का पालन कर रहे हैं

11. इस अध्ययन में भाग लेने के संभावित जोखिम और नुकसान क्या है ?

कोई जोखिम नहीं होगा ।

12. अध्ययन में भाग लेने के संभावित लाभ क्या है ?

अध्ययन में भाग लेने से रोगी /स्वयंसेवक को कोई चिकित्सीय लाभ नहीं होगा , यह स्पष्ट रूप से बताया जाएगा।

13. क्या होगा यदि कोई नई जानकारी उपलब्ध हो जाती है ?

कभी -कभी एक शोध परियोजना के दौरान ,अध्ययन किए जा रहे शोध के बारे में नई जानकारी उपलब्ध हो जाती है। यदि ऐसा होता है , तो आपका शोधकर्ता आपको इसके बारे में बताएगा और आपसे चर्चा करेगा कि क्या आप अध्ययन जारी रखना चाहते हैं। यदि आप वापस लेने का निर्णय लेते हैं , तो आपका शोधकर्ता /अन्वेषक आपकी निकासी की व्यवस्था करेगा। यदि आप अध्ययन जारी रखने का निर्णय लेते हैं , तो आपको एक अद्यतन सहमति फॉर्म पर हस्ताक्षर करने के लिए कहा जा सकता है।

14. क्या होता है जब अध्ययन /शोध परीक्षण बंद हो जाता है।

यदि शोध अध्ययन समाप्त /बंद हो जाता है तो आपके ऑर्थोडॉन्टिक उपचार के पाठ्यक्रम पर कुछ भी असर नहीं पड़ेगा। ।

15. क्या होगा अगर कुछ गलत हो जाता है ?

चूंकि यह गैर -आक्रामक प्रक्रिया है जहां जीसीएफ केवल आपसे एकत्र किया जाता है , इसलिए कुछ भी गलत नहीं हो सकता है , हालांकि किसी भी समस्या के मामले में संस्थान या आईईसी से संपर्क करें।

16. क्या इस अध्ययन में मेरा हिस्सा गोपनीय रखा जाएगा ?

हां।

17. अध्ययन / शोध परीक्षण के परिमाण का क्या होगा ?

परिणामों का उपयोग कंपनयुक्त और गैर-कंपनयुक्त स्थल पर बायोमार्कर के स्तर की तुलना करने के लिए किया जाएगा।

18. इस अध्ययन को कौन आयोजित कर रहा है और इस परीक्षण के लिए धन कहां से आएगा।

यह शोध अध्ययन शैक्षणिक संस्थान (बीबीडीसीओडीएस) द्वारा आयोजित किया जाता है।

19. क्या सेवाएं शोध खत्म हो जाने के बाद उपलब्ध रहेगी या नहीं ?
हां।

20. अध्ययन की समीक्षा किसने की है ?

अध्ययन की समीक्षा की गई है और विभाग के प्रमुख, और आईईसी /आईआरसी के द्वारा अनुमोदित किया गया है। निम्न लोगों से संपर्क करें

21. अधिक जानकारी के लिए संपर्क करें ।

डॉ. आशिमा वर्मा

ऑर्थोडॉंटिक्स और डेंटोफेशियल ऑर्थोपेडिक्स विभाग

बाबू बनारसी कॉलेज ऑफ डेंटल साइंसेज।

लखनऊ-226028

मोब- 9634646929

डॉ. त्रिपुति टिक्कू (प्रोफेसर)

ऑर्थोडॉन्टिक्स और डेंटोफेशियल ऑर्थोपेडिक्स विभाग

बाबू बनारसी कॉलेज ऑफ डेंटल साइंसेज।

लखनऊ-226028

मोब-9554832799

डॉ कामना श्रीवास्तव (पाठक)

ऑर्थोडॉन्टिक्स और डेंटोफेशियल ऑर्थोपेडिक्स विभाग

बाबू बनारसी कॉलेज ऑफ डेंटल साइंसेज।

लखनऊ-226028

मोब-9956099502

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पीआईकाहस्ताक्षर

नाम

दिनांक

पीआई का नाम , पता , ई-मेल पता , टेलीफोन नंबर और नाम , संस्था की
अध्यार समिति के सदस्य सचिव (डॉ . एल . ए.के . एसएचएम आई बी एल ए,
सदस्य सचिव , बीबीडीकोड्स। iec@gmail.com, नैतिकता @bbdu.ac.in) पते
के साथ , ई-मेल पता टेलीफोन नंबर 05226191091 (एक्सटेंशन नंबर
1291) के साथ। अध्ययन में भाग लेने के लिए अपने मरीज को
धन्यवाद देना याद रखें ! मरीज की सूचना पत्रक होना चाहिए
दिनांकप्रतिभागी सूचना दस्तावेज़ में कहा जाना चाहिए कि
प्रतिभागी को सूचना पत्र और हस्ताक्षरित सहमति प्रपत्र
की एक प्रति दी जाएगी। पीआई के
हस्ताक्षर नाम
.....तारीख

ANNEXURE-V**STATISTICAL TESTS USED**

Data were summarised as Mean $\pm$ SD (standard deviation). Groups were compared by paired t test. Groups were also compared by one-way analysis of variance (ANOVA) and the significance of mean difference within (intra) and between (inter) the groups was done. A two-tailed (a-2) $p < 0.05$ was considered statistically significant. Analyses were performed on SPSS software (Windows version 20).

Formula used for the analysis

The Arithmetic Mean

The most widely used measure of central tendency is arithmetic mean, usually referred to simply as the mean, calculated as

$$\bar{X} =$$

$$\frac{1}{n}$$

$$\sum_{i=1}^n X_i$$

$$i=1$$

$$n$$

The Standard Deviation

The standard deviation (SD) is the positive square root of the variance, and calculated as

$$SD = \sqrt{\frac{1}{n-1} \sum_{i=1}^n (X_i - \bar{X})^2}$$

$$SD =$$

$$n-1$$

Where, n= no. of observations

Paired t-test

Paired t-test is used to calculate the differences between two paired samples i.e. when in each observation in Sample 1 is in some way correlated with an observation in Sample 2, so that the data may be said to occur in pairs and calculated as $t = d / S_{\{d\}}$ where d is the mean of difference within each pair of measurements and $S_{\{d\}}$ the standard error of the difference which is calculated as

$$s_i = \sqrt{s^2 * (1/m + 1/n_{\{2\}})}$$

where S^2 is the pooled variance and n_1 and n_2 are number of observations in group 1 and 2 respectively. The degrees of freedom (DF) is calculated as

$$DF = n - 1.$$

Statistical significance

Level of significance "p" is the probability signifies level of significance. The mentioned p in the text indicates the following:

$p > 0.05$ -Not significant (NS)

$p < 0.05$ -Just significant (*)

$p < 0.01$ - Moderate significant (**)

$p < 0.001$ -Highly significant (***)

ANNEXURE – VI

To,
Prof. Dr. Kalpana Singh
KGMU
Lucknow.

Subject: Request to be Co-guide for thesis.

Respected Ma'am,

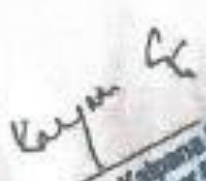
I, Dr Ashima verma ,MDS student,from the Department of Orthodontics and Dentofacial Orthopaedics,BBD is planning to conduct a study titled "To evaluate the effect of vibratory stimulus on levels of biomarkers during orthodontic tooth movement" for which collaboration from your department is needed.

I wish and request you to kindly be my Co-guide for the above study.

I will be highly obliged for the same.

Thanking you,

Yours faithfully,
Dr Ashima verma
MDS 1st Year,
Department of Orthodontics and Dentofacial Orthopaedics,
BBDCODS,Lucknow.


Dr. Kalpana Singh
Professor & Head
Department of Biochemistry
K.G.'s Medical University U.P.
Lucknow


HOD
Dept. of Orthodontics & Dentofacial Orthopaedics
B.B.D. College of Dental Sciences
Lucknow

I ,Prof. Dr. Kalpana Singh(H.O.D- Biochemistry)KGMU Lucknow giving consent to be the Co- guide for the study titled " To evaluate the effect of vibratory stimulus on levels of biomarkers during orthodontic tooth movement" which will be conducted by Dr Ashima verma MDS student, from the Department of Orthodontics and dentofacial Orthopaedics BBDCODS, Lucknow.

Signature 
Dr. Kalpana Singh
Prof. & Head
Department of Biochemistry
KGMU, Lucknow



Date 20/12/23

ANNEXURE-VII

Babu Banarasi Das College of Dental Sciences

(Babu Banarasi Das University)

BBD City, Faizabad Road, Lucknow - 227105 (INDIA)**Consent Form (English)**

Title of the Study-To Evaluate The Effect Of Vibratory Stimulus On Levels Of Biomarker During Orthodontic Tooth Movement

Study Number.....

Subject's Full Name

Date of Birth/ Age

Address of the Subject

Phone no. and e-mail address

Qualification

Occupation: Student / Self Employed / Service / Housewife/

Other (Please tick as appropriate)

Annual income of the Subject

Name and of the nominees(s) and his relation to the subject.....(For the purpose of compensation in case of trial related death).

1. I confirm that I have read and understood the Participant Information Document dated for the above study and have had the opportunity to ask questions. **OR** I have been explained the nature of the study by the Investigator and had the opportunity to ask questions.
2. I understand that my participation in the study is voluntary and given with free will without any duress and that I am free to withdraw at any time, without giving any reason and without my medical care or legal rights being affected.
3. I understand that the sponsor of the project, others working on the Sponsor's behalf, the Ethics Committee and the regulatory authorities will not need my permission to look at my health records both in respect of the current study and any further research that may be conducted in relation to it, even if I withdraw from the trial. However, I understand that my Identity will not be revealed in any information released to third parties or published.
4. I agree not to restrict the use of any data or results that arise from this study provided such a use is only for scientific purpose(s).
5. I permit the use of stored sample (tooth/tissue/blood) for future research. **Yes [] No []**
Not Applicable []
6. I agree to participate in the above study. I have been explained about the complications and side effects, if any, and have fully understood them. I have also read and understood the participant/volunteer's Information document given to me.

Signature (or Thumb impression) of the Subject/Legally Acceptable

Representative:

Signatory's Name

Date

Signature of the Investigator

Date

Study Investigator's Name.....

Date

Signature of the witness

Date

Name of the witness

Received a signed copy of the PID and duly filled consent form

Signature/thumb impression of the subject or legally

Date

Acceptable representative

ANNEXURE-VIII

Babu Banarasi Das College of Dental Sciences (Babu Banarasi Das University) BBD City, Faizabad Road, Lucknow - 226028 (INDIA)

सहमति पत्र

अध्ययन शीर्षक- ऑर्थोडॉंटिक दांत आंदोलन के दौरान बायोमार्कर के स्तर पर कंपन उत्तेजना के प्रभाव का मूल्यांकन करना

अध्ययन संख्या..

प्रतिभागी के पूर्ण नाम.

जन्म तिथि / आयु..

प्रतिभागी का पता

फोन नं. और ई-मेल पता

योग्यता

व्यवसाय: छात्र / स्व कार्यरत / सेवा / ग्रहिणी

अन्य (उचित रूप में टिक करें)

प्रतिभागी की वार्षिक आय

प्रत्याशीयो के नाम और प्रतिभागी से संबंध... (परीक्षण से संबंधित मौत के मामले में मुआवजे के प्रयोजन के लिए)

1. मेरी पुष्टि है कि मैंने अध्ययन हेतु सुचना पत्र दिनांक को पढ़ व समझ लिया तथा मुझे प्रश्न पुछने या मुझे अध्ययन अन्वेषक ने सभी तथ्यों को समझा दिया है तथा मुझे प्रश्न पुछने के समान अवसर प्रदान किए गये।
2. मैंने यहाँ समझ लिया कि अध्ययन में मेरी भागीदारी पूर्णतः स्वैच्छिक है और किसी भी दबाव के बिना स्वतंत्र इच्छा के साथ पदय समझ लिया किसनायकसी भी कारण के बिना, मेरे इलाज या कानूनी अधिकारों को प्रभावित किए बिना • अध्ययन में भाग न लेने के लिए स्वतंत्र हूँ।

लिया है कि अध्ययन के प्रायोजक, प्रायोजक की तरफ से काम करने वाले लोग, आचार समिति 3. मैंने यह समझ लिया और नियामक अधिकारियों को मेरे स्वास्थ्य रिकार्ड को वर्तमान अध्ययन या आगे के अध्ययन के सन्दर्भ देखने के लिए मेरी अनुमति की जरूरत नहीं है, चाहे मैंने इस अध्ययन से नाम वापस ले लिया है। हालांकि मैं यह समझता हूँ कि मेरी पहचान को किसी भी तीसरे पक्ष या प्रकाशित माध्यम में नहीं दी जायेगी।

4. मैं इससे सहमत हूँ कि कोई भी डेटा या परिणाम जो इस अध्ययन से प्राप्त होता है उसका वैज्ञानिक उद्देश्य (ओं) के उपयोग के लिए मेरी तरफ से कोई प्रतिबंध नहीं है। 5. भविष्य के अनुसंधान के लिए भंडारित नमूना (ऊतक / रक्त) पर अध्ययन के लिए अपनी सहमति देता हूँ।

हाँ []

नहीं []अनउपयुक्त []

मैं परीक्षण की अनुमति देता हूँ। मुझे इसके द्वारा यदि कोई परेशानी होती है, इसके बारे में जानकारी दे दी गई है। मैंने रोगी जानकारी सूचना पत्र को पढ़ तथा समझ लिया है।

प्रतिभागी/ कानूनी तौर पर स्वीकार्य प्रतिनिधि का हस्ताक्षर (या अंगूठे का निशान हस्ताक्षरकर्ता का नाम...
हस्ताक्षर अध्ययन अन्वेषक का नाम गवाह के हस्ताक्षर नाम दिनांक दिनांक दिनांक मैंने पीआईडी और
विधिवत भरे सहमति फार्म का एक हस्ताक्षर की नकल प्राप्त की. अन्वेषक के गवाह के

प्रतिभागी कानूनी तौर पर प्रतिनिधि का हस्ताक्षर / अंगूठे का निशान

BBDCOD

ANNEXURE-IX

Submission ID trn:oid:::27450:88557479

Ashima Verma**RE-2022-516067** Peninsula College**Document Details****Submission ID** trn:oid:::27450:88557479**Submission Date**

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