EVALUATION OF SALIVARY BIOMARKERS IN ORTHODONTIC PATIENTS WITH RECENT AND HEALED EXTRACTION SITES DURING EN MASSE RETRACTION - A PILOT STUDY

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AIIMS, JODHPUR **DR. VAGHELA NIRAJ HIMMATLAL**

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CERTIFICATE

This is to certify that thesis entitled "**EVALUATION OF SALIVARY BIOMARKERS IN ORTHODONTIC PATIENTS WITH RECENT AND HEALED EXTRACTION SITES DURING EN MASSE RETRACTION - A PILOT STUDY**" is an original work of **Dr. Vaghela Niraj Himmatlal** carried out under our direct supervision and guidance at Department of Dentistry, All India Institute of Medical Sciences, Jodhpur.

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DECLARATION

I, hereby declare that the work reported in the thesis entitled "**EVALUATION OF SALIVARY BIOMARKERS IN ORTHODONTIC PATIENTS WITH RECENT AND HEALED EXTRACTION SITES DURING EN MASSE RETRACTION - A PILOT STUDY**" embodies the result of original research work carried out by me in the Orthodontics and Dentofacial Orthopaedics section, Department of Dentistry, All India Institute of Medical Sciences, Jodhpur.

I further state that no part of the thesis has been submitted either in part or in full for any other degree of All India Institute of Medical Sciences or any other Institution/ University.

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-Dr. Vaghela Niraj Himmatlal

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INTRODUCTION

Sound orthodontic diagnosis and treatment planning intricates the need for critical evaluation of orthodontic treatment need. If deemed necessary, successful therapy of corrective orthodontics requiring tooth extraction is determined by the orthodontist's judgement based on a series of variables, which being the cephalometric analysis, the study model analysis, variables pertaining to patient's physiology and other diagnostic aids (1,2)

Tooth extraction is a common treatment consideration in orthodontic management of cases such as dental crowding, jaw growth discrepancy, jaw- tooth size discrepancy and tooth pathology or injury (2). The extraction space is closed by either retracting the anterior segment of teeth or mesialization of the posterior segment of teeth. Space closure can be achieved with en masse retraction of anterior segment or retraction of canine into the extraction space followed by retraction of the four anterior teeth. Movement of teeth into an extraction space is depended on the biomechanical procedure of retraction mechanics and other biological factors. The variation in the structure of bone is one such biological factor (3,4).

Previous studies (5–8) have shown that orthodontic tooth movement follows a specific pattern in time. The initial phase takes 24 hrs to 2 days representing initial movement in bony socket; the lag phase lasts for 20-30 days attributed to hyalinization of the periodontal ligament; the post-lag phase accelerates the tooth movement after removal of the hyalinized tissue and the movement continues through the linear phase. The third and fourth phases comprise the real tooth movement. Bohl et al.(8) in their study observed that after 20, 40, and 80 days of orthodontic force application, when the tooth movement had reached its linear phase, many pressure sides showed irregular bone surfaces due to direct bone resorption.

The teeth can be moved into an extraction site where healing has been allowed to take place or tooth movement can start immediately after extraction. According to Amler et al.(9) healing of an extraction alveolus is a rapid process. They found that two-thirds of the alveolus (starting from the base of the socket) was filled with bone trabeculae by the 38^{th} day after extraction. Studies (4–7) have been done on animals where surgically accelerated tooth movement and histological characteristics of

extraction sockets were investigated. Loui and Huang(9) and Hasler et al.(4) found that tooth movement was faster at sites of recent extraction whereas, another animal study done by Samruajbenjakun et al.(12) found that there was no difference in the rate of tooth movement in rats between the recent and healed extraction socket group. They stated that the amount of nonmineralized periodontal space was determined by the time period of tooth movement during the process of bone healing. Depending on the physical characteristics of the applied force, size and biological responses of the periodontal ligament, orthodontic tooth movement can be rapid or slow.

Orthodontic tooth movement is relied on the development of force-induced mechanical strain in the paradental tissues which creates a cellular, molecular and genetic level reaction (13). The cells respond to their change in the environment. The paradental tissues when exposed to varying degrees of force magnitude, frequency and duration of mechanical load, expresses extensive macroscopic and microscopic changes. The nervous network, vascularity and blood flow of the periodontal ligament is altered. This leads to release of various molecules that evoke a plethora of cell responses by many cell types in and around the teeth and therefore, creates a favorable microenvironment for tissue changes and facilitates remodeling of periodontal ligament and alveolar bone (13). Remodeling of alveolar bone is the synchronized interaction of osteoblasts, osteoclasts, osteocytes and periodontal ligament (PDL) cells that contributes to the bone formation and bone resorption cyclic sequence. The types of cells managing the bone remodeling cycle form the bone multicellular unit (BMU) (13,14).

The different cells of the BMU act in a specific sequence composed of four phases (14). During the activation phase, receptor activator of nuclear factor K ligand(RANKL), a homotrimeric protein present on the cell surface of the preosteoclasts from the bone marrow interacts with receptor activator of nuclear factor K (RANK), a homotrimeric transmembrane protein member of the tumor necrosis factor (TNF) family (14,15). Their linkage leads to the activation and differentiation of the preosteoclasts into mature osteoclasts and these cells resorb the bone which lasts for about two weeks. In the reversal phase, preosteoblasts that have migrated into the resorption lacunae, matures and differentiates into mature osteoblast. These osteoblasts secrete osteoprotegerin (OPG), a free-floating soluble decoy receptor belonging to the tumor necrosis factor family, that binds with the RANKL and, thus prevents further

activation of the preosteoclast which in turn inhibit resorption activity by the osteoclast (14,15). In general, when RANKL expression increases, OPG expression decreases or is not induced to the same extent as RANKL, causing the RANKL/OPG ratio to shift in favor of osteoclastogenesis. The new bone is laid and mineralization is achieved in approximately 3-4 months. These activities are spatially and temporally coupled in a cyclic sequence (14–16).

The activity of the BMU can be measured biochemically by determining markers of bone remodeling(15). Oral fluid markers of the mechanisms associated with the paradental tissue changes that account for tooth movement could help identify, assess, and improve tooth movement. Both saliva and gingival crevicular fluid (GCF) are inexpensive potential sources of personalized oral and general health information that are easily and noninvasively collectable. Acquisition methods of saliva requires less manpower and materials than GCF (17–19). Saliva has contributions from the salivary glands, gingival crevicular fluid, blood, mucosa and upper respiratory tract. Salivary testing in research field has advanced and accelerated because of novel approaches that can characterize genotypes, proteins, metabolites, and peptides within an individual sample (16). Previous studies have shown that the use of saliva, provided a sensitive and inexpensive detection technique for determining analytes in the periodontal microenvironment**.** According to Nunes et al.(17) for genetic analysis, saliva collection has the advantages over blood sample as it can be relatively noninvasively collected and storable long term at room temperature with appropriate preservatives.

On clinical level, studies evaluating whole saliva have identified several salivary protein biomarkers in patients during orthodontic tooth movement. Florez et al.(18)have observed time-related variations in soluble form of receptor activator of nuclear factor kappa B ligand (sRANKL) and OPG ratio that increases overtime with the treatment and suggested that their ratio might be linked to the different phases of orthodontic tooth movement. Recently, Reiss et al.(19) observed salivary biomarkers for a period of 3 months and found no difference in the expression of salivary biomarkers and the rate of mandibular anterior alignment when supplemented with the vibratory force. These studies were done on two or more groups with a single or multiple time points during the duration of orthodontic treatment. Histological reports of Murphy et al.(20) observed tension and compression areas of recent extraction site

and healed extraction site in monkeys. They found increased osteoclastic activity at recent extraction site and more osteoblastic activity on healed extraction site. Retraction of teeth into recent extraction site has been claimed to stimulate increased osteoclastic activity process by alternating the levels of pro-inflammatory cytokines(4,11,12).

In the past studies have examined the biochemical markers in whole saliva of patients undergoing fixed appliance therapy during the initial stages of treatment (18,19). However, to the best of our knowledge no study have evaluated these biomarkers during extraction space closure based on the type of extraction socket. In addition, many studies which concentrate on rate of en masse retraction of anterior teeth (21–23), has not made any distinction between healed and recent extraction site. Therefore, the aim of this study was to evaluate the variations in the salivary concentration of RANKL and OPG, during en masse retraction of anterior teeth into recent extraction and healed extraction site.

AIMS AND OBJECTIVES

Aim:

To evaluate and compare the levels of salivary biomarkers (RANKL and OPG) in orthodontic patients during en masse retraction with recent and healed extraction sites.

Objectives:

The study was carried out to assess:

1) Primary outcome:

- To evaluate quantitative variations of RANKL and OPG in orthodontic patients during en masse retraction in recent and healed extraction sites.

2) Secondary outcome:

- To compare the ratio of salivary biomarkers (RANKL/OPG) in orthodontic patients with recent and healed extraction sites during en masse retraction.

-To evaluate and compare the rate of tooth movement in subjects during en masse retraction between recent and healed extraction sites.

REVIEW OF LITERATURE

Diedrich and Wehrbein (11) in 1997, assessed orthodontic retraction into recent and healed extraction sites on three female foxhounds aged 3.5 years. Second incisors were extracted bilaterally, and reciprocal space closure was initiated. In group 1 (6 teeth) retraction was done 12 weeks after extraction and in group 2 (6 teeth) retraction was initiated immediately. Clinical, radiologic and histologic analysis was done to evaluate the advantages of early or late treatment. The histologic findings showed that group 1 had low bone density with more matured lamellar bone whereas, group 2 had high density of bone with less matured bundle bone, broader alveolar process and reduced tendency towards gingival invagination. They suggested that orthodontic retraction should be initiated at an early stage into recent extraction sites.

Hasler et al. (4) in 1997, studied the rate of maxillary canine retraction into healed and recent extraction sites of the first premolar. Twenty-two patients aged 10-27 years requiring first premolar extraction for relieving maxillary crowding and/or an increased overjet were included in the study. The maxillary first premolar on one side of the arch was extracted randomly on these patients (11 patients on right side and 11 patients on left side). Dental impression and intra-oral radiograph were obtained and time point noted as T1. The contralateral side was extracted between 52-151 days and time point noted as T2. The canine was retracted into extraction site using Gjessing canine retraction spring. After the space was closed final recordings were made and time point noted as T3. They found that the canine moved faster on the recent extraction alveolus into the premolar site than the healed extraction site. The canine tipping was increased on the recent extraction site which might have been due to increased rate of tooth movement.

Kanzaki et al. (24) in 2004, conducted a study to detect that local induction of OPG at the compression site of the periodontium might neutralize the RANKL activity induced by the mechanical compression force, inhibiting osteoclastogenesis and diminishing orthodontic tooth movement. Twenty wistar rats divided into three groups were studied. Three rats were used as control, eight rats subjected to orthodontic force and nine rats were subjected to orthodontic force together with local OPG gene transfer. 0.012 in NiTi wire was placed between the right and left upper first molars causing them to move

palatally. Their study reported that OPG gene transfer to periodontal tissue inhibited RANKL-mediated osteoclastogenesis and inhibited orthodontic tooth movement

Nishijima et al. (26) in 2005, did an in vitro study to determine the levels of RANKL and OPG in gingival crevicular fluid during orthodontic tooth movement. They also investigated the effect of compression force on RANKL and OPG production from human periodontal ligament cells. Ten orthodontic patients, four men aged 14.5±2.4 years and six women aged 15.4±3.1 years requiring first premolar extraction were studied. The canines were retracted along an 0.018 in archwire with an elastomeric chain. Study models and gingival crevicular fluid were taken at 0, 1, 24 and 168 hours after force application. They detected that the level of RANKL were significantly higher and OPG levels were significantly lower in gingival crevicular fluid at 24 hours. There were no significant differences at 0,1 or 168 hours. They also found that the compression force increased the secretion of RANKL at approximately 16.7-fold and reduced the secretion of OPG at approximately 2.9-fold compared with the control in human periodontal ligament cells.

Kawasaki, Takahashi, Yamaguchi and Kasai (27) in 2006, conducted a study to compare the receptor activator of nuclear ligand (RANKL) and osteoprotegerin (OPG) levels in the gingival crevicular fluid in response to orthodontic treatment in juvenile and adult patients. Fifteen patients were evaluated in each group. After 3 months of extraction of first premolar, canine retraction was performed using elastomeric chain. GCF was collected at different time points from the distal cervical margins of teeth: 0, 1, 24 and 168 hours after application of a retraction force. The biomarkers were evaluated using enzyme linked immunosorbent assay kits. Their results suggested that RANKL/OPG ratio was lower in adult patients than the juvenile patients. The RANKL levels were increased from the compression side after 24 hour of force application. The rate of tooth movement was larger for the juvenile patients after 168 hours.

Kanzaki et al. (28) in 2006, conducted a study to determine if local RANKL gene transfer would boost RANKL concentration in the periodontal tissue, leading to increased osteoclastogenesis and accelerates orthodontic tooth movement. On wistar rats, the upper first molars were moved palatally using orthodontic wires. Local RANKL gene was induced periodically into the palatal periodontal tissue of the upper first molars during orthodondtic tooth movement. They observed that in the transfer

site, osteoclastogenesis was activated in the periodontal tissue and the rate of tooth movement was significantly increased.

Yamaguchi et al. (29) in 2006, conducted a study to examine the effect of compressive force on the production of these cytokines and on osteoclast formation. Ten patients with Angle's class I crowding requiring extraction cases were divided into the severe resorption group and the non-resorption group. The maxillary central incisors were used for classification of dental root resorption. Periodontal cells were continuously compressed at the site of orthodontic treatment. The premolars were extracted in the group with severe root resorption and the periodontal samples were thawed and made available for study. They observed that RANKL was expressed greatly in the severe root resorption group than in the non-resorption group. The expression of OPG was reduced and the TRAP-positive cells and resorptive pits were increased in the severe root resorption group than in the non-resorption group.

Dunn et al. (30) in 2007, did a study to examine the role of osteoprotegerin in regulating mechanically induced bone modelling in a rat model of orthodontic treatment. Thirty male Sprague-dawley rats were divided into groups of ten and were subjected to two doses of 5.0mg/kg human OPG-Fc, 0.5mg/kg -c,or phosphate buffered saline vehicle. The maxillary first molars were moved mesially using a calibrated nickel-titanium spring attached to the maxillary incisor teeth. Stone casts were used to determine rate of tooth movement, micro-computed tomography and histomorphometric analysis was used to quantify osteoclasts and volumetric parameters and enzyme-linked immunosorbent asssay to detect a bone resorption marker (TRAP-5b) at baseline, 3,7,10,14,17 and 21 days after appliance placement. They concluded that at targeted dental sites, local delivery of OPG-Fc inhibits osteoclastogenesis and tooth movement at days 7, 14 and 21 days.

Kim et al. (31) in 2007, studied the RANKL expression in periodontal ligament of rats subjected to continuous orthodontic force. Fifty-five day old wistar rats were studied. The upper first molars were expanded laterally using NiTi coil spring in the study group and were extracted at days 0,1,3,and 7 after expansion. The periodontal tissues obtained were observed by immunohistostaining with anti-RANKL and the tartrate-resistand acid phosphatase staining. They found that RANKL was seen on the compression side

of the expansion group at 1, 3 and 7 days and TRAP-positive cells was seen on day 3 and 7.

George and Evans (32) in 2009, conducted a study to detect root resorption by determining the levels of OPN (osteopontin), OPG (osteoprotegerin) and RANKL (receptor activator of nuclear factor kappaB ligand) in gingival crevicular fluid (GCF). One control and two study group were studies with twenty patients in each group. One study group had patients with mild root resorption less than 2mm of root loss and the other study group with severe root resorption more than 2mm of bone loss. The eluted GCF was analysed using western blot and enzyme-linked immunosorbent assay techniques. They found that the matrix proteins and cytokines were present in the GCF of root resorbed subjects. The concentrations of OPG and RANKL were higher in the study group. The RANKL/OPG ratio showed higher RANKL concentration than OPG in the study groups but lower than the control group.

Brooks et al. (33) in 2009, did a study to determine if mechanical loading on a tooth activates the proliferation of periodontal ligament cells and specific gene products within the periodontal ligament in the early phases of orthodontic tooth movement. Twenty-two Sprague Dawley rats were divided into two test groups of 3hours and 24 hours of force duration. Force was applied to the maxillary first molar. Immunohistochemical staining was performed to map the spatial expression patterns of three proteins (RANKL, Runx2 and TRAP) at 3 hour and 24 hours. They observed increased expression of KI-67 and RANKL on compression sites of the periodontal ligament after 3 hours of force application. There was increased expression of Ki-67 and Runx2 in the tension areas after 24 hours of force application.

Tan et al. (34) in 2009, conducted a study to investigate whether OPG and RANKL showed differential expression with time related to accelerated tooth movement in ovariectomized rats. Eighty-four semale Sprague-dawley rats were studied. Forty-two rats were ovariectomized bilaterally from the dorsal side and the other forty two were subjected to sham surgery during which ovaries were exteriorized. The molar was moved mesially after three months. After activation of force, the rats were sacrificed at days 0, 1,3,5,7,10 and 14. Immunohistochemistry was done to determine the expression of RANKL and OPG and the rate of tooth movement was measured at each time point as the difference between the initial and the final measurement at day 0. Their study

showed that three phase of tooth movement was seen in both the groups. The expression of RANKL was increased on the compression side and the expression of OPG was decreased on the tension side. The rate of tooth movement was greater in the ovariectomized rats comparatively.

Nakano et al. (35) in 2011, investigated the expression of RANKL/RANK and macrophage colony stimulating factor (M-CSF/c-fms) during root resorption in rats at the time of experimental tooth movement. Forty six-week old wistar rats were studied. Orthodontic force of 10-50gm was applied to the upper first molars for 10 days, inducing a mesial tipping movement of the first molars. After the experimental tooth movement, the sample was sliced into 6 micrometre continuous sections in a horizontal direction and stained biochemically. They found that with orthodontic force of 50g, immunoreactivity for RANKL/RANK and M-CSF/c-fms was detected in odontoclasts on day 7 and 10.

Hart (36) in 2012, studied the levels of RANKL and OPG expression in human gingival crevicular fluid in growing and adult patients in response to orthodontic treatment using enzyme linked immunosorbent assay technique. Fifty-four patients (twenty-six growing patients and twenty-eight adult patients) were studied. Trans-palatal spring was used across left and right pair of premolars. Gingival crevicular fluid was measured from the pressure and tension sides of the maxillary premolars at five different time points: befor force application, 1 day, 2 days and 5 days after force application and 3 days after removal of the trans-palatal spring. They found that RANKL level was increased during force application and OPG level was increased during removal of force. The RANKL/OPG levels were higher in growing patients than the adult patients due to general growth and remodelling in response to orthodontic forces.

Zhang et al. (37) in 2012, in their study compared differences in protein mass peaks from orthodontic patients with different treatment durations using matrix-assisted laser desorption/ ionization time-of-flight mass spectrometry combined with magnetic bead. Peptide mass fingerprints were also created by scanning MS signals. Saliva samples of 40 patients were analysed, where they were divided into four groups: the group without an appliance and groups under treatment for 2, 7, and 12 months. Significant differences with eight protein mass peaks were observed in their study. The findings from the study suggested that complicated changes occur in periodontal tissues during orthodontic

treatment and indicated dynamic interactions between orthodontic treatment and the saliva proteome.

Ellias et al. (38) in 2012**,** did a study to identify salivary protein biomarkers that change in expression during orthodontic treatment. Whole saliva from three female subjects (20-25 years old) undergoing orthodontic treatment were collected before force application using fixed appliance and at 14 days after 0.014" Niti wire was placed. Salivary proteins were resolved using two-dimensional gel electrophoresis over a pH range of 3-10, and the resulting proteome profiles were compared. Differentially expressed protein spots were then identified by MALDI-TOF/TOF tandem mass spectrometry. A total of eight proteins were found to have changed in expression. Four of these eight proteins- Protein S100-A9, immunoglobulin J chain, Ig alpha-1 chain C region, and CRISP-3 have known roles in inflammation and bone resorption. They concluded that these proteins have the potential to be used as potential biomarkers to monitor the progression of orthodontic treatment.

Flórez-Moreno et al. (18) in 2013, did a study on 25 subjects undergoing orthodontic treatment to determine the variations in salivary cooncentrations of soluble receptor activator of nuclear factor kappa B ligand (sRANKL) and osteoprotegerin (OPG). They also assessed if these markers are linked with different phases of orthodontic tooth movement. The selected subjects did not undergo extraction of tooth or any surgical procedures. Unstimulated whole saliva was collected at different time points. Salivary sRANKL and OPG concentrations were determined by enzyme-linked immunosorbent assays. They found that overall, median values of sRANKL showed significant increases, median OPG salivary values showed a significant downward trend, and the sRANKL/OPG ratio tended to increase significantly over time after the activation visit. The immunoenzymatic findings showed clear fluctuations at different sampling times, indicating nonlinear trends in the levels of the biomarkers through time. Their findings indicated that variations in salivary concentrations of sRANKL and OPG and their ratios might be linked to the different phases of the orthodontic tooth movement.

Barbieri et al. (39) in 2013, did a randomized, pilot clinical trial to evaluate the expression of receptor activator of nuclear factor kappa (RANK), osteoprotegerin (OPG), osteopontin (OPN), and transforming growth factor (TGF-B1) in gingival crevicular fluid in response to orthodontic treatment. Ten healthy patients (aged 20-50

years) were studied using split mouth design. Orthodontic elastic separators were placed between the 2nd premolar and the 1st molar on one side. GCF samples was taken from the mesiobuccal and mesiolingual sides of the 1st molar at different time points: before placement of separator, 24 hours and 7 days after placement of separator. The separator was removed after 7 days. The study showed that the concentration of OPG significantly decreased on the compression side and the bone resorptive mediators (RANKL and TGF-B1) increased on the compression side.

Grant et al. (40) in 2013, conducted a controlled longitudinal intervention study to investigate the changes in cytokines and biomarkers of bone and tissue metabolism within gingival crevicular fluid from patients undergoing orthodontic treatment. Twenty-one patients aged 12-20 years requiring upper first premolar extraction were studied. Gingival crevicular fluid, impression and periodontal examination was taken before commencement of treatment, after extraction, after appliance placement and during treatment progress- 4 hour, 7 days and 35 days after distalising force has been applied to maxillary canine. Their data demonstrated that elevated levels of pro-inflammatory cytokines and biomarkers of tissue and bone metabolism were seen at 4hours after force application and it continued upto 6 weeks period.

Navarro-Palacios et al. (41) in 2014, conducted a study to measure myeloperoxidase enzymatic activity in gingival crevicular fluid (GCF) and whole saliva in orthodontic patients with different levels of dental crowding at the alignment phases of orthodontic treatment with the same arch wires. Twenty orthodontic patients were evaluated and grouped into crowding of the mandibular anterior teeth as having severe or minimum crowding according to the irregularity index. GCF and saliva samples were collected immediately before the placement of orthodontic appliances at baseline, 2 hours, 7 days, and 14 days after initial orthodontic activation. Myeloperoxidase (MPO) activity was measured using the modified Bradley-Bozeman technique. They found that the maximum activity was at 2 hours after activation in both the groups. It remained elevated until day 7 and the values were similar to baseline value at day 14. They concluded that although the myeloperoxidase activity was not correlated with dental crowding, the values in GCF reflected the inflammatory changes more accurately than the values in saliva.

Rody et al. (42) in 2014, investigated the differences in the gingival crevicular fluid composition between adults (aged, 21-39 years) and adolescents (aged, 13-15 years) undergoing orthodontic treatment. Twenty patients with class I malocclusion and minor crowding were equally divided into each group. Orthodontic appliance was placed for 20 weeks, arch-wire sequence was changed and GCF sample was collected from labial sides of upper incisors (experimental sites) and lower incisors (control sites) at different time interval: before appliance placement, 3 weeks after placement and insertion of 0.014-in NITi, 6 weeks after insertion of 0.014-in NiTi, 6 weeks after insertion of 0.016x0.022-in NiTi wire and 2 weeks after insertion of 0.019x0.025-in NiTi. Aliquotes from diluted GCF was screened for biomarkers. Their study demonstrated that IL-1, IL-1RA, RANKL and OPG levels may be used to help differentiate tissue response between adults and adolescents in response to orthodontic treatment. RANKL-OPG ratio was at peak in adolescents 6 weeks after placement of 0.016x0.022 NiTi. The ratio of IL-1 to IL-1RA decreased in adults, 3 weeks after appliance placement and first archwire activation (0.014-in NiTi).

Jayachandran et al. (43) in 2017, have assessed and compared the concentration of salivary leptin levels in in normal weight and overweight individuals and have also evaluated the rate of orthodontic tooth movement. Only female subjects were included in the study where they were grouped into groups: group I (control group, age 14-28 years) with body mass index between 18.5 and 25 kg/m^2 and group II (overweight group, age 14-30 years) with body mass index between 25 and 30kg/m^2. Unstimulated whole saliva was collected just before orthodontic force application (T0) and 1 hour (T1) and 1 month (T2) after force application. Distal force was applied in the maxillary right canine using active lace backs. The rate of tooth movement was evaluated over three months and was measured on study models. They found that the mean leptin concentration was two to three times greater in overweight individuals than normal weight individuals at all three-time intervals. The mean leptin concentration was found to increase significantly at 1 hour after force application (T1), compared with the baseline value (T0) and at 1 month after force application (T2), the levels decreased to less than the baseline value (T0). The mean rate of tooth movement was less in overweight group compared with the normal weight group. There was a positive correlation of salivary leptin concentration and rate of tooth movement.

Kaczor-Urbanowicz et al. (44) in 2017, did a study to discover potential diagnostic protein biomarkers for detection of orthodontically induced inflammatory root resorption in whole saliva. Forty-eight patients undergoing orthodontic treatment and twenty-four untreated patients were examined. Unstimulated whole saliva was collected from the subjects and periapical radiographs of 4 upper incisors were taken before and nine months after bonding for radiographic assessment. The pooled saliva was subjected to amylase-depletion device where, ProteoPrep Immunoaffinity Albumin and IgG Depletion Kit was used for albumin and immunoglobulin G removal. They found a unique panel of biomarkers candidates for early identification and monitoring of root resorption in susceptible orthodontic patients.

Wu et al. (45) in 2018, conducted a study to identify a panel of differentially expressed candidate biomarkers for patients undergoing accelerated osteogenic orthodontics. Saliva samples of six class III patients: two males and four females (mean age 20.0$\pm$1.5) were taken at the time of maxillary corticotomy and five time points thereafter preceding orthognathic surgical correction of class III malocclusion. Peptide mass fingerprints were created using matrix-assisted laser desorption/ ionisation time-of-flight mass spectrometry (MALDI-TOFMS) combined with magnetic beads. The study showed that the salivary protein profiles changed with accelerated osteogenic orthodontic treatment duration. The mass peaks after corticotomy predicted to be Apoliprotein A-I precursor that increased sharply in T2 and then decreased, complement component 3 decreased in T2, then gradually increased and declined in T6, vitamin D-binding protein precursor increased in T2, then fell to the preoperative level and Isoform 1 of the fibrinogrn alpha chain precursor first decreased, then increased with time.

Samruajbenjakun, Kanokpongsak and Leethsnskul (12) in 2018, conducted a split-mouth randomized controlled trial to investigate the rate of tooth movement and histological characteristics into recent and healed extraction sites combined with corticotomy. Thirty-two adults male wistar rats were studied, grouped into two groups: healing extraction socket and recent extraction socket. They were sub-grouped into four subgroups based on number of days between corticotomy induction and mandible removal. The maxillary first molar was extracted on one side and allowed to heal for 60 days. The contralateral side was extracted and at the mid alveolar level the alveolus was intervened surgically. Orthodontic retraction force was initiated. The rate of tooth

movement measured showed no significant differences between the healed and recent extraction group at the four time points. The histologic analysis also showed no significant differences between the group but they showed regional acceleration phenomena during every time points. They suggested that the rates of tooth movement did not differ between the groups.

MATERIALS AND METHODOLOGY

Setting and Location

Patients willing to undergo treatment with fixed orthodontic appliances for correction of their malocclusion were recruited from orthodontic OPD of Department of Dentistry, AIIMS Jodhpur. Ethical approval was obtained from the Institutional Ethics Committee, AIIMS Jodhpur (AIIMS/IEC/2019-20/977), Rajasthan, India (Annexure – I). After attaining information about the study, either the patient or the guardian signed the informed consent. This study was registered prospectively at Clinical Trials Registry India, CTRI/2020/12/029660.

Trial Design

This pilot clinical trial was randomized, open label, parallel group, single blinded, active control study with 1:1 allocation ratio.

Study Duration

The recruitment of the subjects was started in January 2020 and the observation period ended in October 2021. The subjects were enrolled before the beginning of orthodontic treatment and observation period was started as patient reached retraction phase.

Sample Size

This trial is a pilot study undertaken in anticipation of conducting a larger randomized clinical trial. At the time of trial initiation, no published estimates had been reported on the concentration of salivary biomarkers in recent and healed extraction site during en masse retraction. A convenient sample of 20 subjects from the target population was used in this pilot study.

Study Population

Patients of both sexes, requiring fixed orthodontic treatment for the correction of their malocclusion, were recruited based on pre-defined inclusion criteria. Patients of age between 12 and 30 years were included. Mean age of all the participants in healed and recent extraction site groups were 19.8(±3.56) and 23.29(±5.28) years, respectively.

Participants

The subjects were included based on the following criteria:

A. Inclusion Criteria-

- Age group of 12-30 years, at the beginning of fixed orthodontic treatment.
- Patients requiring extraction of maxillary first premolars for correction of malocclusion (Class II Division 1 malocclusion, Bimaxillary protrusion).
- Minimal arch length tooth size discrepancy.
- Full permanent dentition with sound first and second molars.
- Good oral hygiene.

B. Exclusion Criteria-

- Patients who did not give informed consent.
- Patients with pregnancy and lactation.
- Patients consuming alcohol or tobacco use.
- Any systemic condition that could affect periodontal status.
- Previous history of orthodontic treatment.
- Patient with disorders of bone metabolism (e.g., osteoporosis, gastrointestinal diseases related to nutrition and mineral metabolism, endocrine diseases, immunologic disorders, and connective tissue diseases).
- Patient already under medication for treatment of conditions like heart ailments, joint replacements, hormonal or bisphosphonate antiresorptive therapies, and chronic therapy with heparin or corticosteroids.
- Patients with developmental anomalies like, cleft lip and palate and other craniofacial abnormalities.

Randomization, Allocation concealment and Blinding

Randomization:

Twenty patients undergoing fixed orthodontic treatment were randomly allocated into two groups equally using variable block randomization scheme. In the healed extraction site (control) group, extraction of premolars was caried out at least three months prior to any application of retraction forces while in the recent extraction site intervention group, retraction force was applied immediately after extraction

of premolars. The randomization sequence was computer generated and the participants were allocated into healed and recent extraction sites group by a single operator as per the randomization sequence.

- Group I: Healed extraction site (Mean age: 19.8 ±3.56 years): Retraction force was applied at least three months after extraction of premolar teeth when the extraction socket were healed.
- Group II: Recent extraction site (Mean age: 23.29 years): Retraction force was applied on the same day of extraction of premolar teeth.

Allocation Concealment:

The allocation concealment was achieved using an opaque sealed envelope (sequentially numbered as per randomization scheme). Clinician (operator) was handed over these envelopes whenever a patient was recruited in the trial. The concealed envelops were handled by a person who was not involved in the trial.

Blinding:

The study was designed as active control, single blind study. Participants and the operator were not blinded to the treatment allocated in each group. The outcome assessor was blinded with regard to allocation groups of salivary samples.

Interventions

All the patients were bonded with 022×028 inch MBT (M.B.T.) bracket appliance system. After the completion of alignment and leveling, when the patients were ready for en masse retraction they were randomly allocated into two groups (Figure 10). In the healed extraction site group, retraction force was applied after three months of healing of premolar extraction site. In the recent extraction site group retraction force was applied immediately (same day of extraction). About 5 ml of patient's unstimulated whole saliva sample was collected after the application of retraction forces. Subjects were told to abstain from eating or drinking for one hour before saliva sample collection. The saliva sample was collected into a sterile tube at all timepoints by a passive drooling for either 5 minutes or until 5 ml was reached. Saliva sample were then centrifuged at 4000rpm for eight minutes. The supernatant was collected and aliquoted into 500 µL volume and frozen at -80º until processed (Figure 4).

Salivary samples were collected at 0 weeks (baseline) after completion of leveling and alignment and at two, eight, twelve weeks respectively after application of retraction force in each group (Figure 1). Salivary samples were later analyzed for RANKL and OPG concentration using human Enzyme-linked immunoassay for RANKL and OPG (Figure 2-3). Maxillary arch impression were made at 0 weeks, 2 weeks, 8 weeks and 12 weeks respectively (Figure 1). Study models were prepared for assessment of amount of tooth movement (Figure 11). Salivary samples were processed as per methodology described below.

Sampling and Processing Preparation

At the time of biomarker analysis, the salivary samples and kit was equilibrated at room temperature, 100µL of standard working buffer was added to each sample and gradually diluted which was subjected to incubation at 37ºC for 80 minutes. The liquid in the plate was discarded and 200µL wash buffer added to each well, and the plate washed three times using automated ELISA plate washer (Figure 5). After spin-drying, 100µL biotinylated antibody working solution added to each well, and incubated at 37ºC for 50 minutes. The liquid in the plate was discarded and 200µL of wash buffer added to each well, and the plate washed three times. After drying, 100µL Streptavdin- horseradish peroxide (HRP) working solution added to each well, and incubation was done at 37°C for 50 minutes. The liquid in the plate was discarded and 200µL of wash buffer added to each well, and the plate washed five times. After spin-drying, 90µL tetramethylbenzidine (TMB) added to each well and incubated at 37°C for 20min. Stop solution of approximately 50µl was added to each well and plates were read immediately at 450nm using absorption reader (Fig.6), followed by which calculation of the results was done.

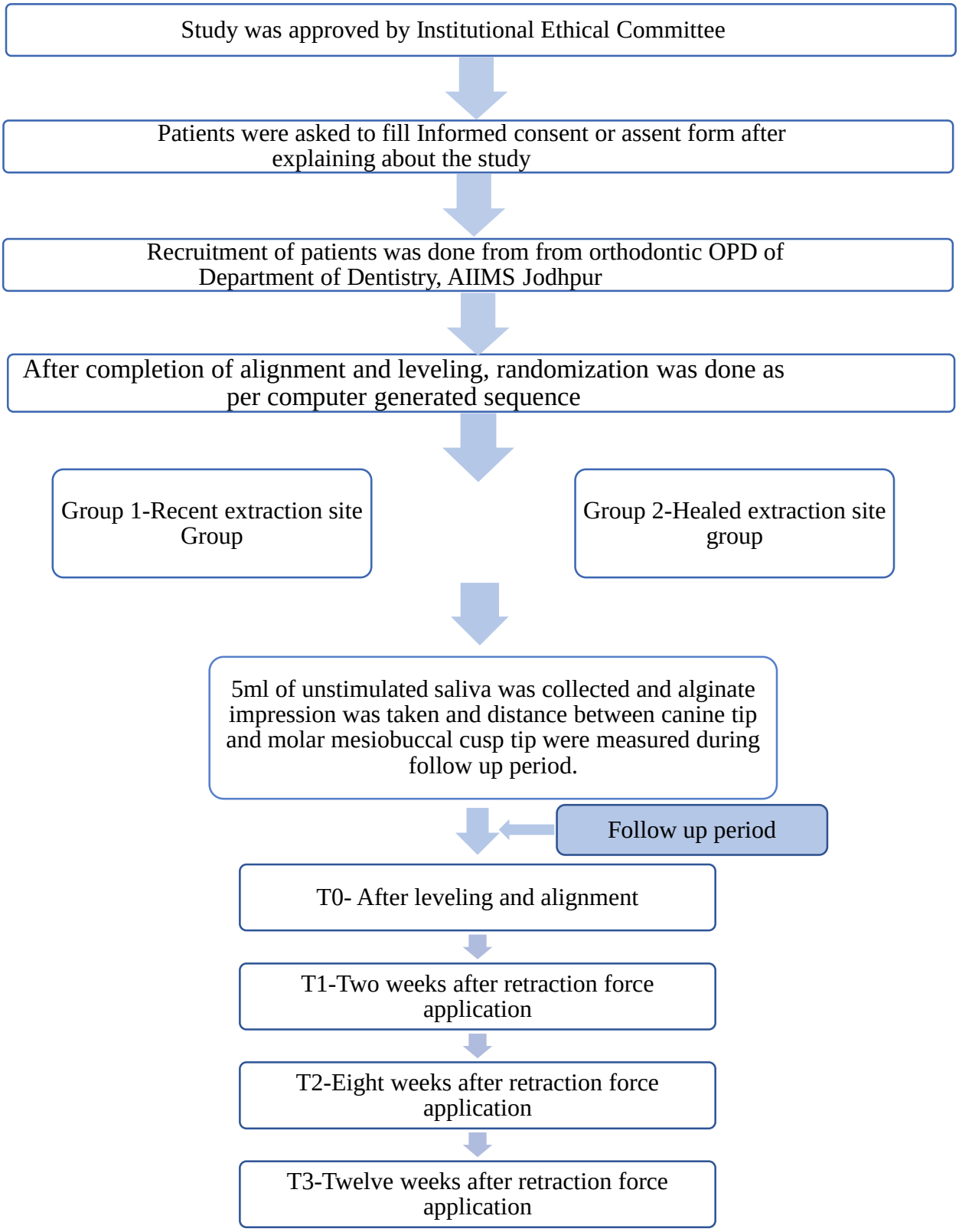


Figure 1: Patients recruitment and follow up flowchart

Outcomes:

1) Primary outcome:

- To evaluate quantitative variations of RANKL and OPG in orthodontic patients during en masse retraction in recent and healed extraction sites.

2) Secondary outcome:

- To compare the ratio of salivary biomarkers (RANKL/OPG) in orthodontic patients with recent and healed extraction sites during en masse retraction.

-To evaluate and compare the rate of tooth movement in subjects during en masse retraction between recent and healed extraction sites.

Method of assessment of salivary biomarker RANKL and OPG;

The concentration of RANKL and OPG in saliva samples were determined by comparing the optimal density of the samples to standard curve (Figure 7-8) given by RANKL human Enzyme-linked immunoassay (RANKL-ELISA) and OPG human Enzyme-linked immunoassay (OPG-ELISA). The ELISA Kit used for the study was manufactured by Biossay Technology Laboratory. The test principle for human RANKL and OPG applied here is sandwich enzyme immunoassay. The microtiter plate was pre-coated with antibody specific to human RANKL and OPG. Samples were added to the appropriate microtiter plate wells then with a biotin-conjugated antibody specific to RANKL and OPG. Next, Avidin conjugated to horseradish peroxidase was added to each microplate well and incubated for eighty minutes. After TMB substrate solution was added, only those wells that contained RANKL and OPG, biotin-conjugated antibody and enzyme-conjugated Avidin showed change in colour. The enzyme-substrate reaction was terminated by the addition of sulphuric acid solution and colour change was measured spectrometrically at a wavelength of 450±10nm immediately. To calculate the concentrations, a nonlinear regression model was performed with Chromate Manager software. The standard curves were constructed by using a 4-parameter logistic calibration curve fit and used to calculate the real concentration of each protein in the samples, standards and internal controls (Figure 8-9). R-squared values for typical standard curves were 0.9995 for RANKL and 0.9998 for OPG. These concentration RANKL and OPG was obtained for each saliva samples of orthodontic patients during en masse retraction at different time points (Figure 7).

Method of assessment of tooth movement;

The distance, amount and rate of tooth movement measured from study models taken after leveling and alignment (T0) until twelve weeks (T3) after retraction force application. The distance between the cusp tip of maxillary canine and mesiobuccal cusp tip of maxillary first molar was measured in millimetre at different timepoints (T0, T1, T2, and T3), individually on right and left side (average of right and left side was taken), with the help of a standard caliper with a sharpened fine edge with accuracy to 0.01 mm (Standard Caliper Series: EC16) (figure 11).

The retraction distance was then subtracted from the pre-retraction distance to calculate the amount of tooth movement (TM) at 2 weeks after retraction force (TM1= T0-TI), 8 weeks (TM2= T1-T2) and at 12 weeks (TM3= T2-T3) after retraction force application.

The rate of tooth movement was measured by difference in amount of tooth movement achieved after en masse retraction of maxillary anterior teeth to the time taken for space closure.

$$\text{Rate of tooth movement (RTM)} = \frac{\text{Amount of tooth movement (mm)}}{\text{Time taken (in weeks)}}$$

Statistical Analysis

Data was analyzed using the Statistical Package for Social Sciences for Windows, version 23.0 (Armonk, NY: IBM Corp.). During the whole study period, there was no dropout. Analysis was done using intention to treat (ITT) principle in all parameters. One calibrated blinded examiner evaluated the ratios and concentration of RANKL, OPG and study models by using standard measurement as described in previous studies. ICC was used to check the intra-examiner and inter-examiner reliability of measurement on study models. Ratios and quantitative variation of salivary biomarkers (RANKL and OPG) in salivary samples and rate of movement of teeth in plaster models were carefully analyzed.

Materials used in the present study

1. Saliva collecting device- 10 ml conical plastic centrifuge tube.
2. Reagents-

a) Pre-coated Microplate
b) Standard (lyophilized)
c) Standard Diluent Buffer
d) Biotinylated Antibody (100×)
e) Biotinylated Antibody Diluent
f) Streptavidin-HRP (100×)
g) HRP Diluent
h) Wash Buffer (25×)
i) TMB Substrate Solution
j) Stop reagent
k) Plate Covers

3. 1.5ml aliquoted plastic tube.
4. Microplate reader capable of measuring absorbance at 450 ± 10 nm.
5. High-speed centrifuge.
6. Electro-heating standing-temperature cultivator.
7. Absorbent paper.
8. Distilled or deionized water.
9. Single or multi-channel pipettes with high precision and disposable tips.
10. Precision pipettes to deliver 2 μL to 1 mL volumes.

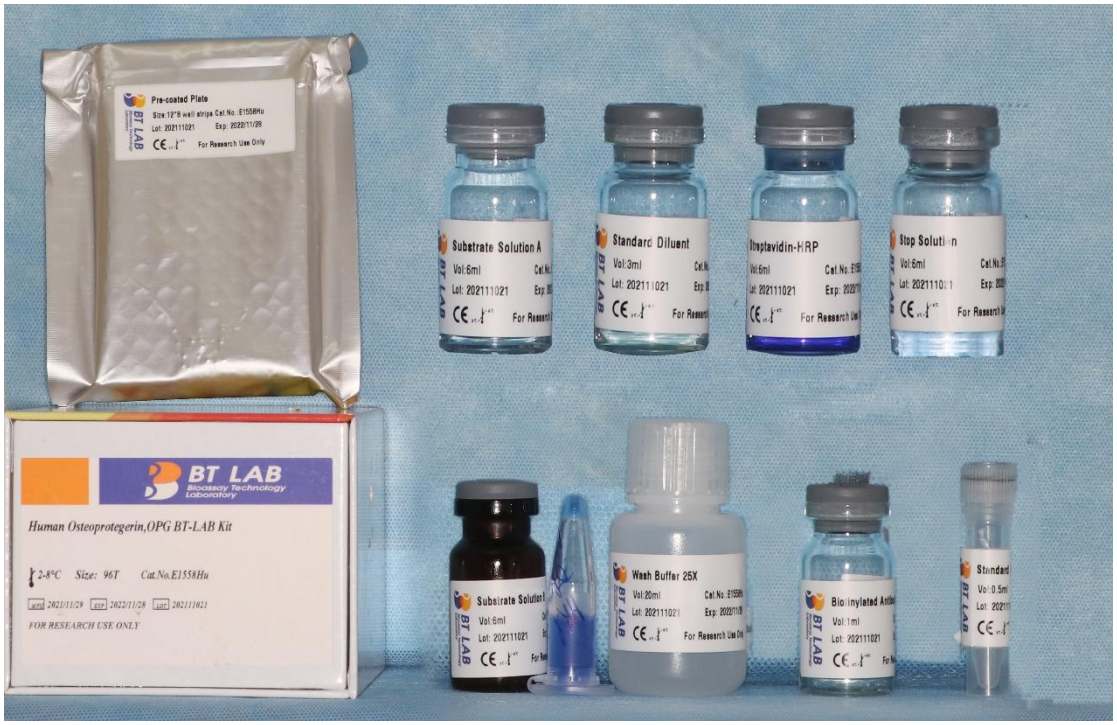


Figure 2: Armamentarium used in OPG ELISA kit

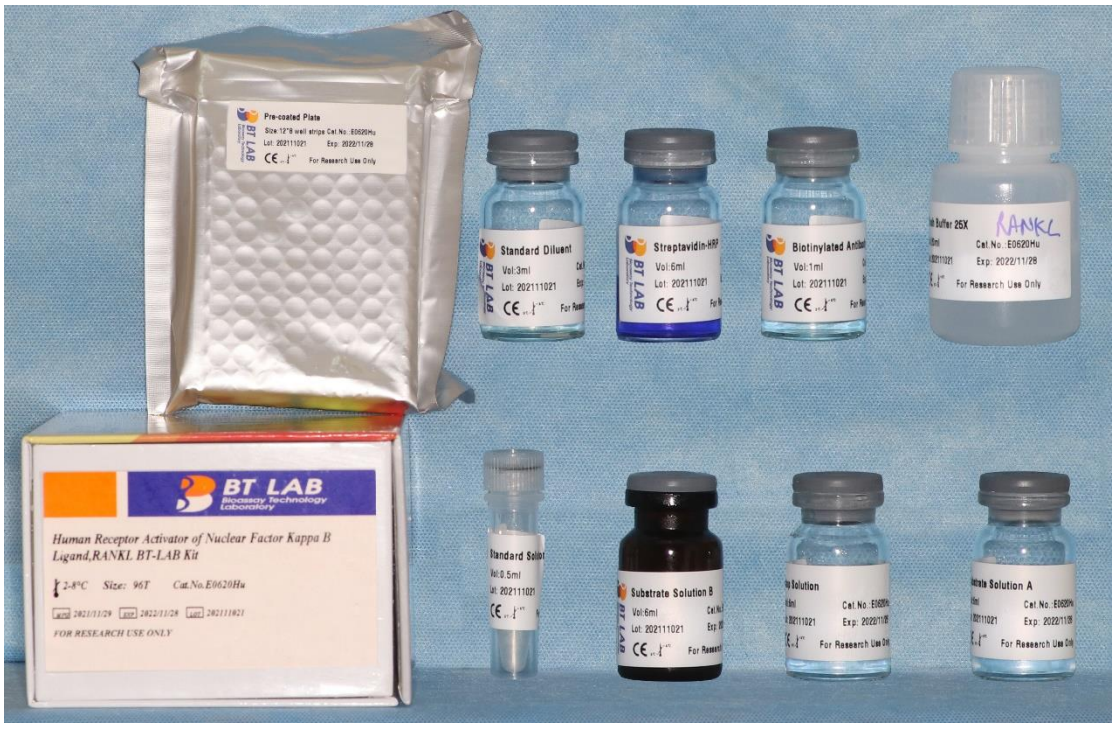


Figure 3: Armamentarium used in RANKL ELISA kit

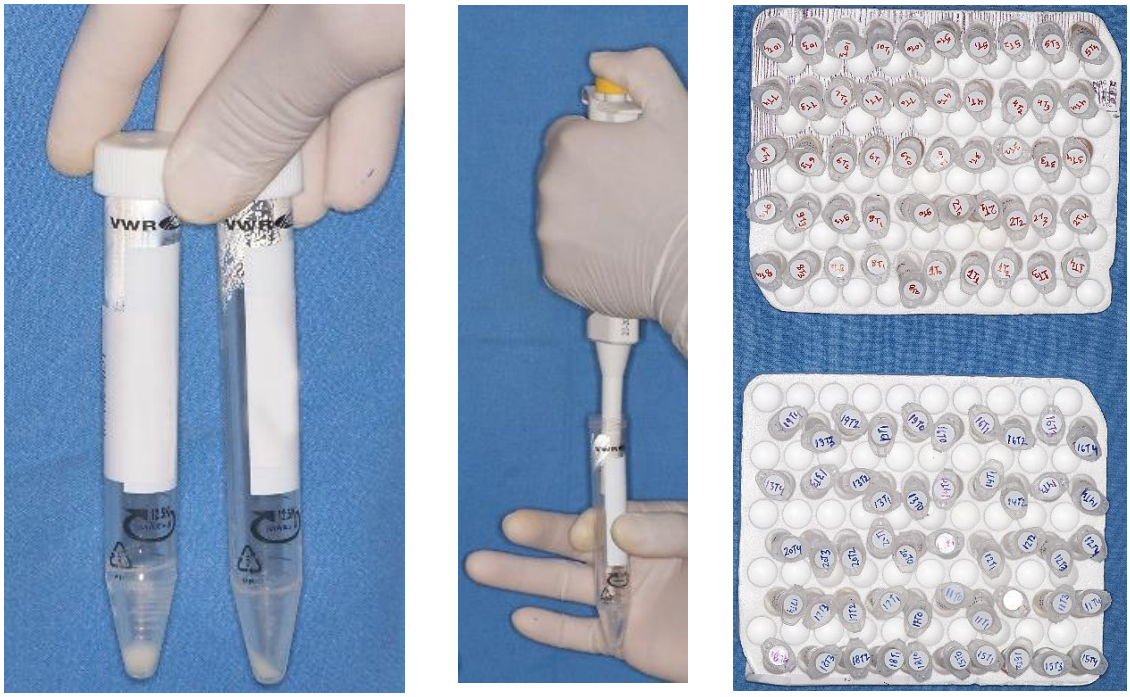


Figure 4: Collection of 5 ml of unstimulated saliva into 10 ml sterile plastic centrifuge tube, supernatant was collected and aliquoted into 500-µl volumes and frozen at -80^0.

Figure 5: Automated ELISA plate washer

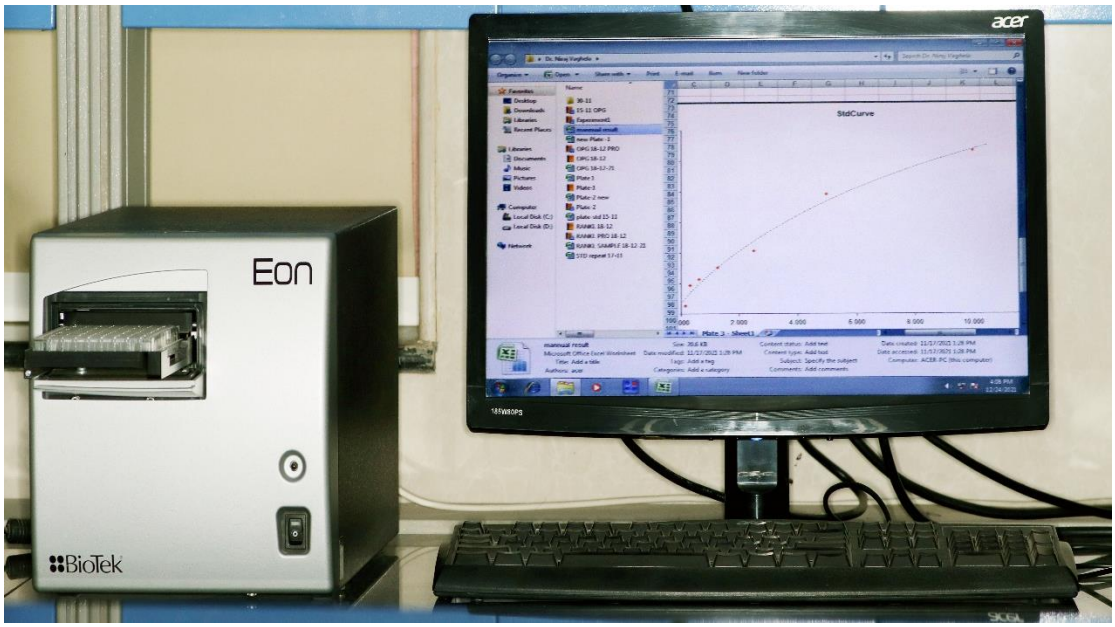


Figure 6: Absorption reader 800TS 1-channel, for 6-96 well microplates, 405,450,490,630nm

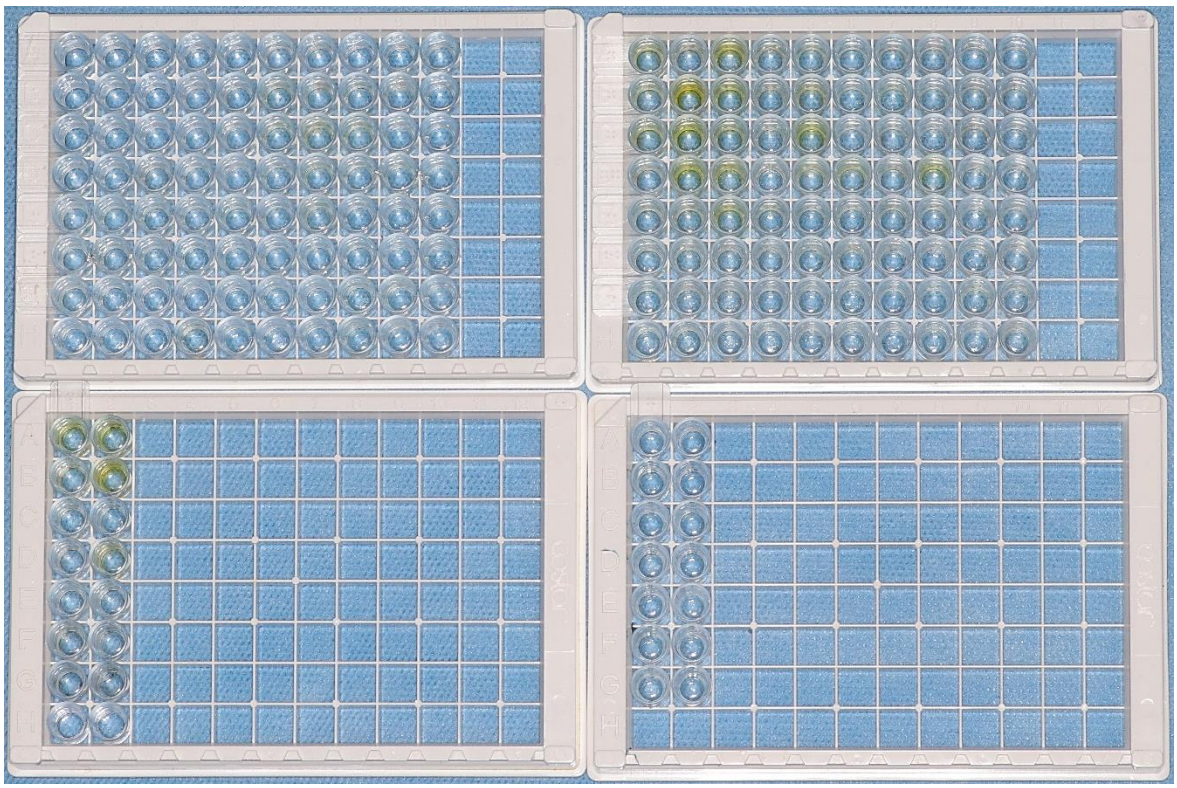


Figure 7: Saliva samples after successfully running ELISA test for RANKL and OPG

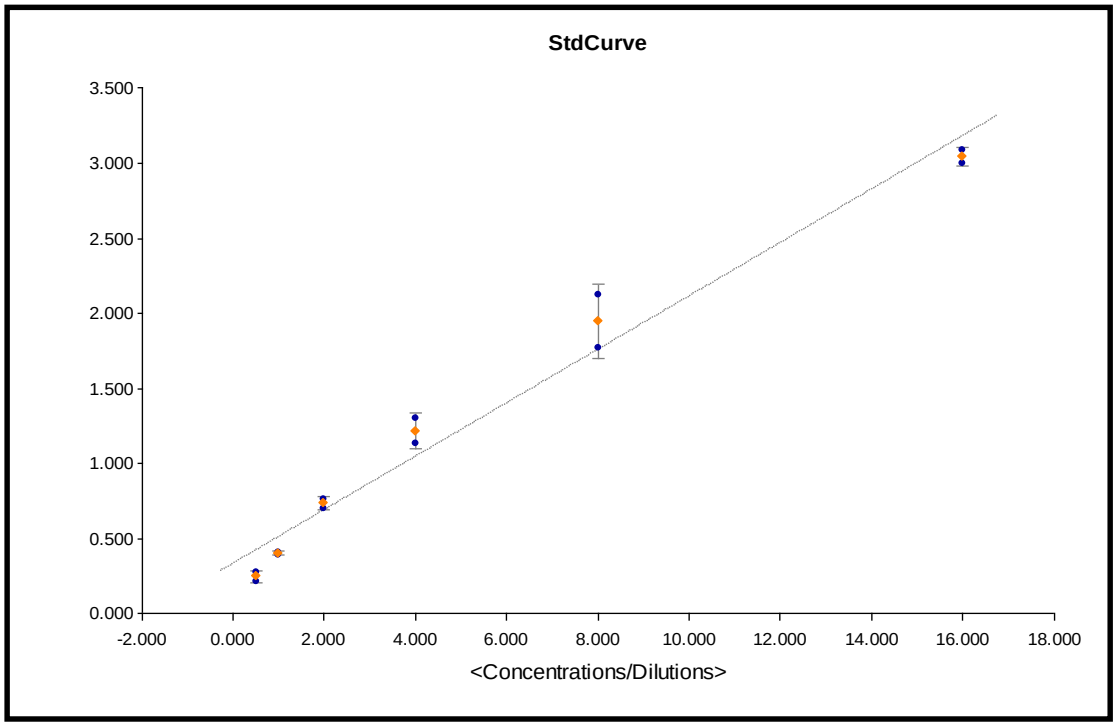


Figure 8: Standard curve obtained for OPG

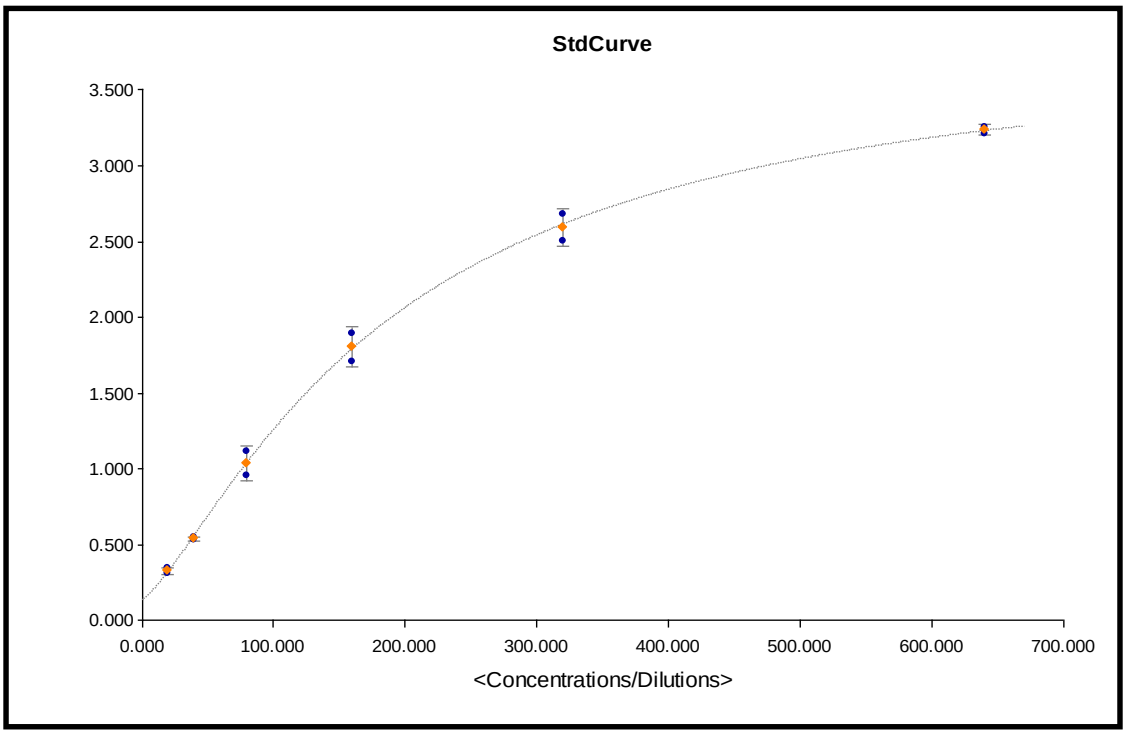
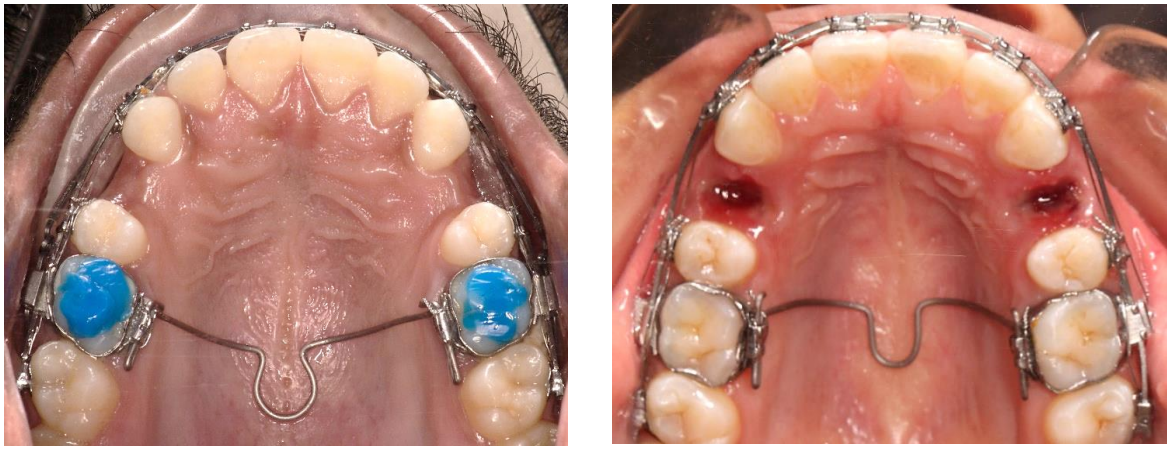


Figure 9: Standard curve obtained for RANKL



(A) **(B)**

Figure 10: Before starting of retraction forces in (A) healed and (B) recent extraction sites

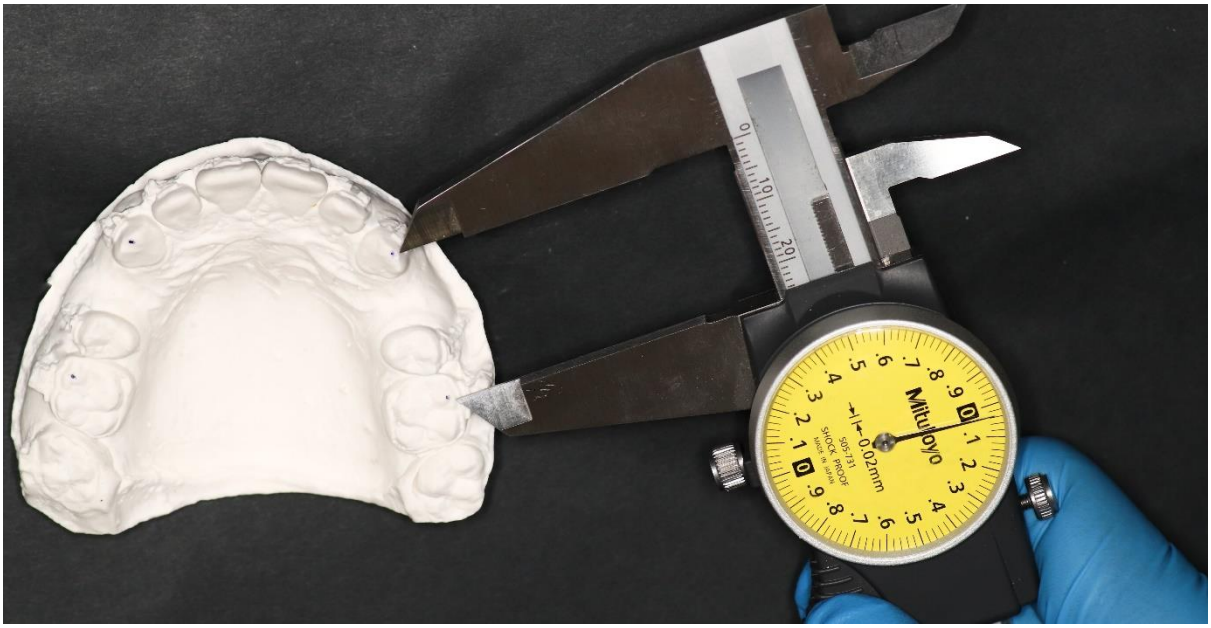


Figure 11: The distance, amount and rate of tooth movement measured from study models with the help of a standard caliper

RESULTS

PARTICIPANT FLOW

Twenty patients having maxillary anterior proclination or bidental protrusion, who required en masse retraction were included in the study. Patients were randomly allocated equally into healed extraction and recent extraction site group. There was no dropout during entire trial phase. The consort flowchart of the participants for this trial is shown in Figure 12.

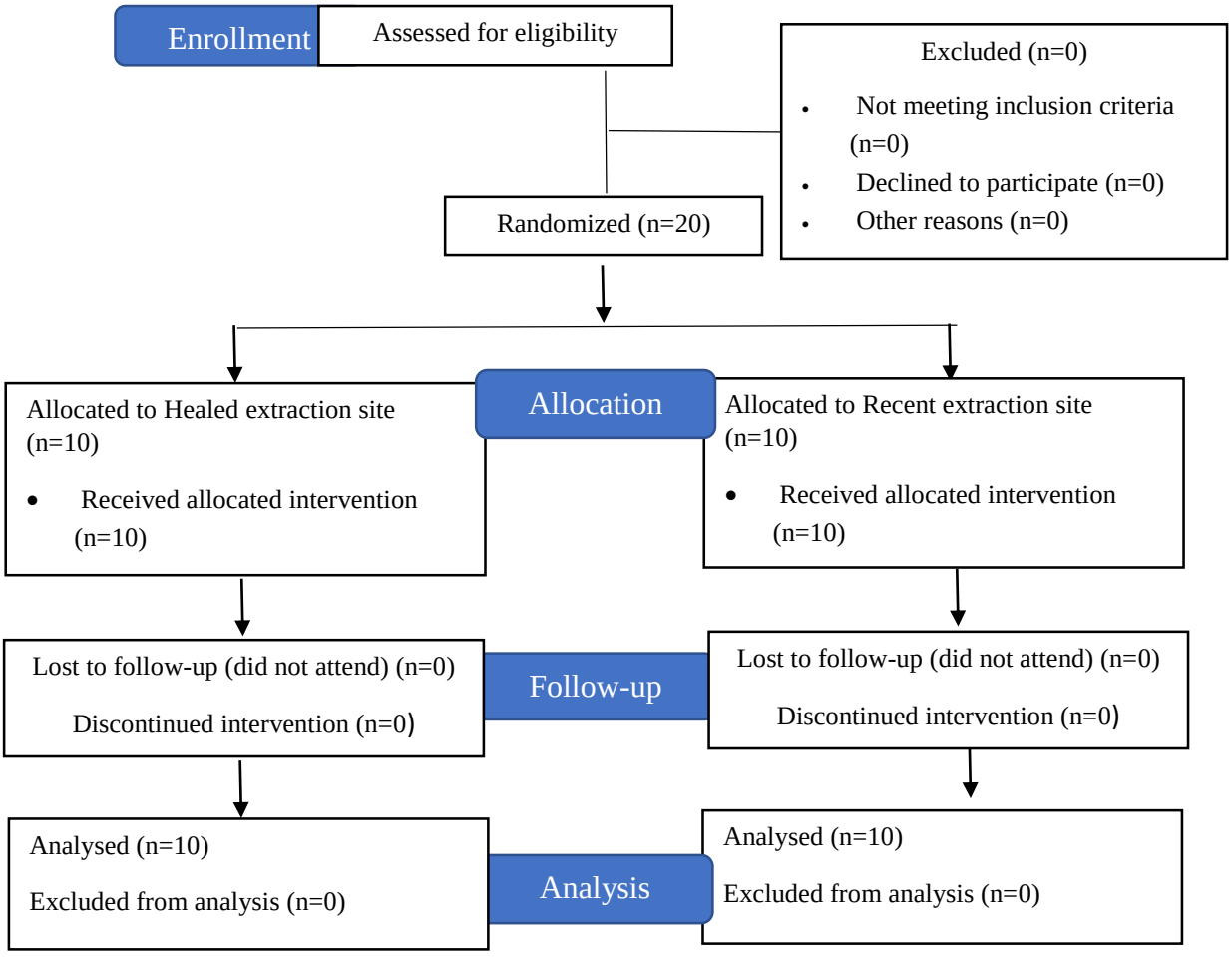


Figure 12. Consort flowchart of participants through each stage of the trial

BASELINE CHARACTERISTICS

Table 1: Baseline characteristics of participants in each study group

Baseline characteristics	Healed extraction site (Group I) n=10	Recent extraction site (Group II) n=10	P-Value*
Mean age (years) Mean±SD	19.8±3.56	23.29±5.28	0.101
Male/Female n (%)	3/7 (30/70)	3/7 (30/70)	1.000
Malocclusion			
Class I bimaxillary protrusion n (%)	4(40)	6(60)	0.656
Class II Division 1 n (%)	6(60)	4(40)	
Salivary biomarkers			
Concentration of RANKL (pg/ml) at T0 Mean±SD	71.75±6.37	76.50±20.67	0.496
Concentration of OPG (pg/ml) at T0 Mean±SD	2.71±0.527	2.56±1.32	0.755
RANKL/OPG ratio at T0 Mean±SD	27.44±6.37	34.58±10.87	0.090
Distance between cusp tip of canine and mesial cusp tip of first molar at T0(mm)	20.78±1.09	21.61±0.81	0.158

*P value for comparison of group means by Student's t-test and categorical data by Fischer's Exact test/ Chi-square test. Values are presented as mean ± SD or n (%), SD indicates Standard deviation, T0 indicate time point after leveling and alignment (Baseline).

Table 1 shows the baseline characteristics of participant in each group. Healed extraction site group consisted of 10 patients (3 males and 7 females) with a mean age of 19.8±3.56 years whereas recent extraction site group consisted of 10 patients (3 males and 7 females) with a mean age of 23.29±5.28 years. The comparison of baseline data between groups was done using Fischer's Exact test for categorical data and Student's t test for numerical data. There was no significant difference between the age (P=0.101) and gender (P=1.000) of the participants in healed extraction site group and recent extraction site group. There was no significant difference in terms of malocclusion of the participants between healed extraction and recent extraction site

group (P=0.656). Baseline value for salivary biomarker RANKL at T0 (after leveling and alignment) was 71.75±6.37 pg/ml and 76.50±20.67 pg/ml in healed extraction site and recent extraction site, respectively. There was no significant difference between concentration of RANKL at T0 (after leveling and alignment) in healed extraction site and recent extraction site (P= 0.496). Similarly, baseline value for salivary biomarker OPG at T0 (after leveling and alignment) was 2.71±0.527 pg/ml and 2.56±1.32 pg/ml in healed extraction site and recent extraction site, respectively. There was no significant difference between concentration of OPG at T0 (after leveling and alignment) in healed extraction site and recent extraction site (P= 0.755). Baseline value for RANKL/OPG ratio at T0 (after leveling and alignment) was 27.44±6.37 and 34.58±10.87 in healed extraction site and recent extraction site, respectively. There was no significant difference between RANKL/OPG ratio at T0 (after leveling and alignment) between healed extraction site and recent extraction site (P= 0.090). The baseline distance between cusp tip of canine and mesiobuccal cusp tip of first molar after alignment was also found to be similar in both the groups (P= 0.158).

TEST OF RELIABILITY

Table 2: Intra-class correlation coefficients for intra-examiner repeatability

Variable	Examiner A (V.N.) (At 0 week) Mean±SD	Examiner A (V.N.) (After 2 weeks) Mean±SD	ICC (95%CI)	P-value
Amount of tooth movement at [Distance between cusp tip of canine and mesiobuccal cusp tip of first molar (mm)]	21.20±0.97	21.19±0.95	1.000 (95% CI 0.999-1.000)	<0.001**

**P-value <0.05 is considered as significant; Intra class correlation was analysed using two-way mixed model with absolute agreement.

Twenty study models were measured for assessment of amount of tooth movement by the same examiner A (V.N.) on two different occasions at interval of two weeks. Table 2 indicates excellent intra-examiner reliability [ICC value, inter-examiner: 1.000 (95% CI 0.999-1.000)].

Table 3: Inter-class correlation coefficients for inter-examiner reproducibility				
Variable	Examiner A (V.N.) Mean±SD	Examiner B (R.S.) Mean±SD	ICC (95%CI)	P-value
Amount of tooth movement at [Distance between cusp tip of canine and mesiobuccal cusp tip of first molar (mm)]	21.19±0.97	21.18±0.96	1.000 (95% CI 0.999-1.000)	<0.001**

**P-value <0.05 is considered as significant; Intra class correlation was analysed using two-way mixed model with absolute agreement.

Twenty study models were measured by two examiners (V.N and R.S.) for assessment of amount of tooth movement. Table 3 indicates excellent inter-examiner reliability [ICC value, inter-examiner: 1.000 (95% CI 0.999-1.000)].

Table 4: Descriptive statistics and comparison of salivary concentration of RANKL *(pg/ml)* measured at different time points during en-masse retraction in two different intervention group.

Trial phase	(Group I) Healed extraction site (n=10)	(Group II) Recent extraction site (n=10)
	Mean ± SD	Mean ± SD
T_0	76.50± 20.67	71.75±6.37
T_1	87.00±26.28	99.11±21.63
T_2	96.00±25.89	99.50±12.04
T_3	112.99±18.23	115.47±20.08
p-value		
A. Time	<0.001	
1. Pairwise comparisons over time	0.009*, 0.001**, <0.001***, 0.313^{I}, 0.001^{II}, 0.013$^{\#}$	
B. Time×groups	0.524	

*Within-group and between group comparison P- values for change from T0 to T1, T2, or T3 against no change by using repeated-measures ANOVA with results of Greenhouse-Geisser have been used. P<0.05 is considered significant, SD indicates Standard deviation. T0 - Baseline test, T1 - 2week test, T2 - 8week test, T3 - 12week test. *(T_0 vs T_1), **(T_0 vs T_2), ***(T_0 vs T_3), ^{I}(T_1 vs T_2), ^{II}(T_1 vs T_3), $^{\#}$(T_2 vs T_3).

Table 4 gives the result of one-way repeated-measures ANOVA for evaluation of the change in salivary concentration of RANKL in healed extraction and recent extraction site group at different time points during en masse retraction. It was observed that during en masse retraction there was significant increase in salivary concentration of RANKL in either group over a period of 12-week (P<0.001). Significant increase in the salivary concentration of RANKL was seen on pairwise comparison in either group over different time points during en masse retraction (T_0 vs T_1) =0.009; (T_0 vs T_2) =0.001; (T_0 vs T_3) = 0.000; (T_1 vs T_3) =0.001; (T_2 vs T_3) =0.013. There was no significant difference with increase in salivary concentration of RANKL over a period of 12 weeks between the healed and the recent extraction site group (P=0.524).

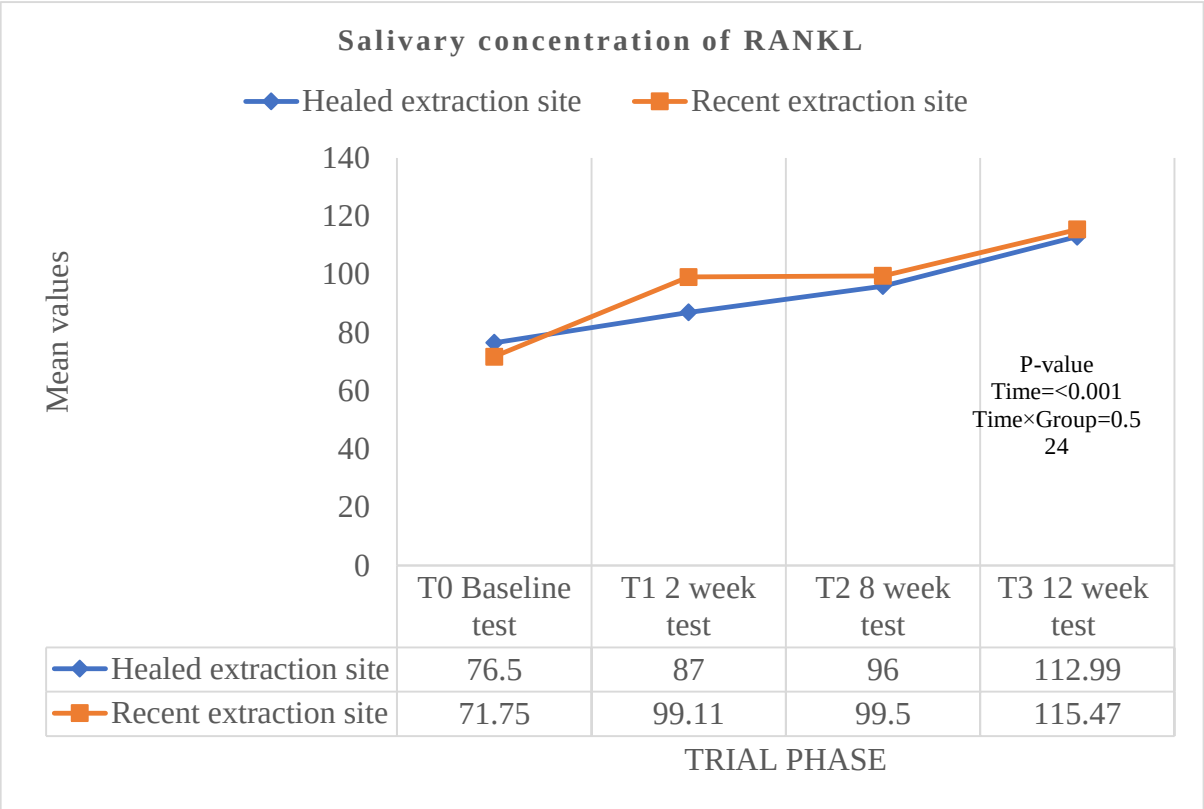


Figure13. Plot for descriptive statistics and comparison of salivary concentration of RANKL (pg/ml) at different time points during en masse retraction in healed extraction and recent extraction site group.

Table 5: Descriptive statistics and comparison of salivary concentration of OPG *(pg/ml)* measured at different time points during en-masse retraction in two different intervention group.

Trial phase	(Group I) Healed extraction site (n=10)	(Group II) Recent extraction site (n=10)
	Mean ± SD	Mean ± SD
T_0	2.57±1.32	2.71± 0.53
T_1	2.38±0.98	2.13±0.41
T_2	2.66±0.56	2.43±0.88
T_3	2.31±0.67	1.78±0.64
p-value		
A. Time	0.035	
I. Pairwise comparisons over time	0.141*, 0.724**, 0.015***, 0.211^{I}, 0.257^{II}, 0.008$^{\#}$	
B. Time×groups	0.498	

*Within-group and between group comparison P- values for change from T0 to T1, T2, or T3 against no change by using repeated-measures ANOVA with results of Greenhouse-Geisser have been used. P<0.05 is considered significant, SD indicates Standard deviation. T0 - Baseline test, T1 - 2week test, T2 - 8week test, T3 - 12week test. *(T_0 vs T_1), **(T_0 vs T_2), ***(T_0 vs T_3), ^{I}(T_1 vs T_2), ^{II}(T_1 vs T_3), $^{\#}$(T_2 vs T_3).

Table 5 gives the result of one-way repeated-measures ANOVA for evaluation of the change in salivary concentration of OPG in healed extraction and recent extraction site group at different time points during en masse retraction. It was observed that significant decrease in salivary concentration of OPG in either group over a period of 12-week during en masse retraction (P=0.035). Significant difference with decrease in the salivary concentration of OPG was seen on pairwise comparison in either group over different time points during en masse retraction (T_0 vs T_1) = 0.141; (T_0 vs T_3) = 0.015; (T_2 vs T_3) =0.008. There was no significant difference with decrease in salivary concentration of OPG over a period of 12 weeks between the healed and the recent extraction site group (p=0.498).

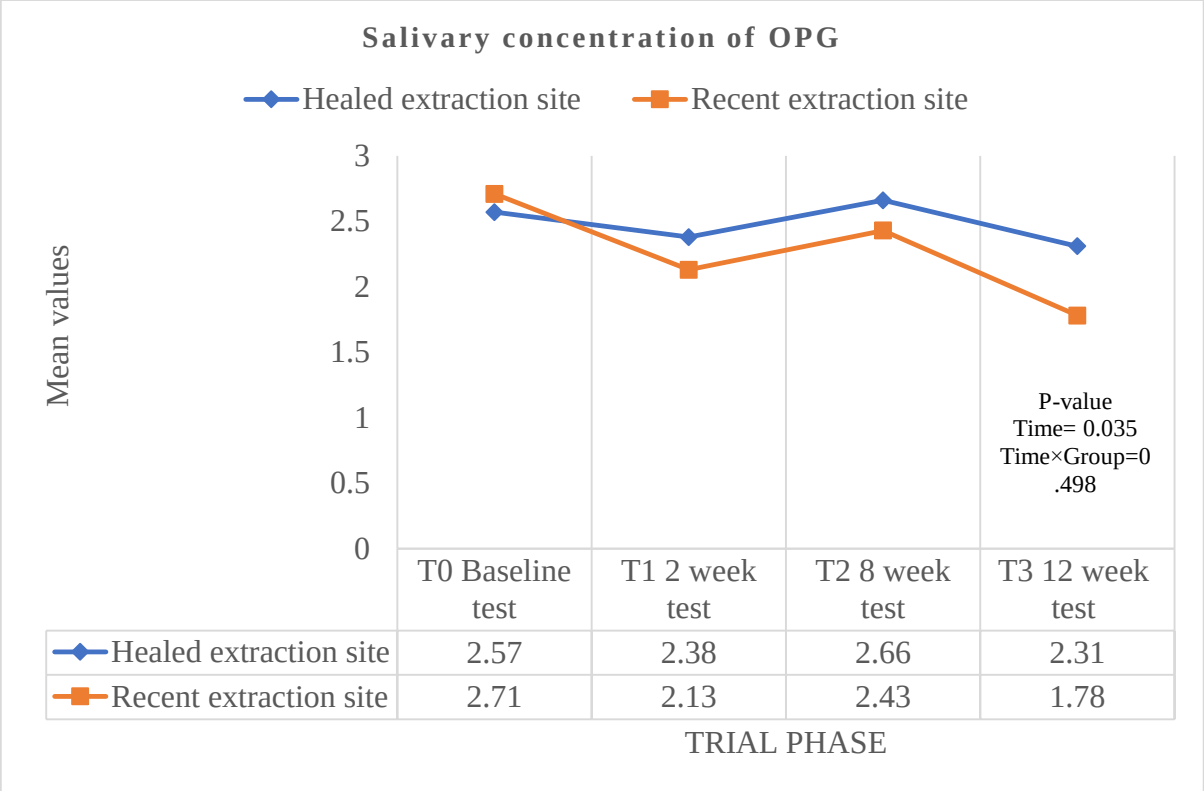


Figure14. Plot for descriptive statistics and comparison of salivary concentration of OPG (pg/ml) at different time points during en masse retraction in healed extraction and recent extraction site group.

Table 6: Descriptive statistics and comparison of salivary concentration of RANKL/OPG ratio measured at different time points during en-masse retraction in two different intervention group.

Trial phase	(Group I) Healed extraction site (n=10)	(Group II) Recent extraction site (n=10)
	Mean ± SD	Mean ± SD
T_0	34.58±10.87	27.45±6.37
T_1	44.49±23.97	45.35±11.44
T_2	34.22±13.12	44.87±13.68
T_3	52.12±14.83	72.89±26.76
p-value		
A. Time	<0.001	
I. Pairwise comparisons over time	0.007*, 0.038**, <0.001***, 0.184^{I}, 0.011^{II}, <0.001$^{\#}$	
B. Time×groups	0.037	

*Within-group and between group comparison P- values for change from T0 to T1, T2, or T3 against no change by using repeated-measures ANOVA with results of Greenhouse-Geisser have been used. P<0.05 is considered significant, SD indicates Standard deviation. T0 - Baseline test, T1 - 2week test, T2 - 8week test, T3 - 12week test. *(T_0 vs T_1), **(T_0 vs T_2), ***(T_0 vs T_3), ^{I}(T_1 vs T_2), ^{II}(T_1 vs T_3), $^{\#}$(T_2 vs T_3).

Table 6 gives the result of one-way repeated-measures ANOVA for evaluation of the change in salivary concentration of RANKL/OPG ratio in healed extraction and recent extraction site group at different time points during en masse retraction. It was observed that significant increase in salivary concentration of RANKL/OPG ratio in either group over a period of 12-week during en masse retraction (P<0.001). Significant difference with increase in the salivary concentration of RANKL/OPG ratio was seen on pairwise comparison in either group over different time points during en masse retraction (T_0 vs T_1)=0.007; (T_0 vs T_2)=0.038 ; (T_0 vs T_3)= 0.000 ; (T_1 vs T_3) =0.011 ; (T_2 vs T_3) =0.000. There was significant increase in salivary concentration of RANKL/OPG ratio observed over 12 weeks in the recent extraction site group as compared to healed extraction site group (P=0.037).

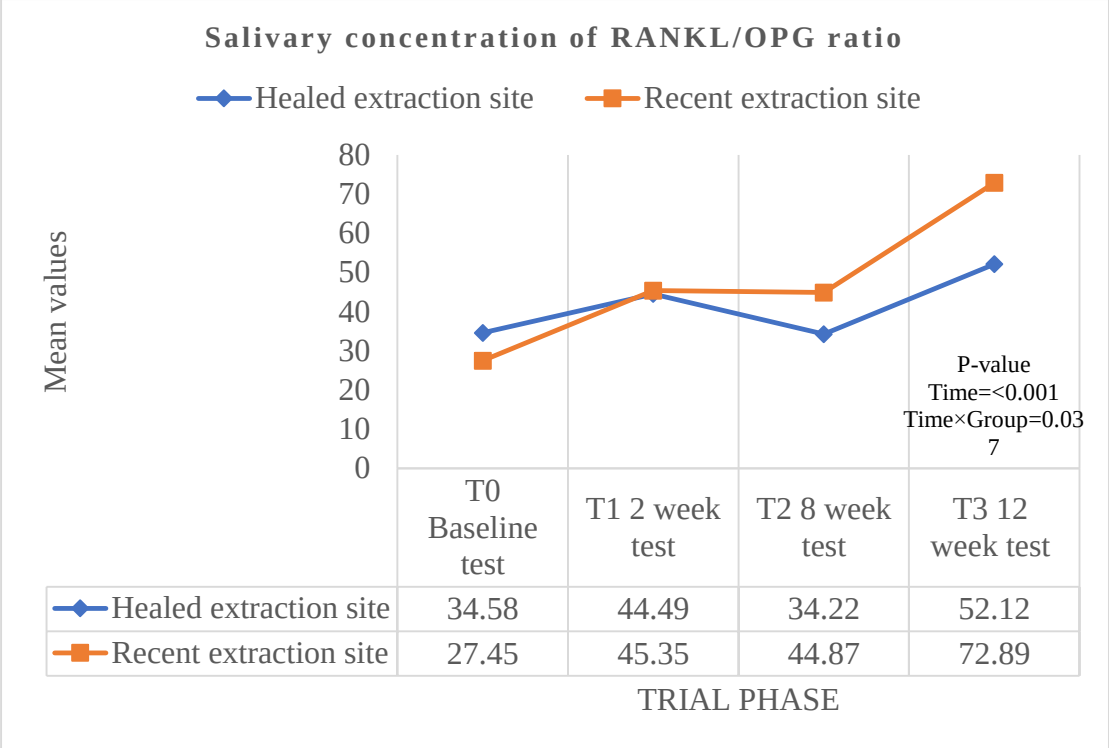


Figure15. Plot for descriptive statistics and comparison of salivary concentration of RANKL/OPG at different time points during en masse retraction in healed extraction and recent extraction site group.

Table 7: Comparison of mean distances between cusp tip of canine and mesial cusp tip of first molar *(mm)* at different time points during en masse retraction in two different intervention group.

Trial phase	(Group I) Healed extraction site (n=10)	(Group II) Recent extraction site (n=10)
	Mean ± SD	Mean ± SD
T_0	20.78±1.09	21.61±0.81
T_1	20.42±1.23	20.89±0.81
T_2	19.39±1.23	19.60±0.73
T_3	18.31±1.28	18.31±0.72
p-value		
A. Time	<0.001	
I. Pairwise comparisons over time	<0.001*, <0.001**, <0.001***, <0.001, <0.001, <0.001	
B. Time×groups	<0.001	

*Within-group and between group comparison P- values for change from T0 to T1, T2, or T3 against no change by using repeated-measures ANOVA with results of Greenhouse-Geisser have been used. P<0.05 is considered significant, SD indicates Standard deviation. T0 - Baseline test, T1 - 2week test, T2 - 8week test, T3 - 12week test. *(T_0 vs T_1), **(T_0 vs T_2), ***(T_0 vs T_3), ^{I}(T_1 vs T_2), ^{II}(T_1 vs T_3), $^{\#}$(T_2 vs T_3).

The result of one-way repeated-measures ANOVA (Table 7) showed that significant decrease in mean distances between cusp tip of canine and mesial cusp tip of first molar *(mm)* at different time points during en-masse retraction in healed extraction and recent extraction site group over a period of 12-week (P<0.001). Significant difference with decrease in distance between cusp tip of canine and medial cusp tip of first molar *(mm)* was observed on pairwise comparison over different time points (T_0 vs T_1)=0.000; (T_0 vs T_2)=0.000 ; (T_0 vs T_3)= 0.000 ; (T_1 vs T_2)= 0.000 ; (T_1 vs T_3) =0.000 ; (T_2 vs T_3) =0.000 in either group during en masse retraction. There was significant decrease in distance between cusp tip of canine and medial cusp tip of first molar *(mm)* observed over a period of 12 weeks in the recent extraction site group as compared to healed extraction site group (P<0.001).

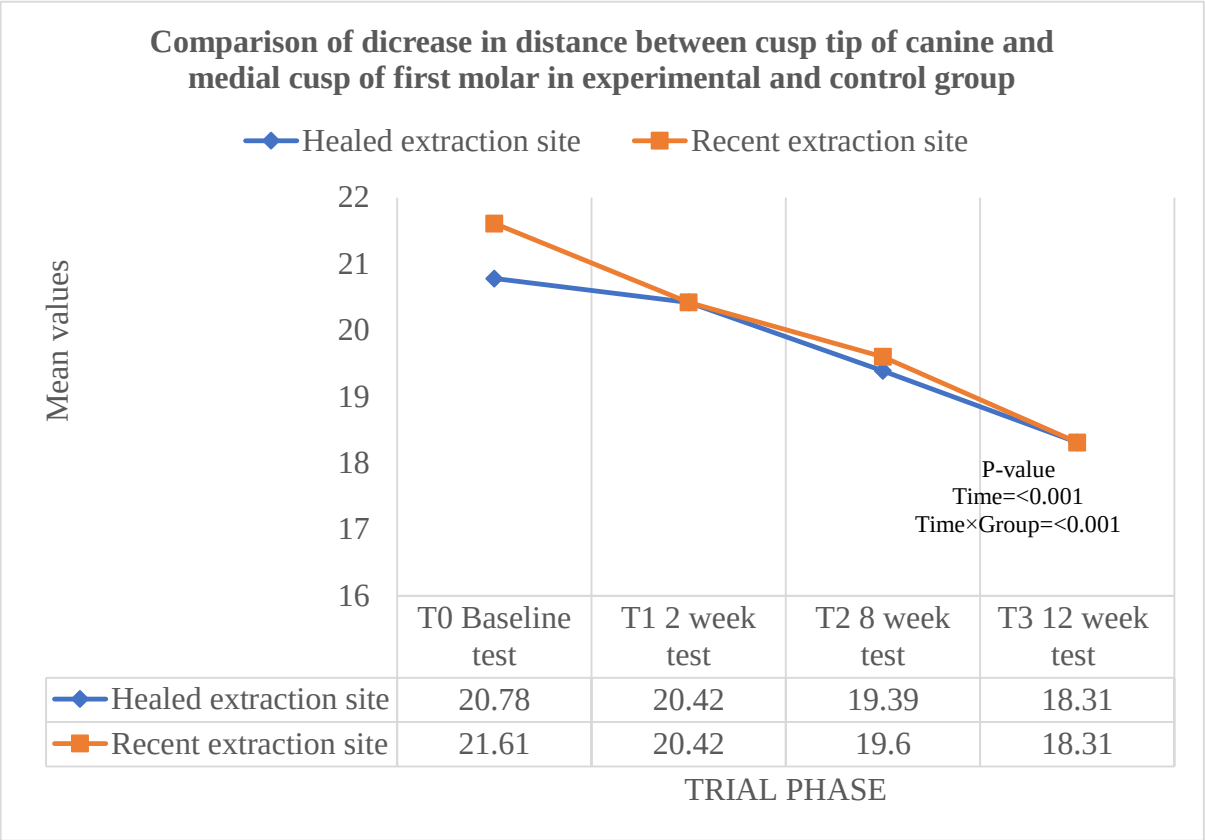


Figure16. Plot for descriptive statistics and comparison of mean distance between cusp tip of canine and medial cusp of maxillary first molar at different time points during en masse retraction in healed extraction and recent extraction site group.

Table 8: Comparison of mean amount of tooth movement *(mm)* at different time points during en masse retraction between two groups.

Time period	(Group I) Healed extraction site (n=10) Mean ± SD	(Group II) Recent extraction site (n=10) Mean ± SD	*p-value*[#]
At Two weeks (TM1)	0.35±0.30	0.72±0.15	0.003*
At Eight weeks (TM 2)	1.38±0.38	2.00±0.24	0.001*
At Twelve weeks (TM 3)	2.46±0.29	3.3±0.26	<0.001*

*P value of difference between groups, at different time points is calculated using student's t test. *P<0.05 is considered significant, SD indicates Standard deviation. TM indicates tooth movement. TM1= T0-T1, TM2= T1-T2, TM3= T2-T3.

Table VIII gives the result of the amount of tooth movement is reflected by decrease in distance (measured at cusp tip of canine to mesial cusp of maxillary first molar) at two weeks (TM1; T0-T1), at 8 weeks (TM2; T1-T2) and 12 weeks (TM3; T2-T3) respectively. The result of student's T test showed the amount of tooth movement was found to significantly higher in recent extraction site group at 2 weeks (0.003), 8 weeks (P= 0.001), and 12 weeks (P= <0.001) as compared to healed extraction site (Table V).

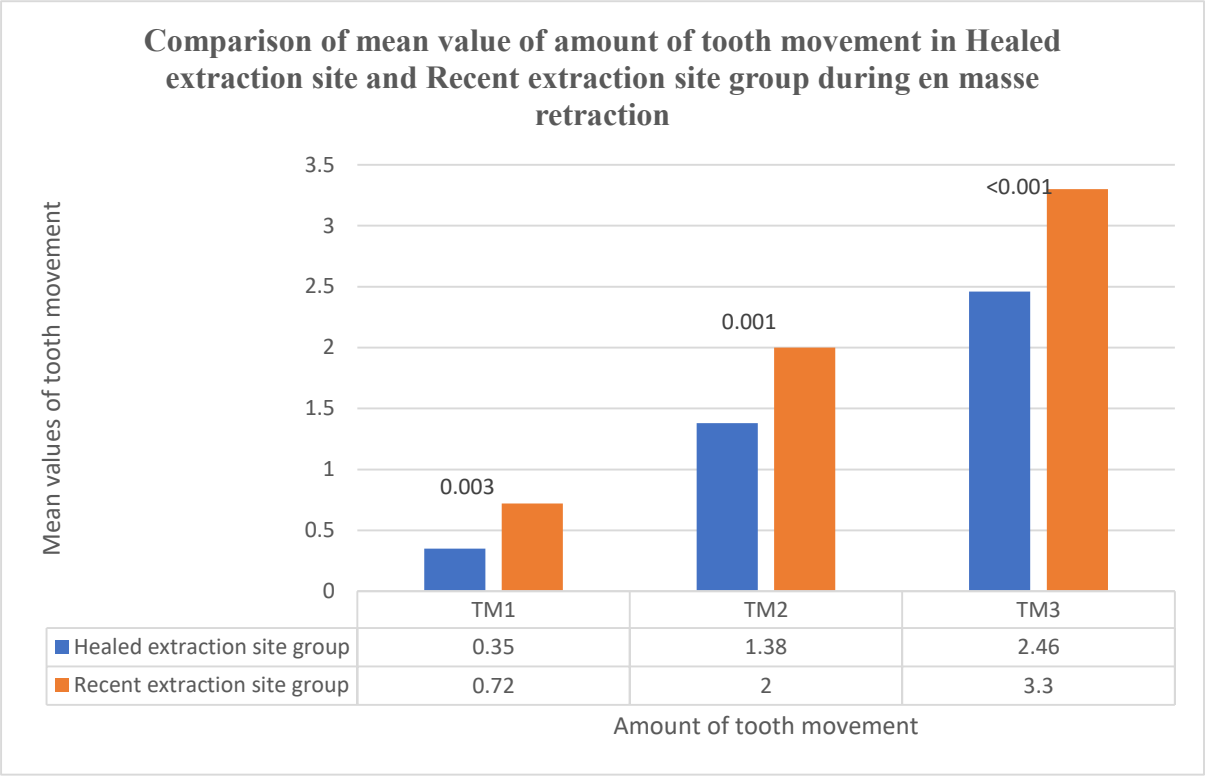


Figure17. Plot for comparison of amount of tooth movement in healed extraction and recent extraction site group during en masse retraction.

Table 9: Comparison of rate of tooth movement (mm/week) during en masse retraction in healed extraction site and recent extraction site group.

Variable	(Group I) Healed extraction site (n=10)	(Group II) Recent extraction site (n=10)	*p-value*[#]
	Mean ± SD	Mean ± SD	
Rate of tooth movement	0.20± 0.02	0.27± 0.02	<0.001*

*P value of difference between groups is calculated using student's t test. *P<0.05 is considered significant, SD indicates Standard deviation. Rate of tooth movement is calculated by T0-T3/12 Weeks.

Table 9 gives the result of the rate of tooth movement was recorded as difference in amount of distance (as measured between T0 and T3 divided by 12 weeks). The result of student's T test illustrated significant increase in the rate of tooth movement in recent extraction site group (P= <0.001) as compared to healed extraction site group during en masse retraction (Table VI).

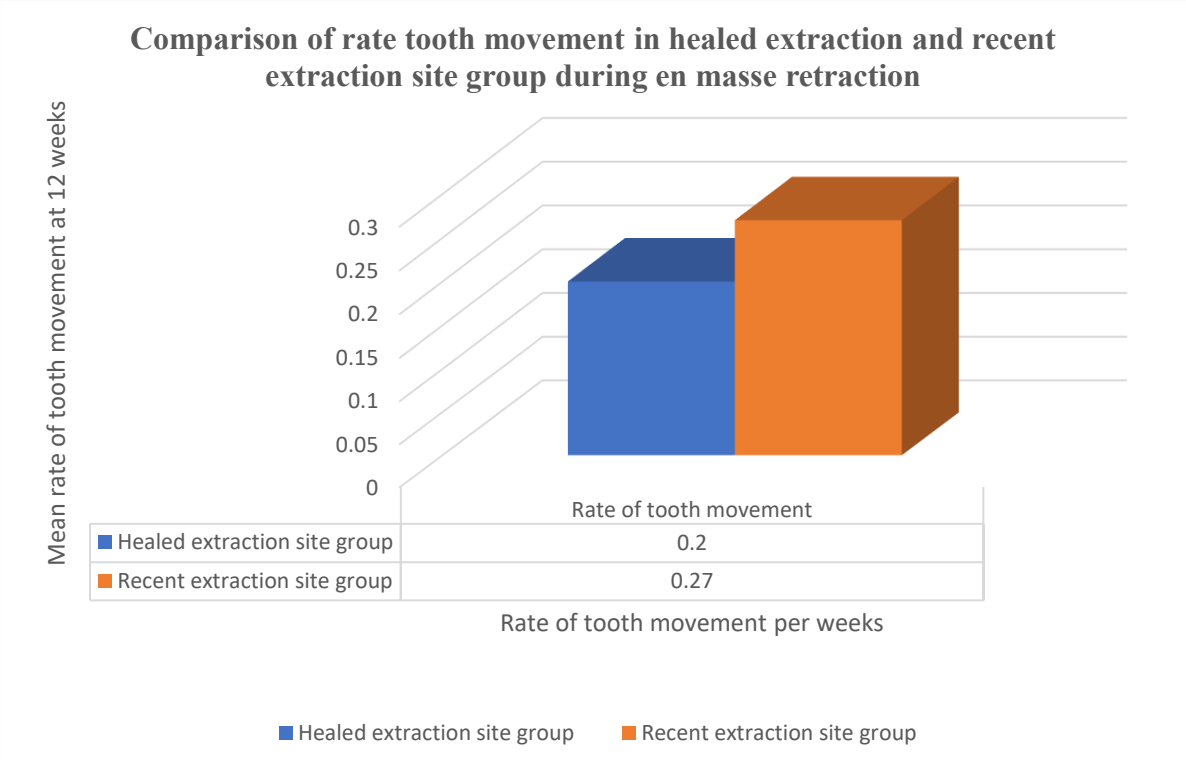


Figure18. Plot for comparison of rate tooth movement in healed extraction and recent extraction site group during en masse retraction at end of 12 weeks.

DISCUSSION

Orthodontic tooth movement results from remodelling of the alveolar bone that is induced by varying degrees of magnitude, frequency and duration of therapeutic mechanical strain. This leads to expression of extensive changes at molecular level (16). Based on studies, it has been thoroughly proven that orthodontic tooth movement has a specific pattern in time with 4 phases: an initial phase; lag phase-lasting 4 to 20 days; postlag phase-during which the rate of tooth movement gradually increases followed by a fourth phase named linear phase, initiating about 40 days after force application during which due to direct bone resorption orthodontic tooth movement continues (7)**.**

Each phase is a result of tissue-specific reactions having recruitment of osteoblast and osteoclast as well as chemotaxis of inflammatory cytokines. It has been proven that upregulation of pro- inflammatory cytokines plays an important role in bone modelling of the alveolus associated with the different phases of orthodontic tooth movement. The activity of the basic multicellular unit of alveolar bone remodelling can be evaluated biochemically by determining markers of bone remodelling. Several important biomarkers have been identified clinically and in animal studies that is associated with orthodontic tooth movement. Among these, the TNF-related ligand receptor activator of nuclear factor kappa B ligand (RANKL) and the decoy receptor osteoprotegerin (OPG) in bone modelling has been observed in studies performed on animals and recently on humans during orthodontic treatment. Moreover, most human studies regarding the biology of tooth movement have analyzed different mediators associated with dental and paradental tissue remodelling which illustrate the complex three-dimensional nature of orthodontic tooth movement (13).

Orthodontic forces change the levels of RANKL and OPG, as well as the RANKL/OPG ratios, in gingival crevicular fluid during orthodontic tooth movement. Various mediators involved in alveolar bone remodelling during orthodontic tooth movement are continuously washed into saliva from gingival crevicular fluid. These salivary RANKL and OPG levels appears to correspond well with those investigated in gingival crevicular fluid samples. Hence collection of unstimulated whole saliva was considered an easy alternative compare to crevicular fluid for sampling.

Previous studies have investigated the biomarkers of bone remodelling during orthodontic tooth movement which are limited to initial stages of orthodontic tooth

movement. No studies have been done on humans that have evaluated the salivary biomarkers RANKL and OPG in orthodontic patients during en masse retraction in recent extraction site and healed extraction site. The aim of the present study was to evaluate and compare the salivary biomarkers (RANKL and OPG) during en masse retraction in recent and healed extraction site. The sampling time recorded in this study were chosen to coordinate with the 4 phases of orthodontic tooth movement during retraction previously described. To evaluate the amount of salivary RANKL and OPG and in orthodontic patients, unstimulated saliva was collected after leveling and alignment (baseline), and at 2 weeks, 8weeks, and 12 weeks respectively after retraction force was applied. The saliva sample collection and its storage was done as protocol described by Fléorez-Moreno et al.(18) These samples were analyzed for RANKL and OPG with help of ELISA.

In present study, it was observed that during en masse retraction there was significant increase in salivary concentration of RANKL in healed extraction and recent extraction site group over a period of 12-week. Subsequent to force application, the strain in the periodontal ligament and the alveolar bone causes cellular and molecular reactions to initiate around the paradental tissues. The initial phase lasts for 24 hr to 2 days. The second phase is depicted by formation of hyalinized tissue that arrest the tooth movement for 4-20 days. The results from the 2 week period might be linked with this phase. The mean concentration of RANKL at 2 weeks time point was increased in both the healed extraction and recent extraction site group from its baseline values. The result was in consensus with that of Florez et al.(18) where they observed that salivary concentration of RANKL was increased after two weeks of application of retraction force as compared to 24-48 hour after the initial force application. These may be related to the fact that during this phase of orthodontic tooth movement, hyalinized tissue is formed on the pressure side of the periodontal ligament space. The necrotic tissue is removed by the osteoclasts from the adjacent viable marrow spaces. Therefore, biochemical mediators are released during these intense cellular activity in response to the hyalinized tissue (13).

The mean RANKL value at 8 week period was also in consensus with the result of Florez et al.(18) where the value was increased as compared to 24-48 hours after the initial force application. However, direct comparison with the present study cannot be done as in their study orthodontic appliance remained inactivated after initial force

application whereas in our study force was reactivated at each timepoint. Our trial was also in agreement with the trial of Reiss et al.(19) where the biomarkers were investigated with fixed appliances in conjunction with vibration appliance therapy. They found that concentration of RANKL was increased after initial bonding appointment to 6 weeks after active orthodontic force application. These may be linked with the linear phase of the orthodontic tooth movement. The RANKL concentration and this phase may be related due to the further differentiation of existing osteoclasts and also recruitment and activation of new osteoclasts. Therefore, maintaining a negative bone balance at the basic multicellular unit level (16).

The mean RANKL value showed increased trend in their concentration up to 12 week period in our study. This may have occurred as a consequence of reactivation of the retraction force to retract the anterior teeth. The finding observed may suggest that the event of remodelling process is not a one-time process. During tooth displacement, the development and removal of the necrotic tissue is a continuous process irrespective of the force magnitude applied. The results are in affirmation with the clinical trial of Reiss et al. (19) where the salivary concentration of RANKL was increased from 10-12 weeks after reactivation of orthodontic force as compared to RANKL concentration at initial force application. The concentration of RANKL increased consistently in both the healed and recent extraction site group over a period of 12 weeks and the concentration of RANKL was found to be higher in the recent extraction site group at every time points comparatively.

Based on previous histological and histochemical studies, these could be related to the fact that at the recent extraction site increased osteoclastic activity is seen and formation of new bone is seen at the healed sites (9). Considering extraction of tooth to be a minor surgical procedure, regional acceleratory phenomenon could be considered at the extraction site. This process causes reduced bone density as it activates a temporary physiologic bone healing process of injured tissue. Therefore, recruiting osteoclasts and osteoblasts by local intercellular mediator mechanisms to model the bone (12)**.**

In contrary to the RANKL values, salivary concentration of OPG showed downward trend in both healed extraction site and recent extraction site during en masse retraction observed over a period of 12 weeks. The mean concentration of OPG at 2

weeks after application of retraction force was decreased in both the healed extraction and recent extraction site group from its baseline concentration. The result was in consensus with that of Florez et al.(18) and Reiss et al.(19)where the concentration of OPG were lower than the baseline level in their study. The 2-week time point is in correlation with the lag phase depicted by hyalinized zone on the compression side in which tooth movement is at standstill. During this phase, biochemical mediators induce recruitment of osteoclasts from adjacent marrow spaces to remove the necrotic tissue. In a time and force-magnitude dependent manner OPG secretion is decreased in human PDL cells during this phase and has also been expressed in our study (13).

The OPG concentration in saliva at 8 week time point was in consensus with the result of Florez et al. (18) and Reiss et al. (19) where the concentration of OPG in saliva decreased during orthodontic tooth movement compared to the baseline concentration. This time point may be linked with the linear phase of tooth movement. This could be related to reactivation of orthodontic appliance leading to another period of acute inflammation which overlap ongoing chronic inflammation and subsequent direct resorption could be considered part of remodelling (13).

The concentration of OPG fell below the baseline concentration at 12-week time-point in both the healed extraction and the recent extraction site group. This finding might suggest that the remodelling process is due to a negative bone balance maintained at the basic multicellular unit level. This result was found to be in agreement with the trial of Reiss et al. (19) where the salivary concentration of OPG was decreased from 10-12 weeks after reactivation of orthodontic force as compare to OPG concentration at initial force application. The salivary concentration of OPG was more in the healed extraction site group at every time point as compared to recent extraction site group. These could be due to decreased osteoblastic activity at the recent extraction site and formation of new bone at the healed sites (8).

Our study showed that the RANKL/OPG concentration increased over a period of 12 weeks in both the healed and the recent extraction site groups. At 2 weeks after retraction force application, the RANKL/OPG ratio increased with respect to the baseline concentration at the beginning of trial, mainly because the concentration of OPG was decreased below the baseline level and increased RANKL value more than the baseline level. Our finding was similar to the findings of Florez et al. (18) and Reiss

et al. (19) where the ratio for RANKL/OPG concentration was more as compare to concentration of RANKL/OPG ratio at beginning of trial. These may be related to increased activity of osteoclast in response to the hyalinized tissue in the periodontal ligament space (13)**.**

At 8 weeks after force application, the RANKL and OPG ratio was increased compared to the baseline level in both the healed and the recent extraction site group. Our finding was similar to the findings of Florez et al. (18) and Reiss et al. (19) where the ratio was more than the baseline level. The ratio was less compared to 2-week time point in both the groups, mainly because the OPG concentration was increased during this time point. This may indicate that over time the tooth attachment is restored after initial deformation along with the reestablishment of the cellular function therefore, maintaining the bone resorption and deposition balance (13)**.**

The RANKL/OPG ratio was increased at 12-weeks after retraction force, majorly because of increased RANKL concentration and less OPG concentration with respect to the baseline. This may have occurred as a consequence of reactivation of the retraction force to retract the anterior teeth. The finding observed may suggest that the event of remodelling process is not a one-time process. During tooth displacement, the development and removal of the necrotic tissue is a continuous process irrespective of the force magnitude applied (13)**.** The salivary concentration of RANKL/OPG ratio was in consensus with the result of Reiss et al.(19) where the value was increased from baseline to 10-12 weeks after reactivation of orthodontic force during orthodontic tooth movement in patients with fixed orthodontic appliance in conjunction with vibration appliance.

Our findings was in consensus with the previous studies on the RANKL/OPG system demonstrating that when the tooth is subjected to compressive forces, the expression of RANKL is upregulated inducing osteoclastogenesis favouring tooth displacement and the secretion of OPG is downregulated thereby, maintaining the remodelling process (25,26,29,35,37).

In the present study, the distance, amount and rate of tooth movement was measured as described in a previous study (46). In our study, significant decrease in distances was observed between the cusp tip of maxillary canine and the mesial cusp tip of maxillary first molar *(mm)* during en-masse retraction in healed extraction and

recent extraction site group over a period of 12-weeks. The amount of tooth movement was greater in recent extraction site group than the healed extraction site group by 0.65mm and 0.74mm at 8 weeks and 12 weeks, respectively. Our findings were similar to the findings of Bauer (3) who reported from animal experiments that tooth movement was faster into a healed extraction site than into a recent extraction site. It was in consensus with the findings of Hasler (4) who also reported that individual canine retraction was faster into recent extraction site than into a healed extraction site by 1.14mm.

The rate of tooth movement calculated during en masse retraction was 0.27mm/week and 0.33mm/week for healed extraction site and recent extraction site, respectively. The rate of tooth movement per week in the present study was slightly higher in recent extraction site group, and the difference was found to be statistically significant when compared to healed extraction site group. However, animal study by Samruajbenjakun et al. (12)reported no statistically significant difference in the rate of tooth movement between the recent and healed extraction sites over a period of 60 days. They stated that timing of extraction and decortication of alveolar bone might not be a factor that could affect the rate of tooth movement. Considering extraction of tooth to be a minor surgical procedure, regional acceleratory phenomenon could be considered at the extraction site. This process activates a temporary physiologic bone healing process of injured tissue recruiting osteoclasts and osteoblasts by local intercellular mediator mechanisms to model the bone thereby, reducing bone density at the extraction site (12)**.** Difference in the rate of tooth movement between healed and recent extraction site could be explained by the histological and histochemical study of Amler and Johnson (9) on undisturbed alveolar socket healing. They found that at the site of tooth extraction, the osteoid starts to appear at the base of the socket by the seventh-day and at least two-thirds of the socket gets filled with trabecular bone by thirty-eight days after exodontia. Diedrich and Wehrbein (11) in their animal study on foxhounds found that regeneration of osseous tissue starts 3 weeks after exodontia and is completed after 4-6 months.

Similarly, Murphey (20) observed tension and compression areas of fresh socket and 6-week healed sockets in macaca rhesus monkeys. They found that osteoclasts appear at the alveolar ridge even at the end of first week and start to smooth the alveolar margins by means of resorption. This regressive restructuring of the bone

was found to be most profound in the first month after extraction and undergoes a gradual decline till the fifth month after exodontia. They concluded that there were more osteoclastic activities at recent sites and new bone formation at healed sites. This states greater restrained tooth movement into healed sockets than into recent sockets (11).

Rate of tooth movement has been studied by various authors either to retract canine individually or to retract the anterior teeth en-masse into the premolar extraction spaces. Rate of canine retraction has been reported to be in the range of 0.21-1.85 mm per month by various authors (47–50). The rate of en masse retraction of anterior teeth has been reported to be in the range of 0.55-1.2mm per month which is slightly lower when compared to canine retraction (21, 22, 50). This is primarily due to more number of teeth involved in en masse retraction. We could not directly compare our study because previous studies have evaluated the efficiency of sliding and loop mechanics, the efficiency of retraction methods like elastomeric-chain, nickel-titanium coil springs (51, 52). None of the above studies were based on recent or healed extraction site socket.

Strength of the study:

This is the first prospective study to evaluate and compare outcome specific biomarker in healed extraction site and recent extraction site during en masse retraction. We have found that biomarkers of bone remodelling process were significantly altered during en masse retraction of anterior teeth. Reactivation of appliance was done at each timepoints and it was observed that RANKL/OPG ratio increased over a period of 12 weeks suggesting that tooth movement is a continuous process maintaining the pool of osteoclastic cells in the alveolar bone. Although this is a small study, our findings has hinted that recent extraction site may promote faster tooth movement. Immediate application of activation forces into the recent extraction site may be considered an advantage and prevent overtaxing of the alveolar bone where bone has been allowed to heal during the tooth movement.

Our study suggested that saliva could be used to analyse response specific biomarkers that could be linked to bone-turnover events during orthodontic tooth movement and not be relied upon gingival crevicular fluid only, as it has been said to be site specific and its collection method is technique sensitive.

Limitations of the study:

A small sample size was used to determine whether the timing of extraction could have a profound effect on orthodontic tooth movement. In our study immediate tooth movement in recent extraction site was faster but the method of retraction to retract the anterior teeth was not specified. Different methods of retraction produce different rate of tooth movement and this was not considered in our study. Future studies with a larger sample size specifying type of mechanics used for en masse retraction should be undertaken. The salivary biomarkers were evaluated during the initial retraction phase of 12 weeks, perhaps evaluation during and after the entire en masse retraction force could provide a better understanding about the role of these biomarkers in orthodontic tooth movement.

CONCLUSION

The following conclusions were drawn from the study:

1. Significant increase in the salivary concentrations of RANKL were noted over a period of 12 weeks in healed extraction site and the recent extraction site groups during en masse retraction however, no significant difference was found between both the groups.
2. Statistically significant decrease in salivary concentration of OPG at 2 weeks and 12 weeks were noted in in both the healed extraction site and the recent extraction site groups during en masse retraction. However, no statistically significant difference in OPG levels was found between both the groups over a period of 12 weeks.
3. There was significant increase in salivary concentration of RANKL/OPG ratio at each time points in both, healed extraction site and recent extraction site groups. Additionally, salivary RANKL/OPG ratio was found to be significantly increased over a period of 12 weeks in recent extraction site group when compared to healed extraction site group.
4. There was a significantly higher rate of en masse retraction of anterior teeth at recent extraction site group (0.27mm/week) when compared to healed extraction site group (0.20mm/week) during twelve week follow up period.

SUMMARY

Introduction: The aim of this study was to evaluate and compare the levels of various salivary biomarkers (RANKL and OPG) during en masse retraction in patients with recent and healed extraction sites during orthodontic treatment.

Design, settings, and participants: Twenty patients between ages of 12-30 undergoing fixed orthodontic treatment requiring extraction for correction of their malocclusion were randomly allocated to recent extraction site group (*n=10)* or healed extraction site group (*n=10).* Salivary biomarker assay was completed to analyse the concentration of RANKL and OPG and their ratios were recorded after leveling and alignment before application of any retraction force (T0), two weeks (T1), eight weeks (T2) and twelve weeks (T3) after retraction force application. Distance, amount, and rate of movement of anterior teeth during en masse retraction in both healed extraction site and recent extraction site groups were assessed by measurement obtained from study models taken at various time points. Data was analyzed on an intention-to-treat basis principle with descriptive statistics, repeated measures analysis of variance, student's 't'- test.

Results: Significant variations were observed in salivary concentration of RANKL, and OPG and their ratios at any time points in both groups over the duration of trial. RANKL/OPG ratio showed a significant increase over time after active retraction forces were applied in both recent extraction site and healed extraction site group. A significant increase in RANKL/OPG ratios were also noted at recent extraction site group when compared to healed extraction site at all time points. There was significant increase in rate of en masse retraction of anterior teeth at recent extraction site group as compare to healed extraction site group after 12 weeks.

Conclusion: There was a significant increase in salivary concentrations of RANKL and decrease in salivary concentrations OPG at various time points during en masse retraction in both recent extraction and healed extraction sites. The ratio of RANKL/OPG increased over a period of 12 weeks in recent extraction site as compare to healed extraction site during en masse retraction of anterior teeth. Also, there was an increase in rate of en masse retraction observed in recent extraction site group (0.27mm/week) as compare to healed extraction site group (0.20mm/week) after 12 weeks.

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ANNEXURES

Annexure I: Institutional Ethical Clearance Certificate

अखिल भारतीय आयुर्विज्ञान संस्थान, जोधपुर
All India Institute of Medical Sciences, Jodhpur
संस्थागत नैतिकता समिति
Institutional Ethics Committee

No. AIIMS/IEC/2020/2043 **Date: 01/01/2020**

ETHICAL CLEARANCE CERTIFICATE

Certificate Reference Number: **AIIMS/IEC/2019-20/977**

Project title: **"Evaluation of salivary biomarkers in orthodontic patients with recent and healed extraction sites during enmasse retraction-A pilot study"**

Nature of Project:	**Research Project**
Submitted as:	**M.D.S. Dissertation**
Student Name:	**Dr.Vaghela Niraj Himmatlal**
Guide:	**Dr.Vinay Kumar Chugh**
Co-Guide:	**Dr.Mithu Banerjee & Dr. Pravin Kumar**

This is to inform that members of Institutional Ethics Committee (Annexure attached) met on **23-12-2019** and after through consideration accorded its approval on above project. Further, should any other methodology be used, would require separate authorization.

The investigator may therefore commence the research from the date of this certificate, using the reference number indicated above.

Please note that the AIIMS IEC must be informed immediately of:

- Any material change in the conditions or undertakings mentioned in the document.
- Any material breaches of ethical undertakings or events that impact upon the ethical conduct of the research.

The Principal Investigator must report to the AIIMS IEC in the prescribed format, where applicable, bi-annually, and at the end of the project, in respect of ethical compliance.

AIIMS IEC retains the right to withdraw or amend this if:

- Any unethical principle or practices are revealed or suspected
- Relevant information has been withheld or misrepresented

AIIMS IEC shall have an access to any information or data at any time during the course or after completion of the project.

On behalf of Ethics Committee, I wish you success in your research.

Dr. Praveen Sharma
Member secretary
Institutional Ethics Committee
AIIMS,Jodhpur

Enclose:
1. Annexure I

Page 1 of 2

Basni Phase-2, Jodhpur, Rajasthan-342005, **Website:** www.aiimsjodhpur.edu.in, **Phone:** 0291-2740741 Extn. 3109
Email: ethicscommittee@aiimsjodhpur.edu.in

Annexure II: Case Record Form

CASE RECORD FORM

Sr. No.: Clinic No.:

Name: CR No.:

Address and contact number: Age/Sex:

Occupation: Date:

Group Allocated-

1 ☐

☐

2

EVALUATION

S.No.	**Procedure**	**Measurements**			
		T_0	**T_1**	**T2**	**T_3**
1.	Salivary levels of OPG				
2.	Salivary levels of RANKL				
3.	RANKL/OPG Ratio				
4.	Amount of Tooth Movement (distance between the cusp tip of maillary canine and mesiobuccal cusp tip of maxillary first molar)				
5.	Rate of Tooth Movement $\left[\frac{\text{Amount of tooth movement (mm)}}{\text{Time taken (in weeks)}}\right]$				

Annexure III: Patient Information Leaflet (English)

All India Institute of Medical Sciences, Jodhpur

Department of Dentistry

Patient Information Leaflet

You are being invited to willing fully participate in the study entitled "EVALUATION OF SALIVARY BIOMARKERS IN ORTHODONTIC PATIENTS WITH RECENT AND HEALED EXTRACTION SITES DURING EN MASSE RETRACTION - A PILOT STUDY"

You have been requested to volunteer for a research study since you have undergone fixed orthodontic treatment. Even though these techniques (orthodontic retraction force applications in recent and healed extraction sites) are commonly used in orthodontic practices, there is less literature describing variations of salivary biomarkers (RANKL/OPG) associated with these procedures, rate of tooth movement. So, this study is aimed to establish these biomarkers association with rate of tooth movement during en masse retraction.

Confidentiality

Your medical records and identity will be treated as confidential documents. They will only be revealed to other doctors/scientists/ monitors/auditors of the study if required. The results of the study may be published in a scientific journal but you will not be identified by name.

Ethics committee approval has been obtained for the study.

Your participation and rights

Your participation in the study is fully voluntary and you may withdraw from the study anytime without having to give reasons for the same. In any case, you will receive the appropriate treatment for your condition. You will not be paid any amount for the participation in the study. You will have to pay for the routine investigations that will be done.

Contact Person: for further queries-

Dr VAGHELA NIRAJ HIMMATLAL

Post Graduate student

Orthodontics and Dentofacial Orthopaedics

Department of Dentistry

AIIMS, Jodhpur

Mobile No: - 8320312500

Email ID: drnirajvaghela@gmail.com

Annexure IV: Patient Information Leaflet (Hindi)

अखिल भारतीय आयुर्विज्ञान संस्थान, जोधपुर
दंत चिकित्सा विभाग

शीर्षक: "EVALUATION OF SALIVARY BIOMARKERS IN ORTHODONTIC PATIENTS WITH RECENT AND HEALED EXTRACTION SITES DURING ENMASSE RETRACTION - A PILOT STUDY"

आपसे एक शोध अध्ययन के लिए स्वयंसेवा करने का अनुरोध किया गया है क्योंकि आपने निश्चित ओर्थोडोंटिक उपचार किया है। भले ही इन तकनीकों (EVALUATION OF SALIVARY BIOMARKERS IN ORTHODONTIC PATIENTS WITH RECENT AND HEALED EXTRACTION SITES DURING ENMASSE RETRACTION - A PILOT STUDY) आमतौर पर ऑर्थोडोंटिक प्रथाओं में उपयोग की जाती हैं, इन प्रक्रियाओं से जुड़े लार बायोमार्कर (रैंकल / ओपीजी) की विविधताओं का वर्णन करने वाला साहित्य कम है, दांतों की गति की दर। तो इस अध्ययन का उद्देश्य इन बायोमार्कर एसोसिएशन को इनमास रिट्रेक्शन के दौरान दांतों की गति की दर के साथ स्थापित करना है।

गोपनीयता

आपके मेडिकल रिकॉर्ड और पहचान को गोपनीय दस्तावेज माना जाएगा। यदि आवश्यक हो तो उन्हें केवल अन्य डॉक्टरों/वैज्ञानिकों/अध्ययन की निगरानी/लेखा परीक्षकों के सामने प्रकट किया जाएगा। अध्ययन के परिणाम एक वैज्ञानिक पत्रिका में प्रकाशित हो सकते हैं लेकिन आपकी पहचान नाम से नहीं होगी।

अध्ययन के लिए आचार समिति की मंजूरी मिल गई है।

आपकी भागीदारी और अधिकार

अध्ययन में आपकी भागीदारी पूरी तरह से स्वैच्छिक है और आप बिना कारण बताए कभी भी अध्ययन से पीछे हट सकते हैं। किसी भी मामले में, आप अपनी स्थिति के लिए उचित उपचार प्राप्त करेंगे। अध्ययन में भाग लेने के लिए आपको किसी भी राशि का भुगतान नहीं किया जाएगा। आपको नियमित जांच के लिए भुगतान करना होगा जो कि किया जाएगा।

संपर्क व्यक्ति: अधिक प्रश्नों के लिए-

डॉ वाघेला नीरज हिम्मतलाल
स्नातकोत्तर छात्र
ऑर्थोडोंटिक्स और डेंटोफेशियल ऑर्थोपेडिक्स
दंत चिकित्सा विभाग
एम्स, जोधपुर
ईमेल आईडी: drnirajvaghela@gmail.com

Annexure V: Patient Consent Form (English)

All India Institute of Medical Sciences, Jodhpur

Department of Dentistry

Informed Consent Form

Subject: "EVALUATION OF SALIVARY BIOMARKERS IN RECENT AND HEALED EXTRACTION SITES DURING ENMASSE RETRACTION- A PILOT STUDY"

Patient OPD No: _____I, _____ S/o or D/o_____ R/o _____give my full, free, voluntary consent to be a part of the study "EVALUATION OF SALIVARY BIOMARKERS IN RECENT AND HEALED EXTRACTION SITES DURING ENMASSE RETRACTION- A PILOT STUDY"

The procedure and nature of which has been explained to me in my own language to my full satisfaction. I confirm that I have had the opportunity to ask questions. I give my permission for the use of orthodontic records, including photographs, made in the process of examinations and treatment for the purposes of research, education, or publication in professional journals.

I understand that my participation is voluntary and I am aware of my right to opt out of the study at any time without giving any reason.

I understand that the information collected about me and any of my medical records may be looked at by responsible individual from AIIMS Jodhpur or from regulatory authorities. I give permission for these individuals to have access to my records.

Date: _____ _____

Place: _____ Signature/Left thumb impression (Patient)

(Caregiver)

This to certify that the above consent has been obtained in my presence.

Date:_____ _____

Place:_____ Signature of Principal Investigator

1. Witness 1 2. Witness 2

Name: _____ Name: _____

Address: _____ Address: _____

_____ _____

Signature Signature

Principle Investigator:

Dr VAGHELA NIRAJ HIMMATLAL
Post Graduate student
Orthodontics and Dentofacial Orthopaedics
Department of Dentistry
AIIMS, Jodhpur Mobile No: - 8320312500

Annexure VI: Patient Consent Form (Hindi)

अखिल भारतीय आयुर्विज्ञान संस्थान, जोधपुर

दंत चिकित्सा विभाग

सूचित सहमति प्रपत्र

विषय: "EVALUATION OF SALIVARY BIOMARKERS IN ORTHODONTIC PATIENTS WITH RECENT AND HEALED EXTRACTION SITES DURING ENMASSE RETRACTION - A PILOT STUDY"

रोगी ओपीडी संख्या: _____ मैं, _____ पुत्र या डी/ओ_____
अध्ययन का एक हिस्सा बनने के लिए मेरी पूर्ण, मुफ्त, स्वैच्छिक सहमति पत्र जिसक EVALUATION OF SALIVARY BIOMARKERS IN ORTHODONTIC PATIENTS WITH RECENT AND HEALED EXTRACTION SITES DURING ENMASSE RETRACTION - A PILOT STUDY.

जिसकी प्रक्रिया और प्रकृति मुझे मेरी अपनी भाषा में मेरी पूर्ण संतुष्टि के लिए समझाया गया है। मैं पुष्टि करता हूं कि मुझे प्रश्न पूछने का अवसर मिला है। मैं अनुसंधान, शिक्षा, या पेशेवर पत्रिकाओं में प्रकाशन के प्रयोजनों के लिए परीक्षाओं और उपचार की प्रक्रिया में बनाए गए फोटो सहित, ऑर्थोडोंटिक रिकॉर्ड के उपयोग के लिए अपनी अनुमति देता हूं।

मैं समझता हूं कि मेरी भागीदारी स्वैच्छिक है और मैं बिना कोई कारण बताए किसी भी समय अध्ययन से बाहर निकलने के अपने अधिकार से अवगत हूं।

मैं समझता हूं कि मेरे और मेरे किसी भी मेडिकल रिकॉर्ड के बारे में एकत्र की गई जानकारी को एम्स जोधपुर के जिम्मेदार व्यक्ति या नियामक अधिकारियों द्वारा देखा जा सकता है। मैं इन व्यक्तियों को अपने रिकॉर्ड तक पहुंच की अनुमति देता हूं।

दिनांक: _____ _____

स्थान: _____ हस्ताक्षर/बाएं अंगूठे का निशान (रोगी) (देखभाल करने वाला)

यह प्रमाणित करने के लिए कि उपरोक्त सहमति मेरी उपस्थिति में प्राप्त की गई है।

दिनांक: _____ स्थान: _____ प्रधान अन्वेषक के हस्ताक्षर

गवाह 1	गवाह 2
हस्ताक्षर	हस्ताक्षर
_____	_____
नाम: _____	नाम: _____
पता: _____	पता: _____
_____	_____
_____	_____

Annexure VII: Patient Assent Form (English)

All India Institute of Medical Sciences, Jodhpur

Department of Dentistry

Informed Assent Form For 12-18 years

Principle Investigator:

Dr VAGHELA NIRAJ HIMMATLAL
Post Graduate student
Orthodontics and Dentofacial Orthopaedics
Department of Dentistry
AIIMS, Jodhpur Mobile No: - 8320312500 Study title:

"EVALUATION OF SALIVARY BIOMARKERS IN ORTHODONTIC PATIENTS WITH RECENT AND HEALED EXTRACTION SITES DURING EN MASSE RETRACTION - A PILOT STUDY"

Part I: Information Sheet

Introduction

My name is Dr Vaghela Niraj Himmatlal and my job is to see which works best to do faster movement of tooth during orthodontic treatment. We want to know if extraction of teeth done before or after application of orthodontic forces which is works best for faster orthodontic tooth movement

I am going to give you information and invite you to be part of a research study. You can choose whether or not you want to participate. We have discussed this research with your parent(s) /guardians and they know that we are also asking for your agreement. If you are going to participate in the research, your parent(s)/guardians also have to agree. But if you do not wish to take part in the research, you do not have to, even if your parents have agreed.

You may discuss anything in this form with your parents or friends or anyone else you feel comfortable talking to. You can decide whether to participate or not after you have talked it over. You do not have to decide immediately.

There may be some words that you do not understand or things that you want me to explain more about because you are interested or concerned. Please ask me to stop at any time and I will take time to explain.

Purpose of this study:

We are doing this research because we want to find whether the extraction done before or after orthodontic force application which will be better for faster orthodontic tooth movement and the clinical outcome.

Choice of participant:

We are doing this research on children who are your age – between 12-18 years old. We are only taking the saliva sample to see the markers which may increase or decrease the orthodontic tooth movement during orthodontic force application.

You don't have to be in this research if you don't want to be. It's up to you. If you decide not to be in the research, its okay and nothing changes. This is still your clinic, everything stays the same as before. Even if you say "yes" now, you can change your mind later and it's still okay.

If applicable: If anything changes and we want you to stay in the research study even if you want to stop, we will talk to you first .

I have checked with the child and they understand that participation is voluntary.

Information on orthodontic tooth movement

Any tooth movement which occurs after force is applied during orthodontic treatment.

Explanation about research:

In this research I will be taking your saliva sample at different time points during the orthodontic treatment. And I will send those saliva sample to the biochemistry laboratory for further evaluation of biomarkers which may cause bone resorption and deposition. At the same time I will be taking alginate impression of your teeth to see clinically whether it increases the tooth movement in extraction done before or after orthodontic force application.

Discomforts:

This will not hurt you as I will be collecting your saliva in plastic saliva collecting vials by spitting only.

I have checked with the child and they understand the risks and discomforts ____(initial).

Benefits:

Nothing good might happen to you. But this research may help to find out which works better for faster orthodontic tooth movement during orthodontic treatment. I have checked with the child and they understand the benefits_____ (initial) Reimbursements:

As in this study we are collecting saliva in salivary collecting vials which will be provided by investigator. So we will not be giving any Reimbursements for your participation in this study. Confidentiality:

We will not tell other people that you are in this research and we won't share information about you to anyone who does not work in the research study. Information about you that will be collected from the research will be put away and no-one but the researchers will be able to see it. Any information about you will have a number on it instead of your name. Only the researchers will know what your number is and we will lock that information up with a lock and key. It will not be shared with or given to anyone.

Compensation:

If you hurt during the research, we will look after you. We have given your parents information about what to do if you are hurt during the research.

Sharing the Findings:

When we are finished the research, I will sit down with you and your parent and I will tell you about what we learnt. I will also give you a paper with the results written down. Afterwards, we will be telling more people, scientists and others, about the research and what we found. We will do this by writing and sharing reports and by going to meetings with people who are interested in the work we do.

Right to Refuse or Withdraw:

You do not have to be in this research. No one will be mad or disappointed with you if you say no. It's your choice. You can think about it and tell us later if you want. You can say "yes" now and change your mind later and it will still be okay.

Who to Contact:

You can ask me questions now or later. You can ask the nurse questions. I have written a number and address where you can reach us or, if you are nearby, you can come and see us. If you want to talk to someone else that you know like your teacher or doctor or auntie, that's okay too.

If you choose to be part of this research I will also give you a copy of this paper to keep for yourself. You can ask your parents to look after it if you want.

You can ask me any more questions about any part of the research study, if you wish to. Do you have any questions?

PART 2: Certificate of Assent

I understand the research is about this study which is being done on me and I will be giving saliva sample and allow to take alginate impression at different time intervals during the study which cause no harm to me.

I have read this information. I have had my questions answered and know that I can ask questions later if I have them.

I agree to take part in the research.

I wish to take part in the research and I have signed the assent below._____(initialled by child/minor)

<u>Only if child assents:</u>

Print name of child _____

Signature of child: _____

Date: _____

Day/month/year

Annexure VIII: Patient Assent Form (Hindi)

अखिल भारतीय आयुर्विज्ञान संस्थान, जोधपुर

दंत चिकित्सा विभाग

***12-18* वर्षों के लिए सूचित सहमति पत्र**

अध्ययन शीर्षक:

"EVALUATION OF SALIVARY BIOMARKERS IN ORTHODONTIC PATIENTS WITH RECENT AND HEALED EXTRACTION SITES DURING ENMASSE RETRACTION - A PILOT STUDY"

भाग I: सूचना पत्र

परिचय

मेरा नाम डॉ वाघेला नीरज हिम्मतलाल है और मेरा काम यह देखना है कि ऑर्थोडोंटिक उपचार के दौरान दांत की गति को तेज करने के लिए कौन सा सबसे अच्छा काम करता है। हम जानना चाहते हैं कि क्या दांतों की निकासी ऑर्थोडोंटिक बलों के आवेदन से पहले या बाद में की जाती है जो कि तेजी से ऑर्थोडोंटिक टूथ मूवमेंट के लिए सबसे अच्छा काम करता है।

मैं आपको जानकारी देने जा रहा हूं और आपको एक शोध अध्ययन का हिस्सा बनने के लिए आमंत्रित करता हूं। आप चुन सकते हैं कि आप भाग लेना चाहते हैं या नहीं। हमने आपके माता-पिता/अभिभावकों के साथ इस शोध पर चर्चा की है और वे जानते हैं कि हम भी आपसे सहमति मांग रहे हैं। यदि आप शोध में भाग लेने जा रहे हैं, तो आपके माता-पिता/अभिभावकों को भी सहमत होना होगा। लेकिन यदि आप शोध में भाग नहीं लेना चाहते हैं, तो आपके माता-पिता के सहमत होने पर भी आपको ऐसा करने की आवश्यकता नहीं है।

आप इस रूप में अपने माता-पिता या दोस्तों या किसी और से बात करने में सहज महसूस कर सकते हैं। बात करने के बाद आप तय कर सकते हैं कि भाग लेना है या नहीं। आपको तुरंत निर्णय लेने की आवश्यकता नहीं है।

कुछ ऐसे शब्द हो सकते हैं जो आपको समझ में नहीं आते हैं या ऐसी चीजें हैं जिनके बारे में आप चाहते हैं कि मैं इसके बारे में अधिक समझाऊं क्योंकि आप रुचि रखते हैं या चिंतित हैं। कृपया मुझे किसी भी समय रुकने के लिए कहें और मुझे समझाने में समय लगेगा।

इस अध्ययन का उद्देश्य:

हम यह शोध इसलिए कर रहे हैं क्योंकि हम यह पता लगाना चाहते हैं कि ऑर्थोडोंटिक बल लगाने से पहले या बाद में किया गया निष्कर्षण जो तेजी से ऑर्थोडोंटिक टूथ मूवमेंट और क्लिनिकल परिणाम के लिए बेहतर होगा।

प्रतिभागी की पसंद:

हम यह शोध उन बच्चों पर कर रहे हैं जिनकी उम्र आपकी उम्र *12-18* साल के बीच है। हम केवल लार के नमूने को मार्करों को देखने के लिए ले रहे हैं जो ऑर्थोडोंटिक बल आवेदन के दौरान ऑर्थोडोंटिक दांतों की गति को बढ़ा या घटा सकते हैं।

यदि आप नहीं बनना चाहते हैं तो आपको इस शोध में शामिल होने की आवश्यकता नहीं है। यह आप पर निर्भर करता है। यदि आप शोध में शामिल नहीं होने का निर्णय लेते हैं, तो ठीक है और कुछ भी नहीं बदलता है। यह अभी भी आपका क्लिनिक है, सब कुछ पहले जैसा ही रहता है। यहां तक कि अगर आप अभी "हां" कहते हैं, तो आप बाद में अपना विचार बदल सकते हैं और यह अभी भी ठीक है।

यदि लागू हो: यदि कुछ भी बदलता है और हम चाहते हैं कि आप शोध अध्ययन में बने रहें, भले ही आप रुकना चाहें, हम पहले आपसे बात करेंगे।

मैंने बच्चे के साथ जाँच की है और वे समझते हैं कि भागीदारी स्वैच्छिक है।

ऑर्थोडोंटिक टूथ मूवमेंट की जानकारी

किसी भी दांत की गति जो बल के बाद होती है उसे ऑर्थोडोंटिक उपचार के दौरान लागू किया जाता है।

अनुसंधान के बारे में स्पष्टीकरण:

इस शोध में मैं ऑर्थोडोंटिक उपचार के दौरान अलग-अलग समय बिंदुओं पर आपके लार के नमूने ले रहा हूं। और मैं उन लार के नमूने को बायोमार्कर के आगे मूल्यांकन के लिए जैव रसायन प्रयोगशाला में भेजूंगा जो हड्डियों के पुनर्जीवन और जमाव का कारण हो सकता है। साथ ही मैं चिकित्सकीय रूप से यह देखने के लिए आपके दांतों का एल्गिनेट इंप्रेशन लूंगा कि क्या यह ऑर्थोडॉन्टिक बल लगाने से पहले या बाद में किए गए निष्कर्षण में दांतों की गति को बढ़ाता है।

असुविधाएँ:

इससे आपको कोई नुकसान नहीं होगा क्योंकि मैं आपकी लार को केवल थूक कर प्लास्टिक की लार में इकट्ठा कर रहा हूं।

मैंने बच्चे के साथ जाँच की है और वे जोखिमों और असुविधाओं को समझते हैं ____(प्रारंभिक)।

लाभ:

आपके साथ कुछ भी अच्छा नहीं हो सकता है। लेकिन यह शोध यह पता लगाने में मदद कर सकता है कि ऑर्थोडोंटिक उपचार के दौरान दांतों की तेज गति के लिए कौन सा बेहतर काम करता है। मैंने बच्चे के साथ जाँच की है और वे लाभों को समझते हैं_____ (प्रारंभिक) प्रतिपूर्ति:

जैसा कि इस अध्ययन में हम लार एकत्र करने वाली शीशियों में लार एकत्र कर रहे हैं जो अन्वेषक द्वारा प्रदान की जाएगी। इसलिए हम इस अध्ययन में आपकी भागीदारी के लिए कोई प्रतिपूर्ति नहीं देंगे। गोपनीयता:

हम अन्य लोगों को यह नहीं बताएंगे कि आप इस शोध में हैं और हम आपके बारे में जानकारी किसी ऐसे व्यक्ति से साझा नहीं करेंगे जो शोध अध्ययन में काम नहीं करता है। आपके बारे में जो जानकारी शोध से एकत्र की जाएगी उसे हटा दिया जाएगा और इसे कोई और नहीं बल्कि शोधकर्ता देख पाएंगे। आपके बारे में किसी भी जानकारी पर आपके नाम की जगह एक नंबर होगा। केवल शोधकर्ताओं को ही पता चलेगा कि आपका नंबर क्या है और हम उस जानकारी को एक ताला और चाबी से बंद कर देंगे। इसे किसी के साथ साझा या दिया नहीं जाएगा।

मुआवज़ा:

यदि शोध के दौरान आपको चोट लगती है, तो हम आपकी देखभाल करेंगे। हमने आपके माता-पिता को इस बारे में जानकारी दी है कि यदि शोध के दौरान आपको चोट लगती है तो क्या करें।

निष्कर्षों को साझा करना:

जब हम शोध समाप्त कर लेंगे, तो मैं आपके और आपके माता-पिता के साथ बैठूंगा और जो कुछ हमने सीखा है उसके बारे में आपको बताऊंगा। मैं आपको नीचे लिखे परिणामों के साथ एक पेपर भी दूंगा। बाद में, हम शोध के बारे में और लोगों, वैज्ञानिकों और अन्य लोगों को बताएंगे.

भाग 2: सहमति का प्रमाण पत्र

मैं समझता हूं कि शोध इस अध्ययन के बारे में है जो मुझ पर किया जा रहा है और मैं लार का नमूना दूंगा और अध्ययन के दौरान अलग-अलग समय अंतराल पर एल्गिनेट इंप्रेशन लेने की अनुमति दूंगा जिससे मुझे कोई नुकसान नहीं होगा।

मैंने यह जानकारी पढ़ ली है। मेरे पास मेरे सवालों के जवाब हैं और मैं जानता हूं कि अगर मेरे पास सवाल हैं तो मैं बाद में पूछ सकता हूं।

मैं शोध में भाग लेने के लिए सहमत हूं।

मैं शोध में भाग लेना चाहता हूं और मैंने नीचे सहमति पर हस्ताक्षर किए हैं।

केवल अगर बच्चा सहमति देता है:

बच्चे का _____
बच्चे के हस्ताक्षर: _____
दिनांक: _____
दिन /महीने/ साल

Annexure IX: Plagiarism Certificate

Evaluation of salivary biomarkers in orthodonti...
By: Vaghela Niraj Himmatlal
As of: Jan 15, 2022 1:41:10 PM
10,159 words - 93 matches - 31 sources

Similarity Index
15%

Mode: Summary Report

sources:

309 words / 3% - Crossref
Gloria Amparo Flórez-Moreno, Diana María Isaza-Guzmán, Sergio Iván Tobón-Arroyave, "Time-related changes in salivary levels of the osteotropic factors sRANKL and OPG through orthodontic tooth movement", American Journal of Orthodontics and Dentofacial Orthopedics, 2013

183 words / 2% - Crossref
Stacey Reiss, Marie Claude Chouinard, Dasha Frias Landa, Ravindra Nanda et al. "Biomarkers of orthodontic tooth movement with fixed appliances and vibration appliance therapy: a pilot study", European Journal of Orthodontics, 2020

105 words / 1% - Crossref
"Biological mechanisms of tooth movement", Wiley, 2015

71 words / 1% - Internet
Bancha Samruajbenjakun, Kaviya Kanokpongsak, Chidchanok Leethanakul, "Comparison of clinical and histological characteristics of orthodontic tooth movement into recent and healed extraction sites combined with corticotomy in rats", Korean Journal of Orthodontics

76 words / 1% - Internet from 24-Oct-2019 12:00AM
boris.unibe.ch

76 words / 1% - Internet from 02-Nov-2020 12:00AM
res.mdpi.com

66 words / 1% - Internet from 08-Jan-2022 12:00AM
ebin.pub

60 words / 1% - Crossref
Angela Quain, Mark Hoyer, Georges Ephrem, W. Aaron Kay, "Non-invasive cardiac output monitoring (NICOM) in adult congenital heart disease patients with Fontan palliation", International Journal of Cardiology Congenital Heart Disease, 2021

21 words / < 1% match - Internet
M. Knaś, M. Maciejczyk, I. Daniszewska, A. Klimiuk et al. "Oxidative Damage to the Salivary Glands of Rats with Streptozotocin-Induced Diabetes-Temporal Study: Oxidative Stress and Diabetic Salivary Glands", Journal of Diabetes Research

44 words / < 1% match - Internet from 27-Aug-2021 12:00AM
coek.info

40 words / < 1% match - Internet from 05-Jan-2022 12:00AM
coek.info

35 words / < 1% match - Internet from 07-Dec-2021 12:00AM

Annexure X: CONSORT 2010 checklist of information to include when reporting a randomised trial

Section/Topic	Item no.	Checklist Item	Reported on page no.
Title and abstract			
	1a	Identification as a randomised trial in the title	No
	1b	Structured summary of trial design, methods, results and conclusions	30
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	1-4
	2b	Specific objectives or hypotheses	5
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	16
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	NA
Participants	4a	Eligibility criteria for participants	17
	4b	Settings and locations where the data were collected	16
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	18
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	21-22
	6b	Any changes to trial outcomes after the trial commenced, with reasons	NA
Sample size	7a	How sample size was determined	16

	7b	When applicable, explanation of any interim analyses and stopping guidelines	NA
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	17
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	16
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	17-18
Implementation	10	Who generated the random allocation sequence, who enrolled participants and who assigned participants to interventions	17-18
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those assessing outcomes) and how	18
	11b	If relevant, description of the similarity of interventions	NA
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	22
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	22
Results			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment and were analysed for the primary outcome	30
	13b	For each group, losses and exclusions after randomisation, together with reasons	NA
Recruitment	14a	Dates defining the periods of recruitment and follow-up	16
	14b	Why the trial ended or was stopped	NA

Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	31
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	30
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group and the estimated effect size and its precision (such as 95% confidence interval)	33
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	NA
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	NA
Harms	19	All important harms or unintended effects in each group	NA
Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision and, if relevant, multiplicity of analyses	54
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	54
Interpretation	22	Interpretation consistent with results, balancing benefits and harms and considering other relevant evidence	47-54
Other information			
Registration	23	Registration number and name of trial registry	16
Protocol	24	Where the full trial protocol can be accessed, if available	16
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	NA