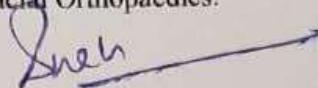


CERTIFICATE BY THE GUIDE/ CO-GUIDE

This is to certify that the dissertation entitled "**COMPARISON OF PROINFLAMMATORY CYTOKINE LEVELS BETWEEN ANTERIOR AND POSTERIOR SEGMENT, DURING ENMASSE RETRACTION OF ANTERIOR TEETH**" is a bonafide work done by **Dr Nidhi Tiwari**, under my direct supervision and guidance in partial fulfilment of the requirement for the degree of MDS in Department of Orthodontics and Dentofacial Orthopaedics.


GUIDE

Dr. Sneh Lata Verma, Professor

Department of Orthodontics and Dentofacial Orthopaedics BBDCODS, BBDU
Lucknow



CO-GUIDE

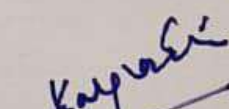
Dr. Shantanu Khattri

Professor & Head

Department of Orthodontics and

Dentofacial Orthopaedics

BBDCODS, BBDU, Lucknow


CO-GUIDE

Dr. Kalpana Singh Professor &

Head Department of Biochemistry

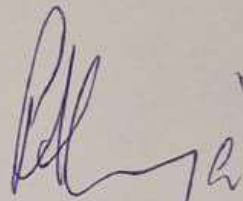
KGMU, LUCKNOW

ENDORSEMENT BY THE HOD / HEAD OF THE INSTITUTION

This is to certify that the dissertation entitled "**COMPARISON OF PROINFLAMMATORY CYTOKINE LEVELS BETWEEN ANTERIOR AND POSTERIOR SEGMENT, DURING ENMASSE RETRACTION OF ANTERIOR TEETH**" is a bonafide work done by **Dr Nidhi Tiwari** under the supervision of **Dr. Sneh Lata Verma** Professor, Department of Orthodontics and Dentofacial Orthopaedics, Babu Banarasi Das College of Dental Sciences, Babu Banarasi Das University, Lucknow, Uttar Pradesh.



Dr. Shantanu Khattri
Professor & Head Department of
Orthodontics and Dentofacial
Orthopaedics. BBDCODS, BBDU
Lucknow



Dr. Puneet Ahuja Principal
BBDCODS, BBDU
Lucknow

COPYRIGHT

I hereby declare that the **Babu Banarasi Das University** shall have the rights to preserve, use and disseminate this dissertation in print or electronic format for academic / research purpose.

Date: 25.03.2025

Place: BBDCDS

Dr Nidhi Tiwari
Dr Nidhi Tiwari

DECLARATION BY THE CANDIDATE

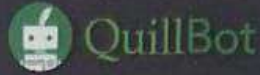
I hereby declare that this dissertation entitled "**COMPARISON OF PROINFLAMMATORY CYTOKINE LEVELS BETWEEN ANTERIOR AND POSTERIOR SEGMENT, DURING ENMASSE RETRACTION OF ANTERIOR TEETH**" is a bonafide and genuine research work carried out by me under the guidance of *Dr. Sneh Lata Verma* Professor, Department of Orthodontics and Dentofacial Orthopedics, Babu Banarasi Das College of Dental Sciences, Babu Banarasi Das University, Lucknow, Uttar Pradesh.

Date: 25 03 . 2025

Place: BBDCDS

Dr Nidhi Tiwari

Dr Nidhi Tiwari



QuillBot

Scanned on: 22:27 March 18, 2025 UTC

- | | |
|---|------|
| DDD Flashcards Quizlet
https://quizlet.com/862456809/ddd-flash-cards/ | 100% |
| Mass spectrometry techniques for qualitative and quantita...
https://www.sciencedirect.com/science/article/abs/pii/S1740674904000204 | 100% |
| Biological Marker - an overview ScienceDirect Topics
https://www.sciencedirect.com/topics/pharmacology-toxicology-and-phar... | 100% |
| Advancements in Drug Discovery and Development Flashca...
https://quizlet.com/862572576/advancements-in-drug-discovery-and-deve... | 100% |
| (PDF) Induction of cytokines, MMP9, TIMPs, RANKL and OPG...
https://www.academia.edu/14322461/Induction_of_cytokines_MMP9_TIMP... | 100% |
| Effects of Matrix Metalloproteinase Inhibitors on Bone Reso...
https://www.researchgate.net/publication/10597945_Effects_of_Matrix_Me... | 100% |
| (PDF) Salivary Levels of Opg and Rankl During Orthodontic T...
https://www.academia.edu/106225530/Salivary_Levels_of_Opg_and_Rankl... | 100% |
| Induction of cytokines, MMP9, TIMPs, RANKL and OPG durin...
https://pubmed.ncbi.nlm.nih.gov/22987319/ | 100% |

**COMPARISON OF PROINFLAMMATORY CYTOKINE
LEVELS BETWEEN ANTERIOR AND POSTERIOR
SEGMENT, DURING ENMASSE RETRACTION OF
ANTERIOR TEETH**

DISSERTATION

Submitted to

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

In the partial fulfillment of the requirements for the degree

of

MASTER OF DENTAL SURGERY

In

ORTHODONTICS AND DENTOFACIAL ORTHOPAEDICS

By

Dr NIDHI TIWARI

Under the guidance of

Dr. SNEH LATA VERMA

Professor

Department of Orthodontics and Dentofacial Orthopaedics

BABU BANARASI DAS COLLEGE OF DENTAL SCIENCES, LUCKNOW

(Faculty of Babu Banarasi Das University)

YEAR OF SUBMISSION: 2025

BATCH: 2022-2025

CERTIFICATE BY THE GUIDE/ CO-GUIDE

This is to certify that the dissertation entitled “**COMPARISON OF PROINFLAMMATORY CYTOKINE LEVELS BETWEEN ANTERIOR AND POSTERIOR SEGMENT, DURING ENMASSE RETRACTION OF ANTERIOR TEETH**” is a bonafide work done by **Dr Nidhi Tiwari**, under my direct supervision and guidance in partial fulfilment of the requirement for the degree of MDS in Department of Orthodontics and Dentofacial Orthopaedics.

GUIDE

Dr. Sneh Lata Verma, Professor

Department of Orthodontics and Dentofacial Orthopaedics BBDCODS, BBDU
Lucknow

CO-GUIDE

Dr. Shantanu Khattri
Professor & Head
Department of Orthodontics and
Dentofacial Orthopaedics
BBDCODS, BBDU, Lucknow

CO-GUIDE

Dr. Kalpana Singh Professor &
Head Department of Biochemistry
KGMU, LUCKNOW

ENDORSEMENT BY THE HOD / HEAD OF THE INSTITUTION

This is to certify that the dissertation entitled “**COMPARISON OF PROINFLAMMATORY CYTOKINE LEVELS BETWEEN ANTERIOR AND POSTERIOR SEGMENT, DURING ENMASSE RETRACTION OF ANTERIOR TEETH**” is a bonafide work done by **Dr Nidhi Tiwari** under the supervision of **Dr. Sneh Lata Verma** Professor, Department of Orthodontics and Dentofacial Orthopaedics, Babu Banarasi Das College of Dental Sciences, Babu Banarasi Das University, Lucknow, Uttar Pradesh.

Dr. Shantanu Khattri
Professor & Head Department of
Orthodontics and Dentofacial
Orthopaedics. BBDCODS, BBDU
Lucknow

Dr. Puneet Ahuja Principal
BBDCODS, BBDU
Lucknow

COPYRIGHT

I hereby declare that the **Babu Banarasi Das University** shall have the rights to preserve, use and disseminate this dissertation in print or electronic format for academic / research purpose.

Date:

Place:

Dr Nidhi Tiwari

DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation entitled “**COMPARISON OF PROINFLAMMATORY CYTOKINE LEVELS BETWEEN ANTERIOR AND POSTERIOR SEGMENT, DURING ENMASSE RETRACTION OF ANTERIOR TEETH**” is a bonafide and genuine research work carried out by me under the guidance of *Dr. Sneh Lata Verma* Professor, Department of Orthodontics and Dentofacial Orthopedics, Babu Banarasi Das College of Dental Sciences, Babu Banarasi Das University, Lucknow, Uttar Pradesh.

Date:

Place:

Dr Nidhi Tiwari

*I bow my head to the **Almighty God**, who blessed me with his blessings, bestowed me with his kind grace, provided me with strength, courage and good health to reach this stage and made it possible for me to bring out this manuscript. The satisfaction and euphoria that accompany the successful completion of a task could be incomplete without the mention of the people who made it possible.*

*It is my privilege and honor to express my most sincere and heartfelt thanks to my guide **Dr. Sneh Lata Verma, MDS, Professor**, Department of Orthodontics and Dentofacial Orthopaedics, Babu Banarasi Das College of Dental Sciences, Lucknow whose brilliant foresight and practical approach have been the guiding force behind the present shape of my project. Her inestimable calm and ineffable wisdom, continuous encouragement and earnest attention have always been a driving force throughout the project. It's an honor to have such teachers and shall forever be indebted to her.*

*I am also extremely thankful to my co-guide **Dr. Shantanu khatti**, MDS, Professor & Head, Department of Orthodontics and Dentofacial Orthopaedics, Babu Banarasi Das College of Dental Sciences, Lucknow. I am also extremely thankful to my co-guide **Dr. Kalpana**, MS, Professor & Head, Department of Biochemistry, King George's Medical University, Lucknow. I am deeply grateful for her patience, and expertise that she shared with me. Most importantly, I am thankful for her collaboration, and mentorship throughout. It was her understanding, valuable suggestions, unstinted help and personal attention that have provided good and smooth basis for this work.*

*I am extremely thankful to **Dr. Tripti Tikku** MDS, Professor, Department of Orthodontics and Dentofacial Orthopaedics, Babu Banarasi Das College of Dental Sciences, Lucknow. She always guided me and gave her valuable advice wherever needed.*

*I am extremely indebted to **Dr. Kamna Srivastava**, MDS, Reader, Department of Orthodontics and Dentofacial Orthopaedics, Babu Banarasi Das College of Dental Sciences, Lucknow for her valuable advice throughout, unbiased support and constant help rendered not just for dissertation, but always. I am deeply grateful for*

her patience, time to time guidance, encouragement, motivation, her unwavering support, and expertise that she shared with me at every stage, her wise and logical suggestions,

personal attention and meticulous attention to the fine details have helped me give this dissertation its final shape. Without her support this would not have been possible for me.

*I would also like to express my gratitude to **Dr. Rana Pratap Maurya**, MDS for his encouragement, constant moral support and inspiration.*

*I would also like to thank all my Senior Lecturers **Dr. Srishti Aditi, Dr. Prateek, Dr. Anjali Dr. Sparsh and Dr Rashi** MDS, Department of Orthodontics and Dentofacial Orthopedics, Babu Banarasi Das College of Dental Sciences, Lucknow, for their valuable suggestions, encouragement and constant moral support during the period of my study.*

*I would like to pay high regards to all my loving seniors. I owe a special debt to my seniors and am very thankful to them, who always guide us. Thank you, my dear seniors, **Dr. Sweety, Dr. Jaya, Dr. Shireen, Dr. Monika, Dr. Aliya, Dr. Sarbajit** for their help and encouragement throughout my work sailing together in the same boat.*

*I would also like to thank my batchmates **Dr. Anupama, Dr. Abhishek E.P, Dr. Sineen, Dr. Ashima, Dr. Ashim.***

*I would also like to thank my juniors, **Dr. Pooja, Dr. Aditya, Dr. Safa, Dr. Upasna, Dr. Ankita, Dr. Mudasir, Dr. Aditya, Dr. Prarthana, Dr. Vaishakha** for their constant support and help in all my work.*

I am dedicating my thesis work to my grandmother Late Smt. Krishna kumari Bajpayee, whatever I have achieved today, the foundation was laid down by her only since my childhood.

*A special thanks to my husband **Mr. Sourabh Mishra** for the unconditional love and support and sacrifice through this journey. This would not be possible without him. I would like to thank my family especially **my mausaji Mr. U.S. Mishra** (not less than*

*father figure), and also to my in laws **Dr. A. k. Mishra and Asha Mishra** for their continuous support and motivation. I would like to express my indebtedness for all the sacrifices they have made to see me succeed in my past, present and future.*

*It is a pleasure at this point to thank my principal, **Dr. Puneet Ahuja** for providing me with the opportunity to conduct the research and use the facilities of this college.*

Last, but not the least, I express my gratitude for all who directly or indirectly provided their esteemed cooperation that made me complete my library dissertation.

Nidhi Tiwari

TABLE OF CONTENT

S. NO.	PARTICULARS	PAGE NO.
1.	ACKNOWLEDGEMENT	VI-VIII
2.	TABLE OF CONTENTS	IX
3.	LIST OF FIGURES	X-XI
4.	LIST OF TABLES	XII
5.	LIST OF BARGRAPHS	XIII
6.	LIST OF ANNEXURES	XIV
7.	LIST OF ABBREVIATIONS	XV
8.	ABSTRACT	XVI-XVII
9.	INTRODUCTION	1-4
10.	AIM & OBJECTIVES	5
11.	REVIEW OF LITERATURE	6-26
12.	MATERIALS & METHOD	27-60
13.	OBSERVATION AND RESULTS	61-70
14.	DISCUSSION	71-82
15.	CONCLUSIONS	83
16.	REFERENCES	84-92
17.	SUMMARY	93-95
18.	ANNEXURES	96-116

LIST OF FIGURES

FIG. NO.	CONTENTS	PAGE NO.
1.	Materials used for Diagnosis and Impression Making	30
2.	Materials used for cephalometric analysis	32
3.	Materials used for fixed orthodontic treatment	34
4.	Materials used For Sliding Mechanics	36
5.	Materials used for GCF collection and storage	38
6.	Materials used for interim and final storage	40
7.	Materials used for assessment of Biomarker	42
8.	Intraoral photograph (Right and Left lateral view) after alignment and levelling of the arch	44
9.	Intraoral photograph (Right and Left lateral view) after 1 st Premolar Extraction	44
10.	Crimpable hook crimped on arch wire	46
11.	Initiation of enmasse retraction of anterior teeth using Niti closed coil spring	46
12.	Retraction force of 200 gm standardized on Dontrix gauge	47
13.	Adequate Isolation of site before GCF collection	49
14.	Absorbent point inserted in distal gingival sulcus of Canine	51
15.	Absorbent point inserted in mesial gingival sulcus of maxillary first molar	51
16.	Soaked absorbent point placed in labeled Eppendorf tube containing Phosphate Buffer Saline (PBS) solution	53
17.	GCF storage and transportation a) Ice Box with Eppendorf tube b) Deep freezer	53

18.	Sample processing foe ELISA analysis a) Centrifuge machine with Eppendorf tube for elution of GCF b) Supernatants were transferred into new Eppendorf tubes. c) Incubator	55
19.	Materials used for ELISA test (ELISA kit for RANKL/IL6)	57
20.	Preparation of assay for sandwich ELISA procedure. a) Addition of sample to each well b) Washing of wells c) Dilution and addition of biotinylated antibody, HRP-conjugated pipetted to wells	59
21.	Showing Colour change a) Positive well colour changed to blue b) Colour changed from blue to yellow	60

LIST OF TABLES

TABLE NO	TITLE OF THE TABLE	PAGE NO.
1.	Time interval at which GCF was collected	50
2.	Descriptive statistics for mean values of IL-6 and RANKL as obtained for Group I and Group II at different time	62
3.	Intragroup comparison of IL6 for Group I at different time intervals	63
4.	Intragroup comparison of IL-6 for Group II at different time intervals	64
5.	Intergroup comparison of IL-6 between Group I & Group II at T0 , T1 ,T2, T3.	65
6.	Intragroup comparison of RANKL for Group I at different time intervals .	66
7.	Intragroup comparison of RANKL for Group II at different time intervals.	68
8.	Intergroup comparison of RANKL between Group I & Group II at T0, T1 ,T2, T3.	69
9.	Overall Comparison between Group I and Group II for RANKL and IL-6.	70

LIST OF BAR GRAPHS

GRAPH NO	TITLE OF THE GRAPH	PAGE NO.
1.	Mean Values Of IL-6 (pg/ml) For Group I And Group II	73
2.	Intragroup Comparison Of IL-6 For Group I at different time intervals.	74
3.	Intragroup Comparison Of IL6 For Group II at different time intervals.	74
4.	Mean values of RANKL(pg/ml) for Group I And Group II	75
5.	Intragroup Comparison Of RANKL for Group I at different time intervals.	75
6.	Intragroup comparison of RANKL for Group II at different time intervals.	76
7.	Intergroup comparison of IL-6 between Group I & Group II at T0, T1, T2, T3.	76
8.	Intergroup comparison of RANKL between Group I & Group II at T0, T1, T2, T3.	77
9.	Overall comparison between Group I and Group II for RANKL and IL-6.	77

LIST OF ANNEXURES

NO.	ANNEXURE	PAGE NO.
1.	ANNEXURE-I - IRC Approval certificate	96
2.	ANNEXURE-II-IEC Approval certificate	97-98
3.	ANNEXURE-III- Approval by Co-guide	99
4.	ANNEXURE-IV-PID-ENGLISH	100-105
5.	ANNEXURE-V – PID -HINDI	106-110
6.	ANNEXURE-VI-CONSENT ENGLISH	111
7.	ANNEXURE-VII-CONSENT HINDI	112
8.	ANNEXURE-VIII - PLAGIARISM REPORT	113-116

LIST OF ABBREVIATIONS

S.NO	ABBREVIATED FORM	FULL FORM
1.	GCF	Gingival crevicular fluid
2.	IL-6	Interleukin-6
3.	RANKL	Receptor activator nuclear kappa B ligand
4.	OTM	Orthodontic tooth movement
5.	PDL	Periodontal ligament
6.	TNF-α	Tumor Necrosis Factor
7.	MMP	Matrix Metalloproteinase
8.	OPG	Osteoprotegrin
9.	SOST	Sclerostin
10.	LDL	Low density lipoprotein
11.	HbA1c	Glycated Haemoglobin
12.	IGF	Insulin Growth Factor
13.	FGF	Fibroblast Growth Factor
14.	IL-1	Interleukin -1
15.	IL-6	Interleukin-6
16.	IL-8	Interleukin-8
17.	IgA	Immunoglobulin A
18.	IgM	Immunoglobulin M
19.	ELISA	Enzyme- linked immunosorbent assay
20.	PBS	Phosphate Buffer Saline
21.	OPG	Orthopantomogram

AIM: Comparison of proinflammatory cytokine (IL-6 & RANKL) levels between anterior and posterior segment, during enmasse retraction of anterior teeth.

MATERIAL AND METHOD: 15 patients (age range of 18-25 yrs) with clinical presentation of class I Bi-maxillary protrusion or Class II Div I malocclusion undergoing fixed orthodontic treatment (in which extraction of all 1st premolar teeth was required) were included in the study. Following standard protocol of wire sequence for alignment and levelling first premolars were extracted. Thereafter enmasse retraction of anterior teeth was initiated on .019x.025 inch SS archwire using sliding mechanics with Niti closed coil spring with force levels of 200 gm. Based on site of GCF collection GCF sample was divided as Group I (included GCF collected from distal gingival sulcus of maxillary Canine) and Group II (included GCF collected from mesial gingival sulcus of maxillary first molar) at different time intervals -T0 (Baseline) immediately after applying the retraction force; T1- (after 24 hrs); T2- (after 20 days), and T3- (after 2 months) using absorbent points. Mean level of IL-6 and RANKL were obtained following standard protocol of collection, storage, transport and analysis using ELISA analyser and Data was tabulated and statistical comparison was done.

RESULT: The trend for IL-6 levels in Group I was T0>T2>T3>T1, with statistically significant difference between T0 - T1 and T1 - T2, in Group II was T1>T0>T3>T2, with statistically significant difference between T0 -T2. The trend for RANKL levels in Group I was T2>T3>T0>T1, in Group II it was T1>T0>T2>T3, and the difference was statistically non-significant between both groups. Levels of both the biomarkers IL-6 and RANKL were higher in Group II than Group I at all time. Intragroup comparison of both biomarkers in Group II did not show significant fluctuation in consequent time interval but Intergroup comparison revealed higher values in Group II than Group I with significant difference only at T0, T1 & T3 for IL-6 and at T1 for RANKL.

CONCLUSION: Cytokines play an important role in the bone remodeling processes, indicate that the changes in level of cytokines in GCF are associated with orthodontic tooth movement. The assessment of biomarker in anterior and posterior segment can be helpful in assessing amount of anchorage loss as well.

KEYWORDS: Enmasse retraction, IL-6, RANKL, Orthodontic tooth movement, Cytokines, Biomarkers.

Extraction of specific teeth is often required in fixed orthodontic treatment to improve facial esthetics, attain good structural balance, and achieve occlusal harmony¹. Space closure after extraction can be accomplished either by frictionless/loop mechanics or by frictional/sliding mechanics. Frictionless/loop mechanics require intricate wire bending and is more technique-sensitive. Sliding mechanics uses lighter forces, is less technique-sensitive, and is more comfortable for the patient. In sliding mechanics, retraction of anterior teeth can be done either en masse or in two steps, where the canine is retracted initially, followed by the remaining four incisors ². Two-step retraction is generally performed in maximum anchorage cases but prolongs overall treatment time and creates unesthetic spaces distal to the lateral incisor after canine retraction³. This limitation is overcome during en masse retraction, which is routinely used in Orthodontics and was selected in the present study for space closure.

The mechanical stress arising from force application during orthodontic tooth movement (OTM) changes periodontal vascularity and induces cellular and molecular changes in the periodontal ligament (PDL), leading to the release of biologically active substances called signaling molecules, which regulate alveolar bone modelling and remodeling ⁴.

Two interrelated biological processes occur during OTM: compression of the PDL on one side (pressure side) and stretching on the other side (tension side), leading to cellular changes at the molecular level and the release of various signaling molecules responsible for OTM. These molecules include neurotransmitters, cytokines, growth factors, colony-stimulating factors, and arachidonic acid metabolites ⁵.

Cytokines are inflammatory mediators released soon after applying force in response to an acute inflammatory reaction, leading to vasodilation, leukocyte migration, and subsequent cytokine release. Among these cytokines, the levels of Interleukin 1 β (IL1 β), IL-1, IL-6, Receptor Activator of Nuclear Factor Kappa-B Ligand (RANKL), Tumor Necrosis Factor (TNF)- α , Epidermal Growth Factor, Matrix Metalloproteinase (MMP), Osteoprotegerin (OPG), and Sclerostin (SOST) are commonly assessed during orthodontic tooth movement. The assessment of these cytokines, neurotransmitters, and arachidonic acid metabolites as biomarkers has significantly enhanced our understanding of molecular changes during OTM ^{5,6}.

A biomarker is a substance that is measured and evaluated objectively as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention. A good biomarker should be specific, sensitive, and capable of providing information about biological conditions ⁷.

Biomarkers have been used as diagnostic tools for disease identification, staging, prognosis, and monitoring clinical responses to interventions in medicine⁸. Their use in dentistry has facilitated the diagnosis and prevention of periodontal diseases, dental caries, and oral cancer, as well as understanding responses to therapeutic interventions⁹. In Orthodontics, biomarkers have helped to assess puberty, bone turnover, and orthodontic tooth movement at the molecular level ¹⁰.

These biomarkers can be derived from different biofluids such as blood/serum, saliva, and Gingival Crevicular Fluid (GCF) ¹¹. Serum biomarkers, such as LDL for cholesterol, P53 gene and MMPs for cancer, and HbA1c for diabetes, are valuable diagnostic and prognostic tools in medicine ¹². In Orthodontics, serum biomarkers such as Insulin-like Growth Factor (IGF) for growth assessment, Fibroblast Growth Factor (FGF) for skeletal development, and Parathyroid Hormone-related Protein for bone turnover have been studied. However, the need for invasive intervention for serum collection is a major limitation ¹³.

Biomarkers can also be derived from saliva, like levels of sorbitol and fructosamine levels in saliva correlate with glucose levels in capillary blood, and increased isoprostane and oxidized low-density lipoproteins are present in individuals with coronary heart disease ¹⁴.

Various salivary biomarkers have been evaluated in dentistry, including TNF- α , IL-1, IL-6, IL-8, IgA, IgM, α -Glucosidase, Acid Phosphatase, Alkaline Phosphatase, MMP-13, MMP-8, MMP-9, and PDGF, as well as cancer antigen-125 (CA-125) in oral cancer subjects ^{15,16}.

Salivary biomarkers have also been assessed during various stages of fixed orthodontic treatment. **Naoumova J et al** ¹⁷evaluated OPG and RANKL levels during different orthodontic stages, including alignment, space closure, and finishing. **Gloria Amparo Florez-Moreno et al**¹⁸ assessed OPG and RANKL levels in saliva at different intervals during initial levelling and alignment. **Vaghela N et al** ¹⁹ studied salivary biomarkers

(RANKL and OPG) during en masse retraction of anterior maxillary teeth at different stages of healing. These studies indicated that biomarker levels change after force application at various stages of orthodontic tooth movement, correlating with different phases (initial, lag, and post-lag) of treatment⁵.

Similar to serum, salivary biomarkers are also present in minimal quantities, requiring highly sensitive tests for quantification. The composition of saliva is influenced by environmental and psychological factors. Thus detection of presence of biomarkers in saliva can be reliably achieved but quantification of these biomarker is a real challenge²⁰. To overcome these limitations, GCF biomarkers are preferred due to their proximity to periodontal tissues, minimizing the influence of systemic inflammatory processes²¹.

Navarro-Palacios et al²² evaluated Myeloperoxidase activity in GCF and saliva, demonstrating that GCF values reflected inflammatory changes more accurately than saliva. GCF is an osmotically mediated inflammatory exudate that increases in volume and capillary permeability during inflammation²³. Considering this, biomarker levels in GCF during orthodontic tooth movement were assessed in the present study.

Biomarkers associated with bone resorption include IL-1, IL-6, and TNF- α , which upregulate RANKL expression in stromal cells and osteoblasts, stimulating bone resorption. IL-6 promotes RANKL activation in the initial force application stages. RANKL, produced by osteoblasts and activated by T-lymphocytes, binds to its receptor (RANK) on osteoclasts, inducing osteoclast maturity and alveolar bone resorption. However, if RANKL binds to OPG, it results in bone deposition⁵.

Several animal and human studies have demonstrated cytokine level changes in periodontal tissues and GCF during orthodontic treatment. Most studies assessed biomarker levels during the alignment and levelling phase. **Madureira et al**²⁴ and **Mohammed A et al**²⁵ showed increased IL-6 levels in the human PDL after 12-15 days of orthodontic force application. **Basaran G et al**²⁶ found increased IL-2, IL-6, and IL-8 levels in GCF during levelling and distalization. **Widayati R et al**²⁷ evaluated OPG levels in early leveling and aligning using different bracket systems.

Uday H Barhate et al²⁸ found a positive but not statistically significant correlation between the rate of canine retraction and the concentrations of cytokines such as IL-1 β

and TNF- α in GCF , **Jayprakash P et al**²⁹ evaluated levels of Interleukin (IL)-1 β , IL-6, tumor necrosis factor- α , epidermal growth factor, and β 2-microglobulin levels in GCF during canine retraction immediately before activation, and at 1, 24, and 168 hours after the initiation of tooth movement.

However, none of the studies have evaluated levels of biomarkers during enmasse space closure using sliding mechanics. As in sliding mechanics there is reciprocal movement of anterior and posterior segment, there by altering the level of biomarkers in both the segments irrespective of method of force application. Movement of posterior segment can be controlled by application of differential force and by anchorage reinforcement, hence level of biomarker might be different in anterior and posterior segment. Thus, in the present study, biomarkers present in GCF was evaluated both in anterior and posterior segment during enmasse retraction of anterior teeth³⁰.

To collect the GCF there are various methods like Intracrevicular washing technique, microcapillary technique, absorption technique. In this study absorption technique was used, advantage is being quick and easy to use, can be used at individual sites, and causing minimal trauma. Absorbent paper points were used and inserted just at the entrance of the crevice, to the base of the pocket or until minimum resistance is felt, and these samples (absorbent points) were stored till further procedures ³¹.

Considering the role of IL-6 and RANKL in orthodontic tooth movement, the present study was conducted to compare the proinflammatory cytokine (IL-6 & RANKL) levels between anterior and posterior segment during enmasse retraction of anterior teeth.

AIM:

To evaluate and compare the levels of cytokine (IL6 and RANKL) in GCF collected from anterior and posterior segment during enmasse retraction of anterior teeth using sliding mechanics at different time intervals.

OBJECTIVES:

1. To evaluate level of biomarkers (IL6 and RANKL) in GCF collected from anterior and posterior segment during en masse retraction at different time intervals.
2. To compare level of biomarkers (IL6) in GCF collected from anterior segment during enmasse retraction at different time intervals.
3. To compare level of biomarkers (IL6) in GCF collected from posterior segment during enmasse retraction at different time intervals.
4. To compare the level of biomarkers (IL6) in GCF between anterior and posterior segments during enmasse retraction at different time intervals.
5. To compare level of biomarkers (RANKL) in GCF collected from anterior segment during enmasse retraction at different time intervals.
6. To compare level of biomarkers (RANKL) in GCF collected from posterior segment during enmasse retraction at different time intervals.
7. To compare the level of biomarkers (RANKL) in GCF between anterior and posterior segments during enmasse retraction at different time intervals.

William G. Grieve, Georgia K. Johnson, Robert N. Moore, c Richard A. Reinhardt, and Linda M. DuBois (1994)³² examined levels of prostaglandin E (PGE) and interleukin-1 β (IL-1 β) in GCF during human orthodontic tooth movement on 10 patients, each having one treatment tooth undergoing orthodontic movement and a contralateral control tooth. The GCF was sampled at control sites and treatment (compression) sites before activation and at 1, 24, 48, and 168 hours, radioimmunoassay is used for levels of PGE and IL-1 β levels. At 1 and 24 hours, mean GCF IL-1 β levels were significantly elevated at treatment teeth compared with control teeth. The GCF levels of PGE for the treatment teeth were significantly higher at 24 and 48 hours than the control teeth. The GCF levels of PGE and IL-1 β remained at baseline levels throughout the study for the control teeth, whereas significant elevations from baseline in GCF IL-1 β (24 hours) and PGE levels (24 and 48 hours) were observed over time in the treatment teeth ($p < 0.05$).

Jennifer J. Lowney, Louis A. Norton, David M. Shafer, Edward F. Rossomando (1995)³³ measured tumor necrosis factor- α (TNF) directly in the human gingival sulcus before and after the application of an orthodontic force. To recover TNF from the sulcus, paramagnetic beads, coated with monoclonal antibodies for TNF, were introduced into the gingival sulcus of 50 teeth undergoing orthodontic tooth movement (by two force systems) in 20 patients. Retrieval was performed by a permanent magnetic device designed to fit the periodontal sulcus. The samples were taken before force application (controls) and at a fixed time after force application. The amount of immunoadsorbed TNF was quantified with an immunochemical assay. There was more than twofold increase in TNF recoverable from the gingival sulcus after application of orthodontic forces (mean of 12.9 ng vs 30.5 ng). A Student's t test for paired samples demonstrated statistical significance at $p < 0.01$.

S Uematsu, M Mogi, T Deguchi (1996)³⁴ investigated levels of biomarker in GCF during orthodontic tooth movement. Twelve patients (mean age, 14.4 years) were used as subjects. An upper canine of each patient having one treatment for distal movement served as the experimental tooth, whereas the contralateral and antagonistic canines were used as controls. The GCF around the experimental and the two control teeth was taken from each subject immediately before activation, and at 1, 24, and 168 hr after the initiation of tooth movement. The concentrations of interleukin (IL)-1 α , IL-

6, tumor necrosis factor-c, epidermal growth factor, and 32- microglobulin were significantly higher in the experimental group than in the controls at 24 hr after the experiment was initiated. All the cytokines remained at baseline levels throughout the experiment for the two control groups.

Sappho Tzannetou, Stella Efstratiadis, Olivier Nicolay, John Grbic, and Ira Lamster (1999)³⁵ examined whether the inflammatory mediators interleukin (IL-1b) and β -glucuronidase (β G) are present in the gingival crevicular fluid (GCF) of children undergoing rapid palatal expansion and whether their levels vary upon activation of the appliance and movement of the maxillary first molars. Nine adolescent patients who needed palatal expansion were considered. After Four weeks of receiving periodontal prophylaxis, a modified Hyrax appliance was inserted in patients. The jackscrew was activated twice daily until the appropriate expansion was achieved. GCF samples were collected with filter paper strips at 2 pre-treatment observation periods and 9 observation periods after placement of the appliance and analyzed by means of ELISA and time-dependent fluorometry for IL-1b and β G, respectively. The values recorded at the observation period 2 weeks after the periodontal prophylaxis were used as baseline. The results indicate that (1) β G and IL-1b are present in GCF of young, healthy individuals, (2) their levels decrease following a strict regimen of plaque control, (3) orthodontic/orthopaedic forces evoke changes in the levels of the inflammatory mediators IL-1b and β G in the periodontal tissues that can be detected in GCF.

Najat Alhashimi, Lars Frithiof, Pongsri Brudvik and Moiz Bakhiet (2001)³⁶ measured the messenger RNA expression of IL-1 β , IL-6, and TNF α at 3, 7, and 10 days after the application of orthodontic force on the maxillary first molars of 12 rats. The contralateral side and 3 untreated rats served as controls. Measurements of the messenger RNA expression were selected as the means to investigate the role of orthodontic force in de novo synthesis of proinflammatory cytokines. After the application of force, the induction of IL-1 β and IL-6 was observed to reach a maximum on day 3 and to decline thereafter. No messenger RNA induction of either cytokine was measured in the control teeth. The messenger RNA expression of TNF- α was not detected at any time point of this study in the experimental or contralateral sides or in the control animals.

Perinetti G et al (2003)³⁷ examined aspartate aminotransferase (AST) activity in GCF in order to assess AST potential in GCF as a possible diagnostic aid to monitor tooth movement and tissue response during orthodontic treatment. 18 patients (mean age, 16.1 years) were taken for sample., during upper first molar distalization served as the test tooth (TT), with its contralateral (CC) and antagonist (AC) first molars used as controls. The CC was included in the orthodontic appliance, but was not subjected to the orthodontic force; the AC was free from any orthodontic appliance. The GCF around the experimental teeth was collected from both mesial and distal tooth sites immediately before appliance activation, 1 hour after, and weekly over the following 4 weeks. Clinical gingival condition was evaluated at baseline and at the end of the experimental period. AST levels were significantly elevated in all sites from the TT and CC groups compared to the AC group where, conversely, AST activity remained at the baseline level.

Serra E et al (2003)³⁸ examined the lactate dehydrogenase (LDH) activity in GCF to assess whether GCF LDH can be proposed as a sensitive marker for periodontal tissue modifications during orthodontic tooth movement on 37 subjects, 16 males and 21 females (mean age 18.7 years, range 14.0 to 26.7 year). Subjects underwent a session of professional oral hygiene and oral hygiene instructions; 2 weeks later, a fixed orthodontic appliance was placed on the maxillary arch and maxillary canine was considered as the test tooth, and its antagonist, which had no appliance, was used as the control tooth. From 2 to 12 weeks after orthodontic appliance placement, GCF was collected from both experimental teeth at the mesiobuccal angle. The results showed that no differences in clinical conditions and GCF volume occurred between the experimental teeth. On the contrary, GCF LDH activity in the test teeth was significantly greater than that of the control teeth (P .01).

Ogasawara T et al (2004)³⁹ examined the in-situ expression of RANKL, RANK, Osteoprotegerin, interleukin-1b (IL-1b) and tumor necrosis factor α (TNF α) in the osteoclasts of rat periodontal tissue. Tooth movement was performed by the Waldo method, and the pathological bone resorption was induced on 4 -week-old Wistar rats. The demineralized maxillae and mandible were embedded with paraffin. RANKL, RANK, osteoprotegerin, IL-1b, and TNF α mRNAs in osteoclasts and other cells using the specific RNA probes, respectively detected in In situ hybridization. Both RANKL

and RANK were expressed in some osteoclasts. RANKL was also positive in osteoblasts and PDLs. No IL-1 β - and TNF α -positive osteoclast found. The positive signals of osteoprotegerin were detected in almost all osteoblasts, PDLs and odontoblasts. The number and the distribution pattern of RANKL- and RANK-expressing osteoclasts changed when orthodontic excessive force was applied to periodontal tissue. In addition, IL-1 β and TNF- α were shown to be expressed in osteoclasts under pathological status.

Nishijima Y, Yamaguchi M, Kojima T, Aihara N, Nakajima R, Kasai K (2005)⁴ examined the secretion of RANKL and OPG from hPDL cells following a compression force (0, 0.5, 1.0, 2.0, or 3.0 g/cm² for 48 h) on 10 adolescent patients. GCF was collected at the distal cervical margins of the experimental and control teeth 0, 1, 24, and 168 h after the retracting force was applied. GCF levels of RANKL were significantly higher, and the levels of OPG significantly lower, in the experimental canines than in the control teeth at 24 h, but there were no such significant differences at 0, 1, or 168 h.

Batra P, Kharbanda OP, Duggal R, Singh N, Parkash H (2005)⁴⁰ – investigated alkaline phosphatase activity in the gingival crevicular fluid (GCF) during orthodontic tooth movement in humans. Ten female patients requiring all first premolar extractions were selected and treated with standard edgewise mechanotherapy. Canine retraction was done using 100 g stainless steel springs. Maxillary canine on one side acted as experimental site while the contralateral canine acted as control. GCF was collected from mesial and distal of canines before initiation of canine retraction (baseline), immediately after initiation of retraction, and on 1st, 7th, 14th and 21st day and the alkaline phosphatase activity was estimated. The results show significant ($p < 0.05$) changes in alkaline phosphatase activity on the 7th, 14th and 21st day on both mesial and distal aspects of the compared experimental and control sides. The peak in enzyme activity occurred on the 14th day of initiation of retraction followed by a significant fall in activity especially on the mesial aspect.

Basaran G et al (2006)⁴¹ evaluated interleukins 2, 6, and 8 levels in GCF during levelling and distalization of the teeth in fifteen patients in which 9 female, 6 male; ages, 15-19 years; mean age, 16.7 $\pm$ 2.3 years. Each underwent a session of professional oral hygiene and received oral hygiene instructions. The patients were seen at baseline,

at days 7 and 21, and as the teeth were levelled. Records of the baseline scores for the distalization forces were taken at the sixth month. Scores of days 7 and 21 after 6 months of the distalization treatment were also recorded. Increases were seen in the volume of gingival crevicular fluid and the concentrations of interleukins 2, 6, and 8. Levelling and distalization of the teeth caused increase in interleukins 2, 6, and 8 levels in the periodontal tissues which can be detected in gingival crevicular fluid. No statistical differences between the observations except at the 7th day of levelling for IL-8 (P .05). Although there were no statistical differences in IL-2 levels, it increased at the 7th and 21st days of levelling and distalization. IL-6 showed no significant alterations either statistically or mathematically.

Masaru Yamaguchi, Mizuho Yoshii and Kazutaka Kasai (2006)⁴² investigated GCF levels of Substance P (S)P and interleukin-1 β (IL-1 β) during human orthodontic tooth movement. On 3 males, with a mean age of 21.3 ± 2.8 years old, and 6 females, with a mean age of 23.1 ± 2.4 years, undergoing orthodontic movement of a single tooth, with the contralateral tooth used as the control. GCF was collected at the control and treatment (compression) sites before and 1, 4, 8, 24, 72, 120, and 168 hours after initiation of orthodontic treatment. Prevention of plaque-induced inflammation allowed assessment of the dynamics of mechanically stimulated SP and IL-1 β levels in the GCF, which were determined using ELISA kits. GCF levels of SP and IL-1 β for the treated teeth were significantly higher (P < 0.001) than for the corresponding control teeth from 8 to 72 hours, and peaked at 24 hours. These results show that the amounts of SP and IL-1 β in GCF increase with orthodontic tooth movement.

Kawasaki K, Takahashi T, Yamaguchi M, Kasai K (2006)⁴³ compared the levels of the receptor activator of nuclear kappa B ligand (RANKL) and Osteoprotegerin (OPG) in the gingival crevicular fluid (GCF) during orthodontic tooth movement in juvenile and adult patients. Fifteen juveniles and 15 adults served as subjects. GCF was collected from the distal cervical margins of the experimental and control teeth at 0, 1, 24, and 168 h after application of a retracting force. ELISA kits were used to determine RANKL and OPG levels in the GCF samples. The amount of tooth movement for juveniles was larger than for adults after 168 h. Further, after 24 h RANKL levels were increased and those of OPG decreased in GCF samples from the compression side during orthodontic tooth movement in both juveniles and adults. The RANKL/OPG ratio in GCF from adult patients was lower than that in the juvenile patient samples.

Hilal Uslu Toygara; Beyza Hancioglu Kircelib; Sule Bulut; Nurzen Sezgin; Bahar Tasdelen (2008)⁴⁴ investigated the level of osteoprotegerin (OPG) in GCF during tooth movement. GCF were taken from 12 patients (13–17 years of age) required canine distalization at baseline, 1 hour, 24 hours, 168 hours, 1 month, and 3 months from the distal sites of the test and with control teeth after the application of force. OPG concentration was detected by ELISA. OPG concentrations in distal sites of the test teeth were decreased in a time-dependent manner. Decrease is significant when compared with the baseline measurements (P .038). Variability was detected in the levels of OPG concentration in the distal sites of the control tooth throughout the experimental period.

Sappho Tzannetou, Stella Efstratiadis, Olivier Nicolay, John Grbic, and Ira Lamster (2008)⁴⁵ compared the responses of IL-1 β and β G in the GCF of the maxillary first molars, first premolars and central incisors during palatal expansion. Four weeks after receiving periodontal prophylaxis, a modified hyrax appliance was placed. The jackscrew was activated twice daily until the appropriate expansion was achieved. GCF samples were collected with filter paper before and after periodontal prophylaxis and during passive wearing of the appliance, active orthodontic treatment, and retention. The values recorded after periodontal prophylaxis were used as the baseline. The results validate that IL-1 β and β G are present in the GCF of adolescents, and, although their level decreases after a strict regimen of plaque control, it increases during orthodontic or orthopaedic movement. Moreover, this study demonstrates that both heavy and light forces evoke increased levels of IL-1 β and β G, stronger forces cause higher levels of inflammatory mediators, and both IL-1 β and β G respond to direct and indirect application of mechanical force to teeth.

N Hamamci et al (2011)⁴⁶ assessed and found insignificant difference in the level of IL-6 during canine distalization, maxillary miniscrew implants were placed bilaterally between the maxillary second premolar and first molars. Peri miniscrew implant crevicular fluid samples were collected from the mesiobuccal aspects of the miniscrew implants and gingival crevicular fluid (GCF) samples were obtained from the distobuccal site of maxillary canines (treatment) and mesial of the maxillary second premolar teeth (controls), on applying 150 g force delivered by a NiTi closed coil spring (7mm) on a 0.016 $\times$ 0.022 inch SS archwire in .018 slot with molar toe-ins and

tip-back bends, and tying second maxillary premolars and first molars with figure of 8 ligature, at baseline (T1) and at 1hr (T2), 24hr (T3), and 48hr (T4), 7 (T5) and 21 (T6) days, and 3 months (T7). For treatment group (Canine) with highest concentration at T4(3.20 ± 1.82)>T3(3.15 ± 1.83)>T2(3.05 ± 1.62)>T5(3.04 ± 0.99)>T6($.99 \pm 1.15$)>T1(2.95 ± 2.66)T7(2.95 ± 1.04) pg/ml. For Control group (2nd premolar) highest was at T3(2.92 ± 1.15)>T4(2.92 ± 1.03)>T2(2.89 ± 1.98)>T1($.80 \pm 0.57$)>T5(2.78 ± 0.75)>T6(2.69 ± 2.57)>T7(2.62 ± 1.20) pg/ml. For miniscrew group was highest at T4(3.42 ± 1.19)>T3(3.36 ± 0.97)>T7(3.25 ± 0.71)>T2(3.23 ± 0.78)>T5(3.05 ± 1.49)>T6(3.02 ± 0.76)>T1(3.01 ± 0.82) pg/ml. None of the intragroup comparison of T1 with T2, T3, T4, T5, T6, T7 were made.

Sarah A. Alfaqeeh and Sukumaran Anil (2011)⁴⁷ analyzing lactate dehydrogenase (LDH) activity in GCF during orthodontic movement on 20 patients all requiring first premolar extractions and treated with conventional straight wire mechanotherapy. Canine retraction was done using 125 g Nitinol closed coil springs. The maxillary canine on one side served as the experimental site while the contralateral canine served as the control. GCF was collected from the canines before initiation of retraction, then 1 hour after initiating canine retraction, followed by 1 day, 7 days, 14 days and 21 days. GCF LDH levels were estimated and compared with the control site. The results revealed significantly higher LDH levels on the 7th, 14th and 21st day at the sites where orthodontic force had been applied. The levels also showed a significant increase from 0 hour to the 21st day. Peak levels were seen on 14th and 21st day following initiation of retraction.

Asma alhusna abang abdullah, Rohaya megat abdul wahab & Shahrul hisham bin zainal ariffin(2011)⁴⁸ investigated the presence of an enzyme called alkaline phosphatase (ALP) in GCF during leveling and alignment stage of orthodontic fixed appliance treatment. 14 patients between the ages of 15 and 27 years old with moderate upper labial segment crowding. GCF from mesial and distal sites of upper canines were collected using paper point before the fixed appliance placement (week 0). The baseline level of ALP (week 0) acted as a control. GCF samples were repeated at 1st, 2nd and 3rd week during orthodontic treatment. The activities of ALP were measured using spectrophotometer (405 nm). Paired sample t-test was used to assess the significance of difference over the 3 weeks. The results showed patterns of ALP

activities on the test teeth throughout the 3 weeks of levelling and alignment stage, however the results were not significant ($p > 0.05$) when compared to control.

Zhao NN, Lin JX, Chen ZB, Liu Y(2012)⁴⁹ evaluated the level of osteoprotegerin (OPG) and RANKL in gingival crevicular fluid (GCF) during orthodontic retention and corresponding expression of OPG and RANKL in periodontal tissues were evaluated. Fifteen male Wistar rats (age, 6 weeks) were divided into three groups with 5 rats for each. GCF samples were collected at the baseline, 14 days after orthodontic force application, and 14 days after orthodontic force removal. ELISA was used to quantify the concentration of OPG and sRANKL (soluble receptor activator of nuclear factor kappa B ligand) in GCF. The concentration of sRANKL in GCF increased statistically significant from baseline to T2 ($P < 0.05$) while decreased significantly from T2 to T3 ($P < 0.05$). The concentration of OPG had minimum fluctuation from baseline to T3 without any statistical significance ($P > 0.05$). The sRANKL/OPG ratio in GCF and that in periodontal tissues during the three time points were similar which showed a prominent increase in T2 ($P < 0.05$ and $P < 0.01$, respectively) and sharp decrease in T3 ($P < 0.05$).

Barbieria G et al (2012)²³ evaluated levels of RANK, OPG, OPN, and TGF- β 1 using ELISA in GCF of 10 healthy volunteers during early orthodontic tooth movement. Orthodontic elastic separators were placed between the second premolar and first molar, with the contralateral quadrant serving as a control. GCF samples were collected at the mesiobuccal and mesiolingual sites of control molars at baseline and at 7 days, and the test molar samples were obtained 24 hours and 7 days after placement of elastic separators at the mesiobuccal (tension side) and disto buccal (compression side) sites. The concentration of OPG significantly decreased at the compression site by 24 hours, and the amount and concentration of RANK differed significantly between the control, compression, and tension sites after 7 days. A significant increase in absolute TGF- β 1 levels was also detected at the compression site versus the control and tension sites after 7 days. Bone metabolism is affected by application of force to the teeth by elastic separators. Both increased expression of bone resorptive mediators (eg, RANK and TGF- β 1) and decreased expression of a bone-forming mediator (eg, OPG) on the compression side were detected.

Cristiane Canavarro, Ricardo Palmier Teles and Jonas Capelli Júnior(2013)⁵⁰ examined the levels of matrix metalloproteinases (MMPs) -1, -2, -3, -7, -8, -12, and -13 in GCF at different time intervals during orthodontic tooth movement. from 16 healthy orthodontic subjects (7 females, 9 males; mean age, 17.7 years) who needed first upper premolars extracted. Maxillary canine was randomly chosen and subjected to distalizing force and was considered the test side. The contralateral canine, which was not subjected to any force but was included in the orthodontic appliance, was used as a control side. GCF sample was collected at both the mesial (tension) and distal (pressure) test and control sites at baseline, immediately before applying the orthodontic appliance, and after 1 and 24 hours and 7, 14, and 21 days. GCF samples were analyzed by multiplexed bead immunoassay. The mean levels of the MMP-1, -2, -3, -7, -8, -12, and -13 were not significantly different between the test and control groups. The comparisons between the tension and pressure sites were also not significantly different at each individual time. Expression of MMP-8 was higher than that of the other MMPs. MMPs are released in sufficient quantities such that tooth movement occurs but there was no significant increase in GCF levels.

Melissa Grant, Jay Wilson, Peter Rock and Iain Chapple (2013)⁵¹ investigated changes in cytokines of bone and tissue metabolism in GCF from 20 patients undergoing orthodontic treatment at baseline, before tooth extraction and appliance placement and then at time intervals during orthodontic treatment. Samples were taken 10 weeks following first appliance placement: at four hours; 7 days; and 42 days after application of distalising forces to maxillary canine teeth. Cytokines (GM-CSF, interferon-gamma, IL-1beta, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10 and TNF- α), and bone metabolism indicators (RANKL and OPG) were measured in GCF using multiple assays. Tension sites adjacent to canines showed significant increases in IL-1beta, IL-8, TNF- α , MMP-9 and TIMPs 1 and 2 across all time points following force application, while compression sites exhibited increases in IL-1beta and IL-8 after 4 hours, MMP-9 after 7 and 42 days and RANKL after 42 days.. Elevated levels were found at 4 hours but continued for periods of up to 6 weeks.

Gloria Amparo Florez-Moreno, Diana Maria Isaza-Guzman, and Sergio Ivan Tobon- Arroyave (2013)¹⁸- evaluated salivary sRANKL and OPG concentrations determined by ELISA in 20 healthy subjects who required fixed appliance therapy not

involving tooth extractions or surgical procedures were selected. Unstimulated whole saliva samples were collected from each patient before fitting the orthodontic appliances, and at 24 to 48 hours, 2 weeks, 5 weeks, and 8 weeks after the activation. 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. However, clear fluctuations in the immunoenzymatic findings were noted at the different sampling times, indicating nonlinear trends in the levels of the biomarkers through time.

Tuncer B et al (2013)⁵² evaluated the levels of osteoprotegerin (OPG) and receptor activator of nuclear factor kappa- β ligand (RANKL) in tension (mesial) and pressure (distal) sides during continuous orthodontic force in GCF. Eighteen canine teeth of 9 adults (5 female, 4 male; age range 17.5-18.9 yrs) with different Angle classifications were included. After first premolar extractions, the maxillary canines were tipped distally by sentalloy closed coil springs with a continuous force of 200 g. Gingival crevicular fluid (GCF) was sampled from mesial and distal gingival crevices of each canine separately at baseline, 1 hour(h), 24 h, 168 h, and 1 month after force application. OPG and RANKL levels were analyzed by ELISA. Concentration of OPG showed an increase in mesial side, while there was a decrease in distal side at 1 h, demonstrating a statistically significant difference, RANKL expression showed no significant changes during study.

Navarro-Palacios A et al (2014)²²- evaluated Myeloperoxidase (MPO) activity in gingival crevicular fluid (GCF) and whole saliva in orthodontic patients with different levels of dental crowding at the alignment. 20 patients were classified according to the irregularity index into 2 groups: severe and minimum crowding (10 in each group). MPO activity was evaluated in GCF and saliva at 0 and 2 hours, and 7 and 14 days after the orthodontic appliances were activated. MPO activity was measured using the modified Bradley-Bozeman technique. In which they found that in both groups, the maximum activity was at 2hours($P \leq 0.05$) after activation. MPO activity remained elevated until day 7, and values similar to base line were found at day 14 in the GCF and saliva samples. Enzymatic activity did not show statistical differences between the groups. They concluded that amount of dental crowding does not seem to influence MPO activity, which showed similar patterns in GCF and saliva, but the values in GCF

showed the inflammatory changes more accurately than did the values in saliva.

Madureira DF et al (2014)²⁴ investigated the kinetics of interleukin-6 and chemokine ligands 2 and 3 levels in periodontal ligaments subjected to orthodontic forces. On 64 premolars teeth. The experimental group consisted of premolars subjected to a force of 0.980 N in the apical direction for 3 hours, 15 hours, 3 days, 12 days, or 21 days with a 0.017 × 0.025-in beta-titanium alloy cantilever. The contralateral teeth, without orthodontic appliances, were used as controls. The premolars were extracted for orthodontic reasons, and the periodontal ligaments were scraped for analysis of cytokine levels by ELISA. Compared with the control group, an increase in chemokine ligand 2 was observed on days 3 and 12, and increases in interleukin-6 and chemokine ligand 3 were observed on day 12 in the experimental group. There is differential expressions of interleukin-6 and chemokine ligands 2 and 3 in periodontal ligaments after mechanical loading; this might reflect the distinct roles of these molecules in the bone remodeling process.

Wahab R et al (2014)⁵³ evaluated Lactate Dehydrogenase (LDH), Aspartate Aminotransferase (AST), Tartrate-resistant Acid Phosphatase (TRAP) and Alkaline Phosphatase (ALP), using Self-ligating Brackets (SLBs), Saliva and Gingival Crevicular Fluid (GCF) were collected from 19 subjects (n=19) before and during orthodontic treatment (5 weeks). The subjects were bonded with SLBs with 100 g or 150 g of orthodontic force. Enzyme assays, ELISA and tooth movement measurements were performed. A statistical analysis showed that compared to baseline values, significant differences (p<0.05). were observed in the saliva levels of AST at week 5, the levels of TRAP at week 2, and the levels of ALP at weeks 1 to 5. In the GCF, LDH showed significant differences (p<0.05).

Leethanakul, C., Suamphan, S., Jitpukdeebodintr, S., Thongudomporn, U. and Charoemratrote, C. (2016)⁵⁴ investigated the effects of application of vibratory stimuli on interleukin (IL)-1 β secretion during maxillary canine distalization in 15 subjects (mean age, 22.9 years; range 19-25 years) whose bilateral maxillary first premolars were extracted. On the experimental side, light force (60 g) was applied to the canine for 3 months in combination with vibratory stimuli provided using an electric toothbrush 15 minutes a day for 2 months; only orthodontic force was applied to the contralateral control canine. GCF was collected from the mesial and distal sides of each

canine at each monthly appointment. IL-1 β levels were analyzed using an ELISA. Canine movement was measured monthly. Overall, enhanced IL-1 β secretion was observed at the pressure sites of experimental canines compared to control canines ($P < .001$). The amount of tooth movement was greater for the experimental canine than for the control canine (mean, 2.85 $\pm$ 0.17 mm vs 1.77 $\pm$ 0.11 mm, respectively, $P < .001$)

Louchian I et al (2016)⁵⁵ determined the level of IL-1 β in the saliva of teeth that are orthodontically tractioned, comparison was made between the levels of IL-1 β in these teeth and the teeth upon which no orthodontic force is applied. The 60 patients considered in the study were divided into three groups, as follows: group A, the control lot, consisting of 16 patients without periodontal disease, without clinical gingival modifications; group B, 22 patients with periodontal disease (chronic periodontitis localized it at least three teeth), who received periodontal treatment; group C, 22 patients with periodontal disease (chronic periodontitis localized it at least three teeth) who received combined orthodontic and periodontal treatment. Saliva was collected in two rounds from the patients in groups B and C, for group B, an initial collection and a second collection six months after the end of periodontal treatment- for group C, an initial collection and a second one six months after periodontal stabilisation and start of orthodontic treatment. The values of IL-1 β dropped significantly both in the patients who had only periodontal treatment ($F = 26.69$, $p < < 0.01$), as well as in those who had periodontal treatment associated with orthodontic treatment ($F = 16.49$, $p < < 0.01$).

Thukral R et al (2017)⁵⁶ evaluated Pentraxin-3 level in GCF of 25 patients that underwent canine retraction as a part of fixed orthodontic treatment. In the maxillary right and left quadrant, active tieback and NiTi coil spring were used respectively. Gingival crevicular fluid samples were collected 1 hour prior to the orthodontic canine retraction procedure followed by collection of sample at 1 hour, 1, 7, and 14 days after the start of canine retraction procedure. ELISA kit was used for analysis of the samples, nonsignificant difference was observed in the values of PTX- 3 at baseline and 1 hour. While comparing the mean PTX-3 values between different time intervals in both the study groups, significant difference was observed.

Andrade A et al (2018)⁵⁷evaluated the hypothesis that treatment with orthodontic appliances evokes significant functional limitations and emotional stress, which can be

detected by salivary biomarkers. Twenty subjects (10 men and 10 women) who needed orthodontic treatment were included. Saliva was collected for detection of alpha-amylase activity and cortisol levels at three different times: before bracket placement (T0), 24 hours after archwire placement (T1), and 30 days after archwire placement (T2). The saliva electrolytes concentrations of calcium, phosphorus, magnesium, sodium and potassium were also evaluated. The orthodontic patients exhibited a significant increase in emotional stress as detected by the alpha-amylase activity at T1, the period in which patients reported the higher values of pain, basal salivary cortisol was not affected by the treatment and the main change detected in electrolyte concentration was a sodium reduction, when these patients were compared to controls.

J Amanda, R Widayati, N Soedarsono and M K Purwanegara et al (2018)⁵⁸ determined the differences in RANKL concentrations in early orthodontic treatment between preadjusted edgewise appliances (PEA) and self-ligating (SL) bracket systems. Gingival crevicular fluid was retrieved before treatment and at 1, 24, and 168 hours after treatment from five maxillary anterior proximal sites on the mesiolabial side of the upper right-to-left canines. An ELISA test was used to determine RANKL concentrations. The results showed differences in RANKL concentrations between the SL and PEA groups at 1, 24, and 168 hours after bracket insertion. The RANKL concentration at 168 hours in the SL group was higher compared to that in the PEA group, whereas that in the PEA group decreased to the baseline value after 24 hours. RANKL concentration in the passive SL bracket system was higher compared to that in the PEA system due to differences in the force and mechanotherapy of the SL brackets. The RANKL concentrations in the experimental group yielded statistically significant results ($p < 0.01$), while those in the control group showed no statistically significant difference ($p > .01$). paper strip, GCF was collected from the study and control teeth prior to orthodontic force application (T0), one hour after (T1) and 28 days after force application (T2), then the level of TNF α and IL-6 was measured using ELISA. Data were analyzed using SPSS version 20 via Friedman and Wilcoxon test, the level of IL-6 and TNF α at both sides of the study teeth was higher than that in the control teeth at T1, but this difference was not statistically significant $p < 0.05$.

Agita Pramustika, Nurtami Soedarsono, Krisnawati, Retno Widayati

(2018)⁵⁹ compared TNF- α concentrations in the gingival crevicular fluid (GCF) between preadjusted edgewise appliance (PEA) and self-ligating (SL) systems during the early leveling stage of orthodontic treatment. Eighteen patients (aged 15–35 years) who participated in this study were divided into two experimental groups (PEA and SL) and control group (without orthodontic treatment). The GCF was taken at five sites in the maxilla anterior teeth from each participant just before bracket bonding and at 1, 24, and 168 h after the initiation of tooth movement. Cytokine levels were determined through ELISA. The concentration of TNF- α was significantly higher in the experimental groups than in the control group at 24 h after force application. TNF- α levels were significantly decreased at 168 h after force application in the PEA group. Meanwhile, in the SL group, the level of TNF- α at 168 h was still increased, although there was no statistically significant difference.

Parviz Padisar, Roya Hashemi, Mohammadreza Naseh, Bahareh Abde Nikfarjam, Mahdi Mohammadi (2018)⁶⁰ determined the level of tumor necrosis factor alpha (TNF α) and interleukin 6 (IL 6) in gingival crevicular fluid (GCF) during OTM. from 10 patients who were undergoing orthodontic treatment. A canine tooth was randomly selected as the study group and subjected to distalization force while the contralateral canine tooth served as control. Using paper strip, GCF was collected from the study and control teeth prior to orthodontic force application (T0), one hour after (T1) and 28 days after force application (T2), then the level of TNF α and IL-6 was measured using ELISA. Data were analyzed using SPSS version 20 via Friedman and Wilcoxon test, the level of IL-6 and TNF α at both sides of the study teeth was higher than that in the control teeth at T1, but this difference was not statistically significant $p < 0.05$.

Gujar A et al (2019)⁶¹ investigated the hypothesis that the levels of IL-1 β and TNF- α increased more and IL-1 α , IL-2, IL 6, IL-8 increased less in GCF, after 3 weeks of treatment with conventional labial fixed appliance and with aligners. 40 patients who were treated either with labial brackets ($n = 20$) or aligners ($n = 20$). GCF samples were collected at baseline and after 21 days. Cytokine levels were evaluated by ELISA. Plaque index (PI), gingival index (GI), and bleeding on probing (POB) were also examined. The levels of IL-1 α , IL-1 β , IL-2, IL-6, IL-8 and TNF- α in the GCF were significantly increased in both groups. The levels of IL-2, IL-6, IL-8 increased more in patients treated with aligners compared to those treated by labial fixed appliances.

There was a statistically significant difference in change of the mean cytokine levels of IL-1a, IL-2, IL-6, IL-8 and TNF-a compared to labial fixed appliances and aligners. The levels of the six studied cytokines in GCF (IL-1a, IL-1 β , IL-2, IL-6, IL-8 and TNF-a) increased after 3 weeks both after treatment with conventional labial fixed appliance and with aligners. IL-1 β and TNF-a showed a prominent increase compared to the other cytokines in the GCF of teeth by both the labial fixed appliance and aligners. However, there were only minor differences in the changes of the cytokine levels from baseline to 3 weeks between the two groups.

Jayaprakash P et al (2019)²⁹ elevated levels of Interleukin (IL)-1 β , IL-6, tumor necrosis factor- α , epidermal growth factor, and β 2-microglobulin levels in gingival crevicular fluid during human Orthodontic tooth movement (OTM) , statistically significant no. of subjects (n = 10 and mean age = 15.6 years) were included in the study. A maxillary cuspid of each subject having one treatment for distal orthodontic tooth movement served as the experimental tooth, whereas the contralateral cuspids were used as controls. Gingival crevicular fluid (GCF) around the experimental and the two control teeth was collected from each subject immediately before activation, and at 1, 24, and 168 hours after the initiation of tooth movement. Result showed Elevated levels of Interleukin (IL)-1 β , IL-6, tumor necrosis factor- α , epidermal growth factor, and β 2- microglobulin levels in gingival... ELISAs were used to determine cytokine levels. The concentrations of interleukin (IL)-1 β , IL-6, tumor necrosis factor- α , epidermal growth factor, and β 2-microglobulin were significantly higher in the experimental group than in the controls at 24 hr after the experiment was initiated. All the cytokines remained at baseline levels throughout the experiment for the control groups.

Bhadrinath Srinivasan, Arun Chitharanjan, Vignesh Kailasam, Vamsi Lavu and Vani Ganapathy (2019)⁶²assessed the concentration of leptin in Gingival Crevicular Fluid (GCF) during orthodontic force application and to correlate its concentration to rate of tooth movement, sample was taken from 20 orthodontic patients (10 males and 10 females) . Leptin concentration was measured at T0, before force application; T1, one hour after force application; T2, one day after force application; T3, one week after force application; T4, one month after force application. GCF was collected using filter paper strips from the distal aspect of gingival sulcus of the right maxillary canine

distalized by an active lace-backs, tooth movement was measured on dental casts, before and one month after force application. The mean GCF leptin concentration increased from T0 to T1, rose to a peak at T2, then declined to a minimum value at T3 and then increased to a value at T4, closer to the base line value (T0), and it was statistically significant ($P < 0.05$). There was positive correlation of the overall mean leptin concentration to rate of tooth movement (correlation coefficient = 0.634).

Mohammad A et al (2019)²⁵ measured the levels of Interleukin-6 (IL-6) expression in the periodontal ligament (PDL) during orthodontic treatment at various intervals of the force application. A sample of 60 premolar teeth were divided into controls and test groups of 30 samples each. Each tooth was assigned a time point for its extraction at 2 hours, 15th day or the 30th day after application of force. Brackets were bonded to the experimental teeth in the mesiodistal center but at 5 degrees to the long axis of the tooth to apply a force of 200–250 g, which was measured by Correx gauge. Gracey's curette was used to scrape off all the periodontal ligament covering the root surface of the extracted teeth. Cytokine IL-6 was measured by ELISA kit at a wavelength of 450 nm. There was no difference in the levels of IL-6 at 2 hours for the test and the control groups. IL-6 concentration showed the maximum levels when measured on the 15th day and showed a decrease on the 30th day. This study depicted a pattern followed by IL-6 after orthodontic force application, and is a step further in establishing IL-6 as a potential biomarker for improving the orthodontic treatment in terms of decreased treatment time and decreased side effects.

Reiss S et al(2020)⁶ investigated the effect of supplemental vibratory force on biomarkers of bone remodeling during orthodontic tooth movement, the rate of mandibular anterior alignment (RMAA), and compliance with a vibration device. Seventeen biomarkers were analysed with the Multiplex analysis: OPN (osteopontin), RANKL, SOST (sclerostin), OPG, MMP1, MMP8, MMP9, MMP13, TNF- α , IL-1 β , IL-3, IL-6, IL-8, IL-11, IL-18, and DKK1 (Dickkopf Related Protein) on 40 patients between the ages 15–35 undergoing fixed appliance treatment, were randomly allocated to supplemental use of an intraoral vibrational device or fixed appliance only ($n = 20$). Salivary multiplex assay was done to analyse the concentration of selected biomarkers of bone remodeling before treatment (T0) and at three following time points (T1, T2, T3), 4–6 weeks apart. Irregularity of the mandibular anterior teeth was

assessed at the same trial time points. No difference in the changes in salivary biomarkers of bone remodeling and RMAA between groups at any time point over the trial duration was observed. No correlation was found between changes in irregularity and biomarker level from baseline to another time point.

Hülya Çevik-Aras ,Fadimana Isik-Altun ,Hatice Kilic-Tok , and Julia Naoumova(2021)⁶³ evaluated Vascular endothelial growth factor (VEGF) and interleukin 1 beta (IL-1 β) during different orthodontic treatment stages:alignment, space closure, and finishing. in saliva. Nine healthy females (15-20 years of age) with crowding requiring four premolar extractions and fixed appliances in both jaws were included. Total 134 stimulated and 134 unstimulated saliva samples were collected during two years of treatment: before tooth extractions (baseline) and then every 6- 8 weeks at follow-up appointments. All saliva samples analysed by the ELISA. The percentage difference in IL-1 β and VEGF between the different treatment stages was analysed by Bland-Altman plots. There was a gradual increase in IL-1 β and VEGF was observed at alignment, reaching significance at space closure ($p=0:002$ and $p=0:025$, respectively). At finishing, both IL-1 β and VEGF declined, however, without reverting to baseline values ($p =0:172$ and $p=0:207$, respectively). The salivary levels of both IL-1 β and VEGF increased following orthodontic treatment and reached their peaks during the treatment stage of space closure.

Sheikh A et al (2021)⁶⁴found statistically significant increase in IL-6 values (from GCF) from day 2 to day 60 during retraction of the anteriors with active tie backs after having a basal 0.019*0.025 SS arch wire providing 150 grams of force activation at an interval of 4 weeks, highest mean level of IL-6 was seen after 2 days $651\pm.242$,then subsequently reduced and least was found at 60 days $477\pm.224$.

Emire Aybuke Erdura, Kuter Karakaslıb, Elif Oncuc, Bahadır Ozturkd, Sema Hakkie (2021)⁶⁵ evaluated the efficiency of injectable platelet-rich fibrin (i-PRF) in accelerating canine tooth movement and to examine levels of the MMP-8, IL-1B), RANKL, and OPG in the gingival crevicular fluid during orthodontic treatment. Twenty patients with Class II Division 1 malocclusion were included in a split-mouth study. The treatment plan for all patients was extraction of maxillary first premolars followed by canine distalization with closed-coil springs using 150 g of force on each side. The study group received i-PRF two times, with a 2-week interval, on one side of

the maxilla. The contralateral side served as the control and did not receive i-PRF. Maxillary canine tooth movement was measured at five time points (T1–T5) on each side. Also, the activity of inflammatory cytokines was evaluated at three time points in the gingival crevicular fluid samples. There was a significant difference in canine tooth movement between the two groups ($P, .001$). i-PRF significantly increased the rate of tooth movement, and stimulation in the levels of inflammatory cytokines supported this result ($P, .001$). The levels of cytokines changed in both groups between T1 and T2. The IL-1b, MMP8, and RANKL values were significantly increased in the study group compared with the control group, while the OPG values were significantly decreased.

Sioustis IA, Martu MA, Aminov L, Pavel M, Cianga P, Kappenberg-Nitescu DC, et al(2021)⁶⁶ assessed MMP-8, MMP-9 in saliva on 111 patients who were about to receive fixed orthodontic treatment, salivary levels of MMP-8 and MMP-9 and bleeding on probing (BOP) determined before applying the orthodontic fixed appliance (T1), one week after appliance placement (T2), and during orthodontic treatment, one month after non-surgical periodontal treatment (T3). Patients showed a significant increase in BOP, MMP-8, and MMP-9 levels one week after orthodontic appliance placement (T2) and a decrease in these parameters one month after periodontal treatment (T3). Statistically significant correlations were found between MMP-8 levels and BOP values at T1, T2, and T3. Strong positive statistically significant correlations were found between MMP-8 levels and BOP and medium positive statistically significant correlations between MMP-9 and BOP values before and after orthodontic treatment and periodontal treatment.

Chelărescu S et al (2021)⁶⁷ investigated IL1 β and IL6 in the gingival crevicular fluid of clinically healthy adolescents and young adults during the acute phase of orthodontic treatment. Gingival crevicular fluid was collected from 20 patients (aged between 11 and 28) undergoing orthodontic treatment. Measurements were taken before (T0) and after 24 h after distalization forces were activated (T1). IL1 β and IL 6 were analyzed using ELISA tests. The statistical tests used were two sided t tests. Between the two time periods there was a significant raise both in the crevicular fluid rate (0.57 μ L at T0 vs. 0.95 μ L at T1, $p = 0.001$) and in IL1 β levels (15.67 pg/ μ L at T0 vs. 27.94 pg/ μ L at T1, $p = 0.009$). We were able to identify IL6 only in a third of the sites. There is a significantly increased level of IL β at T1 in adolescents, more than in young adults

(42.96 pg/ μ L vs. 17.93 pg/ μ L, $p = 0.006$). Conclusion of this study was the periodontal tissues of adolescents are more responsive to orthodontic forces than those of young adult .

Barhate U et al (2022)²⁸ validated the rate of maxillary canine retraction by identifying the levels of Interleukin-1 β (IL-1 β) and Tumour Necrosis Factor- α (TNF- α) in the Gingival Crevicular Fluid (GCF), on 15 females (age range, 18-25 years) with Class I bimaxillary dentoalveolar protrusion malocclusions were included. After levelling and alignment of maxillary arches, 1st premolars were extracted from both sides. Canines were retracted immediately after the extraction of 1st premolars in control sides and placement of L-PRF plugs in the experimental sides. The amount of canine retraction was evaluated from study models recorded before the extraction of 1st premolars (T_0) and at 1-week (T_1), 2-weeks (T_2), 4-weeks (T_3), and 8-weeks (T_4) after the beginning of canine retraction by using digital model superimpositions. The concentrations of IL-1 β and TNF- α were evaluated from the GCF collected at T_0 , T_1 , T_2 , T_3 , and T_4 by using commercially available human IL-1 β and TNF- α ELISA kits. The concentration of IL-1 β increased significantly in the experimental sides at T_1 ($P=0.000$), T_2 ($P=0.000$), and T_3 ($P=0.032$). The concentration of TNF- α increased both in the control and experimental sides, but it was statistically greater in the experimental sides compared to control sides at T_1 ($P=0.000$) and T_2 ($P=0.006$). A positive but not statistically significant correlation was noted between the rate of canine retraction and the concentrations of cytokines such as IL-1 β and TNF- α in GCF.

Julia Naumova ,Richard Olofsson ,Annica Almståhl ,Hülya Cevik-Aras (2022)¹⁷- evaluated mean levels of OPG and RANKL according to the different orthodontic treatment stages: alignment, space closure and finishing in saliva. In 9 healthy females (15-20 years of age), who had crowding required four premolar extractions and fixed appliances in both jaws were included. In total, 134 stimulated and 134 unstimulated saliva samples were collected: before tooth extractions (baseline), and then every 6-8 weeks at follow-up appointments during the whole treatment period of two years. All saliva samples were analysed by ELISA. RANKL was not detectable either in stimulated or unstimulated saliva. OPG levels were measured in stimulated saliva due to low levels in unstimulated saliva. A significant increase in OPG was observed at all treatment stages: alignment, space closure and

finishing compared with baseline ($p = 0.002$, $p = 0.039$, $p = < 0.001$, respectively). A small non-significant decrease was seen in space closure compared with alignment. OPG reached peak levels during the finishing stage.

Bhardwaj A et al (2022)⁶⁸ analyzed IL1b and IL18 in saliva of 28 patients requiring orthodontic treatment of both genders were divided into 2 groups of 14 each. Group I was vibrational and fixed appliance group and group II was fixed appliance group only. Collection of unstimulated whole saliva in a sterile test tube was done were analyzed using ELISA assay test. Group I had 5 males and 9 females and group II had 6 males and 8 females. The mean IL-1B level at T0 was 41.5 and 82.5, at T1 was 26.3 and 57.4, at T2 was 29.7 and 106.3 and at T3 was 26.8 and 131.5. The mean IL-8 level at T0 was 265.4 and 250.5, at T1 was 190.7 and 178.2, at T2 was 198.2 and 261.5 and at T3 was 296.4 and 385.2 in group I and II respectively. The difference was significant ($P < 0.05$).

Shetty A et al(2022)⁶⁹ assessed level of IL-6 in a split mouth study from the premolar region just before the extraction on control side whereas on the experimental site brackets (0.022 slot MBT mechanotherapy) were bonded on premolar to be extracted after 3 days and an initial intrusive force of 100–150 g was applied using a cantilever spring on this premolar for 3 days. They found insignificant difference in mean level of IL-6 in GCF after 3rd day of force application (4.5950 ± 1.6668 in the control group and 4.3975 ± 1.5948 in the experimental group).

Alnazeh et al (2023)⁷⁰ evaluated level Of IL-6, IL-10, 13, 17, and GM-CSF in GCF of patients receiving fixed orthodontic therapy (FOT) and Invisalign. 60 participants were enrolled after meeting the predefined inclusion criteria. Patients then underwent either FOT or Invisalign by allocating them randomly to each group ($n = 30$). The basic periodontal assessment was performed, including the plaque index (PI), gingival index (GI), and bleeding on probing (BoP), at baseline and again after 4 weeks. GCF samples were taken from each individual at baseline and after 4 weeks. ELISA technique was used to determine the cytokine and BMB levels. An unpaired t-test compared the FOT and Invisalign group's means and SDs. Paired t- tests examined the difference between T0 baseline and T1. The levels of IL-6 were significantly higher in patients treated with FOT as compared to Invisalign at T1 ($p < 0.05$) The other tested cytokines, IL-10, 13, 17, and GM-CSF, were not significantly different in either the FOT or Invisalign group

at baseline and 4 weeks follow-up ($p > 0.05$). FOT and Invisalign displayed comparable outcomes in terms of cytokine and BMB levels. However, only IL-6 and NTx (BMPS) were significantly different at week 4 from baseline.

Cazzolla A et al (2024)⁷¹ evaluated changes in the concentration of N-terminal type I collagen extension pro-peptide (PINP), tartrate-resistant acid phosphatase (TRAcP), and parathyroid hormone-related protein (PTHrP) in saliva during orthodontic treatment in order to evaluate whether changes in bone turnover marker (BTM) concentration can help highlight the effects of orthodontic mechanical loading in the absence of clinical evidence of tooth movement in terms of tooth movement. Saliva samples from 25 apparently healthy young subjects (10 females and 15 males) were collected and the concentrations of PTHrP, TRAcP 5b, and PINP were analyzed at time 0 (T1), 25 days (T2), and at 45 days (T3). Differences in the median value of biomarker levels between baseline T1 and follow-up of the different groups (T2 and T3) were assessed. Trough concentrations of PINP, PTHrP, and TRAcP were 0.80 µg/L, 0.21 ng/mL, and 0.90 U/L above the method LOD. All subjects evaluated had a statistically significant difference between T1 vs. T3.

Vaghela N et al(2024)¹⁹, evaluated concentrations of salivary biomarkers; receptor activator of nuclear factor kappa-B ligand (RANKL) and osteoprotegerin (OPG) in 20 patients with protruded anterior maxillary teeth requiring en-masse retraction, they were randomly allocated into two groups. In the healed extraction (HE) group, 1st premolar extraction was performed at the beginning of treatment while in the recent extraction (RE) group, it was done just before the initiation of en-masse retraction. Saliva and study model collection was done at start of treatment, after levelling, and after 2, 8 and 12-weeks of retraction, respectively. An increase in concentration of RANKL and decrease in OPG concentration was observed in both the RE and HE groups at 12 weeks; however, the intergroup comparison showed no statistically significant differences ($P = 0.517$). RANKL/OPG ratio was significantly higher in the RE group ($P = 0.015$) at 12 weeks. A significantly higher rate of en-masse retraction was found in the RE group ($P < 0.001$).

The present clinical study was conducted in the Department of Orthodontics and Dentofacial Orthopaedics, Babu Banarasi Das College of Dental Sciences, BBDU Lucknow, in collaboration with department of Biochemistry, King George's Medical University, Lucknow, with the aim to evaluate and compare proinflammatory cytokine (IL-6 & RANKL) levels between anterior and posterior segment, during enmasse retraction of anterior teeth.

MATERIALS

Sample

15 patients (age range of 18-25 yrs) with clinical presentation of class I Bi-maxillary protrusion or Class II Div I Malocclusion who required fixed orthodontic treatment with extraction of all 1st premolar teeth were included in the study. In all the patients fixed orthodontic treatment was initiated and alignment & levelling was accomplished, and then 1st Premolar extraction was done, and enmasse retraction of anterior teeth was initiated using sliding mechanics. GCF was collected from gingival sulcus (distal to maxillary canine and mesial to maxillary first molar) at different time intervals (Baseline - immediately after application of retraction force; after 24 hrs; after 20 days; after 2 months) and level of biomarkers IL-6 & RANKL was assessed using ELISA test.

Criteria for Sample collection

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

Inclusion criteria

1. Patients with age range of 18-25 years.
2. All subjects in good general health with healthy periodontal tissues
3. Patients with Angle's class I Bi-maxillary protrusion/Angle's class II division 1 malocclusion, in whom all first premolar extractions are advised as a part of fixed orthodontic treatment.

4. Patients having full complement of teeth till 2nd Molar.

Exclusion criteria

1. Patients having history of chronic diseases like diabetes, hypertension.
2. History of taking drugs changing the hormonal balance such as contraceptives, antihistamines or non-steroidal anti-inflammatory drugs, bisphosphonate in the past four months or during the fixed orthodontic treatment.
3. Patient advised asymmetric extraction.
4. Patient not willing for fixed orthodontic treatment.

Ethical Committee Approval

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

A total of 15 subjects with Angle's Class 1 bimaxillary protrusion or Class II div 1 malocclusion who fulfilled above mentioned inclusion and exclusion criteria were selected for the study.

Materials and Equipments used in the study:

1. Diagnosis and Impression Making :(Fig.1)

1. Mouth -mirror
2. Explorer
3. Tweezer
4. Gloves
5. Kidney tray
6. Bowl, spatula
7. Impression trays
8. Photographic Mirror
9. Cheek retractor
10. Alginate impression material
11. Orthokal



Fig.1: Materials used for Diagnosis and Impression

- | | | |
|------------------|------------------------|----------------------------------|
| 1. Mouth -mirror | 5. Kidney Tray | 9. Cheek Retractor |
| 2. Explorer | 6. Bowl, Spatula | 10. Alginate Impression material |
| 3. Tweezer | 7. Impression Trays | 11. Orthokal |
| 4. Gloves | 8. Photographic Mirror | |

2.Materials used for Cephalometric Analysis (Fig.2)

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

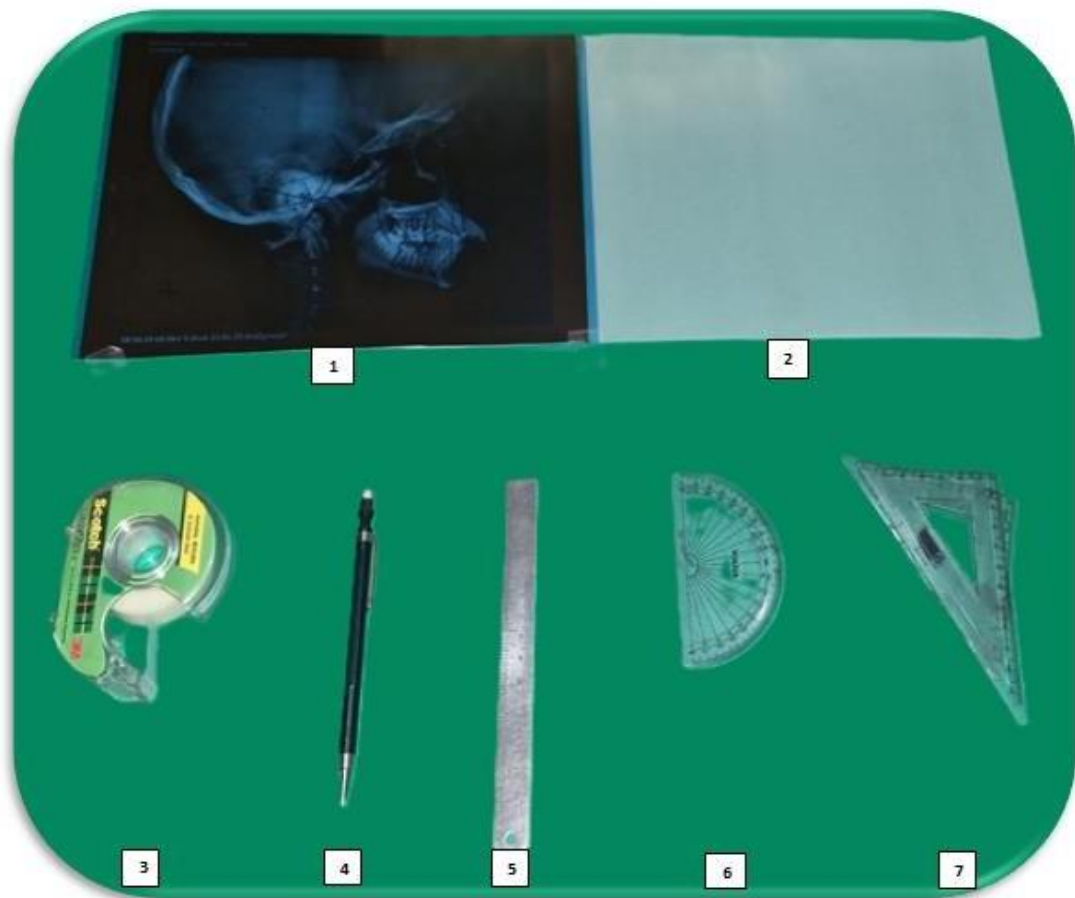


Fig.2: Materials used for cephalometric analysis

- | | |
|------------------------|---------------|
| 1. Lateral cephalogram | 5. Scale |
| 2. Tracing sheet | 6. Protractor |
| 3. Masking Tape | 7. Set square |
| 4. Pencil | |

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

1. Separators
2. Modules
3. Ligature wire
4. Cotton roll
5. Cheek retractor
6. Preformed bands on molar as per size (3M, unitek) with buccal tube
7. MBT brackets (.022*.028)”
8. Bonding materials (etchant, primer, bonding adhesive)
9. Glass Ionomer cement (luting)
10. Arch wire NiTi and SS (round and rectangular)
11. MBT Gauge
12. Bracket Holding Forcep
13. Ligature wire cutter
14. Distal End cutter
15. Hoe Plier
16. Mathieu Haeden plier
17. Dontrix Gauge
18. Light cure Gun



Fig.3: Materials used for fixed orthodontic treatment

- | | |
|---|----------------------------|
| 1. Separators (Garmy) | 10. Arch Wires |
| 2. Modules (Garmy) | 11. MBT Gauge |
| 3. Ligature wire | 12. Bracket holding forcep |
| 4. Cotton roll | 13. Ligature wire cutter |
| 5. Cheek retractor | 14. Distal end cutter |
| 6. Preformed bands on molar with buccal tube (3M, unitek) | 15. Hoe plier |
| 7. MBT brackets (.022" x .028" 3M) | 16. Mathieu Haiden plier |
| 8. Bonding material (D-tech) | 17. Dontrix gauge |
| 9. Glass Ionomer Cement (d-tech) | 18. Light cure gun |

3. Material used for Sliding Mechanics:(Fig.4)

1. .019"x.025" ss arch wire
2. Crimpable hook
3. Crimpable Plier
4. Niti closed coil spring

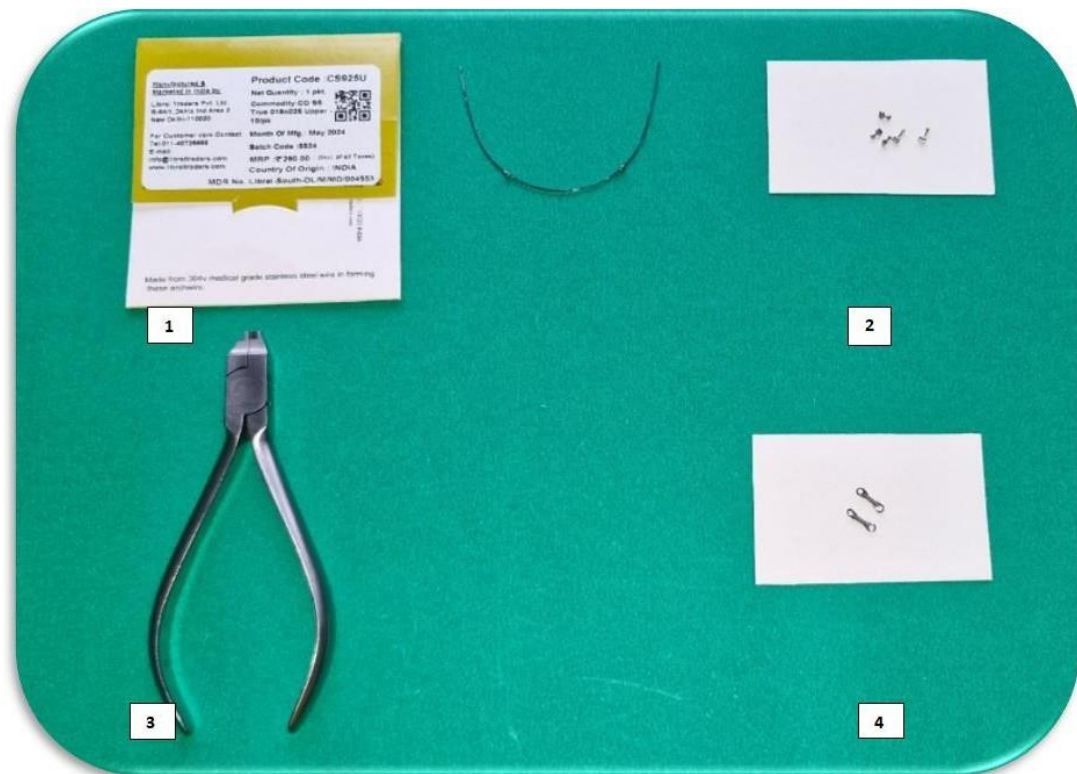


Fig.4: Material used For Sliding Mechanics

- | | |
|-------------------------------|---------------------------------|
| 1. 0.019" x.025" ss arch wire | 3. Crimpable plier |
| 2. Crimpable hook | 4. Niti closed coil spring(6mm) |

4. Material used for GCF Collection (Fig.5)

1. Mouth wash
2. Gloves
3. Cotton rolls
4. Cheek retractor
5. Phosphate buffer solution
6. Absorbent points
7. Eppendorf tube



Fig.5: Material used for GCF collection and storage

- | | |
|-------------------|----------------------------|
| 1.Mouth wash | 5. Phosphate buffer saline |
| 2. Gloves | 6. Absorbent points |
| 3.Cotton rolls | 7. Eppendorf tube |
| 4.Cheek retractor | |

5. Material used for Interim and final storage(Fig.6)

1. Ice Box for interim storage of sample (at 4 °C) during transportation.
2. Deep freezer for final storage of sample till ELISA test(-80°C).



Fig.6: Materials used for interim and final storage

1. Ice Box for interim storage of sample (at 4 °C)
2. Deep freezer for final storage of sample till ELISA test(-80 °C)

4. Assessment of biomarker (ELISA TEST) (Fig.7)

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



Fig.7: Material used for assessment of Biomarker

- | | |
|------------------------|-------------------------|
| 1. Centrifuge Machine | 4.ELISA kit for IL-6 |
| 2. Incubator | 5.ELISA analyser system |
| 3. ELISA kit for RANKL | |

METHODOLOGY:

A) START AND PROGRESSION OF FIXED ORTHODONTIC TREATMENT

1. Patient coming to the department for fixed orthodontic treatment were screened for Angle's class I Bimaxillary protrusion or Angle's class II Div 1 malocclusion.
2. Clinical history and diagnosis was done and all the diagnostic records were taken .After evaluating the records treatment plan was finalized and patients in whom all 1st Premolar extractions were indicated for treatment purpose were selected for the study.
3. As per the treatment plan, anchorage consideration was maximum. Seperator placement was done distal and mesial to 2nd molar, mesial to 1st molar and left for 4- 5 days on selected cases. After space creation appropriate band size with buccal tube was selected and a transpalatal arch was fabricated and soldered with maxillary 1st molar bands. Bands were cemented with Glass ionomer cement (luting GIC) to maxillary 1st and 2nd molars.
4. MBT brackets were bonded following standard protocol.
5. Initial alignment and levelling was done by following Sequential NiTi arch wire insertion as per case requirement till .019"x.025" Stainless steel can be placed passively.(Fig.8)
6. Before starting the anterior enmasse retraction 1st premolar was extracted as part of fixed orthodontic treatment. (Fig.9)



Fig.8: Intraoral photograph (Right and Left lateral view) after alignment and levelling.



Fig.9: Intraoral photograph (Right and Left lateral view) after first Premolar Extraction

B) FOR ENMASSE RETRACTION

1. For enmasse retraction crimpable hooks (4mm length) were crimped on .019"x.025" stainless steel wire between lateral incisor and canine.(Fig.10)
2. Enmasse retraction was initiated using Niti closed coil spring extended from crimpable hook, to the hook of buccal tube on maxillary 1st Molar band. (Fig.11)
3. Force of 200 gm, standardized on both sides using dontrix gauge. (Fig.12)

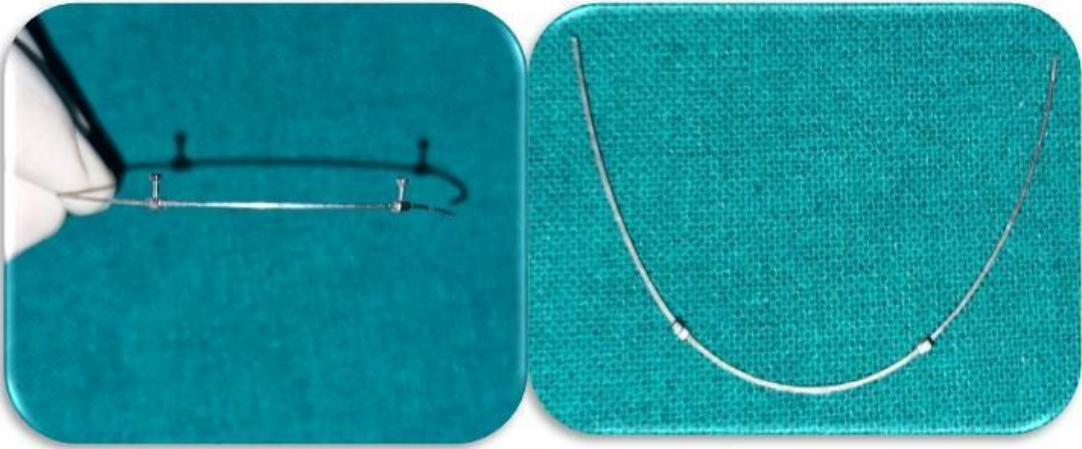


Fig.10: Crimpable hook crimped on arch wire



Fig.11: Initiation of enmasse retraction of anterior teeth using Niti closed coil spring



Fig.12: Retraction force of 200 gm standardized on Dontrix gauge

C) PROCEDURE BEFORE COLLECTING GCF

1. The participants were seated comfortably in a dental chair and the procedure for GCF collection was explained to them.
2. Before collection of GCF, Supragingival plaque were carefully removed from the site , thoroughly washed and patient was instructed to do mouth wash rinses with 0.012% chlorhexidine mouthwash.
3. The site was dried with an air syringe and isolated from salivary and blood contamination with the aid of cotton rolls. For retraction cheek retractor and mirror was used. (Fig.13)



Fig.13: Adequate Isolation of site before GCF collection

D) FOR GCF SAMPLE COLLECTION

1. Initially GCF was collected from 2 sites at each time. The absorbent / paper points were gently inserted into the gingival sulcus of the selected sites (distal to maxillary canine and mesial to maxillary first molar) at a depth of 1-2mm for approximately 40 sec. Thereafter absorbent points were removed gently from the sulcus avoiding saliva/ blood contamination. GCF was collected at T0 Baseline -immediately after applying the retraction force. (Fig.14 & 15)
2. The same protocol for GCF collection was followed at different time selected in the study- T1- After 24 hrs; T2- After 20 days; T3 -after 2 months . Based on site of GCF collection GCF sample was divided as Group I that included GCF collected from distal gingival sulcus of maxillary Canine and Group II that included GCF collected from mesial gingival sulcus of maxillary first molar.

TABLE-1 TIME INTERVAL AT WHICH GCF WAS COLLECTED

Number of subjects N=15	GCF detection site	GROUP-I Anterior segment (Distal to Maxillary Canine)				GROUP -II Posterior segment (Mesial to Maxillary 1st Molar)			
		T0	T1	T2	T3	T0	T1	T2	T3
	Time at which GCF samples were collected								
		Baseline	After 24hr	After 20days	After 2Month	Baseline	After 24hr	After 20days	After 2Month

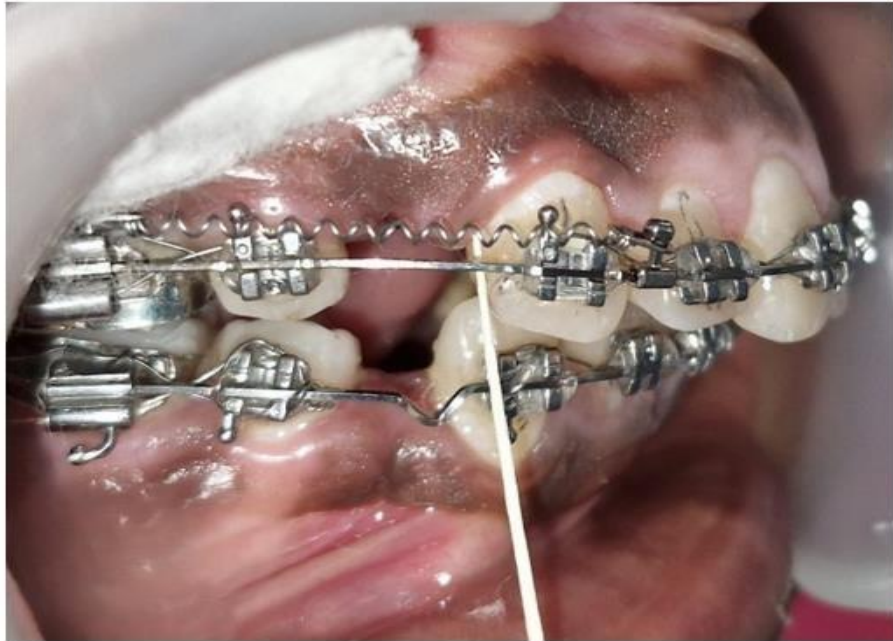


Fig.14: Absorbent point inserted in distal gingival sulcus of Canine tooth



Fig.15: Absorbent point inserted in mesial gingival sulcus of maxillary first molar

E) GCF STORAGE AND TRANSPORTATION

1. These Absorbent points soaked with GCF were placed in Eppendorf tube containing 250ul phosphate buffered saline. (Fig. 16)
2. Eppendorf tube was stored in a dry ice box maintaining 4 °C temperature till its transportation to the laboratory of Biochemistry department of KGMU, Lucknow for its storage at – 80 °C temperature till further procedures.(Fig 17a&b)



Fig.16: Soaked Absorbent point placed in labeled Eppendorf tube containing Phosphate Buffer Saline (PBS) solution



a. Ice Box with Eppendorf tube



b. Deep freezer

Fig.17: (a&b) GCF storage and transportation solution

F) FOR PROCESSING OF SAMPLE

1. After collection of all samples of study, samples were processed at laboratory.
2. All frozen samples were thawed at room temperature (18-25⁰ C). All samples were centrifuged and mixed fully at time of testing for 30 seconds to ensure the complete elution of GCF from paper points.(Fig.18a)
3. After being centrifuged, the paper points were removed and supernatants were transferred into a new Eppendorf tube and kept in incubator. (Fig.18 b&c)

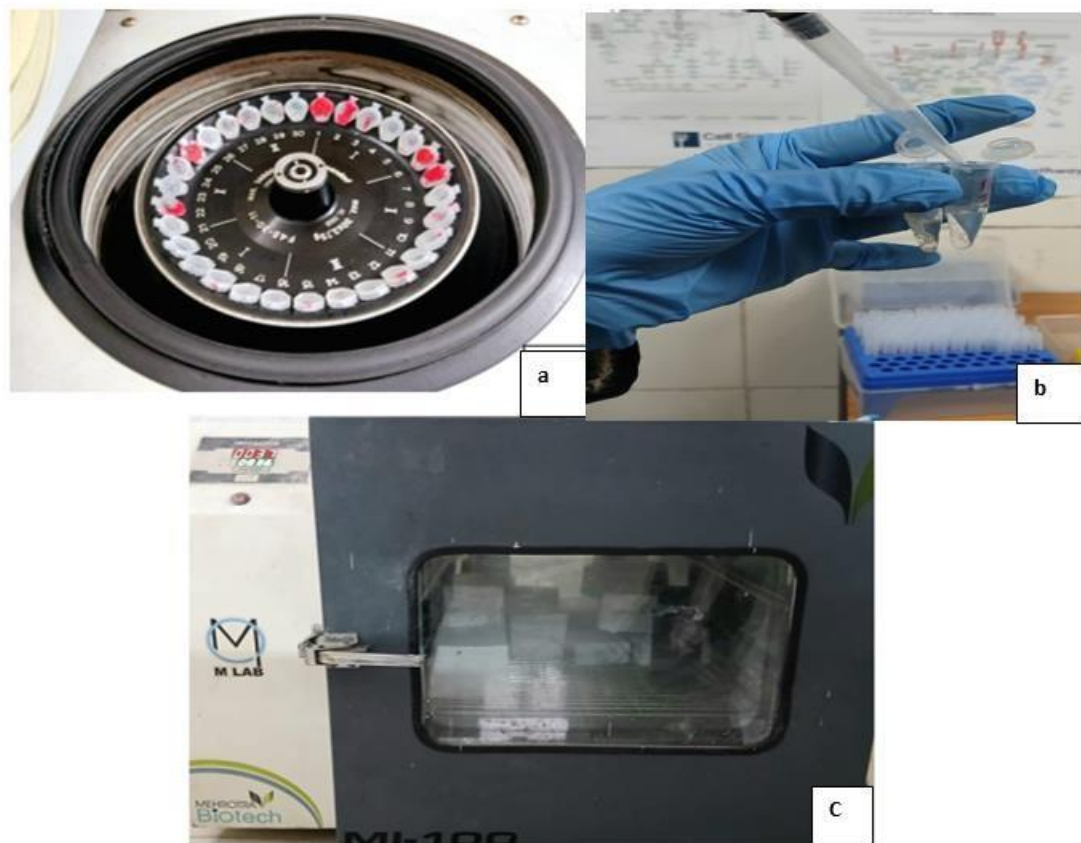


Fig.18(a-c): Sample processing for ELISA analysis

- a. Centrifuge machine with Eppendorf tube for elution of GCF
- b. Supernatants were transferred into new Eppendorf tubes.
- c. Incubator

G). FOR BIOMARKER ANALYSIS (ELISA):

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

1. The kit for either of the biomarker to be tested was taken from the refrigerator 20 minutes in advance to bring all reagents to room temperature (18-25°C) before use. (Fig 19)
2. Dilute the 25x Concentrated Wash Buffer to 1x working solution.
3. Dilute the Reference Standard to different concentration.
4. Dilute the 100x Biotinylated Detection Ab/Ag to 1x working solution 15 minutes earlier before.
5. Dilute the 100x Concentrated HRP Conjugate to 1x working solution 15 minutes earlier before.
6. After the preliminary steps of reagent preparation microplate reader of ELISA analyser system was turned on and Assay procedure for ELISA was done as shown in following flow chart.



**Fig.19 Material used for ELISA test
(ELISA kit for RANKL/ IL6)**

Assay procedure summary

Add 100 μ l of standard or sample to each well. Incubate for 90 minutes at 37°C. (Biomarker molecule present in the sample is bound to the wells by the immobilized antibody) (Fig.20a)

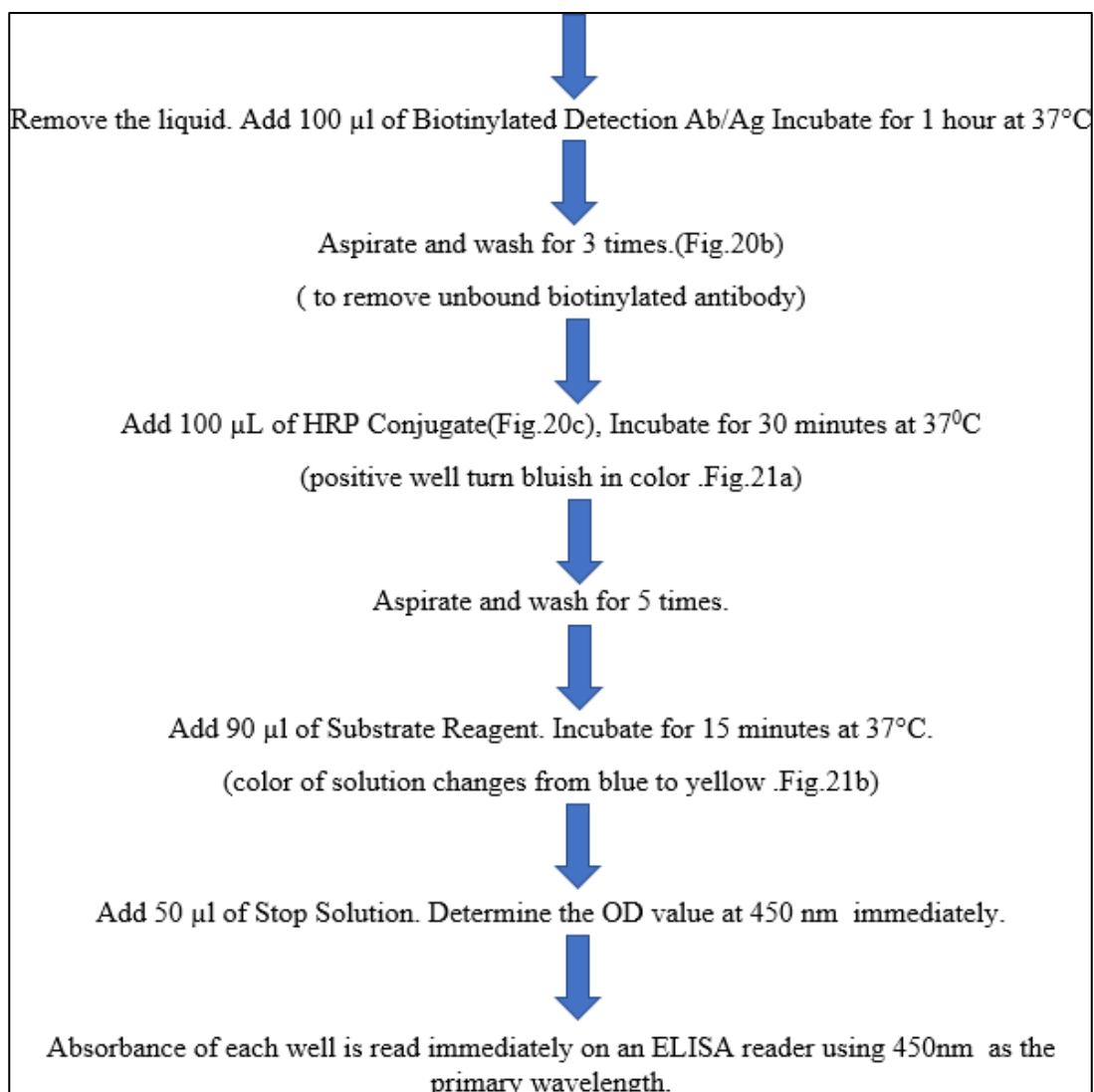




Fig.20(a-c): Preperation of assay for sandwich ELISA procedure.

- a. Addition of sample to each well
- b. Washing of wells
- c. Dilution and addition of biotinylated antibody, HRP- conjugated pipetted to wells

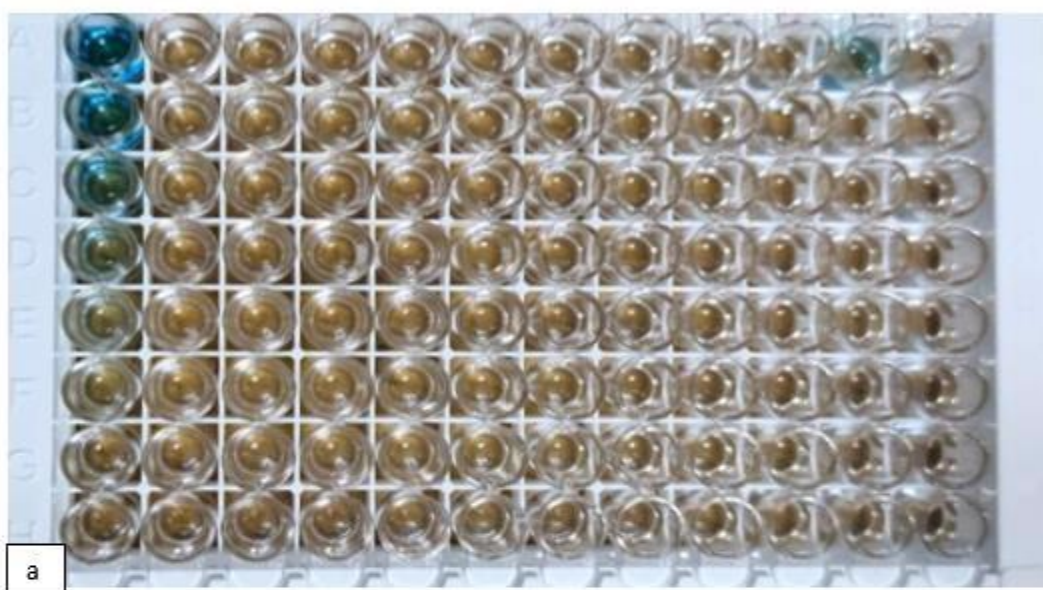


Fig.21(a&b): Showing Colour change

- a. Positive well colour changed to blue
- b. Colour changed from blue to yellow

The present clinical study was conducted in the Department of Orthodontics and Dentofacial Orthopaedics, Babu Banarasi Das College of Dental Sciences, BBDU Lucknow, in collaboration with department of Biochemistry King George's Medical University, Lucknow, with an aim to evaluate and compare proinflammatory cytokine (IL-6 & RANKL) levels between anterior and posterior segment, during enmasse retraction of anterior teeth .

The sample for the study included collected sample(GCF) from anterior and posterior segments that were grouped as Group I and Group II respectively .GCF was collected at different time -T0 (Baseline) immediately after applying the retraction force; T1- (after 24 hrs) ; T2- (after 20 days) ; T3 -(after 2 months). The results of the present study are summarized in the following tables :-

TABLE 2: Descriptive statistics for mean values of IL-6 and RANKL as obtained for Group I and Group II at different time.

TABLE 3: Intragroup comparison of IL6 for Group I at different time intervals.

TABLE 4: Intragroup comparison of IL-6 for Group II at different time intervals.

TABLE 5: Intergroup comparison of IL-6 between Group I & Group II at T0, T1, T2, T3.

TABLE 6: Intragroup comparison of RANKL for Group I at different time intervals

TABLE 7: Intragroup comparison of RANKL for Group II at different time intervals.

TABLE 8: Intergroup comparison of RANKL between Group I & Group II at T0, T1 ,T2, T3.

TABLE 9: Overall Comparison between Group I and Group II for RANKL and IL-6.

TABLE 2: DESCRIPTIVE STATISTICS FOR MEAN VALUES OF IL6 AND RANKL AS OBTAINED FOR GROUP I AND GROUP II AT DIFFERENT TIME.

N=15	IL-6			RANKL		
GROUP I	MIN (pg/ml)	MAX (pg/ml)	Mean±SD (pg/ml)	MIN (pg/ml)	MAX (pg/ml)	Mean±SD (pg/ml)
T0	2.539	3.119	2.829±.290	21.109	24.785	22.947±1.838
T1	2.185	2.851	2.518±.333	20.215	23.775	21.995±1.780
T2	2.486	2.962	2.724±.238	20.753	25.661	23.207±2.454
T3	2.325	3.013	2.669±.344	20.835	25.569	23.202±2.367
GROUP II	MIN (pg/ml)	MAX (pg/ml)	Mean±SD (pg/ml)	MIN (pg/ml)	MAX (pg/ml)	Mean±SD (pg/ml)
T0	2.486	4.412	3.449±.963	19.278	30.574	24.926± .648
T1	2.8	4.194	3.497±.697	20.844	31.132	25.988±5.144
T2	2.254	4.138	3.196±.942	19.544	30.144	24.844±5.300
T3	2.343	4.493	3.418±1.075	20.891	27.031	23.961± .070

For Group I ,mean level of IL6 was highest at T0(2.829±.290pg/ml) ,followed by T2(2.724±.238pg/ml)then T3(2.669±.344pg/ml) and least was in T1(2.518±.333pg/ml).(T0>T2>T3>T1).

For Group II ,mean level of IL6 was highest at T1(3.497±.697pg/ml) followed by T0 (3.449±.963pg/ml), then T3(3.418±1.075pg/ml)and least was in T2 (3.196±.942pg/ml).(T1>T0>T3>T2).

For Group I , mean level of RANKL was highest at T2(23.207 ± 2.454 pg/ml), followed by T3(23.202 ± 2.367 pg/ml) then T0(22.947 ± 1.838 pg/ml) and least was in T1(21.995 ± 1.780 pg/ml). ($T2 > T3 > T0 > T1$).

For Group II, mean level of RANKL was highest at T1(25.988 ± 5.144 pg/ml), followed by T0(24.926 ± 5.648 pg/ml) then T2(24.844 ± 5.300 pg/ml) and least was in T3(23.961 ± 3.070 pg/ml). ($T1 > T0 > T2 > T3$).

TABLE 3: INTRAGROUP COMPARISON OF IL6 FOR GROUP I AT DIFFERENT TIME INTERVALS

		Mean $\pm$ Std.D (pg/ml)	Std. Error Mean	t	df	P value
GROUP I	T0 - T1	.310 $\pm$.324pg/ml	.083	3.705	14	.002**
	T0 - T2	.104 $\pm$.282pg/ml	.073	1.436	14	.173
	T0 - T3	.160 $\pm$.331pg/ml	.085	1.871	14	.082
	T1 - T2	-.205 $\pm$.274pg/ml	.070	-2.908	14	.011*
	T1 - T3	-.150 $\pm$.352pg/ml	.090	-1.656	14	.120
	T2 - T3	.055 $\pm$.419pg/ml	.108	.510	14	.618

P $\geq$.05 nonsignificant ;P $\leq$.05 just significant* ;P $\leq$.01 significant** ;P $\leq$.001 highly significant***

On comparing mean levels of IL-6 between T0 and T1 ,it was seen that values were higher for T0 ($2.829 \pm .290$ pg/ml)than T1($2.518 \pm .333$ pg/ml), with mean difference of $.310 \pm .324$ pg/ml and that difference was statistically significant.(p=.002)

On comparing mean levels of IL-6 between T0 and T2 ,it was seen that values were higher for T0 ($2.829 \pm .290$ pg/ml)than T2($2.724 \pm .238$ pg/ml) with mean difference of $.104 \pm .282$ pg/ml but that difference was not statistically significant.(p=.173)

On comparing mean levels of IL-6 between T0 and T3 ,it was seen that values were higher for T0 ($2.829 \pm .290$ pg/ml)than T3($2.669 \pm .344$ pg/ml) ,with mean difference of $.160 \pm .331$ pg/ml but that difference was not statistically significant.(p=.082)

On comparing mean levels of IL-6 between T1 and T2, it was seen that values were higher for T2 ($2.724 \pm .238$ pg/ml) than T1 ($2.518 \pm .333$ pg/ml) with mean difference of $-.205 \pm .274$ pg/ml and that difference was statistically significant. ($p=.011$)

On comparing mean levels of IL-6 between T1 and T3, it was seen that values were higher for T3 ($2.669 \pm .344$ pg/ml) than T1 ($2.518 \pm .333$ pg/ml), with mean difference of $-.150 \pm .352$ pg/ml but that difference was not statistically significant. ($p=.120$)

On comparing mean levels of IL-6 between T2 and T3, it was seen that values were higher for T2 ($2.724 \pm .238$ pg/ml) than T3 ($2.669 \pm .344$ pg/ml), with mean difference of $.055 \pm .419$ pg/ml but that difference was not statistically significant. ($p=.618$)

TABLE 4: INTRAGROUP COMPARISON OF IL6 FOR GROUP II AT DIFFERENT TIME INTERVALS

		Mean $\pm$ Std. D (pg/ml)	Std. Error Meant		df	P value
GROUP II	T0 - T1	$-.048 \pm .465$ pg/ml	.120	-.400	14	.695
	T0 - T2	$.253 \pm .389$ pg/ml	.100	2.519	14	.025*
	T0 - T3	$.031 \pm .504$ pg/ml	.130	.243	14	.812
	T1 - T2	$.301 \pm .575$ pg/ml	.148	2.027	14	.062
	T1 - T3	$.079 \pm .736$ pg/ml	.190	.418	14	.682
	T2 - T3	$-.221 \pm .453$ pg/ml	.117	-1.893	14	.079

$P \geq .05$ nonsignificant ; $P \leq .05$ just significant* ; $P \leq .01$ significant** ; $P \leq .001$ highly significant***

On comparing mean levels of IL-6 between T0 and T1, it was seen that values were higher for T1 ($3.497 \pm .697$ pg/ml) than T0 ($3.449 \pm .963$ pg/ml), with mean difference of $-.048 \pm .465$ pg/ml but that difference was not statistically significant. ($p=.695$)

On comparing mean levels of IL-6 between T0 and T2, it was seen that values were higher for T0 ($3.449 \pm .963$ pg/ml) than T2 ($3.196 \pm .942$ pg/ml) with mean difference of $.253 \pm .389$ pg/ml and that difference was statistically significant. ($p=.025$)

On comparing mean levels of IL-6 between T0 and T3 ,it was seen that values were higher for T0 ($3.449 \pm .963$ pg/ml)than T3(3.418 ± 1.075 pg/ml) ,with mean difference of $.031 \pm .504$ pg/ml but that difference was not statistically significant.($p=.812$)

On comparing mean levels of IL-6 between T1 and T2 ,it was seen that values were higher for T1 ($3.497 \pm .697$ pg/ml pg/ml)than T2($3.196 \pm .942$ pg/ml) with mean difference of $.301 \pm .575$ pg/ml but that difference was not statistically significant.($p=.062$)

On comparing mean levels of IL-6 between T1 and T3 ,it was seen that values were higher for T1 ($3.497 \pm .697$ pg/ml)than T3(3.418 ± 1.075 pg /ml), with mean difference of $.079 \pm .736$ pg/ml but that difference was not statistically significant .($p=.682$)

On comparing mean levels of IL-6 between T2 and T3 ,it was seen that values were higher for T3(3.418 ± 1.075 pg g/ml)than T2($3.196 \pm .942$ pg /ml) ,with mean difference of $-.221 \pm .453$ pg/ml but that difference was not statistically significant.($p=.079$)

TABLE 5: INTERGROUP COMPARISON OF IL6 BETWEEN GROUP I & GROUP II AT T0 T1 T2 T3.

N=15	TIME	Group	Mean $\pm$ Std. D.(pg/ml)	Std. Error Mean	P value
	T0	I	2.829 $\pm$.290pg/ml	.074	0.024*
		II	3.449 $\pm$.963pg/ml	.248	
	T1	I	2.518 $\pm$.333pg/ml	.086	0.001***
		II	3.497 $\pm$.697pg/ml	.180	
	T2	I	2.724 $\pm$.238pg/ml	.061	0.070
		II	3.196 $\pm$.942pg/ml	.243	
	T3	I	2.669 $\pm$.344pg/ml	.089	0.016**
		II	3.418 $\pm$ 1.075pg/ml	.277	

$P \geq .05$ nonsignificant ; $P \leq .05$ just significant* ; $P \leq .01$ significant** ; $P \leq .001$ highly significant***

On comparing Group I with Group II at T0 , mean value of IL-6 was higher in Group II ($3.449 \pm .963$ pg/ml) than Group I ($2.829 \pm .290$ pg/ml) and difference was statistically significant ($p=0.024$)

On comparing Group I with Group II at T1 , mean value of IL-6 was higher in Group II ($3.497 \pm .697$ pg/ml) than Group I ($2.518 \pm .333$ pg/ml) and difference was statistically significant. ($p=0.001$)

On comparing Group I with Group II at T2 , mean value of IL-6 was higher in Group II ($3.196 \pm .942$ pg/ml) than Group I ($2.724 \pm .238$ pg/ml) but difference was not statistically significant. ($p=.070$)

On comparing Group I with Group II at T3 , mean value of IL-6 was higher in Group II (3.418 ± 1.075 pg/ml) than Group I ($2.669 \pm .344$ pg/ml) and difference was statistically significant. ($p=.016$)

TABLE 6: INTRAGROUP COMPARISON OF RANKL FOR GROUP I AT DIFFERENT TIME INTERVALS

		Mean $\pm$ Std. D. (pg/ml)	Std. Error Mean	t	df	P value
GROUP I	T0 - T1	.952 $\pm$ 1.997pg/ml	.515	1.845	14	.086
	T0 - T2	-.260 $\pm$ 2.213pg/ml	.571	-.455	14	.656
	T0 - T3	-.254 $\pm$ 3.145pg/ml	.812	-.314	14	.758
	T1 - T2	-1.212 $\pm$ 2.563pg/ml	.661	-1.831	14	.088
	T1 - T3	-1.206 $\pm$ 2.837pg/ml	.732	-1.647	14	.122
	T2 - T3	.005 $\pm$ 2.585pg/ml	.667	.008	14	.994

$P \geq .05$ nonsignificant ; $P \leq .05$ just significant* ; $P \leq .01$ significant** ; $P \leq .001$ highly significant***

On comparing mean levels of RANKL between T0 and T1 ,it was seen that values were higher for T0 (22.947 ± 1.838 pg/ml)than T1(21.995 ± 1.780 pg/ml), with mean difference of $.952 \pm 1.997$ pg/ml but this difference was not statistically significant.($p=.086$)

On comparing mean levels of RANKL between T0 and T2 ,it was seen that values were higher for T2 (23.207 ± 2.454 pg/ml)than T0(22.947 ± 1.838 pg/ml) with mean difference of $-.260 \pm 2.213$ pg/ml but this difference was not statistically significant.($p=.656$)

On comparing mean levels of RANKL between T0 and T3, it was seen that values were higher for T3 (23.202 ± 2.367 pg/ml) than T0(22.947 ± 1.838 pg /ml) ,with mean difference of $-.254 \pm 3.145$ pg/ml but this difference was not statistically significant.($p=.758$)

On comparing mean levels of RANKL between T1 and T2, it was seen that values were higher for T2 (23.207 ± 2.454 pg/ml) than T1(21.995 ± 1.780 pg /ml) with mean difference of -1.212 ± 2.563 pg/ml but this difference was not statistically significant.($p=.088$)

On comparing mean levels of RANKL between T1 and T3, it was seen that values were higher for T3 (23.202 ± 2.367 pg /ml) than T1 (21.995 ± 1.780 pg /ml) ,with mean difference of -1.206 ± 2.837 pg/ml but this difference was not statistically significant.($p=.122$)

On comparing mean levels of RANKL between T2 and T3, it was seen that values were higher for T2(23.207 ± 2.454 pg /ml) than T3 (23.202 ± 2.367 pg /ml), with mean difference of $.005 \pm 2.585$ pg /ml but this difference was not statistically significant.($p=.994$)

**TABLE 7: INTRAGROUP COMPARISON OF RANKL FOR GROUP I I
AT DIFFERENT TIME INTERVALS**

		Mean ± Std. D. (pg/ml)	Std. Error Mean	t	df	P value
GROUP II	T0 - T1	-1.062 ± 4.564pg/ml	1.178	-.901	14	.383
	T0 - T2	.082 ± 4.498pg/ml	1.161	.071	14	.944
	T0 - T3	.965 ± 4.366pg/ml	1.127	.856	14	.406
	T1 - T2	1.144 ± 6.486pg/ml	1.674	.684	14	.505
	T1 - T3	2.027 ± 5.467pg/ml	1.411	1.436	14	.173
	T2 - T3	.882 ± 2.707pg/ml	.699	1.262	14	.227

P ≥ .05 nonsignificant ; P ≤ .05 just significant* ; P ≤ .01 significant** ; P ≤ .001 highly significant***

On comparing mean levels of RANKL between T0 and T1 ,it was seen that values were higher for T1 (25.988±5.144pg/ml)than T0(24.926±5.648pg/ml) , with mean difference of -1.062± 4.564pg/ml but this difference was not statistically significant.(p=.383)

On comparing mean levels of RANKL between T0 and T2 for ,it was seen that values were higher for T0 (24.926±5.648pg/ml)than T2(24.844±5.300pg/ml) with mean difference of .082± 4.498pg/ml but this difference was not statistically significant.(p=.944)

On comparing mean levels of RANKL between T0 and T3 ,it was seen that values were higher for T0 ((24.926±5.648pg/ml)than T3(23.961±3.070pg /ml) with mean difference of .965± 4.366pg/ml but this difference was not statistically significant.(p=.406)

On comparing mean levels of RANKL between T1 and T2, it was seen that values were higher for T1(25.988±5.144pg/ml) than T2(24.844±5.300pg/ml) with mean difference of 1.144± 6.486 pg/ml but this difference was not statistically significant. (p=.505)

On comparing mean levels of RANKL between T1 and T3 ,it was seen that values were higher for T1 (25.988 ± 5.144 pg/ml)than T3(23.961 ± 3.070 pg /ml) ,with mean difference of 2.027 ± 5.467 pg/ml but this difference was not statistically significant.(p=.173)

On comparing mean levels of RANKL between T2 and T3 ,it was seen that values were higher for T2(24.844 ± 5.300 pg /ml)than T3(23.961 ± 3.070 pg /ml) ,with mean difference of $.882 \pm 2.707$ pg /ml but this difference was not statistically significant.(p=.227)

TABLE 8: INTERGROUP COMPARISON OF RANKL BETWEEN GROUP I AND GROUP II AT T0 ,T1, T2 ,T3

N=15	N=15	N=15	N=15	N=15	N=15	N=15
	T0	I	$22.947 \pm$	1.838 pg/ml	.474	0.207
		II	$24.926 \pm$	5.648 pg/ml	1.458	
	T1	I	$21.995 \pm$	1.780 pg/ml	.459	0.008**
		II	$25.988 \pm$	5.144 pg/ml	1.328	
	T2	I	$23.207 \pm$	2.454 pg/ml	.633	0.287
		II	$24.844 \pm$	5.300 pg/ml	1.368	
	T3	I	$23.202 \pm$	2.367 pg/ml	.611	0.455
		II	$23.961 \pm$	3.070 pg/ml	.792	

$P \geq .05$ nonsignificant; $P \leq .05$ just significant*; $P \leq .01$ significant** ; $P \leq .001$ highly significant***

On comparing Group I with Group II at T0, mean value of RANKL was higher in Group II (24.926 ± 5.648 pg/ml) than Group I (22.947 ± 1.838 pg/ml), however difference was not statistically significant. (p= .207)

On comparing Group I with Group II at T1, mean value of RANKL was higher in Group II (25.988 ± 5.144 pg/ml) than Group I(21.995 ± 1.780 pg/ml), and difference was statistically significant. (p= .008)

On comparing Group I with Group II at T2 , mean value of RANKL was higher in Group II (24.844 ± 5.300 pg/ml) than Group I (23.207 ± 2.454 pg/ml) ,however difference was not statistically significant .($p = .287$)

On comparing Group I with Group II at T3 ,mean value of RANKL was higher in Group II (23.961 ± 3.070 pg/ml) than Group I (23.202 ± 2.367 pg/ml) ,however difference was not statistically significant.($p = .455$)

TABLE 9: OVERALL COMPARISON BETWEEN GROUP I AND GROUP II FOR IL-6 AND RANKL .

	GROUP	Mean $\pm$ Std. D (pg/ml)	Std. Error Mean	p VALUE
IL-6	I	10.741 $\pm$.902pg/ml	.232	0.05*
	II	13.562 $\pm$ 3.483pg/ml	.899	
RANKL	I	91.352 $\pm$ 5.712pg/ml	1.475	0.061
	II	99.720 $\pm$ 15.624pg/ml	4.034	

$P \geq .05$ nonsignificant; $P \leq .05$ just significant*; $P \leq .01$ significant** ; $P \leq .001$ highly significant***

On overall comparison of IL-6 level in Group I with Group II at , mean value of IL-6 was higher in Group II (13.562 ± 3.483 pg/ml) than Group I ($10.741 \pm .902$ pg/ml) and difference was statistically significant. ($p = .05$)

On overall comparison of RANKL level in Group I with Group II at ,mean value of RANKL was higher in Group II (99.720 ± 15.624 pg/ml) than Group I (91.352 ± 5.712 pg/ml) ,however difference was not statistically significant. ($p = .061$)

Space closure after extraction can be accomplished either by frictionless/loop mechanics or by frictional/sliding mechanics. Frictionless/loop mechanics require intricate wire bending, and is more technique sensitive. Sliding mechanics uses lighter forces, less technique sensitive, and is comfortable for the patient².

The mechanical stress arising as a result of force application used for any orthodontic tooth movement (OTM) changes periodontal vascularity and induces changes at cellular and molecular level in the PDL to form biologically active substances termed as signalling molecules or biomarkers, which are responsible for modelling and remodelling of alveolar bone (e.g. IL-1 β , IL-1, IL-6, RANKL, TNF- α , MMP, OPG, Sclerostin)^{4,6}.

A Biomarker is a substance that is measured and evaluated objectively as an indicator of normal biologic processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention⁷. Biomarkers can be derived from different biofluids such as blood/serum, saliva and Gingival crevicular fluid (GCF)¹¹. In serum biomarkers are present in minimal quantity and collection method is invasive and levels are influenced by other inflammatory condition of body. The salivary biomarkers are also present in minimal quantities and there is need of highly sensitive test to assess lesser amount of biomarker present, so quantification is a real challenge²⁰. In the present study GCF biomarkers are preferred, because of their close proximity to periodontal tissues, and are least effected by other inflammatory processes in the body²¹.

Various studies have been conducted on biomarkers during various stages of orthodontic tooth movement. However, none of the studies have evaluated levels of biomarkers (IL-6 and RANKL) during en masse space closure using sliding mechanics. IL-6 is a multifunctional cytokine, that promotes activation of RANKL cascade in initial stages of force application. RANKL is a proinflammatory cytokine produced by osteoblasts and it is activated by lymphocyte T as a regulating factor. RANKL activates its specific receptor (RANK), which is located on the surface of the osteoclast and induces osteoclast maturity, resulting in resorption of the alveolar bone, if it binds with OPG resulting in bone deposition⁵. Hence we have chosen IL-6 and RANKL for the present study.

The present clinical study was conducted in the Department of Orthodontics and

Dentofacial Orthopaedics, Babu Banarasi Das College of Dental Sciences, BBDU Lucknow, in collaboration with department of Biochemistry, King George's Medical University, Lucknow, with the aim to evaluate and compare proinflammatory cytokine (IL-6 & RANKL) levels between anterior and posterior segment, during enmasse retraction of anterior teeth .

15 patients (age range of 18-25 yrs) with clinical presentation of class I Bi-maxillary protrusion or Class II Div I Malocclusion who required fixed orthodontic treatment with extraction of all 1st premolar teeth were included in the study. In all the patients first Premolar extraction was done and fixed orthodontic treatment was initiated. Initial alignment and levelling was done by following sequential NiTi arch wire insertion as per case requirement till .019"x.025" Stainless steel can be placed passively . Enmasse retraction of anterior teeth was initiated on .019x.025SS wire using Niti closed coil spring extended from crimpable hook placed between Lateral incisor and Canine to the hook of buccal tube on maxillary 1st Molar band and force level was kept at 200 gm using dontrix gauge.

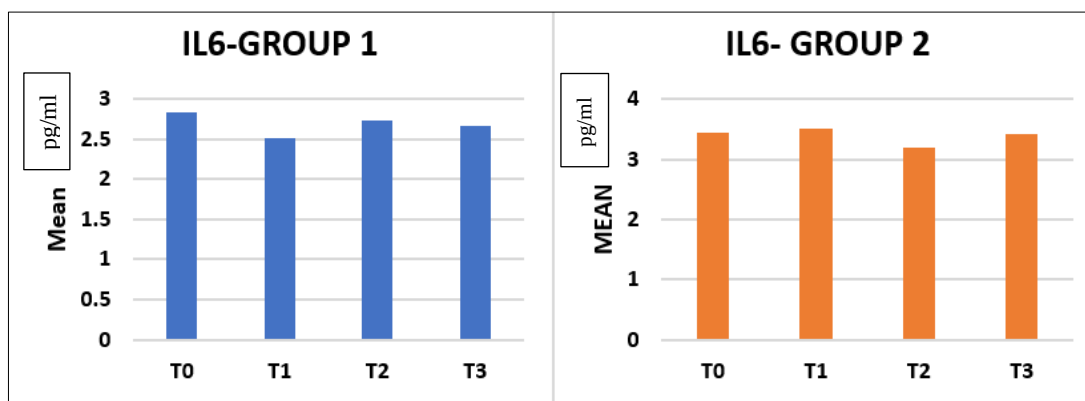
GCF was collected at different time intervals -T0 (Baseline) immediately after applying the retraction force; T1- (after 24 hrs) ; T2- (after 20 days) ;T3 -(after 2 months); from gingival sulcus. Site of GCF collection were grouped as Group I that included distal gingival sulcus of maxillary Canine and Group II that included mesial gingival sulcus of maxillary first molar using absorbent points following standard protocol. These absorbent points soaked with GCF were placed in Eppendorf tube containing 250ul phosphate buffered saline, transported to the laboratory at department of Biochemistry, KGMU, Lucknow for its storage at – 80 °C temperature till further analysis.

After collection of all samples of study, samples were centrifuged at laboratory, the paper points were removed and supernatants were transferred into a new Eppendorf tube and kept in incubator.

After elution, GCF samples were analyzed for IL-6 and RANKL levels. Reagents were prepared by bringing the kit out 20 minutes before use to room temperature (18- 25°C) and preparation was done as per manufacturers instruction. The ELISA analyser was then turned on and the assay procedure was followed as per protocol. The

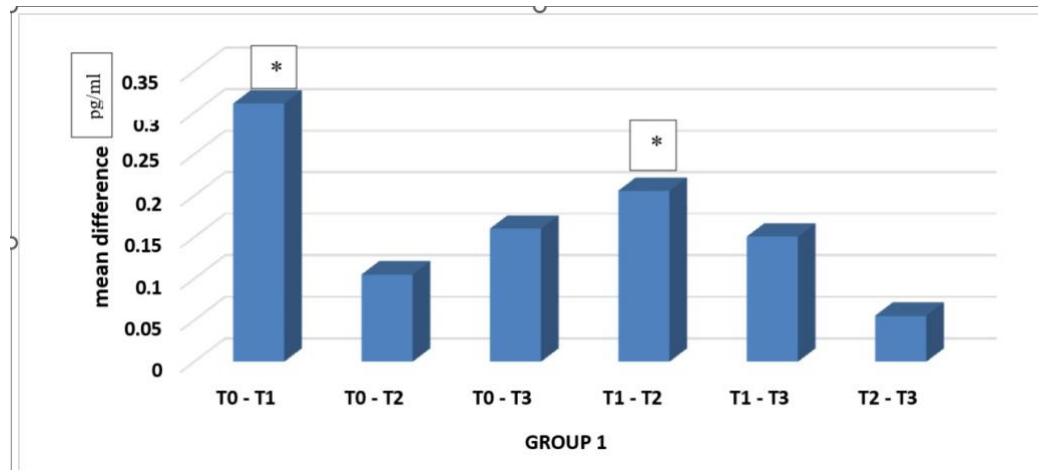
concentration of biomarker was calculated from optical density of samples to standard curve as obtained for each ELISA kit. Mean level of IL-6 and RANKL was obtained as pg/ml. Data was tabulated and adequate statistical comparison was made.

The results of the present study showed variable levels of biomarkers, both in anterior (Group I) and posterior (Group II) segment at different time intervals (T0, T1, T2, T3), suggestive of the fact that fluctuation of these biomarkers are a part of intricate process of OTM at molecular level. The trend for levels of IL-6 in Group I was $T_0(2.829 \pm .290 \text{ pg/ml}) > T_2(2.724 \pm .238 \text{ pg/ml}) > T_3(2.669 \pm .344 \text{ pg/ml}) > T_1(2.518 \pm .333 \text{ pg/ml})$, whereas for Group II trend was $T_1(3.497 \pm .697 \text{ pg/ml}) > T_0(3.449 \pm .963 \text{ pg/ml}) > T_3(3.418 \pm 1.075 \text{ pg/ml}) > T_2(3.196 \pm .942 \text{ pg/ml})$. (Bargraph-1)

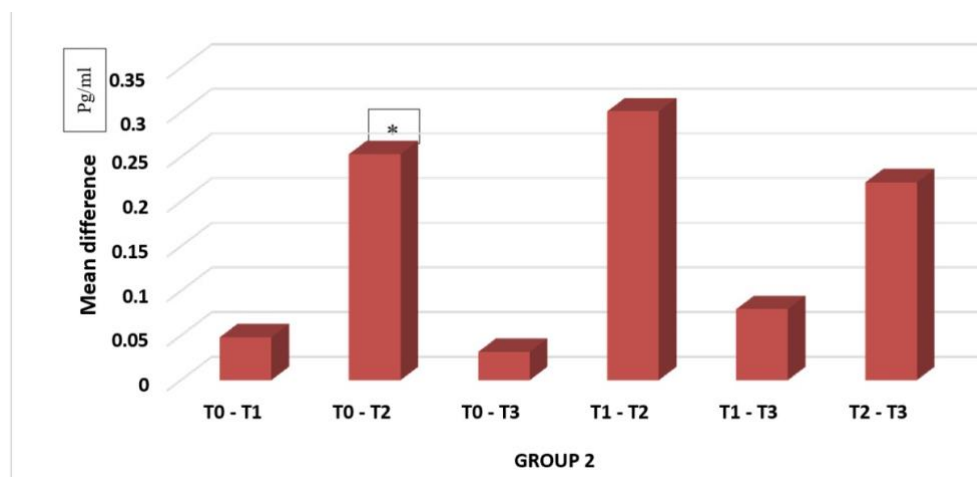


BARGRAPH 1- MEAN VALUES OF IL-6(pg/ml) FOR GROUP I AND GROUP II

On intragroup comparison of levels of IL-6 statistically significant difference was noted between T0 & T1 ($.310 \pm .324 \text{ pg/ml}$, $p=.002$) and T1 & T2 ($-.205 \pm .274 \text{ pg/ml}$, $p=.011$) for Group I whereas in Group II statistically significant difference was seen only between T0 & T2 ($.253 \pm .389 \text{ pg/ml}$, $p=.025$) (Bargraph-2,3)

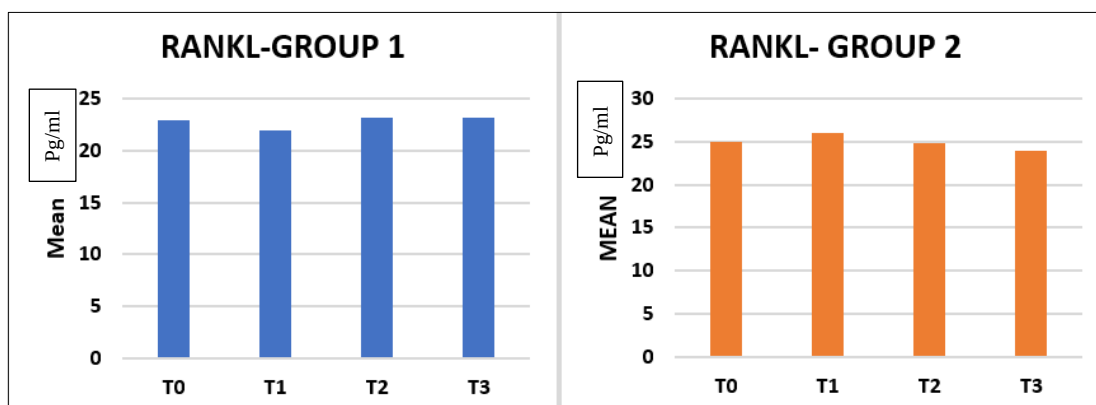


BAR GRAPH 2-INTRAGROUP COMPARISON OF IL6 FOR GROUP I AT DIFFERENT TIME INTERVALS.



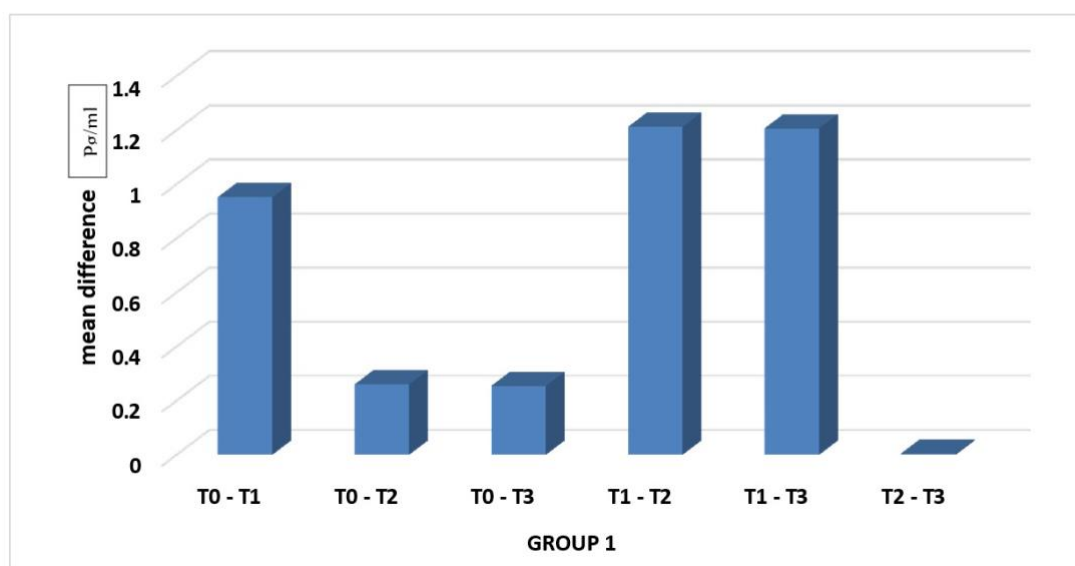
BAR GRAPH 3-INTRAGROUP COMPARISON OF IL6 FOR GROUP II AT DIFFERENT TIME INTERVALS.

The trend for levels of RANKL in Group I was $T2(23.207 \pm 2.454 \text{ pg/ml}) > T3(23.202 \pm 2.367 \text{ pg/ml}) > T0(22.947 \pm 1.838 \text{ pg/ml}) > T1(21.995 \pm 1.780 \text{ pg/ml})$, whereas for Group II trend was $T1(25.988 \pm 5.144 \text{ pg/ml}) > T0(24.926 \pm 5.648 \text{ pg/ml}) > T2(24.844 \pm 5.300 \text{ pg/ml}) > T3(23.961 \pm 3.070 \text{ pg/ml})$. (Bargraph-4)

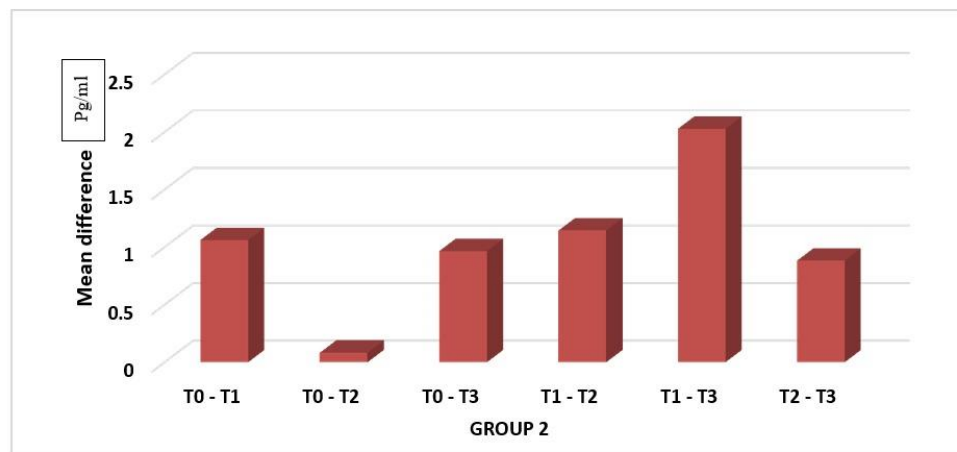


BARGRAPH 4- MEAN VALUES OF RANKL(pg/ml) FOR GROUP I AND GROUP II

On intragroup comparison of levels of RANKL, statistically significant difference was not noted between any time interval for Group I as well as for Group II. (Bargraph 5,6)

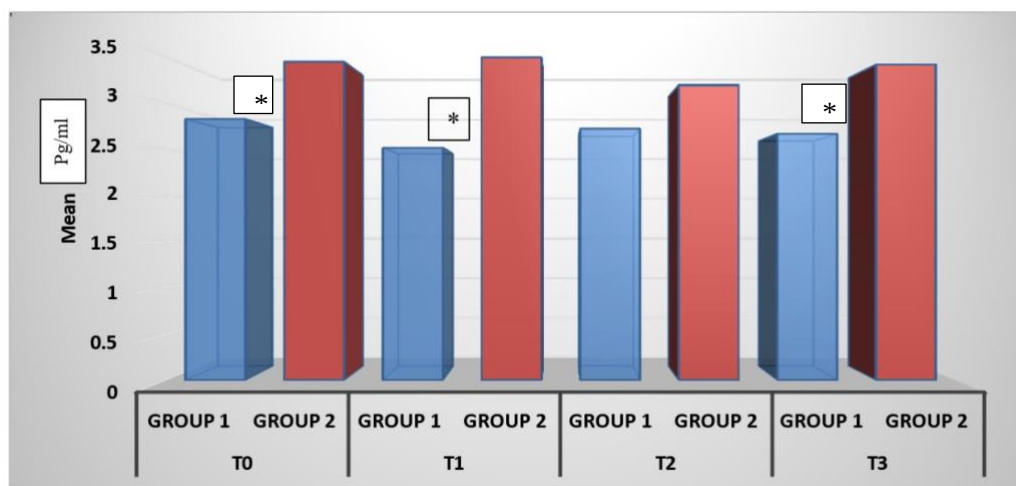


BAR GRAPH 5- INTRAGROUP COMPARISON OF RANKL FOR GROUP I AT DIFFERENT TIME INTERVALS.

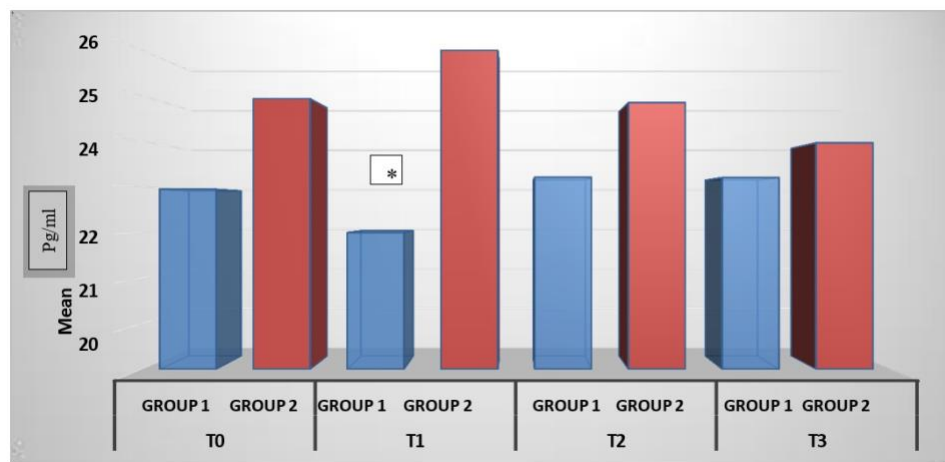


BAR GRAPH 6- INTRAGROUP COMPARISON OF RANKL FOR GROUP II AT DIFFERENT TIME INTERVALS.

On intergroup comparison it was seen that levels of both the biomarkers IL-6 and RANKL were higher in Group II than Group I at all time that is T0 ,T1,T2,T3 however difference was statistically significant only at T0,T1 &T3 for IL-6 and at T1 for RANKL.(Bargraph-7,8). Peak levels of RANKL were attained later than IL-6 in Group I suggestive of the fact that IL-6 is a initial cytokine which modulate the release of RANKL to continue with osteoclastogenesis during orthodontic tooth movement.(Bargraph 7,8)

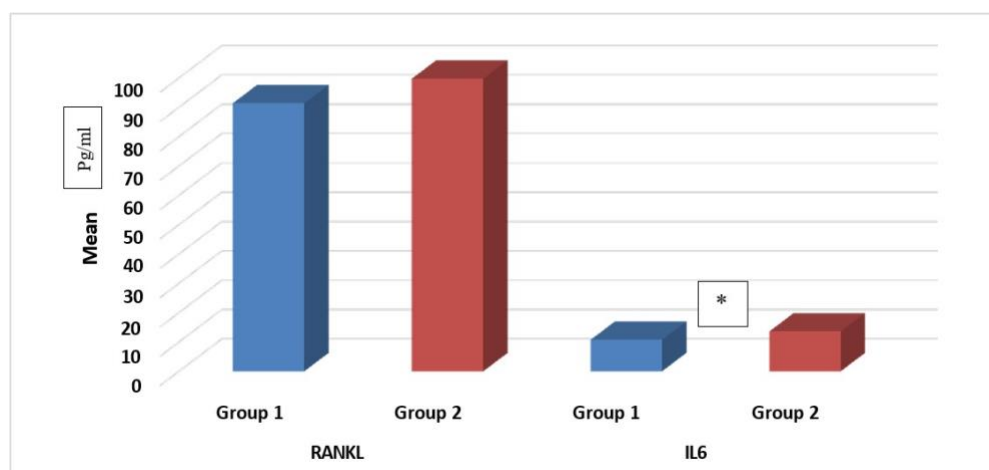


BARGRAPH 7- INTERGROUP COMPARISON OF IL-6 BETWEEN GROUP I & GROUP II AT T0 ,T1 ,T2, T3.



BARGRAPH 8- INTERGROUP COMPARISON OF RANKL BETWEEN GROUP I & GROUP II AT T0,

On overall comparison of IL-6 level and RANKL in Group I with Group II at ,mean value of both biomarkers was higher in Group II than Group I .However difference was statistically significant only for IL-6 between Group I and Group II .(Bargraph-9)



BARGRAPH 9- OVERALL COMPARISON BETWEEN GROUP I AND GROUP II FOR RANKL AND IL-6.

Though biomarker levels of Group II were higher than Group I, however the values of Group II did not change significantly between consecutive time interval, that is suggestive of the fact that molars were maintained in their respective position and force of 200 gms was adequate for moving anterior segment. The reason for higher values in Group II could be that molars are always being subjected to reciprocal forces exerted in anterior segments and could be due to bends in wires or soldering of TPA/Nance to prevent anchorage loss. Also addition of inflammatory component owing to elimination of self-cleansing process, due to bands on molars, could be responsible for the higher levels of biomarker in this area.

For Group I the peak concentration of IL-6 was at T0 as anterior teeth were already consolidated by figure of 8 ligature wire at each appointment on progression to higher dimension archwire, whereas peak concentration of RANKL was at T2 in Group I, suggestive of the fact that IL-6 is an initial biomarker released on application of force that initiates inflammatory process in the concerned area, this further stimulates RANKL release that subsequently controls resorption of bone in direction of force application.

The result of present study was compared with previous studies done at different phases of orthodontic treatment like enmasse retraction of anterior teeth by **Sheikh et al⁶⁴**, during distalization of canine by **Basaran et al⁴¹**, **Jayprakash et al²⁹**, by **S. Uematsu et al³⁴**, **Tuncer et al⁵²**, **Grant M et al⁵¹**, and **Nishijima et al⁴** or during application of other forces on premolar to be extracted by **Shetty et al⁶⁹**, **Madureira et al²⁴**, **Mohammad A et al²⁵** or after placement of separators during alignment and levelling by **Barbieria G et al²³**.

Sheikh A et al⁶⁴ had evaluated level of IL-6 between control and experimental group where physical stimulation to accelerate tooth movement was applied using LASER during enmasse retraction of anteriors with active tie backs after keeping a basal 0.019*0.025 SS arch wire for 6 weeks using 150 grams of force. The activation was at an interval of 4 weeks. The mean level of IL-6 in control group could be similar Group I of our study. It followed a trend of highest levels at day 2 (651 ± 242) > at day 9 (894 ± 218) > at day 29 (586 ± 254) > then subsequently reduced at day 46 (549 ± 240) and least was found at day 60 (477 ± 224), however they did not show any statistically significant difference.

Jayprakash P. et al²⁹ found significantly elevated levels of IL-6 on moving canine distally by elastic chain exerting an initial force of 250 gms, at 24 hours 0.065 ± 0.005 pg/ μ g than at 1hr (baseline) that had IL-6 concentration as 0.030 ± 0.008 pg/ μ g. At 168 hr (7days) mean level of IL-6 $.044 \pm .007$ pg/ μ g were higher than baseline but difference was not significant. **S. Uematsu et al³⁴** found significantly elevated levels of IL-6 during separate canine retraction, by elastic chain exerting an initial force of 250 g. At 24 hr (0.063 ± 0.004 pg/ μ g) mean levels of IL-6 was significantly higher than at baseline $0.020 \pm .007$ pg/ μ g ($p=0.0441$). At 168 hr (7days) mean level of IL-6 was $.009 \pm .006$ pg/ μ g ($p=0.0147$) was significantly lesser than baseline values. **Basaran G et al⁴¹** assessed the levels of IL-6 during canine distalization on 0.016 0.022-in SS archwire using 7-mm sentalloy closed coil spring with 150 g of force and found highest values at 21days T2($2.433 \pm .399$, $p=.996$) followed by 7th day T1($2.387 \pm .594$, $p=.594$) and least was at baseline T0($2.320 \pm .528$ pg/ml, $p=.535$) i.e. just before starting canine retraction. However difference was not statistically significant at any time interval.

Shetty A et al⁶⁹ assessed level of IL-6 in a split mouth study from the premolar region just before the extraction on control side whereas on the experimental site brackets (0.022 slot MBT mechanotherapy) were bonded on premolar to be extracted after 3 days and an initial intrusive force of 100–150 g was applied using a cantilever spring on this premolar for 3 days. They found insignificant difference in mean level of IL-6 in GCF after 3rd day of force application (4.5950 ± 1.6668 in the control group and 4.3975 ± 1.5948 in the experimental group).

Madureira et al²⁴ assessed levels of IL-6, with a 0.017×0.025 -in beta-titanium alloy cantilever attached on premolars that had to be extracted and were subjected to a force of 0.980 N in the apical direction for 3 hours, 15 hours, 3 days, 12 days, or 21 days. They found that there was an increase in IL-6 levels from 15hrs to day 3 and after that there was a subsequent decrease in IL-6 level, at day 7 with mild increase till day 14 and concentration of IL-6 was levelled from day 14 to day 21.

Mohammed A et al²⁵ evaluated IL-6 in PDL of extracted 1st premolar, each tooth was assigned a time point for its extraction at 2 hours, 15th day or the 30th day after application of force. They found a significant increase on experimental teeth in the level of IL-6 on 15th day (14.91) than on 30th day (11.1) and least was at 2hrs (3.4) which was non-significant in comparison to control.

Grant M et al⁵¹ evaluated IL-6 level at pretreatment , four hours; 7 days; and 42 days after application of distalising forces to maxillary canine teeth using 9 mm nickel-titanium closed coil spring on .018SS wire and force produced was 100 g .IL-6 level was highest at T1(25.79 ± 13.91)>T2(9.71 ± 3.15)>T0(4.48 ± 1.10)T3(4.44 ± 0.86)in pg/30s, however there was statistically nonsignificant difference in levels of IL-6 with time. The difference in the trend found for level of IL-6 from the present study (T0>T2>T3>T1 for Group I) may be attributed to methodological differences.

Barbieria G et al²³evaluated RANKL level and found elevated but not statistically significant increase after the placement of separators at 24 hours (6.58 ± 7.22 pg/ul) and at 7 days (4.75 ± 7.65 pg/μl) with p =.552.

Tuncer et al⁵² evaluated and found non -significant difference in levels of RANKL with time ,On retracting the maxillary canines by sentalloy closed coil springs with a continuous force of 200gm on the segmental .016x.022SS wire, four weeks after 1st premolar extraction .The mean value was highest at 7 days (1.29 ± 1.0 pmol/l9)>at baseline(pre-treatment- $1.19 \pm .71$ pmol/l)>at 1st month($1.04 \pm .50$ pmol/l)>then at 24hrs($.96 \pm .55$ pmol/l)>least at 1 hr ($.83 \pm .30$ pmol/l).

Grant M et al⁵¹ evaluated RANKL level at four hours; 7 days; and 42 days after application of distalising forces to maxillary canine teeth using 9 mm nickel-titanium closed coil spring on .018SS wire and force produced was 100 g, there was slight increase in RANKL level within 4 hrs of force application ,after that it reduced till 7th day and then there was significant increase in RANKL level from 7th to 42 days(highest) after force application.

Nishijima et al⁴ evaluated RANKL level at 0, 1, 24, and 168 h after the retracting force was applied (canine retraction) along a 0.018 inch archwire with an elastomeric chain, that delivered an initial force of 250gm. The mean values of RANKL were significantly higher at experimental site after 24 h (219.3 ± 26.9 pg/μl) in comparison to control site (7.7 ± 5.8 pg/μl).

The fluctuation in levels of biomarker (IL-6 and RANKL)as seen in above mentioned studies was also seen in present study .However the variation in the results of the present study regarding level of biomarker at different time interval could be due to the difference in methodologies, archwires sizes, the times,appliances

(mechanotherapy), directions and magnitudes of the forces (heavy/light, interrupted/continuous) and also could be related to variations in age, sex, bone mineral density, anatomic structures or cellular activity in the PDL and alveolar bone of that particular individual.

The major limitation of present study was that other sources of cytokines such as bone, gingivae, and blood vessels, might interfere with GCF and PDL microenvironments. Also release of multiple other cytokines that can modulate the levels of selected biomarker (IL-6 & RANKL) of present study is a limitation, the variation in orthodontic treatment status of participants of present study could also alter biomarker levels. The results must be interpreted considering the wide range of biologic responses and interfering factors in both the PDL and GCF.

Despite of the limitation it could be stated that there is change in levels (high or low) of the biomarkers in GCF, in present study suggestive of biological process of bone remodeling on alteration of mechanical stress leading to orthodontic tooth movement.

The result of present study suggested that force of 200gm were light and adequate to alter biomarker in anterior segment to greater extent than in posterior segment that did not show considerable fluctuation in the levels. Excessive forces compress the roots and cause undermining resorption and hyalinisation resulting in prolonged lag phase, hence should be avoided.

On applying heavy forces, tooth movement resumes only after removal of necrotic tissue and acquiring cells from adjacent marrow spaces to initiate bone resorption. Hence light forces are always preferred in orthodontics. The underlying molecular mechanism can be understood by corroborating the results of present study to pressure tension theory. According to this theory, cellular differentiation is produced by alterations in blood flow (in which blood flow is decreased on compression site and increased at tension site). These alterations may have created changes in the chemical environment and creates difference in cellular response on tension and compression site⁵.

The collection site of GCF in present study was compression site where on application of forces, alteration of blood flow must have happened, causes change in O₂ tension, that altered levels of biomarker like IL-6 and RANKL.

The most important clinical implication of assessing biomarker level at different time interval would be to judge magnitude of forces as light and optimum for each individual and comparing to baseline levels of that individual. This would help clinically in avoiding hyalinisation or undermining resorption thereby keeping Orthodontic treatment comfortable and allowing its completion in stipulated time as per the case.

The future scope of the study would be to validate result of present study on larger sample size. Various other biomarkers can also be analysed beside Il-6 and RANKL that play an important role at various stages of tooth movement. RANKL and OPG ratio might be evaluated as it is linked to bone remodelling with RANKL increasing osteoclastic activity whereas OPG increases osteoblastic activity. Also biomarker levels during various stages of orthodontic treatment could be assessed both in growing and non-growing individuals.

Following conclusions are drawn from the present study conducted with the aim to compare proinflammatory cytokine (IL-6 & RANKL) levels between anterior and posterior segment, during enmasse retraction of anterior teeth using sliding mechanics :-

1. Application of mechanical stress during orthodontic treatment altered the level of biomarkers (IL-6 & RANKL) both in anterior and posterior segment at different time intervals.
2. On Intragroup comparison in Group I, mean levels of IL-6 decreased significantly from T0-T1 and increased significantly from T1-T2, whereas in Group II, mean levels of IL-6 increased significantly from T0-T2.
3. On Intragroup comparison for RANKL mean level did not fluctuate significantly between Group I & Group II.
4. Intragroup comparison of both biomarkers in Group II did not show significant fluctuation in consecutive time interval suggestive of the fact that 200gms of force was optimum for causing movement in anterior segment but not for posterior segment, thus maintaining posterior segment in its position.
5. Intergroup comparison revealed higher values in Group II than Group I with significant difference only at T0 T1 & T3 for IL-6 and at T1 for RANKL.

The most important clinical implication of assessing biomarker level at different time interval would be to judge magnitude of forces as light and optimum for each individual and comparing to baseline levels of that individual. This would help making orthodontic treatment comfortable for the patient and allowing its completion in stipulated time as per the case.

1. Proffit WR, Fields HW. Contemporary Orthodontics. Mosby Elsevier; 2018.
2. Ribeiro GLU, Jacob HB. Understanding the basis of space closure in orthodontics for a more efficient orthodontic treatment. Dent Press J Orthod. 2016;21:115–25
3. Rizk MZ, Mohammed H, Ismael O, Bearn DR. Effectiveness of en masse versus two-step retraction: a systematic review and meta-analysis. Prog Orthod. 2018 Jan 5;18(1):41.
4. Nishijima Y, Yamaguchi M, Kojima T, Aihara N, Nakajima R, Kasai K. Levels of RANKL and OPG in gingival crevicular fluid during orthodontic tooth movement and effect of compression force on releases from periodontal ligament cells in vitro. Orthod Craniofac Res 9, 2006; 63–70.
5. Krishnan V, Davidovitch Z. Cellular, Molecular, and Tissue-Level Reactions to Orthodontic Force. Am J Orthod Dentofacial Orthop. 2006;129:469e.1-460e.32.
6. Reiss S et al Biomarkers of orthodontic tooth movement with fixed appliances and vibration appliance therapy: a pilot study. European Journal of Orthodontics, 2020, 1–9.
7. Biomarkers and surrogate endpoints: Preferred definitions and conceptual framework. Clinical pharmacology & therapeutics: MARCH 2001 :69(3);89-95.
8. Sergievsky Gertrude H. Biomarkers: Potential Uses and Limitations. The Journal of the American Society for Experimental neurotherapeutics. 2004: Vol. 1;182-188
9. Priyanka et al., Biomarkers in Gingival Crevicular Fluid. OSR Journal of Dental and Medical Sciences (IOSR-JDMS). (Oct. 2015), Volume 14, Issue 10 ; 104-109.
10. Bhat.Sagar S. Et.al. Orthodontic Therapeutic Biomarkers in Saliva and Gingival Crevicular Fluid .Current trends in orthodontics.
11. Girish Kumar .RANK-RANKL-OPG: A current trends in orthodontic tooth

- movement and its role in accelerated orthodontics .International Journal of Applied Dental Sciences 2022; 8(2): 630-635.
12. Anand Kumar. Biomarkers in orthodontic tooth movement. J Pharm Bioall Sci 2015;7:S325-30.
 13. Gv.Veena andt. Tulika. Non-invasive methods for the assessment of biomarkers and their correlation with radiographic maturity indicators— a scoping review. GV and Tripathi Progress in Orthodontics (2021) 22:26;2-10.
 14. P.Naina ,Mohammad Jalaluddin,Abhitosh debata, Biomarkers in Dentistry.2021
 15. Biomarker Technology Platforms for Cancer Diagnoses and Therapies". Trimark Publications, LLC. July 2014. Archived from the original on 2020-07-05. Retrieved 2014-08-19
 16. G.Noha Ayman .Diagnostic potential and future directions of biomarkers in gingival crevicular fluid and saliva of periodontal diseases: Review of the current evidence. Archives of Oral Biology 87 (2018) 115–12.
 17. Naoumova J, Olofsson R, Almstahl A, Cevik-Aras H , Salivary levels of Osteoprotegerin and receptor activator of nuclear factor-kappa ligand during orthodontic tooth movement-A prospective pilot study.2022,10:1-11.
 18. Flórez-Moreno GA, Isaza-Guzmán DM, Tobón-Arroyave SI. Timerelated changes in salivary levels of the osteotropic factors sRANKL and OPG through orthodontic tooth movement. Am J Orthod Dentofacial Orthop. 2013;143:92-100.
 19. Vaghela N et al , Evaluation of salivary biomarkers during en-masse retraction of protruded teeth into recent and healed extraction sites– a Randomized Clinical Trial. All India Institute of Medical Sciences Jodhpur:1-11.
 20. Hayashi H, Tanimoto K, Hirai H, et al. Orthodontic force induces proliferation and differentiation of osteoblasts in rats. Eur J Orthod. 2004.
 21. Garlet TP, Coelho U, Repeke CE, et al. Cytokine expression pattern in compression and tension sides of the periodontal ligament during orthodontic

- tooth movement. *J Periodontol*. 2007.
22. Navarro-Palacios A, García-López E, Meza-Rios A, ArmendarizBorunda J, Sandoval-Rodríguez A. Myeloperoxidase enzymatic activity is increased in patients with different levels of dental crowding after initial orthodontic activation. *Am J Orthod Dentofacial Orthop*. 2014;146:92-97.
 23. Barbieri G, Solano P, Alarcón JA, Vernal R, Rios-Lugo J, Sanz M, Martín C. Biochemical markers of bone metabolism in gingival crevicular fluid during early orthodontic tooth movement. *Angle Orthod*. 2013 Jan;83(1):63-9.
 24. Madureira DF et al. Cytokine measurements in gingival crevicular fluid and periodontal ligament: Are they correlated? *Am J Orthod Dentofacial Orthop* 2015;148:293-301.
 25. Mohammed A et al, Assessment and Measurement of Interleukin 6 in Periodontal Ligament Tissues during Orthodontic Tooth Movement . *World Journal of Dentistry* . 2019(3-4);10(2):88-92.
 26. Basaran G, Ozer T, Kaya FA, Hamamci O. Interleukins in gingival crevicular fluid and nickel levels in serum during orthodontic treatment. *Am J Orthod Dentofacial Orthop*. 2006;130(2):e9-e14.
 27. Widayati R, Adiwirya M, Soedarsono N. Osteoprotegerin Level Differences in Orthodontic Treatment with Self-ligating and Conventional Preadjusted Brackets at Early Aligning and Leveling Phase: *World J Dent* 2018;9(1):2-7.
 28. Barhate UH, Duggal I, Mangaraj M, Sharan J, Duggal R, Jena AK. Effects of autologous leukocyte-platelet rich fibrin (L-PRF) on the rate of maxillary canine retraction and various biomarkers in gingival crevicular fluid (GCF): A split mouth randomized controlled trial. *Int Orthod*. 2022 Dec;20(4):100681.
 29. Jayaprakash PK , Basavanna JM , Grewal H , Modi P , Apawat P , Bohara. Elevated levels of Interleukin (IL)-1 β , IL-6, tumor necrosis factor- α , epidermal growth factor, and β microglobulin levels in gingival crevicular fluid during human Orthodontic tooth movement. *J Family Med Prim Care*. 2019 May; 8(5): 1602– 1606.

30. Mohammed Nahidh, Arkan M. Al Azzawi , Sajid C. Al-Badri , Arkan M. Al Azzawi, Sajid C. Al-Badri .Understanding anchorage in orthodontics.ARC Journal of Dental Science. 2019(3-4)4, 3.
31. Nazar Majeed Z, Philip K, Alabsi AM, Pushparajan S, Swaminathan D. Identification of Gingival Crevicular Fluid Sampling, Analytical Methods, and Oral Biomarkers for the Diagnosis and Monitoring of Periodontal Diseases: A Systematic Review. Dis Markers;. Epub 2016 Dec :1-23.
32. Grieve W, Johnson G, Moore R, Reinhardt R, and DuBois L. Prostaglandin E (PGE) and interleukin-I[3 (IL-lfi) levels in gingival crevicular fluid during human orthodontic tooth movement. Am J Orthod Dentofac Orthop 1994;105:369-74.
33. Lowney JJ, Norton LA, Shafer DM, Rossomando EF. Orthodontic forces increase tumor necrosis factor alpha in the human gingival sulcus. Am J Orthod Dentofacial Orthop. 1995 Nov;108(5):519-24.
34. Uematsu S, Mogi M, Deguchi T. Interleukin (IL)-6 expression during tooth movement. Arch Oral Biol. 1996;41(11):1091-1095. doi:10.1016/s0003-9969(96)00066-3.
35. Tzannetou S, Efstratiadis S, Nicolay O, Grbic J, Lamster I. Interleukin-1beta and beta-glucuronidase in gingival crevicular fluid from molars during rapid palatal expansion. Am J Orthod Dentofacial Orthop. 1999 Jun;115(6):686-96.
36. Alhashimi N, Frithiof L, Brudvik P, Bakhiet M. Orthodontic tooth movement and de novo synthesis of proinflammatory cytokines. Am J Orthod and Dentofacial Orthop2001;119(3): 307-312.
37. Perinetti G et al. Aspartate Aminotransferase Activity in Gingival Crevicular Fluid During Orthodontic Treatment. A Controlled Short-Term Longitudinal Study. *J Periodontol* 2003;74:145-152.
38. Serra E, Perinetti G, D'Attilio M, Cordella C, Paolantonio M, Festa F, Spoto

- G. Lactate dehydrogenase activity in gingival crevicular fluid during orthodontic treatment. *Am J Orthod Dentofacial Orthop.* 2003 Aug;124(2):206-11.
39. Ogasawara T, Yoshimine Y, Kiyoshima T, Kobayashi I, Matsuo K, Akamine A, Sakai H. In situ expression of RANKL, RANK, osteoprotegerin and cytokines in osteoclasts of rat periodontal tissue. *J Periodontal Res.* 2004 Feb;39(1):42-9.
40. Batra P, Kharbanda O, Duggal R, Singh N, Parkash H. Alkaline phosphatase activity in gingival crevicular fluid during canine retraction. *Orthod Craniofac Res.* 2006 Feb;9(1):44-51.
41. Basaran G, Ozer T, Kaya FA, Hamamci O. Interleukins in gingival crevicular fluid and nickel levels in serum during orthodontic treatment. *Am J Orthod Dentofacial Orthop.* 2006;130(2):e9-e14.
42. Yamaguchi M, Yoshii M, Kasai K. Relationship between substance P and interleukin-1 β in gingival crevicular fluid during orthodontic tooth movement in adults. *Eur J Orthod.* 2006 Jun;28(3):241-6.
43. Kawasaki K, Takahashi T, Yamaguchi M, Kasai K. Effects of aging on RANKL and OPG levels in gingival crevicular fluid during orthodontic tooth movement. *Orthod Craniofac Res* 9, 2006; 137–142.
44. Toygar H, Kircellib BH, Bulut S, Sezgin N, Tasdelen B .Osteoprotegerin in Gingival Crevicular Fluid under Long-Term Continuous Orthodontic Force Application. *The Angle Orthodontist* 2008.
45. Tzannetou S, Efstratiadis S, Nicolay O, Grbic J, Lamster I. Comparison of levels of inflammatory mediators IL-1 β and β G in gingival crevicular fluid from molars, premolars, and incisors during rapid palatal expansion, *Am Journal Orthod and Dentofacial Orthop* 2008,; 133(5); 699-707.
46. Hamamci N, Başaran G, Tümer C. Interleukin-6 levels in the gingival crevicular fluid during orthodontic movement: A pilot study. *Eur J*

- Orthod. 2006;28(6):517- 521.
47. Alfaqeeh, Sarah A. et al..Osteocalcin and N-telopeptides of type I collagen marker levels in gingival crevicular fluid during different stages of orthodontic tooth movement.Am J Orthod and Dentofacial Orthopedics.2011;139(6); e553 - e559
 48. Abdullah A, Wahab R & Ariffin S. Pattern of Crevicular Alkaline Phosphatase During Orthodontic Tooth Movement: Leveling and Alignment Stage. Sains Malaysiana2011: 40(10);1147–1151.
 49. Zhao N, Lin J, Kanzaki H, Ni J, Chen Z, Liang W, Liu Y. Local osteoprotegerin gene transfer inhibits relapse of orthodontic tooth movement. Am J Orthod Dentofacial Orthop. 2012 Jan;141(1):30-40.
 50. Canavarro C, Teles RP, Capelli Júnior J. Matrix metalloproteinases -1, -2, -3, -7, - 8, -12, and 13 in gingival crevicular fluid during orthodontic tooth movement: a longitudinal randomized split-mouth study. Eur J Orthod. 2013 Oct;35(5):652-8.
 51. Grant M, Wilson T, Rock P, et al. Levels of interleukin-6 and RANKL in orthodontic patients undergoing canine retraction. Am J Orthod Dentofacial Orthop. 2014;146(4):500-507.
 52. Tuncer BB, Ozmeric N, Tuncer C, et al. Levels of interleukin-1 β and RANKL in gingival crevicular fluid and their relationship with the rate of tooth movement during orthodontic treatment. Am J Orthod Dentofacial Orthop. 2011;140(6):e423-e430.
 53. Wahab R et al.Enzyme Activity Profiles and Elisa Analysis of Biomarkers from Human Saliva and Gingival Crevicular Fluid during Orthodontic Tooth Movement Using Self-Ligating bracket.OHDM 2014 June: 13 (2);193-199.
 54. Leethanakul, C., Suamphan, S., Jitpukdeebodintr, S., Thongudomporn, U. and Charoemratrote, C. Vibratory stimulation increases interleukin-1

- beta secretion during orthodontic tooth movement. March 2015; The Angle Orthodontist 86(1).
55. Louchian I et al. Salivary IL-1 β : A Biochemical Marker that Predicts Periodontal Disease in Orthodontic Treatment. REV.CHIM.(Bucharest):67(12);2479-2483.
 56. Thukral R, Mangat S, Ganguly A, Agarkar SS, Bali H, Grover S. Pentraxin-3 Levels in Gingival Crevicular Fluid during Canine Retraction with Nickel–Titanium Coil Spring and Active Tieback. J Contemp Dent Pract 2017; 18(8):710-713.
 57. Silva Andrade A, Marcon Szymanski M, Hashizume LN, Santos Mundstock K, Ferraz Goularte J, Hauber Gameiro G. Evaluation of stress biomarkers and electrolytes in saliva of patients undergoing fixed orthodontic treatment. Minerva Stomatol. 2018 Aug;67(4):172-178.
 58. Amanda, J., Widayati, R., Soedarsono, N., & Purwanegara, M. K. (2018). RANKL concentrations in early orthodontic treatment using passive self-ligating and preadjusted edgewise appliance bracket systems. *Journal of Physics: Conference Series*, 1073(4), Article 042002.
 59. Pramustika A, Soedarsono N, Krisnawati, Widayati R. Comparison of Tumor Necrosis Factor- α Concentrations in Gingival Crevicular Fluid between Self- Ligating and Preadjusted Edgewise Appliances in the Early Leveling Stage of Orthodontic Treatment. Contemp Clin Dent. 2018 Jan-Mar;9(1):92-96.
 60. Padisar P, Hashemi R, Naseh M, Nikfarjam BA, Mohammadi M. Assessment of tumor necrosis factor alpha (TNF α) and interleukin 6 level in gingival crevicular fluid during orthodontic tooth movement: a randomized split-mouth clinical trial. Electron Physician. 2018 Aug 25;10(8):7146-7154.
 61. Gujar A et al (2019) Cytokine levels in gingival crevicular fluid during

- orthodontic treatment with aligners compared to conventional labial fixed appliances: a 3-week clinical study. April 2019; *Acta odontologica Scandinavica*.
62. Srinivasan B, Chitharanjan A, Kailasam V, Lavu V, Ganapathy V. Evaluation of leptin concentration in Gingival Crevicular Fluid (GCF) during orthodontic tooth movement and its correlation to the rate of tooth movement. *J Orthod Sci*. 2019 May 23;8:6.
 63. Çevik-Aras H, Isik-Altun F, Kilic-Tok H, Naoumova J. Monitoring Salivary Levels of Interleukin 1 Beta (IL-1 β) and Vascular Endothelial Growth Factor (VEGF) for Two Years of Orthodontic Treatment: A Prospective Pilot Study. *Mediators Inflamm*. 2021 May 24;2021:9967311.
 64. Sheikh A, Amjad F, Javaid MA, et al. Effect of laser stimulation on interleukin-6 levels during orthodontic treatment: A randomized controlled trial. *Angle Orthod*. 2019;89(5):746-752.
 65. Erdur EA, Karakaslı K, Oncu E, Ozturk B, Hakkı S. Effect of injectable platelet- rich fibrin (i-PRF) on the rate of tooth movement. *Angle Orthod*. 2021 May 1;91(3):285-292.
 66. Sioustis IA, Martu MA, Aminov L, Pavel M, Cianga P, Kappenberg-Nitescu DC, Luchian I, Solomon SM, Martu S. Salivary Metalloproteinase-8 and Metalloproteinase-9 Evaluation in Patients Undergoing Fixed Orthodontic Treatment before and after Periodontal Therapy. *Int J Environ Res Public Health*. 2021 Feb 8;18(4):1583.
 67. Chelărescu S et al (2021) Evaluation of IL1 β and IL6 Gingival Crevicular Fluid Levels during the Early Phase of Orthodontic Tooth Movement in Adolescents and Young Adults. *Applied Sciences*. January 2021;11(2):521.
 68. Bhardwaj A et al .Assessment of biomarkers of orthodontic tooth movement with fixed appliances-original research: September 2022.
 69. Shetty A, Suriyanarayanan R, Sreekumar K, et al. IL-6 and TNF- α levels

- in gingival crevicular fluid during orthodontic intrusion. *Contemp Clin Dent*. 2013;4(4):402- 406.
- 70.** Alnazeh et al. Levels of Inflammatory and Bone Metabolic Markers in the Gingival Crevicular Fluid of Individuals Undergoing Fixed Orthodontic Treatment in Comparison to Those Utilizing Invisalign. *Medicina* 2023, 59, 2107.
- 71.** Cazzolla AP, Brescia V, Lovero R, Fontana A, Giustino A, Dioguardi M, Di Comite MS, Di Serio F, Ciavarella D, Crincoli V. Evaluation of Biomarkers of Bone Metabolism on Salivary Matrix in the Remodeling of Periodontal Tissue during Orthodontic Treatment. *Dent J (Basel)*. 2024 Jul 9;12(7):209.

Space closure after extraction can be accomplished either by frictionless/loop mechanics or by frictional/sliding mechanics. Sliding mechanics uses lighter forces, is less technique-sensitive, and is more comfortable for the patient. In sliding mechanics, retraction of anterior teeth can be done either en masse or in two steps, where the canine is retracted initially, followed by the remaining four incisors. Two-step retraction is generally performed in maximum anchorage cases but prolongs overall treatment time and creates unesthetic spaces distal to the lateral incisor after canine retraction. This limitation is overcome during en masse retraction, which is routinely used in Orthodontics and was selected in the present study for space closure.

The mechanical stress arising as a result of force application used for any orthodontic tooth movement (OTM) changes periodontal vascularity and induces changes at cellular and molecular level in the PDL to form biologically active substances termed as signalling molecules or biomarkers, which are responsible for modelling and remodelling of alveolar bone (e.g. IL-1 β , IL-1, IL-6, RANKL, TNF- α , MMP, OPG, Sclerostin). The fluctuation of these biomarkers are a part of intricate process of OTM at molecular level.

In sliding mechanics there is reciprocal movement of anterior and posterior segment, thereby altering the level of biomarkers in both the segments irrespective of method of force application. Movement of posterior segment can be controlled by application of differential force and by anchorage reinforcement, hence level of biomarker might be different in anterior and posterior segment. Thus in the present study, biomarkers present in GCF were evaluated both in anterior and posterior segment during en masse retraction of anterior teeth.

IL-6 and RANKL were chosen for the study. IL-6 is a multifunctional cytokine that promotes activation of RANKL cascade in initial stages of force application. RANKL is a proinflammatory cytokine produced by osteoblasts and it is activated by lymphocyte T as a regulating factor. RANKL activates its specific receptor (RANK), which is located on the surface of the osteoclast and induces osteoclast maturity, resulting in resorption of the alveolar bone, if it binds with OPG resulting in bone deposition. Hence we have chosen IL-6 and RANKL for the present study.

The present clinical study was conducted in the Department of Orthodontics and

Dentofacial Orthopaedics, Babu Banarasi Das College of Dental Sciences, BBDU Lucknow, in collaboration with department of Biochemistry, King George's Medical University, Lucknow, with the aim to evaluate and compare proinflammatory cytokine (IL-6 & RANKL) levels between anterior and posterior segment, during enmasse retraction of anterior teeth .

Samples for the study included 15 patients (age range of 18-25 yrs) with clinical presentation of class I Bi-maxillary protrusion or Class II Div I Malocclusion who required fixed orthodontic treatment with extraction of all 1st premolar teeth. In all the patients 1st Premolar extraction was done and fixed orthodontic treatment was initiated. Initial alignment and levelling was done by following sequential NiTi arch wire insertion as per case requirement till .019"x.025" inch Stainless steel can be placed passively . Enmasse retraction of anterior teeth was initiated on .019x.025SS wire using Niti closed coil spring extended from crimpable hook placed between Lateral incisor and Canine to the hook of buccal tube on maxillary 1st Molar band and force level was kept at 200 gm using dontrix gauge.

GCF was collected at different time intervals -T0 (Baseline) immediately after applying the retraction force; T1- (after 24 hrs) ; T2- (after 20 days) ; T3 -(after 2 months), from gingival sulcus. Site of GCF collection were grouped as Group I that included distal gingival sulcus of maxillary Canine and Group II that included mesial gingival sulcus of maxillary first molar using absorbent points following standard protocol. These absorbent points soaked with GCF were placed in Eppendorf tube containing 250ul phosphate buffered saline transported to the laboratory at department of Biochemistry, KGMU, Lucknow for its storage at – 80 °C temperature till further analysis. Level of biomarkers IL-6 & RANKL was assessed using ELISA test . After collection of all samples of study, samples were centrifuged at laboratory, the paper points were removed and supernatants were transferred into a new Eppendorf tube and kept in incubator.

After elution, GCF samples were analyzed for IL-6 and RANKL levels. Reagents were prepared by bringing the kit out 20 minutes before use to room temperature (18-25°C) and preparation was done as per manufacturers instruction. The ELISA analyser was then turned on and the assay procedure was followed as per protocol. The concentration of biomarker was calculated from optical density of samples to standard curve as

obtained for each ELISA kit. Mean level of IL-6 and RANKL was obtained as pg/ml. Data was tabulated and adequate statistical comparison was made. Following conclusion can be drawn from the results of the present study: -

1. Application of mechanical stress during orthodontic treatment altered the level of biomarkers (IL-6 & RANKL) both in anterior and posterior segment at different time intervals.
2. On Intragroup comparison in Group I, mean levels of IL-6 decreased significantly from T0-T1 and increased significantly from T1-T2, whereas in Group II, mean levels of IL-6 increased significantly from T0-T2.
3. On Intragroup comparison for RANKL mean level did not fluctuate significantly between Group I & Group II.
4. Intragroup comparison of both biomarkers in Group II did not show significant fluctuation in consecutive time interval suggestive of the fact that 200gms of force was optimum for causing movement in anterior segment but not for posterior segment, thus maintaining posterior segment in its position.
5. Intergroup comparison revealed higher values in Group II than Group I with significant difference only at T0 T1 & T3 for IL-6 and at T1 for RANKL.

The most important clinical implication of assessing biomarker level at different time interval would be to judge magnitude of forces as light and optimum for each individual and comparing to baseline levels of that individual. This would help making orthodontic treatment comfortable for the patient and allowing its completion in stipulated time as per the case.

ANNEXURE-I

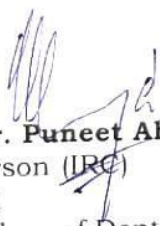


BABU BANARASI DAS COLLEGE OF DENTAL SCIENCES
(Faculty of BBD University, Lucknow)

INSTITUTIONAL RESEARCH COMMITTEE APPROVAL

The project titled **“Comparison of proinflammatory cytokines levels between anterior and posterior unit, during Enmasse retraction of anterior teeth.”** submitted by **Dr. NIDHI TIWARI** Postgraduate student in the **Department of Orthodontics and DentofacialOrthopaedics** for the Thesis Dissertation as part of MDS Curriculum for the academic year 2022-2025 with the accompanying proforma was reviewed by the Institutional Research Committee in its meeting held on **16th October, 2023** at BBDCODS.

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


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


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

15/10/24

ANNEXURE-II


BBD UNIVERSITY

BABU BANARASI DAS UNIVERSITY
BBD City, Faizabad Road, Lucknow – 226028 (INDIA)

Dr. Lakshmi Bala
Professor and Head, Deptt of Biochemistry and
Member-Secretary, Institutional Ethics Committee (IEC) of BBD University, Lucknow

Communication of the Decision of the VIIIth Institutional Ethics Committee Meeting

IEC Code: 33 BBDU/MDS/33/2024

Date: 07/03/2024

Title of the Project: Comparison of proinflammatory cytokines levels between anterior and posterior unit, during Enmasse retraction of anterior teeth.

Principal Investigator: Dr. NIDHI TIWARI **Department:** Department of Orthodontics and Dentofacial Orthopaedics

Name and Address of the Institution: BBD University, Lucknow
Type of Submission: New, MDS Dissertation proposal

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

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

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

ANNEXURE-II

Following comments were suggested and communicated.

Comments:

1. Hindi PID – more appropriate translation in Hindi is required for 'non-invasive'.

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

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

Lakshmi Bala
07/03/24
(Dr. Lakshmi Bala)

Member-Secretary IEC
BBD University
Ethics Cell (Vth Floor, BBDCODS)
ethics@bbdu.ac.in

Dr. LAKSHMI BALA
Member-Secretary
Institutional Ethics Committee
BBD University, Lucknow-28

ANNEXURE-III

To,
Prof. Dr. Kalpana Singh
KGMU
Lucknow.

Subject: Request to be Co-guide for thesis.

Respected Ma'am,

I, Dr Nidhi Tiwari, MDS student, from the Department of Orthodontics and Dentofacial Orthopaedics, BBD is planning to conduct a study titled **"Comparison of proinflammatory cytokines levels between anterior and posterior unit, during Enmasse retraction of anterior teeth"**, for which collaboration from your department is needed.

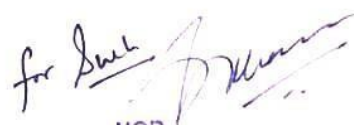
I wish and request you to kindly be my Co-guide for the above study.

I will be highly obliged for the same.

Thanking you,

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


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


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

ANNEXURE-IV

**Babu Banarasi Das College of Dental Sciences
(Babu Banarasi Das University)**

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

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

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

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

The Information

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

1. Study Title

Concentration of biomarkers in GCF during movement of forwardly placed front teeth inside.

2. Invitation Paragraph

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

3. What is the purpose of the study?

To evaluate and Compare the level of biomarker (RANKL and IL6) in GCF collected from anterior and posterior segment during enmasse retraction of anterior teeth using sliding mechanics at different time intervals.

4. Why have I been chosen?

As you fulfill the criteria needed for this study .

5. Do I have to take part?

Taking part in the research is entirely voluntary. States:

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

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

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

7. What do I have to do?

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

What is the procedure that is being tested?

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

8. What are the interventions for the study?

Not an intervention study.

9. What are the side effects of taking part?

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

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

No Risk .

11. What are the possible benefits of taking part?

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

12. What if new information becomes available?

—Sometimes during the course of a research project, new information becomes available about the research being studied. If this happens, your researcher will tell you about it and discuss with you whether you want to

continue in the study. If you decide to withdraw, your researcher/investigator will make arrangements for your withdrawal. If you decide to continue in the study, you may be asked to sign an updated consent form.

13. What happens when the research study stops?

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

14. What if something goes wrong?

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

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

–Yes

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

The results will be used to compare the rate of tooth movement of both anterior and posterior tooth.

17. Who is organizing the research?

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

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

yes

19. Who has reviewed the study?

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

20. Contact for further information

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

Dr Nidhi Tiwari

Department of Orthodontics and Dentofacial Orthopaedics Babu Banarasi

College of Dental Sciences.

Lucknow-227105 Mob-

9514621351

Dr. Shantanu Khattri (HOD)

Department of Orthodontics and Dentofacial Orthopaedics Babu Banarasi

College of Dental Sciences.

Lucknow-227105 Mob-

9936424084

Dr. Snehlata Verma(Reader)

Department of Orthodontics and Dentofacial Orthopaedics Babu Banarasi

College of Dental Sciences.

Lucknow-227105 Mob-

8960943326

Name of the PI, Address, e-mail address, Telephone Numbers and name, Member Secretary of Ethics Committee of the institution (D r . L a k s h m i B a l a, Member Secretary, bbdcods.iec@gmail.com, ethics@bbdu.ac.in) with address, e- mail address with telephone numbers 05226191091 (ext. no. 1291).

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

The patient information sheet should be dated

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

Signature of PI.....

Name.....

Date.....

ANNEXURE-V

Babu Banarasi Das College of Dental Sciences

(Babu Banarasi Das University, Lucknow)

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

प्रतिभागी के लिए सूचना पत्र

1. अध्ययन शीर्षक

अंदर की ओर आगे की ओर रखे गए दांतों की गति के दौरान जीसीएफ में बायोमार्कर की सांद्रता

2. निमंत्रण अनुच्छेद

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

3. अध्ययन का उद्देश्य क्या है ?

अलग-अलग समय अंतराल पर स्लाइडिंग यांत्रिकी का उपयोग करके पूर्वकाल के दांतों के सामूहिक प्रत्यावर्तन के दौरान पूर्वकाल और पीछे के खंड से एकत्र किए गए जीसीएफ में बायोमार्कर (आरएएनसीएल और आईएल 6) के स्तर का मूल्यांकन और तुलना करना।

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

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

5. क्या इसमें मुझे भाग लेना चाहिए ?

अध्ययन का विवरण जानने के बाद यह आपका निर्णय होगा।

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

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

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

7. मुझे क्या करना है?

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

8. किस प्रक्रिया का अध्ययन किया जा रहा है?

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

9. इस शोध में कौन से हस्तक्षेप दिए जाएंगे?

हस्तक्षेप अध्ययन नहीं.

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

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

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

कोई जोखिम नहीं होगा।

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

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

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

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

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

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

15. क्या होगा अगर कुछ गलत हो जाता है?

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

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

हां ।

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

परिणाम आएंगे इसका उपयोग आगे और पीछे दोनों दांतों की गति की दर की तुलना करने के लिए किया जा सकता है

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

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

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

हां।

20. अध्ययन की समीक्षा किसने की है?

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

21. अधिक जानकारी के लिए संपर्क करें।

डॉ निधि तिवारी

ऑर्थोडॉंटिक्स और डेंटोफेशियल ऑर्थोपेडिक्स विभाग

बाबू बनारसी कॉलेज ऑफ डेंटल साइंसेज।

लखनऊ-226028

मोब- 9514621351

एचओडी

ऑर्थोडॉंटिक्स और डेंटोफेशियल ऑर्थोपेडिक्स विभाग

बाबू बनारसी कॉलेज ऑफ डेंटल साइंसेज।

लखनऊ-226028

मोब- 9936424084

डॉ स्नेह लता वर्मा (पाठक)

ऑर्थोडॉंटिक्स और डेंटोफेशियल ऑर्थोपेडिक्स विभाग

बाबू बनारसी कॉलेज ऑफ डेंटल साइंसेज।

लखनऊ-226028

मोब- 8960943326

bbdcods.iec@gmail.com

पीआईकाहस्ताक्षर

नाम

दिनांक.....

पीआई का नाम, पता, ई-मेल पता, टेलीफोन नंबर और नाम, संस्था की आचार समिति के सदस्य सचिव (डॉ. एल. ए.के. एसएचएम आई बी एल ए, सदस्य सचिव, बीबीडीकोड्स।

iec@gmail.com, नैतिकता @bbdu.ac.in) पते के साथ, ई-मेल पता टेलीफोन नंबर

05226191091 (एक्सटेंशन नंबर 1291) के साथ। अध्ययन में भाग लेने के लिए अपने मरीज

को धन्यवाद देना याद रखें! मरीज की सूचना पत्रक होना चाहिए दिनांकप्रतिभागी सूचना

दस्तावेज़ में कहा जाना चाहिए कि प्रतिभागी को सूचना पत्र और हस्ताक्षरित सहमति प्रपत्र

की एक प्रति दी जाएगी। पीआई के

हस्ताक्षर.....नाम.....

.....तारीख.....

ANNEXURE-VI

Babu Banarasi Das College of Dental Sciences

(Babu Banarasi Das University)

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

Consent Form (English)

Title of the Study

Study Number.....

Subject's Full Name.....

Date of Birth/Age

Address of the Subject.....

Phone no. and e-mail address.....

Qualification

Occupation: Student / Self Employed / Service / Housewife/

Other (Please tick as appropriate)

Annual income of the Subject.....

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

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

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

Representative:.....

Signatory's Name.....

Date

Signature of the Investigator.....

Date.....

Study Investigator's Name.....

Date.....

Signature of the witness.....

Date.....

Name of the witness.....

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

Signature/thumb impression of the subject or legally

Date.....

Acceptable representative

ANNEXURE-VII

Babu Banarasi Das College of Dental Sciences

(Babu Banarasi Das University)

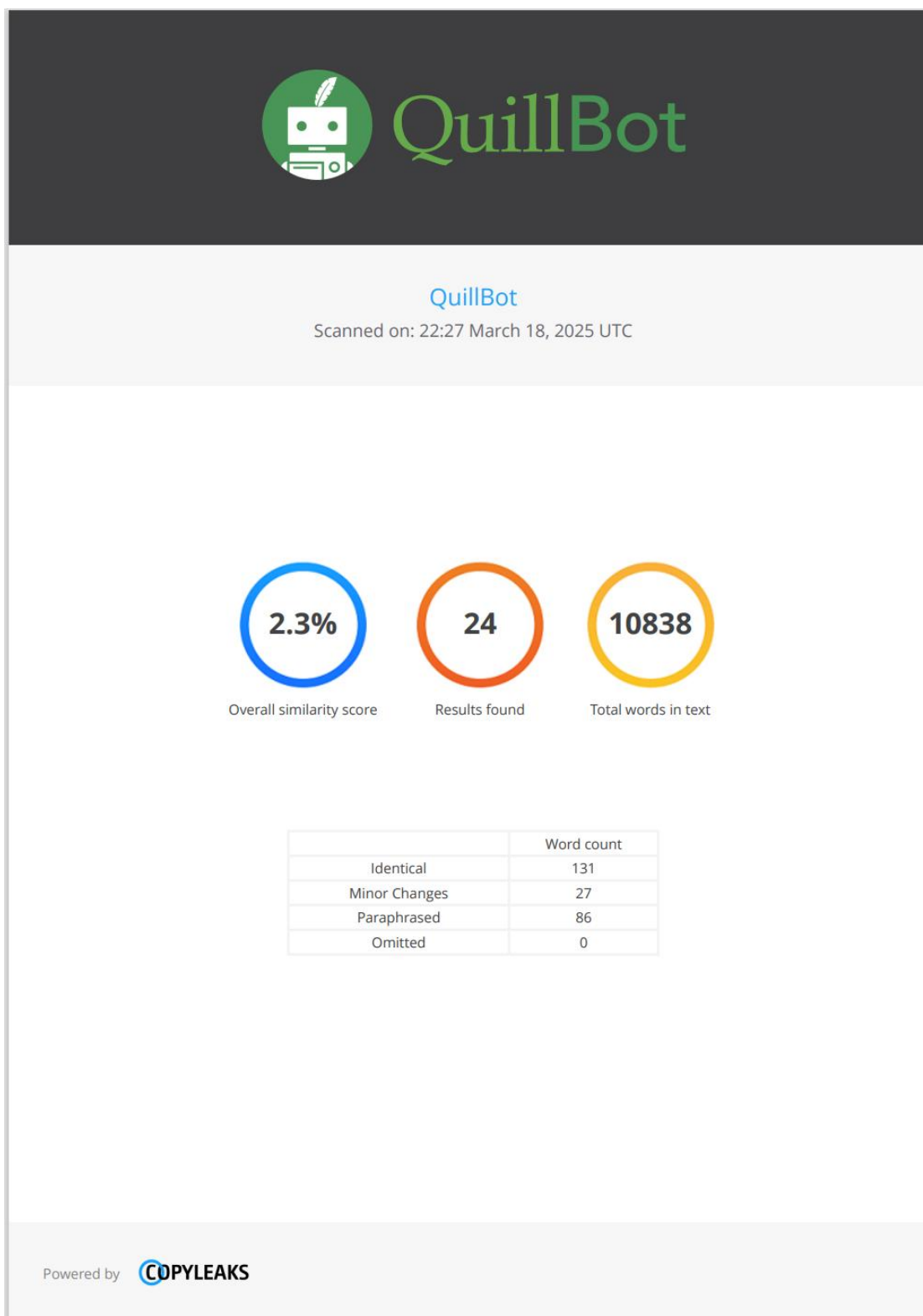
BBD City, Faizabad Road, Lucknow – 227105 (INDIA)

सहमति पत्र

अध्ययन शीर्षक.....
 अध्ययन संख्या.....
 प्रतिभागी के पूर्ण नाम.....
 जन्म तिथि / आयु.....
 प्रतिभागी का पता
 फोन नं. और ई-मेल पता
 योग्यता
 व्यवसाय: छात्र / स्व कार्यरत / सेवा / ग्रहिणी
 अन्य (उचित रूप में टिक करें)
 प्रतिभागी की वार्षिक आय
 प्रत्याशीयो के नाम और प्रतिभागी से संबंध...(परीक्षण से संबंधित मौत के मामले में मुआवजे के प्रयोजन के लिए)

- मेरी पुष्टि है कि मैंने अध्ययन हेतु सूचना पत्र दिनांक को पढ़ व समझ लिया तथा मुझे प्रश्न पुछने या मुझे अध्ययन अन्वेषक ने सभी तथ्यों को समझा दिया है तथा मुझे प्रश्न पुछने के समान अवसर प्रदान किए गये।
- मैंने यहाँ समझ लिया कि अध्ययन में मेरी भागीदारी पूर्णतः स्वैच्छिक है और किसी भी दबाव के बिना स्वतंत्र इच्छा के साथ दिया है किसी भी समय किसी भी कारण के बिना, मेरे इलाज या कानूनी अधिकारों को प्रभावित किए बिना, अध्ययन में भाग न लेने के लिए स्वतंत्र हूँ।
- मैंने यह समझ लिया है कि अध्ययन के प्रायोजक, प्रायोजक की तरफ से काम करने वाले लोग, आचार समिति और नियामक अधिकारियों को मेरे स्वास्थ्य रिकार्ड को वर्तमान अध्ययन या आगे के अध्ययन के सन्दर्भ देखने के लिए मेरी अनुमति की जरूरत नहीं है, चाहे मैंने इस अध्ययन से नाम वापस ले लिया है। हालांकि मैं यह समझता हूँ कि मेरी पहचान को किसी भी तीसरे पक्ष या प्रकाशित माध्यम में नहीं दी जायेगी।
- मैं इससे सहमत हूँ कि कोई भी डेटा या परिणाम जो इस अध्ययन से प्राप्त होता है उसका वैज्ञानिक उद्देश्य (ओं) के उपयोग के लिए मेरी तरफ से कोई प्रतिबंध नहीं है।
- भविष्य के अनुसंधान के लिए भंडारित नमूना (ऊतक/रक्त) पर अध्ययन के लिए अपनी सहमति देता हूँ।
 हाँ [] नहीं [] अनउपयुक्त []

ANNEXURE-VIII





QuillBot
 Scanned on: 22:27 March 18, 2025 UTC

Results

The results include any sources we have found in your submitted document that includes the following: identical text, minor changed text, paraphrased text.

 World Journal of Dentistry https://www.wjoud.com/abstractArticleContentBrowse/WJOD/12/10/2/15...	1%	IDENTICAL Text that is exactly the same.
 (PDF) Levels of IL-8 during tooth movement https://www.academia.edu/53703949/Levels_of_IL_8_during_tooth_movem...	1%	
 (PDF) Assessment of tumor necrosis factor alpha (TNFα) and... https://www.academia.edu/113229161/Assessment_of_tumor_necrosis_fac...	1%	MINOR CHANGES Text that is nearly identical, yet a different form of the word is used. (i.e 'slow' becomes 'slowly')
 (PDF) Evaluation of the Serum Level of Interleukin-6 in Patie... https://www.academia.edu/65731078/Evaluation_of_the_Serum_Level_of_I...	1%	
 World Journal of Dentistry https://www.jaypeedigital.com/toc/WJOD/10/2	1%	PARAPHRASED Text that has similar meaning, yet different words are used to convey the same message.
 Interleukins 2, 6, and 8 levels in human gingival sulcus duri... https://www.researchgate.net/publication/6936911_Interleukins_2_6_and_...	1%	
 Elevated levels of Interleukin (IL)-1β, IL-6, tumor necrosis fa... https://pmc.ncbi.nlm.nih.gov/articles/PMC6559102/	1%	
 jfmmpc.jfmmpc_204_19 https://journals.lww.com/jfmmpc/Fulltext/2019/08050/Elevated_levels_of_Int...	1%	

Unsure about your report?

The results have been found after comparing your submitted text to online sources, open databases and the Copyleaks internal database. If you have any questions or concerns, please feel free to contact us atsupport@copyleaks.com

[Click here to learn more about different types of plagiarism](#)

Powered by



QuillBot

QuillBot
 Scanned on: 22:27 March 18, 2025 UTC

 Journal of Family Medicine and Primary Care https://journals.lww.com/jfmpc/fulltext/2019/08050/elevated_levels_of_int...elevated_levels_of_interleukin__il__1__il_6,.17.aspx	1%
 IL-1β and compressive forces lead to a significant induction ... https://www.researchgate.net/publication/230810956_IL-1b_and_compres...	1%
 3.3.3-Upload-2-Publications-year-wise-during-the-last-five-y... https://sids.ac.in/wp-content/uploads/2023/01/3.3.3-Upload-2-Publication...3.3.3-Upload-2-Publications-year-wise-during-the-last-five-years.pdf	1%
 Cytokine levels in crevicular fluid are less responsive to ort... https://www.researchgate.net/publication/227950949_Cytokine_levels_in_c...	1%
 Induction of cytokines, MMP9, TIMPs, RANKL and OPG durin... https://www.researchgate.net/publication/230871215_Induction_of_cytoki...	1%
 Mechanisms of Osteoclastogenesis in Orthodontic Tooth Mo... https://pmc.ncbi.nlm.nih.gov/articles/PMC10721376/	1%
 Pharmacogenetics of Treatment Response in Psoriatic Arthr... https://link.springer.com/article/10.1007/s11926-015-0518-z	1%
 ES2535723T3 - Methods for determining the patient's respo... https://patents.google.com/patent/ES2535723T3/en	1%

Powered by



QuillBot

QuillBot
 Scanned on: 22:27 March 18, 2025 UTC

 DDD Flashcards Quizlet https://quizlet.com/862456809/ddd-flash-cards/	1%
 Mass spectrometry techniques for qualitative and quantita... https://www.sciencedirect.com/science/article/abs/pii/S1740674904000204	1%
 Biological Marker - an overview ScienceDirect Topics https://www.sciencedirect.com/topics/pharmacology-toxicology-and-phar...	1%
 Advancements in Drug Discovery and Development Flashca... https://quizlet.com/862572576/advancements-in-drug-discovery-and-deve...	1%
 (PDF) Induction of cytokines, MMP9, TIMPs, RANKL and OPG... https://www.academia.edu/14322461/Induction_of_cytokines_MMP9_TIMP...	1%
 Effects of Matrix Metalloproteinase Inhibitors on Bone Reso... https://www.researchgate.net/publication/10597945_Effects_of_Matrix_Me...	1%
 (PDF) Salivary Levels of Opg and Rankl During Orthodontic T... https://www.academia.edu/106225530/Salivary_Levels_of_Opg_and_Rankl...	1%
 Induction of cytokines, MMP9, TIMPs, RANKL and OPG durin... https://pubmed.ncbi.nlm.nih.gov/22987319/	1%

Powered by
