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Effect of sequential Injectable Platelet Rich Fibrin (i-PRF) following Leukocyte-Platelet Rich Fibrin (L-PRF) on maxillary canine retraction: split-mouth randomized controlled trial

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ABSTRACT

Objective: To evaluate the efficacy of injectable platelet-rich fibrin (i-PRF) following autologous leukocyte platelet-rich fibrin (L-PRF) placement on retraction of maxillary canine, and RANKL and OPG expression in gingival crevicular fluid (GCF) during comprehensive orthodontic treatment.

Materials and methods: Sixteen subjects (age range, 18-25 years) requiring all 1st premolar extractions were enrolled. L-PRF plugs were placed in both maxillary 1st premolar extraction sockets. The i-PRF was injected submucosally after 4-weeks of L-PRF placement on the experimental sides. Canine retraction was initiated immediately after 1st premolars extraction and L-PRF placement using NiTi closed coil springs. Amount of canine retraction was evaluated from the study models prepared immediately before the 1st premolars extraction and 4-weeks, 8-weeks and 12-weeks after the start of canine retraction. The RANKL and OPG levels in GCF were measured immediately before the 1st premolars extraction and at 4-weeks, 6-weeks, 8-weeks, 10-weeks and 12-weeks after the beginning of canine retraction.

Results: Over 12-weeks period, significantly greater amount of canine retraction was observed on experimental (4.77 ± 0.35 mm) sides compared to control (3.6 ± 0.48 mm) ($P < 0.001$). On experimental sides, a significant increase in the RANKL ($P < 0.001$) and decrease in the OPG levels ($P < 0.001$) were observed. In control sides, there was a negative, and in experimental sides, a positive correlation between the amount of canine retraction and RANKL and OPG levels in the GCF.

Conclusion: Injection of i-PRF after 4-weeks of L-PRF application significantly enhanced the amount of canine movement and modulated bone metabolism by increasing RANKL and decreasing OPG levels in GCF.

1. Introduction

Orthodontic treatment often requires 18-24 months to achieve desired outcomes. Prolonged treatment duration increases the risks of root resorption, enamel decalcification, and patient non-compliance. The application of blood-derived products to accelerate tooth movement during orthodontic treatment is gaining attention.[1–7] Leukocyte-Platelet Rich Fibrin (L-PRF) and Injectable-Platelet Rich Fibrin (i-PRF) are autologous blood-derived products rich in growth factors, which promote tissue healing and bone remodelling. Cytokines released from leukocytes in L-PRF plugs play a critical role in cell signalling and bone remodelling.[8,9] Bone-resorbing osteoclasts are regulated by the relative ratio of the differentiation factor, receptor activator NF-kappa B ligand (RANKL), and its decoy receptor, Osteoprotegerin (OPG).[10] The biomarkers are readily expressed in the gingival crevicular fluid (GCF) and are used to correlate with the active OTM.

L-PRF, a second-generation platelet concentrate, has been shown to enhance bone remodelling and accelerate OTM.[3,4,6,7] Injectable-PRF, a liquid-phase derivative, delivers growth factors directly to the periodontal ligament and alveolar bone and has been shown to shorten orthodontic treatment duration by increasing the rate of OTM.[11] However, the combined effects of L-PRF and i-PRF on OTM and their impact on bone metabolism biomarkers remain unexplored. Thus, the present

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study was designed to assess the effects of i-PRF injection following placement of L-PRF on maxillary canine retraction and the RANKL and OPG expression in the GCF during comprehensive orthodontic treatment.

2. Materials and Methods

2.1. Study design

Single-center, split-mouth randomized controlled trial with 1:1 allocation.

2.2. Patients, eligibility, and ethical approval

Institutional Ethics Committee approved the study protocol (IEC/AIIMS-BBSR/PG Thesis/2022-23/127), and it was registered with the Clinical Trials Registry of India (CTRI/2024/12/077699). A cohort of female subjects with malocclusion were screened and selected. Each subject had age between 18 and 25 years, Class I bimaxillary dentoalveolar protrusion malocclusion on Class 1 skeletal bases, normal growth pattern (FMA, 20°-28°), minimal spacing or crowding in the maxillary arch (≤2-3 mm), full complement of teeth in all quadrants except 3rd molars and fair oral hygiene (Probing depth ≤2 mm, Gingival index <1). Each subject required the extraction of all 1st premolars for the treatment of malocclusion.

Subjects who had received orthodontic treatment, with any known bone metabolism disorder, pregnancy, syndrome, long-term medications like steroids or NSAIDs, or were under treatment for any systemic problems were excluded. Participants were also excluded if they had a known blood disorder, limited manual dexterity, or failed to maintain adequate oral hygiene throughout the study. All eligible subjects provided a written informed consent following a thorough explanation of the study protocol.

2.3. Sample size

Assuming the mean difference of 1 mm in the total amount of tooth movement between experimental and control sides, standard deviation (SD) of 0.75 mm, power 95%, alpha error of 0.05, a sample of 15 in each side was calculated using Open Epi version 3, an open-source calculator. Assuming a 20% drop in follow-up, 18 subjects were enrolled, each contributing one control and one experimental site.

2.4. Randomization

Computer random number generator software (Microsoft Excel, Microsoft, Washington, USA) was used for randomization. Allocation concealment was not possible due to the split-mouth study design. The CONSORT flowchart (Fig. 1) details the participant flow, including screening, randomization, and allocation.

2.5. Blinding

The outcome assessor was blinded, as it was impossible to blind the subject and the treatment provider.

2.6. Treatment sequence

The same investigator (DB) provided treatment to each subject. Anchorage was saved by involving the second molars in the mechanics. A standard edgewise (slot, 0.018″) (American Orthodontics, WI, USA) appliance was used in each subject. Maxillary arch was aligned and levelled by sequentially ligating the 0.014″ nickel-titanium (NiTi) followed by 0.014″, 0.016″, and 0.016″ × 0.022″ stainless steel (SS) archwires. Following leveling, extraction of 1st premolars was done atraumatically under local anesthesia [Lignocaine hydrochloride (2%) with adrenaline bitartrate (1:80,000)].

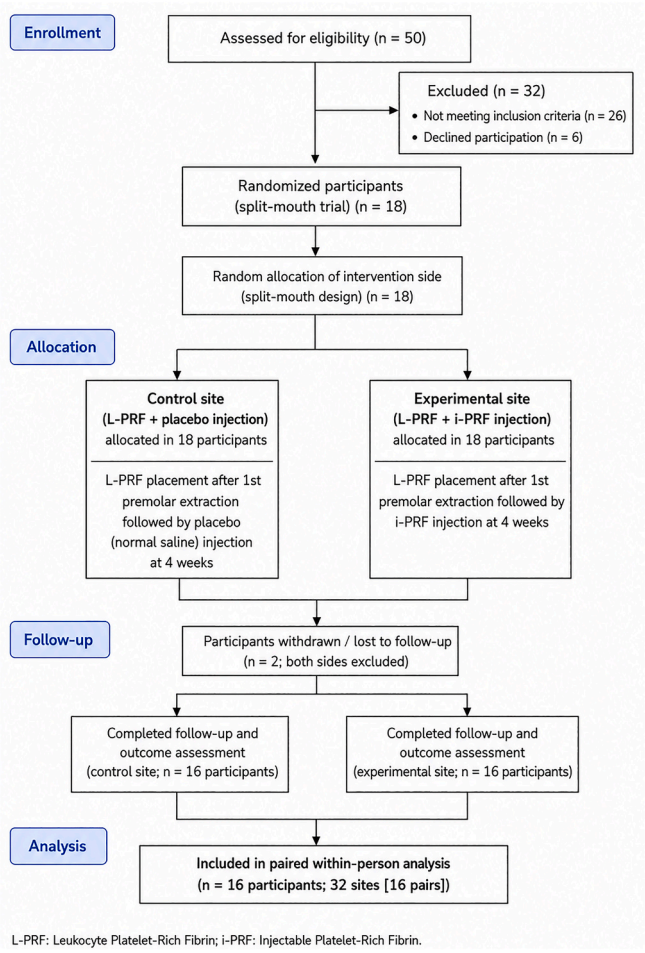

Figure-1. CONSORT flowchart.

2.7. L-PRF preparation and placement

Choukroun et al. [2] guidelines were followed for L-PRF plug preparation. Of 12 ml of venous blood, 2 ml was sent for the evaluation of blood components, and the remaining 10 ml was put in a centrifugation tube and centrifuged in the centrifuge machine (Electra Lab Centrifuge, EURO Diagnostic Systems, Tamil Nadu, India) at 2700 rpm for 12-min to prepare L-PRF plugs. L-PRF plugs were placed in both the extraction sockets immediately after premolar extractions, and stabilized with a 3-0 silk suture. Acetaminophen (650 mg) tablets were prescribed for pain management.

2.8. I-PRF preparation and injection

After 4-weeks of L-PRF placement, i-PRF was injected around the experimental sides' canines. A fresh 10 ml venous blood sample was collected from each subject at 4-weeks after L-PRF placement for preparation of i-PRF. The i-PRF was prepared by centrifuging 10 ml venous blood at 2700 rpm for 3-min.[12] After 5-min of local anesthesia infiltration, approximately 0.6 ml of i-PRF was injected submucosally at each of the buccal, distal, and palatal aspects of canines. Control sides received placebo (normal saline) injections.

2.9. Canine retraction

Canine retraction was commenced on the control and experimental

sides of maxilla immediately after the extraction of 1st premolar and the placement of L-PRF plugs. Canine retraction was accomplished via sliding mechanics on a 0.016″ × 0.022″ SS wire. NiTi closed coil springs (0.9mm × 12 mm) were activated to deliver a constant force of 150g (Koden, Kozhikode, Kerala, India). The force was measured and maintained equal on both sides with the help of a Dontrix gauge (Dentmark, Ludhiana, India). Subjects were instructed to perform high-standard oral hygiene.

2.10. Evaluation of canine retraction

Study models recorded immediately before (T_0) and at 4-weeks (T_1), 8-weeks (T_2) and 12-weeks (T_3) after the initiation of canine retraction were used for the evaluation of canine retraction. The 3D model STL files were generated by Maestro 3D scanner (MDS 400, AGE Solutions, S.r.I, Pisa, Italy). Measurements were done in Dolphin Imaging 11.95 Premium software (Dolphin Imaging & Management Solutions, Patterson Inc., Chatsworth, CA, USA). A set of 2 models was superimposed, one kept fixed and the other floating. Incisive papilla and the medial end points of the third palatal rugae were used as registration points.[13] The best fit of the superimposition was made with a color map to evaluate the canine displacement.[13] The same method was used to obtain the canine displacement at various time intervals. The amount of canine tip displacement was evaluated by using the landmark plotting technique indicated on the X-axis (Fig. 2). The same was also verified in the Y and Z axes to increase plot dependability.

2.11. Biomarker analyses

GCF samples were collected immediately before (T_0) and 4-weeks ($T_{1),\ 6\text{-weeks}}$ (T_2), 8-weeks (T_3), 10-weeks (T_4), and 12-weeks (T_5) after the beginning of canine retraction. Collection of GCF was performed at the distal gingival sulcus of the canines using calibrated micropipettes (Accupipette T-10, Tarson, Kolkata, India). The sulcus was isolated, and moisture was controlled using cotton rolls and air syringe drying. About 5 μL GCF was collected as per the Griffiths guidelines.[14] Phosphate buffer solution (245 μL, pH 7.4) was added to the GCF collected and stored at −80°C until the enzyme-linked immunosorbent assay (ELISA) of RANKL and OPG was done. Quantitative detection of RANKL and OPG was performed using commercially available ELISA kits (BT lab, Shanghai, China) as per the manufacturer's instructions.

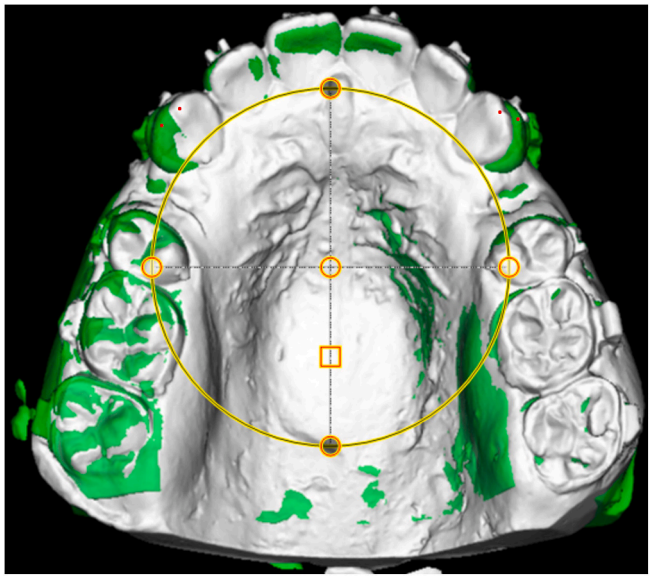

Figure-2. Evaluation of maxillary canine retraction.

2.12. Statistical analysis

Statistical package for social sciences (SPSS 28, Chicago, IL) was used. Shapiro-Wilk test, descriptive statistics, repeated measures ANOVA, and Pearson correlation tests were used. P-value <0.05 was considered a level of significance.

3. Results

3.1. Participant flow

This study involves 18 subjects with 36 extraction sockets, randomly allocated to the experimental and control sides, with 18 subjects per side. Two subjects withdrew from the study. Sixteen subjects were included for outcome assessment.

3.2. Baseline data

The mean age of the subjects at the beginning of the study was 21.73 ± 2.34years. The haematological and dentoskeletal characteristics are mentioned in Supplemental Table 1. The mean gingival index and the probing depth of the gingival sulcus were 0.37 ± 0.09 and 0.36 ± 0.08, and 1.81 ± 0.31 mm and 1.83 ± 0.32 mm at the beginning and end of the study, respectively.

3.3. Canine retraction

Table 1 summarizes the mean canine retraction for both sides. Over 12-weeks, experimental sides had significantly greater canine retraction (4.77 ± 0.35 mm) compared to the control (3.6 ± 0.48 mm) ($P < 0.001$). During the first 4-weeks, the mean canine retraction was comparable between control and experimental sides ($P = 0.999$); whereas in the next 4-weeks, the canine retraction in the experimental sides (1.77 ± 0.15 mm) was significantly more compared to control (1.06 ± 0.21 mm) ($P < 0.001$). During 8- and 12-weeks period, canine retraction was also considerably more on the experimental sides (1.51 ± 0.19 mm) compared to the control (1.09 ± 0.14 mm) ($P < 0.001$).

3.4. Expression of RANKL

The concentration of RANKL in the GCF is mentioned in Table 2. At T_0, the concentration was 48.14 ± 2.49 pg/μl in the control and 49.10 ± 2.43 pg/μl in the experimental sides ($P = 0.999$). At T_1, the concentration was 53.47 ± 2.16 pg/μl in the control and 52.90 ± 2.63 pg/μl in the experimental sides ($P = 0.999$). At T_2, the concentration increased significantly to 60.1 ± 3.4 pg/μl in the experimental sides compared to 53.5 ± 2.5 pg/μl in the control ($P < 0.001$). At T_3, T_4, and T_5, the concentration was significantly higher in the experimental sides ($P < 0.001$).

3.5. Expression of OPG

The concentration of OPG at various time points of the study period is described in Table 2. The mean concentration of OPG at T_0 and T_1 was comparable between the control and experimental sides. After 2-weeks of i-PRF injection (T_2), the concentration in the control and experimental sides was 83.75 ± 4.43 pg/μl and 79.05 ± 4.53 pg/μl, respectively ($P = 0.068$). After 4-weeks (T_3) and 6-weeks (T_4) of i-PRF injection, the concentration was significantly less in the experimental sides ($P < 0.001$). At the end of 8-weeks of i-PRF injection (T_5), the concentration was statistically lower in the experimental sides (76.33 ± 3.67 pg/μl) compared to the control (82.24 ± 3.83 pg/μl) ($P < 0.01$).

Table-1
Comparison of amount of canine retraction at various time intervals of observation in control and experimental sides.

Various time intervals of observation	Amount of canine retraction (mm)			
	Control side (Mean ± SD)	Experimental side (Mean ± SD)	Mean Difference (95% CI) (mm)	Comparison (P-value)
T_0-T_3 (12-weeks)	3.6 ± 0.48	4.77 ± 0.35	1.17 (0.86–1.48)	<0.001
T_0-T_1 (4-weeks)	1.5 ± 0.16	1.47 ± 0.22	−0.03 (−0.17–0.11)	0.999
T_1-T_2 (4-weeks)	1.06 ± 0.21	1.77 ± 0.15	0.71 (0.58–0.84)	<0.001
T_2-T_3 (4-weeks)	1.09 ± 0.14	1.51 ± 0.19	0.42 (0.30–0.54)	<0.001
T_0-T_2 (8-weeks)	2.56 ± 0.34	3.26 ± 0.28	0.70 (0.48–0.92)	<0.001
T_1-T_3 (8-weeks)	2.14 ± 0.29	3.30 ± 0.23	1.16 (0.97–1.35)	<0.001

Bonferroni correction (α value/Type I error) = 0.0045.
T_0, Prior to the start of canine retraction; T_1, 4-weeks after the commencement of canine retraction; T_2, 8-weeks after the commencement of canine retraction; T_3, 12-weeks after the commencement of canine retraction.
The reported P-values represent the exact Bonferroni-adjusted outputs generated by the statistical software.

Table-2
Mean concentration of RANKL and OPG at various time points of observation in control and experimental sides.

Various time points of observation	Concentration of RANKL			Concentration of OPG		
	Control side (Mean ± SD)(pg/µl)	Experimental side (Mean ± SD)(pg/µl)	Comparison (Control Vs. Experimental side) (P-value)	Control side (pg/µl) (Mean ± SD)	Experimental side (pg/µl) (Mean ± SD)	Comparison (Control Vs. Experimental side) (P-value)
Overall	51.8 ± 2.78	57.1 ± 5.31	<0.001	84.7 ± 5.27	80.5 ± 7.08	<0.001
T_0	48.14 ± 2.49	49.10 ± 2.43	0.999	91.88 ± 3.94	91.15 ± 4.30	0.999
T_1	53.47 ± 2.16	52.90 ± 2.63	0.999	84.31 ± 5.10	84.19 ± 4.28	0.999
T_2	53.5 ± 2.5	60.1 ± 3.4	<0.001	83.75 ± 4.43	79.05 ± 4.53	0.068
T_3	52.51 ± 2.56	60.43 ± 2.64	<0.001	82.94 ± 4.21	75.89 ± 4.82	<0.001
T_4	51.10 ± 3.50	59.83 ± 3.32	<0.001	83.33 ± 3.81	76.44 ± 5.21	<0.001
T_5	51.20 ± 1.22	59.55 ± 2.53	<0.001	82.24 ± 3.83	76.33 ± 3.67	0.003

Bonferroni correction (α value/Type I error) = 0.0045.
T_0, before start of canine retraction; T_1, 4-weeks following the start of canine retraction; T_2, 6-weeks following the start of canine retraction; T_3, 8-weeks following the start of canine retraction; T_4, 10-weeks following the start of canine retraction; T_5, 12-weeks following the start of canine retraction.
The reported P-values represent the exact Bonferroni-adjusted outputs generated by the statistical software.

3.6. RANKL to OPG ratio

The mean RANKL:OPG at various time points of observation is mentioned in Table 3. The overall concentration of RANKL:OPG in control and experimental sides was 0.61 ± 0.05 and 0.71 ± 0.11, respectively ($P < 0.001$). The mean RANKL:OPG at T_0 and T_1 in the control and experimental sides was comparable ($P = 0.999$). At T_2, T_3, T_4, and T_5, the mean RANKL:OPG was significantly higher in the experimental sides compared to the control ($P < 0.001$).

3.7. Correlation between canine retraction and RANKL and OPG concentrations

Table 4 highlights the correlation between the mean difference of canine retraction and the mean difference of RANKL and OPG concentrations. A weak, positive correlation was observed between canine retraction and RANKL levels ($R = 0.21$), and a weak, negative correlation with OPG levels. A negative correlation ($R < 0$) was found between the amount of canine retraction and change in the RANKL:OPG in the control sides, whereas a positive correlation ($R > 0$) was found in the experimental sides.

Table-3
Mean RANKL to OPG ratio at various time points of observation in control and experimental sides.

Various time intervals of observation	Control side (Mean ± SD) (pg/µl)	Experimental side (Mean ± SD) (pg/µl)	Comparison (Control Vs Experimental side) (P-value)
Overall	0.61 ± 0.05	0.71 ± 0.11	<0.001
T_0	0.53 ± 0.03	0.54 ± 0.04	0.999
T_1	0.64 ± 0.04	0.63 ± 0.05	0.999
T_2	0.64 ± 0.03	0.77 ± 0.06	<0.001
T_3	0.63 ± 0.05	0.80 ± 0.07	<0.001
T_4	0.61 ± 0.05	0.79 ± 0.06	<0.001
T_5	0.62 ± 0.04	0.78 ± 0.05	<0.001

Table-4
Correlation between amount of canine retraction and relative changes in RANKL and OPG concentrations from baseline.

Rate of canine retraction Vs change in RANKL levels from baseline to various time points of observation	Control side		Experimental side	
	R-value	P-value	R-value	P-value
T_0-T_1	−0.17	0.54	0.53	0.04
T_0-T_3	−0.42	0.11	0.34	0.20
T_0-T_5	−0.29	0.28	0.36	0.17
T_1-T_3	−0.22	0.42	0.59	0.02
T_1-T_5	−0.01	0.97	0.21	0.44
T_3-T_5	−0.08	0.77	0.24	0.37
Rate of canine retraction Vs change in OPG levels from initial to various time points of observation	Control side		Experimental side	
	R-value	P-value	R-value	P-value
T_0-T_1	−0.04	0.87	0.54	0.03
T_0-T_3	−0.27	0.32	0.48	0.06
T_0-T_5	0.26	0.32	0.26	0.33
T_1-T_3	−0.21	0.45	0.69	0.003
T_1-T_5	0.34	0.19	−0.31	0.24
T_3-T_5	−0.08	0.78	−0.43	0.10

T_0, Prior to the start of canine retraction; T_1, 4-weeks after the commencement of canine retraction; T_2, 6-weeks after the commencement of canine retraction; T_3, 8-weeks after the commencement of canine retraction; T_4, 10-weeks after the commencement of canine retraction; T_5, 12-weeks after the commencement of canine retraction.

3.8. Harms

No adverse effects or complications occurred during the trial period.

4. Discussion

Long duration is one of the significant problems of comprehensive orthodontic treatment. Many conservative and surgical methods have been introduced to decrease the treatment duration. At present, blood concentrates are being used to accelerate the OTM.[1–7,15] Of various blood concentrates, L-PRF and i-PRF, which are purely autologous, are frequently used to expedite the OTM. The OTM is faster in younger individuals.[16] To avoid age-related bias, all subjects in the age range of 18 to 25 years were considered. Also, to avoid the effect of sex hormones on tooth movement, only female participants were considered in the present study. As the rate of tooth movement varies according to the jaws,[17] only the maxillary arch was considered. Hematological disorders can affect the quality of L-PRF and i-PRF, therefore, complete blood count (CBC) of all participants was performed. All the participants in this study had normal CBC at the time of L-PRF placement.

The results of the current study showed faster canine retraction following the injection of i-PRF after 4-weeks of L-PRF plug placement. Further, the average total canine retraction on the experimental sides was 4.77 mm, whereas it was 3.6 mm on the control sides. The fibrin matrix of L-PRF enriched with leukocytes and platelets, promotes sustained release of growth factors and cytokines, creating an environment conducive to bone remodelling.[6] Also, the i-PRF retains a higher concentration of platelets, leukocytes, and growth factors, enhancing localized cellular activity.[18] Thus, the faster movement of the canines on experimental sides could be due to the modulation of the biological activities by delivering a concentrated dose of biologically active factors following the injection of i-PRF.[18] The liquid nature of i-PRF allows for its precise infiltration into the periodontal ligament and surrounding alveolar bone, amplifying the biological response initiated by orthodontic forces and L-PRF. The sequential application of L-PRF and i-PRF creates a biologic synergy, where L-PRF ensures sustained growth factor release, and i-PRF provides immediate and localized stimulation, resulting in a significantly higher rate of tooth movement.

Gingival crevicular fluid is an appropriate tool to assess the cytokine activities in the periodontal ligament (PDL) during OTM. The RANKL and OPG axis is critical in regulating osteoclastogenesis and bone remodelling. Orthodontic forces induce an imbalance in this pathway, with increased RANKL expression promoting osteoclast activity on the compression side of the PDL. The release of various growth factors from L-PRF and i-PRF amplifies this response by further elevating RANKL levels while suppressing OPG expression, thereby accelerating bone turnover and facilitating faster tooth movement.[19]

The present study revealed increased RANKL and decreased levels of OPG in the experimental and control sides, respectively. This could be due to the amplification of the RANKL and OPG axis following the application of L-PRF and i-PRF. Nishijima et al. highlighted the pivotal role of RANKL in bone resorption and its direct correlation with the rate of tooth movement. [19] Thus, RANKL can be considered as a validated biomarker for the assessment of the biological efficacy of orthodontic intervention.[19] The concentration of OPG in control and experimental sides was comparable after 4-weeks of L-PRF application. However, following the application of i-PRF, the concentration of OPG level in the GCF decreased and remained relatively constant till the end of 12-weeks. The increased level of RANKL and reduced level of OPG on the i-PRF injection sides favoured rapid bone remodelling and accelerated the canine retraction. Angel et al. also observed a similar observation.[11] The controlled increase in the RANKL/OPG ratio achieved through the application of i-PRF also ensured efficient bone turnover. This modulation of biomolecular pathways is particularly beneficial in adult patients, where slower bone remodelling and reduced cellular activity often hinder treatment progress.

Unlike traditional orthodontic forces, which rely solely on mechanical stimulation, the application of blood concentrates provides a biologically active microenvironment that enhances cellular response to orthodontic forces. This sustained activity ensures continuous bone remodelling throughout the treatment period, strengthening the tooth movement. The integration of L-PRF and i-PRF into orthodontic practice represents a paradigm shift toward biologically driven treatment strategies.

The weak correlation between biomarker levels and canine retraction may reflect the complex interplay of biological and mechanical factors in OTM. Future studies with larger sample sizes, including subjects from both the sexes and longer follow-up periods are needed to validate these findings and explore the long-term effects of blood concentrates on periodontal health and treatment stability.

The isolated effects of L-PRF and i-PRF could not be evaluated independently due to lack of true control. Future parallel-arm randomized clinical trials including untreated controls and isolated intervention groups are recommended.

5. Conclusions

The following conclusions were drawn from the present study.

1. Injection of i-PRF adjacent to the distal aspect of the canine in maxillary 1st premolar extraction cases after 4-weeks of L-PRF placement in 1st premolar extraction sockets increased the amount of canine retraction by 1.16 mm (54%) over the period of 8 weeks compared to the placement of the L-PRF only.
2. Injection of i-PRF after 4-weeks of L-PRF placements in 1st premolar extraction sockets enhanced bone metabolism by increasing RANKL and decreasing OPG concentrations in GCF compared to the L-PRF placement only.
3. A positive correlation was found between the magnitude of canine retraction and RANKL concentration, contrasted by a negative correlation with the OPG levels.
4. The degree of canine retraction showed a weak inverse relationship with the RANKL/OPG ratio.

Clinical study registration number

The study was registered with the Clinical Trials Registry of India (CTRI/2024/12/077699).

Informed consent

An informed written consent was obtained from each participant.

Ethics approval

The study was approved by the Institutional Ethical Committee (IEC/AIIMS-BBSR/PG Thesis/2022-23/127).

Data availability statement

Data are available on request from the corresponding author.

Author contribution

Damodar Bhat contributed to data extraction, statistical analysis and manuscript writing. Ashok Kumar Jena contributed to study design, data extraction, result interpretation, manuscript writing and revision and manuscript correspondence. Manaswini Mangaraj contributed to data extraction and manuscript revision. Jitendra Sharan contributed to study design and manuscript revision.

Human and animal rights statement

All procedures performed in this study involving human participants were in accordance with the ethical standards of the Institutional Ethics Committee of All India Institute of Medical Sciences, Bhubaneswar (IEC/AIIMS-BBSR/PG Thesis/2022-23/127), and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. No animal subjects were involved in this study.

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None.

Declaration of competing interest

The undersigned author declares that there is no competing interest of any author involved in the study.

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None.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jobcr.2026.101510.

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