

Original Article

Effect of leukocyte platelet-rich fibrin on the rate of canine movement – A prospective and randomized control trial

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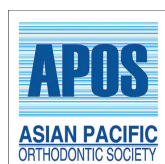
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ABSTRACT

Objectives: The present study was conducted to investigate the effects of leukocyte and platelet-rich fibrin (L-PRF) on the rate of maxillary canine retraction for a period of 5 months.

Material and Methods: A split-mouth study was conducted on 16 patients (32 extraction sockets) (Nine males and seven females; age ranging 17–25 years) with Class II Div 1 malocclusion or Class I bimaxillary protrusion, requiring therapeutic extraction of bilateral maxillary first premolars. After the initial leveling and alignment, L-PRF plugs were placed immediately in the randomly selected socket (Experimental Group) and the other side served as control for secondary healing (Control Group). This was followed by the activation of nickel-titanium closed coil springs for canine retraction. The rate of canine movement, canine rotation, tipping, root resorption, and molar movement was assessed at monthly intervals for 5 months (T0–T5). Data were collected from study models. Assessment of pain accompanying the procedure was done using a Likert scale. The trial was registered at the Clinical Trials Registry of India (REF/2022/02/051837).

Results: The study revealed that there was a significant increase in the rate of canine movement on the experimental side in the first 2 months and significant molar anchorage loss was seen only in the 1st month. There were statistically non-significant differences in canine rotation, tipping, probing depth, root resorption, and pain perception between the groups.

Conclusion: The use of L-PRF plugs in the extraction sockets enhanced the rate of canine movement in the first 2 months of treatment with less anchorage loss and no deleterious effects on periodontium.

Keywords: Leukocyte and platelet-rich fibrin plugs, Platelet concentrate, Space closure, Orthodontic treatment, Canine retraction

INTRODUCTION

The duration of orthodontic treatment is a very important concern for adult patients who want their treatment to be over as quickly as possible. Due to their busy schedules, they desire a shorter course and less chair-side time.^[1] Therefore, attempts have been made to accelerate orthodontic tooth movement (OTM). Various surgically assisted procedures have been used, but they had the disadvantage of being invasive in nature.^[2,3] On the other hand, non-surgical approaches^[2] show conflicting results of systemic complications as their side effects.

One of the many strategies believed to be helpful in increasing OTM by enhancing the production of a variety of growth factors^[4] and effectively shortening the treatment duration is platelet-

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rich fibrin (PRF), as explored for the 1st time in France by Choukroun *et al.*^[5] Platelet concentrates are divided into two major categories depending on the presence of leukocytes and fibrin: platelet-rich plasma (Pure form-PRP, and leukocyte form-PRP) and PRF (pure form-PRF, and leukocyte and platelet-rich fibrin [L-PRF]).^[6] L-PRF has demonstrated more regular growth factor release from the delicate and flexible fibrin matrix, cost effectiveness, and longer effects than PRP. There are currently less studies on humans and more animal studies on canine retraction using PRF plugs. Only six studies have been found in the literature, studying the effects of PRF on OTM and only four studies supported their acceleration effect on OTM.^[7] However, these studies were conducted only for few months and the differences in the preparation methods of L-PRF, platelet concentrates, and observation periods also led to the controversial results found in these studies. None of the studies evaluated anchorage loss and pain assessment in the patients. Therefore, this study aimed to determine the effects of L-PRF on the rate of canine movement, anchorage loss, and pain perception over a period of 5 months.

MATERIAL AND METHODS

Study design

A single-center, randomized control trial with a split-mouth design having 1:1 allocation, was employed on subjects, recruited from the Department of Orthodontics, Surendera Dental College and Hospital, Rajasthan (January 2021–July 2021). The ethical clearance was obtained from the Institution's Ethical Committee (approval no-SDCRI/IEC/2020/012) and the trial was registered at the Clinical Trials Registry of India (REF/2022/02/051837). The informed consents were obtained from the patients and/or legal guardians before recruitment. The consolidated standards of reporting trials statement was followed as a guide for the study [Figure 1].

Sample size calculation

In this study, GPOWER statistical software (Ver. 3.1 Franz Faul, Universität Kiel, Kiel, Germany), assuming a mean difference and standard deviation of 0.55, type 1 error (α) of 0.05, and type 2 error (β) of 0.1 to achieve a statistical power of 90% was used to evaluate the sample size.^[4] The resultant sample size was 15 but considering the sample attrition, it was decided to increase the sample size to 20. Four patients were lost during follow-up due to change of their residence; hence, the study was completed on 16 subjects.

Randomization, allocation, and patient selection

1:1 allocation and a simple randomization procedure of drawing lots was used to allocate the side of the maxilla for placement of L-PRF plugs (Experimental Group; $n = 16$), while the opposing

side served as the split-mouth control inducing secondary healing (Control Group; $n = 16$). The split-mouth design was conducted to prevent bias due to interpersonal variations. All the patients were asked about their dietary habits and side preference of chewing. All the patients were vegetarians and chew on both the sides, with no side preference.

PICOS criteria

- Population: The Class II division 1 or Class I bimaxillary protrusion patients requiring fixed mechanotherapy with first premolar extractions
- Intervention: L-PRF plugs on the experimental side
- Comparator: Control side with no L-PRF plug placement
- Outcome: Primary outcome: Assessment of canine movement rate; secondary outcome: rate of molar movement, assessment of canine angulation, root resorption, and pain perception
- Study design: Randomized controlled trial.

The inclusion criteria were as follows: Subjects with Class II Division 1 malocclusion or Class I bimaxillary protrusion, with relatively well-aligned arches, normodivergent growth pattern frankfort mandibular plane angle (FMA of $25^\circ \pm 5^\circ$), postpubertal as assessed by cervical vertebral maturation index stage >5 , requiring therapeutic bilateral first premolar extractions with subsequent retraction of the canine, healthy oral and systemic conditions (probing depth <3 mm, plaque index <1 mm, no bleeding on probing), and no previous history of orthodontic treatment. Patients taking medications that could interfere with OTM (non-steroidal anti-inflammatory drugs, cortisone, hormones, and anticoagulants), smoking, pregnancy, restorations, or endodontic treatments on maxillary canines, and patients with platelet disorders were excluded from the study.

Method

After taking complete pre-treatment records, all the patients were started with orthodontic treatment procedure using pre-adjusted edgewise appliance mcLaughlin, bennett, and trevisi bracket system (MBT 0.022-inch slot). Transpalatal arch, fabricated with 0.036-inch titanium molybdenum alloy (TMA), was placed for anchorage reinforcement. Leveling and alignment was started with 0.014-inch nickel-titanium (NiTi) wire and completed until 0.016 \times 0.022-inch SS wire was placed for 1 month, following which, the appointment was given for atraumatic extractions of maxillary first premolars and placement of L-PRF plugs. NiTi closed-coil springs (Ormco[®], Orange, California, USA) with a constant force of 150 gms were used to retract canines on both sides.

L-PRF preparation and placement

Using a 10 mL syringe, the whole venous blood sample was drawn from the brachial vein and placed into two sterile

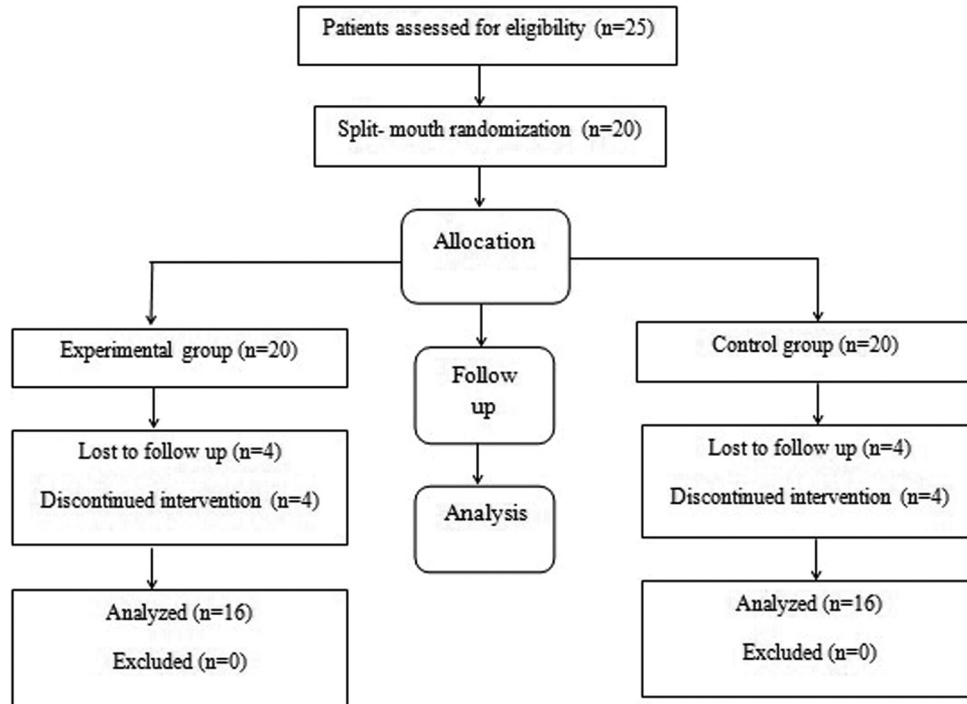


Figure 1: Consolidated standards of reporting trials flow diagram.

tubes without the use of an anticoagulant. These tubes were then immediately centrifuged at 3000 rpm (about 400 g based on our estimations) for 10 min. This caused a three-layer structure to form, with red blood cells at the bottom, cellular plasma of a straw color at the top, and platelets and a fibrin clot in the middle. The middle portion (L-PRF) was collected, 2 mm below the lower dividing line, after the upper straw-colored layer was removed, with sterile tweezers and L-PRF plugs were placed in the socket and compressed with amalgam condenser. The sockets were sutured using 4-0 Vicryl sutures. Adhering to the protocols with proper management of time period is very critical and, therefore, carefully followed in the present study to prevent dose or procedure dependent errors in the study.^[8]

Outcomes

The measurement of rate of canine retraction was our primary outcome, whereas measurements of first molar anchorage loss, canine rotation, tipping, and assessment of pain perception were counted as secondary outcomes. All the required records such as orthopantomograms (OPGs), intraoral periapical radiographs, and probing depths for maxillary canines were taken before the retraction (T0) and after the end of 5 months (T5). Questionnaires for pain assessment were given to each patient to fill out at home and bring back on the next visit. Patients were discouraged from taking analgesics; if taken in case of severe pain, then they were advised to note it down. Patients were recalled at

intervals of 21 days for 5 months (T1–T5). Measurements were made on dental casts according to the procedure described in our previous study.^[9] The procedure is as follows:

Measuring procedure

Evaluation of anteroposterior movement of canine and first molar

The amount of movement of canine and first molar was assessed using the method described by Zigler and Ingervall.^[10] Photographs of the study models were clicked by placing them vertically on a glass plate at a distance of 30 cm from the lens of the digital camera as described by Azevedo *et al.*^[11] Before taking the photographs of the study model, the cusp tip of canine, the median palatal raphae, and the rugae were marked with a pencil. A perpendicular projection of the cusp tip of the canine and the central fossae of the first molar was drawn on the median line. The distance was measured from the medial rugae point of the third palatal rugae to assess movements of the canine and first molar monthly for 5 months. All measurements were made using sliding digital calipers to the nearest 0.1 mm. Angles were measured using a protractor to the nearest 0.5°.

Evaluation of root resorption and periodontal health of the canine

The assessment was done at T0 and T5. The signs of root resorption were assessed by taking the index scores from 0 to 4

as described by Levander and Malmgren.^[12] Probing depth and attachment loss was assessed in the maxillary canine region on all the four surfaces using a UNC 15 periodontal probe.

Evaluation of rotation and mesiodistal tipping of canine

On the standardized photographs of the study models, the angle between the median raphe and the line through the mesial and distal edges of canine were measured for assessment of rotation. Angulation of canine was assessed using an OPG with a line through the orbital plane as a reference plane as described by Ursi *et al.*^[13]

Assessment of patient's perception of pain, discomfort, and satisfaction toward the procedure

A questionnaire was designed and provided to the patients for the assessment of pain and discomfort following the procedure. The questionnaire comprised six questions out of which five questions were four-point Likert scale and one question with three-point scale. Patients were asked to provide their subjective opinion about pain during eating, pain during the daytime and night times, and their feeling of swelling on the surgical side at 2 time points, T1 and T2, where T1 was after 24 h of surgical procedure and T2 was 3 days after the surgical procedure.

Error of method

To determine the errors associated with measurements, measurements were repeated at 2 weeks apart by same investigator on ten subjects. The intraclass correlation coefficient using Dahlberg and paired *t*-tests was used to assess random and systematic errors in the study, respectively.

Blinding

It was a single-blind study where the statistician was blinded as regards to the origin and grouping of data. The coinvestigator who did the measurements was also blinded about the groups. The principal investigator and patients could not be blinded. The same orthodontist provided the orthodontic treatment. The preparation and placement of L-PRF plugs was also performed by same oral surgeon for the purpose of standardization.

Statistical analysis

The findings of measurements were analyzed statistically using the Statistical Package for the Social Sciences (SPSS) software (SPSS for windows, release 7.51 Chicago, USA version 23). The Shapiro–Wilk normality test was applied to study the normality of the data. All variables except pain were found to be normally distributed; therefore, independent *t*-test was used to compare the mean differences of the two

groups for monthly and overall canine and molar movements as well as overall changes in canine rotation, angulation, root resorption, and probing depths at the end of 5 months. The chi-square test was applied to compare the pain, swelling, and discomfort scores across the two groups. Statistical significance was set at $P \leq 0.05$.

RESULTS

The moderate to high reliability was observed with intraclass correlation coefficients between 0.88 and 0.93 for all measurements. No statistically significant differences were found between the repeated measurements for any variable. The mean age of the subjects at the start of the treatment was 21.85 ± 2.45 years. The rate of canine movement, molar movement, canine tipping and rotations, probing depth of canines, and root resorption of the canines were assessed at an interval of 21 days, from T1 to T5 in 16 orthodontic patients.

Primary outcome

The total amount of canine retraction was 6.407 ± 0.336 mm on the experimental side and 5.546 ± 0.663 mm on the control side, and the difference between the two was statistically significant ($P \leq 0.05$). The greater rate of canine movement was observed only in first 2 months (T0–T1 and T1–T2) in the experimental group when compared to the control group ($P \leq 0.05$) [Table 1].

Secondary outcome

On comparison of both the groups, it was noticed that the control group displayed more anchor loss compared to the experimental group which was statistically significant only in 1st month of treatment (T0–T1) ($P \leq 0.05$), and thereafter, it was non-significant for remaining time period ($P \geq 0.05$) [Table 2].

The study revealed non-significant changes in the amount of root resorption, mean probing depth, amount of canine rotation, and tipping in both the groups during canine retraction ($P \geq 0.05$) [Tables 3 and 4].

Assessment of pain, swelling, and discomfort following the surgical intervention and overall perception of discomfort showed non-significant results. None of patient reported use of analgesics [Table 5].

DISCUSSION

Due to lack of sufficient data on long-term efficacy of L-PRF on rate on canine movement,^[7] the present was conducted to determine the effects of L-PRF on rate of canine movement. Most of studies on platelet concentrates were conducted

Table 1: Comparison of rate of canine movement (mm) between the groups.

Rate of canine retraction	Experimental group Mean±SD	Control group Mean±SD	T-value	P-value	Significance
T0-T1	1.806±0.404	1.294±0.297	4.084	0.000*	Sig
T1-T2	2.184±0.297	1.875±0.331	2.779	0.009*	Sig
T2-T3	1.147±0.442	0.906±0.441	1.543	0.133	NS
T3-T4	0.531±0.329	0.528±0.413	0.022	0.982	NS
T4-T5	0.739±0.303	0.943±0.424	-1.566	0.128	NS
Total	6.407±0.336	5.546±0.663	4.633	0.000*	Sig

SD: Standard deviation, NS: Not significant, *P≤0.05: Significant

Table 2: Comparison of rate of molar movement (mm) between the groups.

Rate of molar movement (mm)	Experimental group Mean±SD	Control group Mean±SD	T-value	P-value	Significance
T0-T1	0.407±0.0719	0.473±0.0705	-2.6217	0.0136*	Sig
T1-T2	0.306±0.0609	0.321±0.0802	-0.5496	0.5797	NS
T2-T3	0.359±0.2017	0.399±0.2334	1.0318	0.4660	NS
T3-T4	0.500±0.1204	0.486±0.1250	-2.4048	0.3531	NS
T4-T5	0.239±0.3371	0.243±0.3230	0.3733	0.6613	NS
Total	1.811±0.3819	1.922±0.3824	-0.8216	0.4178	NS

SD: Standard deviation, NS: Not significant, *P≤0.05: Significant

Table 3: Comparison of canine root resorption index and mean probing depths between both the groups.

	Root resorption index		P-value	Probing depth		P-value
	Experimental Group Mean±SD	Control group Mean±SD		Experimental group Mean±SD	Control group Mean±SD	
Pre	1.25±0.433	1.188±0.390	0.681	2.219±0.655	2.328±0.629	0.37
Post	1.375±0.484	1.313±0.464	0.721	2.234±0.664	2.375±0.619	2.17

SD: Standard deviation, P≤0.05: Significant

Table 4: Comparison of mean differences in the degrees of canine rotation and canine tipping between the experimental and control group.

Canine rotation (Degrees)		P-value	Canine tipping (Degrees)		P-value
Experimental group Mean±SD	Control group Mean±SD		Experimental group Mean±SD	Control group Mean±SD	
5.063±3.614	4.938±3.733	0.926	8.969±0.760	9.000±2.179	0.959

SD: Standard deviation, P≤0.05: Significant

using PRP and injectable-PRF (i-PRF). Different doses of PRP promoted OTM, according to animal studies.^[14] Submucosal injection of PRP and i-PRF has disadvantages of pain, discomfort, swelling of the mucosa after the injection, and the potential leakage during the injection.^[15] In contrast, L-PRF has the advantages of simpler preparation and prolonged effects.^[16,17]

In our study, L-PRF plugs were used as they behave as true fibrin tissue, maintain their fibrin structure even after 7 days of placement, and slowly release more growth factors, mainly

transforming growth factor-beta (TGF-β), which has been shown to have anti-inflammatory properties, stimulate neoangiogenesis, increase proliferation of osteoblasts, and collagen synthesis, which triggers bone regeneration and accelerates tooth movement. In addition, protease enzymes, other growth factors such as vascular endothelial growth factor, platelet derived growth factor AB, and matrix proteins such as fibronectin are also released by L-PRF plugs.^[17] As stated in the PRP studies, the effects of L-PRF could also be related to the timing of release, concentration, and content of its growth factors. As the preparation of L-PRF plugs is

Table 5: Assessment of pain, swelling, and discomfort following surgical intervention using a four-point Likert scale and overall perception of discomfort for the experimental and control group.

Question	Group	Time	Scores				Chi value	P-value	Significance
			1	2	3	4			
Q1	Experimental	T1	5	9	2	0	4.3	0.222	NS
	Control		3	6	5	2			
	Experimental	T2	3	7	5	1	1.57	0.664	NS
	Control		5	6	5	0			
Q2	Experimental	T1	9	4	3	0	5.38	0.06	NS
	Control		3	5	8	0			
	Experimental	T2	3	7	5	1	0.17	0.981	NS
	Control		3	8	4	1			
Q3	Experimental	T1	4	8	4	0	0.88	0.641	NS
	Control		4	10	2	0			
	Experimental	T2	9	6	1	0	0.54	0.765	NS
	Control		8	8	1	0			
Q4	Experimental	T1	4	9	3	0	0.54	0.76	NS
	Control		3	11	2	0			
	Experimental	T2	8	7	1	0	0.133	0.98	NS
	Control		7	8	1	0			
Q5	Experimental	T1	4	8	4	0	2.39	0.301	NS
	Control		4	10	2	0			
	Experimental	T2	4	8	4	0	0.376	0.828	NS
	Control		4	10	2	0			
Q6	Experimental	T1	4	8	4	0	1.47	0.478	NS
	Control		4	10	2	0			
	Experimental	T2	4	8	4	0	3.31	0.067	NS
	Control		4	10	2	0			

SD: Standard deviation, NS: Not significant, $P \leq 0.05$: Significant

very technique sensitive and their acceleration effects are directly related to their dosage and method of preparation, a precise method as suggested by Ehrenfest *et al.*^[8] was used in the present study. After coming into contact with the glass, the blood sample lacking an anticoagulant almost instantly began to coagulate, which cut down on the amount of time needed to centrifuge fibrinogen. To get therapeutically useful L-PRF plugs charged with serum and platelets, the proper preparation technique must be followed, and quick handling is essential.

Immediately after the careful extraction of first premolars, L-PRF plugs were inserted into the extraction socket to trigger the regional acceleratory phenomenon. The experimental side of the trial, where L-PRF plugs were inserted, displayed a higher rate of canine retraction than the control side, which was temporary only for first 2 months. The finding of our study was in accordance with the previous studies.^[18,19] The acceleration of canine movement was seen more in 2nd month, compared to 1st month which was similar to the previous studies.^[16,18,19] This may be due to the slow release of BMP2 and TGF- β after a period of 7 days. The original L-PRF clots remained in good shape for a longer period of time.^[8] However, our finding was in disagreement

with findings of Pacheco *et al.*,^[20] who found a decreased rate of retraction in 15 out of 17 patients. The disparity in the results might be due to the fact that they conducted the study on adults with a mean age of 33 years, who presented less periodontal response compared to young adults in our study. Moreover, they used the maxillary dental midline, as reference landmark for assessment of canine movement, which itself is not a stable landmark, and this would be highly influenced by forces acting on the entire arch. Zeitounlouian *et al.*^[21] also did not find any acceleration effects of PRF in their study. They used i-PRF in their study and these conflicting results may be related to the different centrifugation protocols and methodology (700 rpm for 3 min). The centrifuge characteristics have a direct impact on the architecture and cell content of L-PRF clots. The different centrifugation speeds could result in a considerable flaw in all PRP/PRF studies.^[8] Tehranchi *et al.*, in 2018,^[4] found accelerated tooth movement in the L-PRF group compared to the control group at all time intervals. However, their study was conducted for a period of 2 months and also had confounding bias in the method of assessment of movement of canine, which might have affected the outcome.^[4] In our study, all measurements were taken from the third palatal

rugae, which are considered as stable structure. The reason for the short-term increase in OTM might be due to the short-term increase in the number of cells and production of cytokines, enhancing bone remodeling immediately following PRF application.^[17] However, the actual effects and mechanism of PRF need to be elucidated in further well-designed studies using standard protocols. Similar to PRP, L-PRF can also have dose-dependent effects; therefore, it is highly recommended to determine the concentrations of platelets and leukocytes in whole blood and L-PRF samples using Enzyme-Linked Immunosorbent Assay before their application.

During tooth movement, force application away from the center of resistance results in unwanted tipping and rotation. There were no appreciable alterations in canine rotation and inclination between both the groups. The findings were consistent with Zeitounlouian *et al.*,^[21] Karc and Baka,^[22] Naji *et al.*,^[23] and Mheissen *et al.*^[24] Instead, Pacheco *et al.*^[20] showed larger canine rotation on the control side than the experimental side. The reason for conflicting results may be due to difference in methodology and platelet concentration.

By comparing the probing depth and root resorption before and after the investigation, there were no discernible differences between the experimental and control groups. This was in accordance with the previous studies.^[20,21]

Assessment of perception of pain, swelling, and discomfort showed non-significant results in our study, in consistent with the findings of the previous studies.^[5,20,25] This is due to anti-inflammatory effects of PRF.^[26]

Limitations

The present study was a split-mouth, single-center randomized control trial, which requires a small sample size compared to parallel group studies. Therefore, multi-center and parallel group studies with larger sample size and standardized methodology are required in future.

Clinical implications

Our study has shown an overall acceleration in the rate of canine retraction by 1.5 mm in 5 months, and almost all the patients completed their canine retraction on the experimental side, compared to the control, with less tipping, anchorage loss, and maintenance of periodontal health. None of the patients reported any significant pain or discomfort associated with the procedure. Similar results of acceleration have been reported with the use of surgical techniques such as corticotomies. For orthodontic purposes, L-PRF plugs can be effectively used to accelerate tooth movement as they are minimally invasive, cost-effective, totally autogenous, and more acceptable to patients. However, the requirement of centrifugation machine and drawing of patient's blood could be the limiting factors for their use.

CONCLUSION

The movement of canine and molar, tipping, rotation, anchor loss, and probing depth were evaluated, and it was concluded that the rate of canine retraction was found to be statistically greater on the experimental side in the first 2 months with the use of L-PRF. The anchor loss was more in control group; only in 1st month of treatment. The canine tipping, rotation, root resorption, probing depths, and pain perception showed statistically insignificant values in both the groups.

Authors' contributions

Dr. Seema Gupta – Manuscript writing, review, editing, and investigation. Dr. Eenal Bhambri – Manuscript review and supervision of project. Dr. Monika Sorokhaibam – Methodology, review, and investigation. Dr. Namit Nagar – Data analysis, manuscript writing, and review. Dr. Deepali Nagar – Manuscript review, data curation, and formal analysis. Dr. Manish Sharma – Manuscript editing, review, and data analysis.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent.

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Conflicts of interest

There are no conflicts of interest

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