Original Article

Effects of submucosally administered platelet-rich plasma on the rate of tooth movement:
A single-center, split-mouth, randomized controlled trial with clinical and biochemical analysis

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ABSTRACT

Objectives: To evaluate the effects of submucosally administered platelet-rich plasma (PRP) on the rate of maxillary canine retraction. Levels of soluble receptor activator of nuclear factor-κb ligand (sRANKL) and osteoprotegerin (OPG) in the gingival crevicular fluid (GCF) were also measured over 2 months.

Materials and Methods: This split-mouth trial involved 20 sites in 10 subjects randomly assigned to PRP (experimental) side and control side. After alignment, the freshly prepared PRP was injected submucosally distal to the experimental side maxillary canine, and retraction was performed using NiTi closed-coil springs (150 g) on 0.019 × 0.025-inch stainless steel wire. The rate of canine movement was assessed using digital model superimposition at 0, 30, and 60 days. The OPG and sRANKL were assayed using enzyme-linked immunosorbent assay from GCF collected at 0, 1, 7, 21, 30, and 60 days.

Results: Twenty sites were analyzed using paired *t* test. The rate of tooth movement increased significantly by 35% on the PRP side compared with the control side in the first month ($P = .0001$) and by 14% at the end of the second month ($P = .015$). Using the Mann–Whitney *U* test, OPG levels were found to be significantly decreased on the 7th ($P = .003$) and 30th day on the PRP side ($P = .01$), while sRANKL became detectable by the third week postinjection on the PRP side ($P = .069$).

Conclusions: Submucosal injection of platelet-rich plasma significantly increased tooth movement during the 60-day observation period. Local injection of PRP significantly altered the levels of OPG and sRANKL in GCF. (*Angle Orthod.* 2022;92:73–79.)

KEY WORDS: PRP; Acceleration; 3D superimposition

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INTRODUCTION

Orthodontists have always attempted to increase the rate of tooth movement by increasing osteoclastic activity, which in turn increases bone resorption.[1] Techniques such as corticotomy, piezocision, and micro-osteoperforation lead to traumatic inflammation and release of cytokines locally.[2–4] Surgical procedures are much less preferred[5] than less invasive approaches such as injection of biomodulating agents,[6–9] nonsteroidal anti-inflammatory drugs (NSAIDs), and growth factors to mimic the body's immune response to increase local osteoclast production.[10–12] Biomodulating agents are painful and may carry a risk of local adverse reactions.[13] The need for repeated administration and an unknown effective dose have limited their use.

Platelet-rich plasma (PRP) is a concentrated plasma containing a fivefold more amount of autologous platelets than whole blood, approximating more than 1 million/μL in 6-mL aliquots.[14] PRP is a ready-made source of growth factors, proteases, antiproteases, and inflammatory mediators.[15–17] Disparities in action of PRP exist due to variation in yield concentration, centrifugation speed and time, method of activation, and the net functional efficacy of growth factors.[14] Hence, the efficacy of PRP in inducing sustained inflammation triggering osteoclastogenic action that alters bone metabolism is not well established.

Receptor activator of nuclear factor-κb ligand (RANKL) and osteoprotegerin (OPG) are paracrine regulators of bone metabolism. Soluble RANKL (sRANKL) has an especially influential role in osteoclastogenesis.[18] OPG counteracts the osteoclastic activity induced by RANKL competitively to maintain homeostasis.[19]

Studies in animal models and humans lack information about the concentration and effectiveness of PRP in tooth movement.[5,17] Therefore, this trial was designed with the primary objective of determining the effects of autologous PRP injected submucosally on the rate of maxillary canine retraction. The secondary goal was to study its influence on OPG and sRANKL levels in gingival crevicular fluid (GCF).

MATERIALS AND METHODS

Trial Design

This study was a single-center, randomized controlled clinical trial with a split-mouth study design with an allocation ratio of 1:1.

Participants, Eligibility, and Settings

The subjects were enrolled from the orthodontic clinic (September 2018 to March 2019) at the Centre for Dental Education and Research, AIIMS, New Delhi, after obtaining ethical clearance from the Institute Ethics Committee for Postgraduate Research, AIIMS, New Delhi (IECPG-184/23.08.2017). The trial was registered prospectively in Clinical Trial Registry– India (CTRI/2018/08/015257).

Ten chosen subjects were healthy with a full complement of dentition and good periodontal health, between 16 and 24 years of age, with no significant medical history or metabolic bone disease. The selected subjects had either bimaxillary protrusion or Class II division 1 malocclusion and required bilateral maxillary first premolar extraction for orthodontic therapy. Patients with recent major illnesses resulting in reduced platelet count; patients on regular antibiotics, steroids, or anti-inflammatory drugs; and patients with poor oral hygiene were excluded from the study.

Sample Size

No data were available on the effects of PRP on the rate of tooth movement in the human population during the commencement of the study. Hence, a convenient sample size of 10 subjects was used.

Randomization

Randomization was performed by the first investigator using a computer-generated program (Research Randomizer, version 4, Urbaniak, G.C., & Plous, S.). To minimize selection bias, 30 random numbers were generated, concealed in opaque sealed envelopes, and shuffled every time before being picked by the patient.

Blinding

Blinding of the patient and principal investigator was not possible during the trial. The blinding of data was done during biochemical and dental cast assessment stages.

Interventions

After obtaining informed consent, bonding with 0.022-inch slot brackets (Roth prescription) was accomplished. A Nance palatal button, soldered to first molars, was used for enhancing anchorage. Leveling and alignment were carried out until 0.019 × 0.025-inch stainless steel wire was engaged passively.

PRP Preparation

Autologous blood (36 mL) was collected from the patient's medial cubital vein into four 10-mL vacutainer vials, each containing 1 mL ACD-A anticoagulant. A total of 0.5 mL of whole blood was collected separately

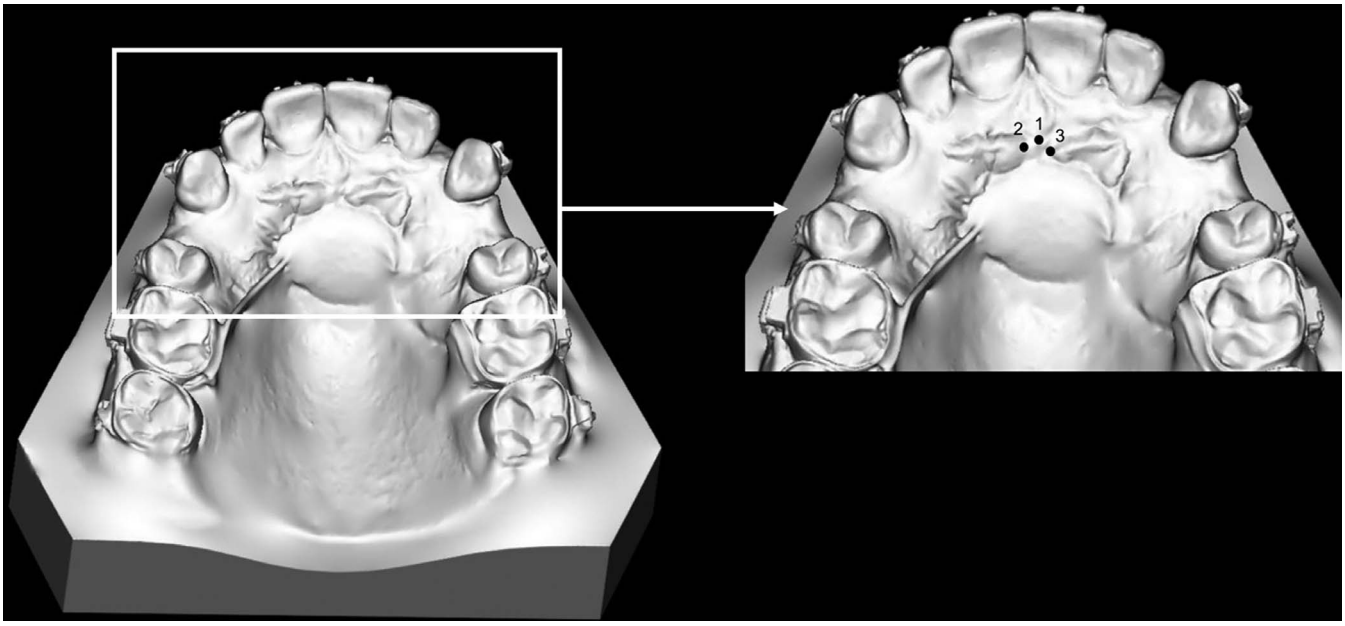

Figure 1. Rugae points used for superimposition.

into a vial coated with EDTA for blood cell counting. According to the double centrifugation protocol, PRP was freshly prepared using a Sorvall Legend XTR centrifuge (Thermo Scientific Inc, Waltham, Mass).[20] The pelleted platelets were homogenously mixed in 1.5 mL of plasma and carefully pipetted into two aliquots: 1.25 mL for injection and 0.25 mL separately for platelet count estimation (BeneSphera, Avantor Inc, Radnor, Penn).

PRP Injection

Local anesthetic infiltration of 0.5 mL lignocaine (1:100 000) was given on each side. The experimental side received an additional PRP injection. After infiltration, 0.6 mL of PRP was slowly administered at each of three sites around the experimental canine: buccally, palatally, and distally. In case of resistance during injection, either the needle was redirected or paused for 10 seconds before removing the syringe to prevent rebound of PRP. Then the adjacent area was injected. Canine retraction was initiated bilaterally using an 8-mm NiTi closed-coil spring (Dentos Inc, Daegu, Korea), delivering 150 g constant force. Patients were followed for 2 months.

Alginate impressions were obtained after removal of arch wires at baseline (T_0), at 30 days (T_{30}), and at 60 days (T_{60}). The study models were scanned using the Maestro 3D scanner (MDS 400, AGE Solutions S.r.l., Pisa, Italy) with an accuracy of <8 μm. Superimposition of digital models was accomplished using Dolphin 3D software (version 11.9, Patterson Inc, Chatsworth, Calif). The inferior tip of the incisive papilla and the medial end of the first rugae bilaterally close to the median raphe were used as reference points (Figure 1). The distance between the canine tips in T_0–T_{30} and T_0–T_{60} models was measured using a 3D ruler. The values for T_{30}–T_{60} were obtained by subtracting the T_0–T_{30} from T_0–T_{60} values.

GCF samples were collected using PerioPaper strips (Oraflow Inc, Smithtown, NY) inserted passively 1 mm into the distal sulcus of each maxillary canine for 60 seconds under cotton roll isolation. Each sample was collected in an Eppendorf tube. The samples were collected at six time points: before injection (T_0), day 1 (T_1), day 7 (T_7), day 21 (T_{21}), day 30 (T_{30}), and day 60 (T_{60}) postinjection, and they were preserved at −80 °C until processing.

The samples were assayed using commercially available human OPG kits (E-EL-H1341) with a sensitivity of 0.10 ng/mL and detection range of 0.16 to 10 ng/mL and human sRANKL kits (E-EL-H5558) with a sensitivity of 9.83 pg/mL and detection range of 15.63–1000 pg/mL (Elabscience Biotechnology Inc, Houston, Tex).

Statistical Analysis

Statistical analysis was accomplished using SPSS software (version 20.0; IBM, Armonk, NY). The first investigator and a junior colleague repeated the measurements after 1 week for cast analysis to assess the intra- and interclass coefficient and determine reliability. All variables were checked for

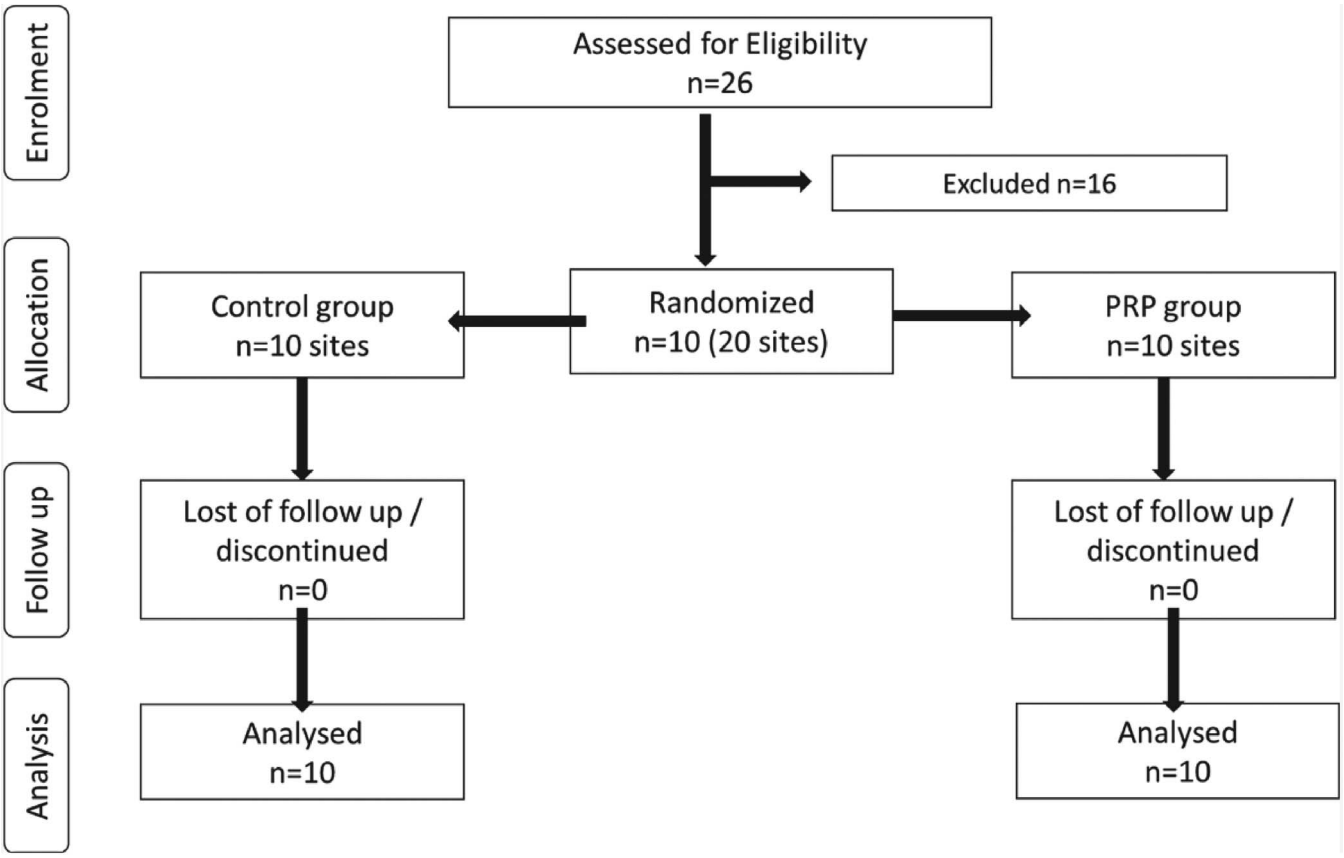

Figure 2. CONSORT flowchart.

normality by the Shapiro-Wilk test. Comparison of intergroup differences was tested using paired-sample *t* test and Mann-Whitney *U* test for normal and nonnormal distributions. The significance level was set at $P \leq .05$.

RESULTS

Participant Flow

The study involved 10 subjects with 20 sites, with 10 sites each randomly allotted to the control and experimental (PRP) groups. All patients were followed until the end of the study period with no loss to follow-up (Figure 2).

Table 1. Distribution and Baseline Data of Participants

Total patients (n = 10)	Male: n = 4 Female: n = 6
Age	Range: 15–25 y Mean: 19.05 ± 3.3 y
Malocclusion	Bimaxillary protrusion: n = 7 Class II division 1: n = 3
Mean platelet concentration	Whole blood: 234 ± 51 × 10^9 /L PRP: 1471 ± 576.7 × 10^9 /L

Baseline Data

Demographic data, including the age, sex, and medical history, were obtained. Baseline impressions for assessing the rate of tooth movement and the GCF samples were collected before injection of PRP (Table 1).

Numbers Analyzed for Each Outcome

Twenty sites assigned to the PRP group (n = 10) and control group (n = 10) were analyzed for primary and secondary outcomes.

Primary Outcome

Maxillary canine retraction at the end of 1 month was 1.34 ± 0.28 mm in the control group and 2.06 ± 0.36 mm in the experimental group. During the second month, the control group retracted by 0.96 ± 0.2 mm and the experimental group by 1.12 ± 0.32 mm, which was significantly different at both time points. The amount of canine retraction for 2 months in the control and PRP groups was 2.30 ± 0.2 mm and 3.19 ± 0.27 mm and was significantly different between groups

Table 2. Canine Retraction Measured on the Digital Superimposed Models Between the Canine Tips in Control and PRP Group Over the Period of 60 Days[a]

			Paired Differences				
					95% CI of the Difference		
Time Interval	Control Group	PRP Group	Mean ± SD	SE Mean	Lower	Upper	*P* Value
T_0–T_{30}	1.34 ± 0.28 mm	2.06 ± 0.36 mm	0.73 ± 0.32 mm	0.10	−0.959	−0.501	.0001**
T_{30}–T_{60}	0.96 ± 0.2 mm	1.12 ± 0.32 mm	0.16 ± 0.17 mm	0.05	−0.281	−0.039	.015*
T_0–T_{60}	2.30 ± 0.20 mm	3.19 ± 0.27 mm	0.89 ± 0.30 mm	0.09	−1.102	−0.678	.0001**

[a] PRP, platelet-rich plasma; T_0, baseline; T_{30}, 30th day; T_{60}, 60th day.
* $P = .05$; ** $P = .0001$.

(Table 2; Figure 3). The inter- and intraobserver reliability coefficient was >0.9.

Secondary Outcome

OPG and RANKL levels (ng/mL) were analyzed at six time points during the experiment. The levels of OPG on the experimental side were significantly lower than on the control side at the 7th day and 30th day. Most of the control samples had sRANKL in the undetectable range, but levels became detectable in the PRP group after the 21st day (Figures 4 and 5).

Harms

No adverse reaction was noted.

DISCUSSION

Bone viability studies using PRP have debated its proinflammatory action at higher concentrations and anti-inflammatory actions at lower concentrations, leading to contradictory results.[21–23] Therefore, this study focused on achieving moderate to high platelet concentrations to achieve proinflammatory action. Platelet concentration was estimated before and after PRP preparation to evaluate the protocol's validity. The mean concentration of the platelets obtained increased significantly from 4 times up to 9 times among the samples, almost equivalent to or more than the concentrations achieved in previous studies.[17–24] Freshly prepared, nonactivated platelets were preferred, to benefit from the slow, sustained release of growth factors during their lifespan of 5–7 days on contact with soluble type 1 collagen.[25] Since dental extraction itself induces a regional acceleratory phenomenon, all premolar extractions were performed atraumatically and at least 3 months before the injection intervention. The medial end of the first rugae and the inferior tip of the incisive papilla were used as reference points for model superimposition.[26,27] Rugae locations can be altered with incisor tooth movement.[28] Since the study was limited to canine retraction, the rugae were a reliable landmark.

The results showed a significant increase of 35% of maxillary canine distal movement during the first month, which decreased to 14% at the end of the second month. The average increase in the rate of maxillary canine retraction was approximately 27% over 2 months. El Timamy et al.[29] reported a similar trend with 15% faster movement in the first month and 5% in the second month. Gulec et al.[17] demonstrated that the high PRP group tooth moved 1.7 times faster while the moderate PRP group moved 1.4 times faster than the control group. Another study reported a percentage change ratio of 2.13:1 between PRP and the control group.[30] Contrary to this, the acceleratory potential of PRP was questioned in one of the related studies.[31] This disparity may be attributed to the variation in the study population, the protocol of administration, and the allogenic nature of PRP.

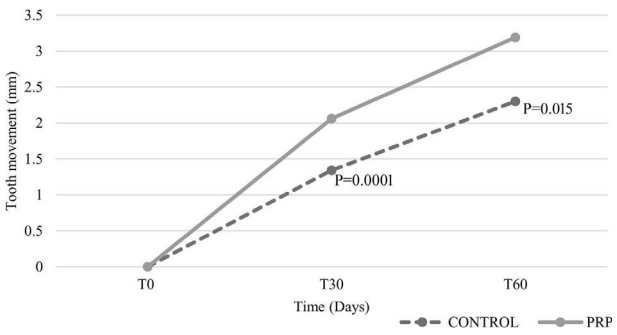

Figure 3. Amount of canine retraction in the control and platelet-rich plasma group over 60 days.

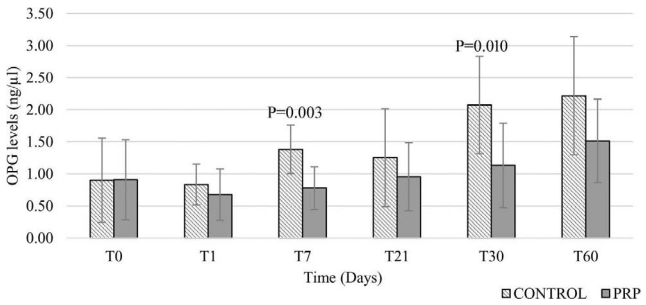

Figure 4. Comparison of osteoprotegerin levels in control and platelet-rich plasma group.

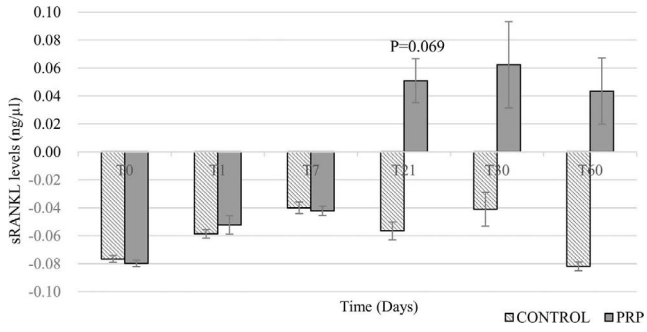

Figure 5. Comparison of soluble receptor activator of nuclear factor-κb ligand L levels in control and platelet-rich plasma group.

A decrease in OPG levels was seen at all observation points. Previous studies reported an immediate decrease in OPG on day 1,[32,33] but the decline was more pronounced in the PRP group than in the control group. OPG levels were reduced significantly on day 7 and day 30 in the PRP group, suggestive of suppression in osteoblastic activity. OPG levels in the PRP group failed to rise to similar levels as the control group, leading to the assumption that PRP had an active role in altering bone homeostasis. Levels of sRANKL were below the detectable range in the control group and only detectable in the PRP group, similar to a previous study on rats.[34] The rationale for low levels is unknown but may be correlated with the subjects' age group.[35] The sRANKL rise was close to achieving significance during the third week postinjection in the PRP group. This peculiar finding may have been because sRANKL is usually released by activated T cells and cells of osteoblastic lineage cleaved by proteases and tumor necrosis factor alpha converting enzyme, marking the onset of a chronic inflammatory response.[31] RANKL in soluble form is a potent inducer of osteoclastic differentiation, proliferation, and survival, attributed to the increased rate of tooth movement achieved.[18] sRANKL's association signified ongoing osteoclastogenesis, which was not a result of periodontal breakdown, reasoning its transient osteopenic effect.[36,37]

Several authors reported severe pain in patients after injecting PRP.[5,29] In this study, almost 90% of patients experienced only slight pain during injection, and they were almost normal within 10 minutes of injection, which was confirmed verbally. Only one patient reported a sense of slight heaviness on the first day of follow-up. None of the patients reported consumption of NSAIDs during the trial period, which indirectly signified the tolerability of PRP injections. The gingiva appeared completely normal, with no significant changes during subsequent visits. This implied that the submucosal injection of PRP was safe and well-tolerated with minimal pain and discomfort.

Limitations and Generalizability

Small sample size, combined sex groups, and a 2-month observation period were the limitations of this study. The study's generalizability was also limited by the learning curve and equipment availability. Similar studies with uniform protocol and adequate sample size may be needed to strengthen the existing hypothesis.

CONCLUSIONS

- Local administration of PRP synergistically increased the rate of tooth movement after orthodontic force application.
- PRP significantly decreased OPG and increased sRANKL levels in the GCF during the study period.

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