Research Article

Comparative Evaluation of Leukocyte Platelet - Rich Plasma and Platelet - Rich Plasma on the rate of Orthodontic Tooth Movement: A Split Mouth Study

Dr. Peddi Sunayana[1], Dr. Ranjit Kumar Manne[2], Dr. Gandikota Chandrasekhar[3]

[1]Private Practitioner, Hyderabad, Telangana, India.

[2]Professor and Head, [3]Professor, Department of Orthodontics and Dentofacial Orthopedics, Panineeya Mahavidyalaya Institute of Dental Sciences and Research Centre, Hyderabad, Telangana, India.

Corresponding author: Dr. Peddi Sunayana; Email: sunayanapeddi25@gmail.com

ABSTRACT

Introduction: One of the most common concerns among orthodontic patients is the duration of treatment for which the rate of orthodontic tooth movement must be accelerated. Corticotomy, piezocision, micro-osteoperforation and platelet-rich plasma injection are a few examples to accelerate tooth movement. Limited data is available on the use of leukocyte platelet-rich plasma to accelerate orthodontic tooth movement in humans. Therefore, this study aimed to evaluate and compare the effects of platelet-rich plasma and leukocyte platelet-rich plasma on the rate of orthodontic tooth movement.

Materials and Method: This study included 12 adult patients undergoing orthodontic treatment requiring extraction of all four first premolars. It used a split mouth design where one side of the oral cavity was injected with platelet-rich plasma and the other side was injected with leukocyte platelet-rich plasma in both the arches, distal to the canine. The injections were given at the end of every 4 weeks for 3 months consecutively and the amount of space closure was measured every 4 weeks using digital vernier calipers.

Result: The results showed that the rate of tooth movement was accelerated between different time intervals when platelet-rich plasma and leukocyte platelet-rich plasma were injected. However, the comparison between platelet-rich plasma and leukocyte platelet-rich plasma did not show statistical significance in upper and lower arches.

Conclusion: Platelet-rich plasma and leukocyte platelet-rich plasma increase the rate of tooth movement and reduce the treatment duration overall. However, when platelet-rich plasma and leukocyte platelet-rich plasma were compared, the rate of acceleration by both products was nearly identical.

KEYWORDS: Platelet-rich plasma, Space closure, Tooth movement techniques

INTRODUCTION

Malaligned teeth can influence patients' quality of life by affecting functions like mastication, speech, esthetics, and psychological well-being, thus necessitating orthodontic care. However, due to the longer duration of treatment, the majority of patients are hesitant to receive fixed orthodontic therapy. Nevertheless, orthodontics has advanced significantly in getting the desired results both clinically and technically.[1] Orthodontic tooth movement occurs as a biological response to the externally applied force. It involves a biphasic process of remodelling of the alveolar bone and the periodontal ligament i.e., bone resorption and bone deposition on the pressure and tension sides respectively.[2]

Orthodontic therapy typically lasts for two years, and in some complex situations, much longer. Several factors, including extraction of the first premolars, can increase treatment time in adult patients because the retraction of the anterior teeth can take between 10 months to 1 year.[3,4] These differences could be because adults have a higher bone density, decreased bone turnover,

and periodontal ligament with fewer cells than younger patients. Adults require more time to complete the stages of tooth movement during orthodontic treatment as they have more areas of periodontal ligament hyalinization and therefore more root resorption. The prolonged duration of orthodontic treatment has side effects like white spot lesions, caries, periodontal disease, and root resorption. Therefore, accelerating tooth movement and shortening the treatment time could be of great importance.[5]

Orthodontists have always attempted to accelerate the rate of tooth movement by enhancing osteoclastic activity, which in turn promotes bone resorption. Techniques such as osteotomy, corticotomy, piezocision, and micro-osteoperforation cause traumatic inflammation and the release of cytokines locally. Surgical procedures are much less preferred than less invasive approaches such as injection of biomodulating agents, nonsteroidal anti-inflammatory drugs (NSAIDs), and growth factors to mimic the body's immune response to increase local osteoclast production.[6]

One of the recently used local agents to accelerate the rate of orthodontic tooth movement is platelet-rich plasma (PRP). Platelet-rich plasma is defined as an autologous concentration of platelets in a small volume of plasma. It is known to be a rich source of autologous growth factors and secretory cytokines provided by the concentrated platelet suspension. The ability of growth factors and cytokines to mediate the differentiation, activation, and survival of all bone cells warrants its significance in orthodontic tooth movement.[7]

Recently, PRP formulations along with leukocytes have been introduced called leukocyte platelet-rich plasma (L-PRP). It promotes bone regeneration by angiogenesis and osteogenesis.[8] A literature review showed that little data is available on the use of L-PRP to accelerate orthodontic tooth movement in humans. Therefore, the current study aimed to evaluate and compare the effects of PRP and L-PRP on the rate of orthodontic tooth movement.

MATERIALS AND METHOD

Subjects:

The Inclusion criteria were as follows:

(1) Angle's Class I malocclusion with Bimaxillary protrusion,
(2) Well-aligned arches,
(3) Patients with good general and oral health,
(4) Patients requiring first premolars extractions.

The Exclusion Criteria were as follows:

(1) Patients with any systemic disease or syndrome,
(2) Patients on anti-inflammatory medications,
(3) History of previous orthodontic treatment,
(4) Patients who require asymmetric extractions.

The sample consisted of 12 adult subjects of age ranging from 18 to 30 years (3 males, 9 females) with permanent dentition, requiring extractions of all first premolars. The study was conducted after obtaining ethical clearance from the institution and informed consent from the patients. (IEC number: PMVIDS&RC/IEC/ORTHO/DN/366-20)

Full preoperative records were obtained, and 0.022-inch preadjusted MBT brackets (Ormco, California, United States) were bonded. Levelling and alignment was done until 0.019 X 0.025-inch nickel titanium archwire was reached. NiTi closed-coil springs of 9mm delivering a retraction force of 1.5 N per side, extending from the retraction hook to the molar hook were used with 0.019X0.025-inch stainless steel wire for retraction (Fig 1).

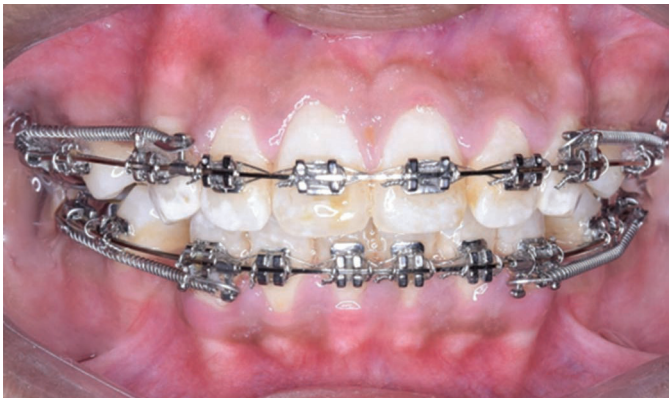

Fig 1: Retraction using NiTi coil springs

PRP and L-PRP Preparation and Injection:

Preparation was done using the double spin technique as described by Zhaoyuan Z et al.[9] 6ml of whole blood was collected from the medial cubital vein of each patient using a 10ml syringe. Out of the 6ml collected, 3ml each was then drawn into two separate sodium citrate-containing vacutainers. Three layers were obtained following the first spin of centrifugation: an upper layer that is mainly composed of platelets, an intermediate thin layer known as the buffy coat that is rich in WBCs, and a bottom layer that is primarily made up of RBCs. For the preparation of PRP, the upper layer and superficial buffy coat were transferred to an empty sterile tube. For the preparation of L-PRP, the upper layer, the entire layer of buffy coat, and a few RBCs were

transferred to another empty sterile tube. The second spin was then performed. After the second spin, the upper 2/3rd of the volume which is composed mostly of platelet-poor plasma was removed from both tubes. The lower 1/3rd that contains L-PRP and PRP was then collected separately in two different insulin syringes.

0.3ml (30 units) of PRP and 0.3ml (30 units) of L-PRP each were obtained. Out of which, 0.15ml (15 units) of PRP was injected on the right side of the oral cavity in the upper and lower arches respectively for half of the sample and 0.15ml (15 units) of L-PRP was injected on the left side of the oral cavity in the upper and lower arches respectively for the other half of the sample. In the remaining half of the sample, the same quantities of PRP and L-PRP was injected on the left and right sides respectively in both the arches. This was done to maintain randomization throughout the trial. The intervention site was anaesthetized using 2% Lidocaine and then PRP and L-PRP were injected using insulin syringes distal to the canine submucosally as given by Liou EJ10 (Fig 2-3).

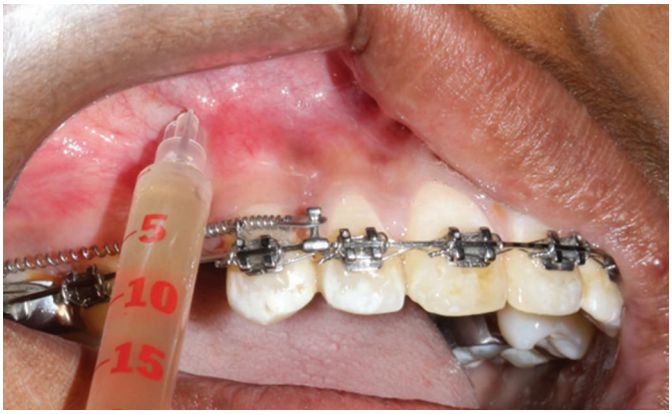

Fig 2: Plasma injected distal to canine submucosally in upper arch

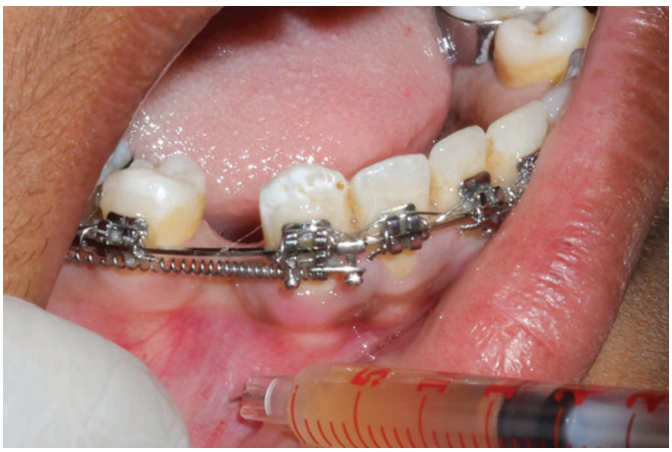

Fig 3: Plasma injected distal to canine submucosally in lower arch

It has no specific injection pattern and is analogous to local anaesthetic injection. The injections were given at 4-week intervals for 3 consecutive months. The rate of space closure was checked every 4 weeks clinically by measuring the inter bracket distance (from the distal tie wing of the canine bracket to the mesial tie wing of the second premolar bracket) using digital vernier caliper (Fig 4).

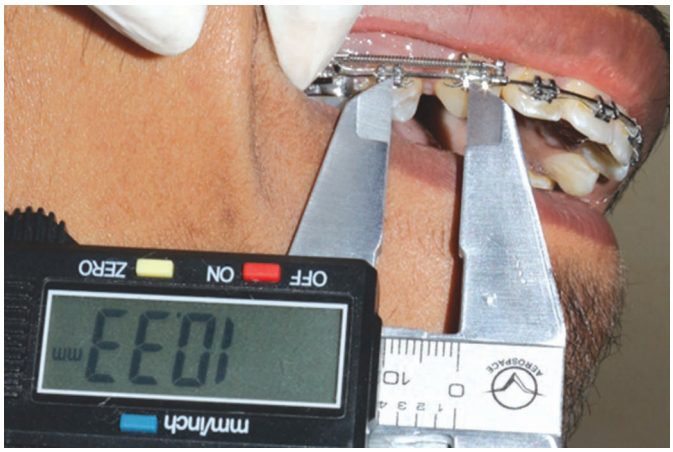

Fig 4: Rate of space closure assessed using digital vernier caliper

RESULTS

The data was analysed using SPSS (Statistical Package for the Social Sciences) version 22 software. Independent t-test was performed to compare the mean differences in the amount of extraction space between the two groups i.e., PRP and L-PRP. Dependent t-test was performed to compare the mean difference in the amount of extraction space in the given group at different time intervals.

The rate of extraction space closure showed statistical significance in the PRP and L-PRP groups in both upper and lower arches between different time intervals (T1-T2, T2-T3, T3-T4) (Table 1 and Table 2). This indicates the acceleration of the rate of orthodontic tooth movement by both products. However, the mean comparison of the two groups for the rate of space closure at different time points did not show statistical significance in either arch. (Table 3 and Table 4). This indicates the rate of extraction space closure by both groups was nearly identical.

Table 1: Comparison of the rate of orthodontic tooth movement in upper arch at different treatment time intervals for two groups

Groups	Measurements	Mean Diff.	SD Diff.	% of change	p-value
PRP	T1-T2	1.25	1.15	8.50	**0.0032***
	T2-T3	0.90	0.69	6.74	**0.0008***
	T3-T4	1.40	0.89	11.17	**0.0002***
LPRP	T1-T2	0.97	0.88	7.19	**0.0029***
	T2-T3	1.34	0.86	10.70	**0.0002***
	T3-T4	1.22	0.62	10.91	**0.0001***

Diff: difference, LPRP: leukocyte platelet-rich plasma, PRP: platelet-rich plasma, SD: standard deviation

Table 2: Comparison of the rate of orthodontic tooth movement in lower arch at different treatment time intervals for two groups

Groups	Measurements	Mean Diff.	SD Diff.	% of change	p-value
PRP	T1-T2	1.06	0.64	7.68	**0.0001***
	T2-T3	1.40	1.20	11.03	**0.0019***
	T3-T4	1.12	0.71	9.88	**0.0002***
LPRP	T1-T2	1.31	0.60	9.98	**0.0001***
	T2-T3	0.97	0.77	8.21	**0.0012***
	T3-T4	1.01	0.38	9.31	**0.0001***

Diff: difference, LPRP: leukocyte platelet-rich plasma, PRP: platelet-rich plasma, SD: standard deviation

Table 3: Comparison of rate of orthodontic tooth movement between PRP and LPRP in upper arch at different treatment time points

Measurements	PRP			LPRP			Mean Diff.	95% CI		p-value
	Mean	SD	SE	Mean	SD	SE		Lower	Upper	
T1	14.65	1.36	0.39	13.49	2.23	0.65	1.16	-0.41	2.72	0.1394
T2	13.40	1.82	0.53	12.52	2.21	0.64	0.88	-0.83	2.60	0.2979
T3	12.50	1.87	0.54	11.18	2.18	0.63	1.32	-0.40	3.04	0.1258
T4	11.10	1.77	0.51	9.96	2.14	0.62	1.14	-0.52	2.80	0.1684

CI: confidence interval, Diff: difference, LPRP: leukocyte platelet-rich plasma, PRP: platelet-rich plasma, SD: standard deviation, SE: standard error

Table 4: Comparison of rate of orthodontic tooth movement between PRP and LPRP in lower arch at different treatment time points

Measurements	PRP			LPRP			Mean Diff.	95% CI		p-value
	Mean	SD	SE	Mean	SD	SE		Lower	Upper	
T1	13.76	1.48	0.43	13.07	2.12	0.61	0.69	-0.86	2.24	0.3674
T2	12.70	1.65	0.48	11.77	2.06	0.60	0.94	-0.65	2.52	0.2329
T3	11.30	1.97	0.57	10.80	1.85	0.53	0.50	-1.12	2.12	0.5270
T4	10.18	2.12	0.61	9.79	1.69	0.49	0.39	-1.24	2.02	0.6237

CI: confidence interval, Diff: difference, LPRP: leukocyte platelet-rich plasma, PRP: platelet-rich plasma, SD: standard deviation, SE: standard error

DISCUSSION

Upon orthodontic force application, remodelling occurs in tissues such as periodontal ligament, alveolar bone, and gingiva leading to tooth movement. The orthodontic forces alter the periodontal ligament's vascularity and blood flow, which cause the local production and release of important molecules such as neurotransmitters, cytokines, growth factors, colony-stimulating factors, and arachidonic acid metabolites. The rate of tooth movement is regulated by the rate of bone resorption, which in turn is governed by osteoclast activity. Osteoclasts should therefore be the primary focus to accelerate the tooth movement. The production of inflammatory mediators like prostaglandin E2 and interleukin-1 (IL-1), which interact with bone cells, plays a major role in bone resorption.[11]

There are various approaches to accelerate tooth movement. Some of these include corticotomy, osteotomy, piezocision, microosteoperforation, low-level laser therapy and PRP. Platelet-rich plasma is a plasma concentrate that contains an autologous concentration of platelets. The concentrated platelet suspension is extensively used as a source of autologous growth factors and secretory cytokines. Recently, leukocytes have been added to PRP formulations called leukocyte-platelet-rich plasma (L-PRP), and these formulations have received increased attention for their therapeutic effect on tissue regeneration. Additionally, L-PRP triggers the bone-resorbing nuclear factor-B (NF-B) pathway, resulting in osteoclastogenesis thereby leading to decreased bone density and increased tooth movement.[8]

In two animal studies conducted by Rashid et al[7] and Güleç et al[2], they evaluated the effects of PRP on tooth movement. Both studies showed a positive correlation between local injection of PRP and acceleration of orthodontic tooth movement. These results are in line with the present study which indicated a statistically significant increase in the rate of tooth movement between different time intervals. However, the present study cannot be compared to the rat model used by Güleç et al[2] as the whole blood used to obtain PRP was not autologous since a rat's blood volume is insufficient to produce autologous PRP. This positive correlation is not in concordance with the results achieved in the study conducted by Akbulut et al[12] where the application of PRP was not beneficial as an adjunct to the orthodontic treatment.

In the study done by El-Timamy et al[11], the results showed a statistically significant increase in the rate of canine retraction which is similar to the present study. However, they also concluded that PRP did not exhibit any long-term acceleration effects as they failed to administer repeated PRP injections throughout the duration of the study.

Angel et al[5] evaluated the effect of submucosal injections of 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. The results obtained were similar to the present study which indicates that PRP has accelerated the rate of tooth movement synergistically. PRP significantly decreased OPG and increased sRANKL levels in the GCF during the study period. Contrary to this, Chandak et al[13] found that PRP was ineffective in accelerating the rate of orthodontic tooth movement during en-masse retraction.

Corticotomy recorded a rate change ratio of (2.04:1)[14] compared to a ratio of (1.6:1) reported with vitamin D3 injection[15] and (1.3:1) reported with low-level laser therapy (LLLT) application for accelerating the rate of orthodontic tooth movement.[16] According to Angel et al[5], maxillary canine distal movement significantly increased in the first month by 35% before declining to 14% at the end of the second month. Over a period of two months, the average rate of maxillary canine retraction increased by about 27%. El Timamy et al[11] showed a comparable pattern with 15% faster tooth movement in the first month and 5% in the second month. According to Güleç et al[2], the moderate PRP group's teeth moved 1.4 times faster than the control group's teeth, while the high PRP group's teeth moved 1.7 times faster. Rashid et al[7] reported a rate change ratio of 2.13:1 between PRP and the control group in their investigation. This potential was contradicted by Akbulut et al[12]. This discrepancy may be attributed to the variation in the study population, the protocol of administration in different studies, and the allogenic nature of PRP.

Recently Nakornnoi et al[8] evaluated the effect of L-PRP on the rate of orthodontic tooth movement in a rabbit model where leukocytes were included in PRP and these formulations were said to stimulate the nuclear factor κB (NF-κB) pathway via IL-1, TNF-α, and receptor activation of the NF-κB ligand. Therefore, tooth movement is accelerated by this pathway as it leads to osteoclastogenesis and enhances bone resorptive activity. The study showed that tooth movement was 1.2 times higher in the L-PRP group compared to the control group. The study concluded that L-PRP accelerated the orthodontic tooth movement which can be attributed to the burst of pro-inflammatory cytokines. There are very few human studies on the use of L-PRP to accelerate orthodontic tooth movement, therefore, the present study determined the effect of local administration of L-PRP on accelerating orthodontic tooth movement in humans, and a comparative evaluation between PRP and L-PRP was also done. The results obtained in the present study concerning L-PRP were similar to those obtained by Nakornnoi et al[8] which indicated an acceleration of the rate of orthodontic tooth movement between different time intervals.

The results of the present study indicate that submucosal injections of PRP and L-PRP accelerate the rate of orthodontic tooth movement. However, comparative evaluation of PRP and L-PRP indicated that the acceleration by both the products was nearly identical and the mean difference between the rates of tooth movement by PRP and L-PRP was not statistically significant. This could be credited to the compositions of PRP and L-PRP which have both growth factors that have regenerative potential and cytokines that have an inflammatory effect which further induces rapid acceleratory phenomenon. This is because of the PRP contents, which have several overlapping biological effects.

El-Sharkawy et al[17] reported that increased production of growth factors may reduce cytokine release, limit inflammation, and thereby promote healing and tissue regeneration. Other plasma elements like fibrinogen, fibronectin, and hyaluronan can induce sterile inflammation; PRP might, therefore, promote inflammation.[18] The leukocytes in L-PRP are also said to cause an initial release of pro-inflammatory cytokines thereby encouraging inflammation. Therefore, it may be claimed that the efficiency of PRP may be determined by its cellular makeup, platelet counts and balance between catabolism and anabolism. Thus, it would be ideal to check the composition of PRP and L-PRP using ELISA to know the amount of each molecule and to understand how long these molecules maintain their biological activity because this is the basis behind accelerated tooth movement by PRP and L-PRP.

CONCLUSION

Both PRP and L-PRP significantly accelerated the rate of orthodontic tooth movement across different time intervals, with nearly identical results. Therefore, PRP and L-PRP can be used to accelerate the rate of tooth movement for reducing the overall treatment duration.

Future research can be done with increased sample size and repeated injections until the closure of the entire extraction space to obtain optimal results. Also, measurement of biological molecules (growth factors and cytokines) can be done for a better understanding of the cellular make up of PRP and L-PRP.

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Conflict of Interest:

The authors declare that they have no competing interests that could have influenced the work reported in this paper.

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