Original Article

The Effect of Submucosal Injection of Plasma Rich Platelets on the Rate of Orthodontically Induced Canine Retraction in Subject with Bimaxillary Protrusion

Trefa M. Ali[1], Omar F. Chawshli[2]*

Abstract

Objective: The present study aimed to reveal the effects of submucosal injection of Plasma Rich Platelets (PRP) on the rate of canine retraction.

Methods: Eighteen females with bimaxillary protrusion were selected from patients seeking orthodontic treatment, College of the Dentistry/University of Sulaimani, whose maxillary and mandibular first premolars were decided to be extracted after proper diagnosis. It's a split-mouth design; the upper left side was the control side while the upper right side served as the intervention side (submucosal injection of PRP); after aligning and leveling, the retraction phase was initiated on .017× .025 Stainless steel archwire with power chain from the canine bracket to temporary anchorage device inserted between the upper 2nd premolar and 1st molar at the same level for both sides. The elastomeric chains were changed every two weeks. Scanned intraoral images were obtained by intra-oral CEREC omnicam scanner before retraction and at the end of retraction to measure the amount of canine movement using inLab CAM 15.0 software 2015.

Results: A highly significant acceleration of canine retraction on the intervention side compared with the control side at $p < .0001$ with a rate of 29.1% higher overall retraction phase (108 days).

Conclusions: Submucosal injection of PRP is a minimally-invasive and low-cost method that can be used for accelerating orthodontic tooth movement.

Keywords: *Canine retraction, Acceleration, Digital models, Platelet-rich plasma, Tooth movement.*

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1. *Department of Pedodontics, Orthodontics, Preventive Dentistry, College of Dentistry, Sulaimani University, Sulaimani, Iraq.*
2. *Department of Pedodontics, Orthodontics, Preventive Dentistry, College of Dentistry, Hawler Medical University, Erbil, Iraq.*

** Corresponding author: trefa.ali@univsul.edu.iq.*

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Introduction

The main objective of the orthodontic treatment is to provide ideal smile, aesthetics, as well as orofacial function[1]; to achieve these objectives, orthodontic treatment should be completed within a certain period. According to previous studies; orthodontic treatment may take a long period up to 2 to 3 years on average[2,3] in today's quick-paced time and age, such a long duration of orthodontic treatment has become a primary concern for most patients seeking the treatment and orthodontists, concerns arose regarding the long term disadvantages of conventional orthodontic treatment like predisposing the patient to caries, gingival recession and resorption of roots[4]. Still, reducing the duration of orthodontic treatment is challenging, as it is one of both orthodontists and patients[5].

Since the inception of orthodontics, one of the domains in the research has been the tooth movement and associated biological reaction. Research has been done to study various approaches to achieve tooth movement most physiologically but with maximum pace[6].

Many techniques have been developed and implemented to accelerate orthodontic tooth movement and shorten orthodontic treatment duration[7-9]; one of these techniques is the use of submucosal injection of PRP.

PRP was first introduced to the dental literature in 1998, combined with autogenous bone grafts to reconstruct mandibular defects[10], having the potential and capability to promote periodontal regeneration through various mechanisms[6].

PRP has recently been considered an ortho biological adjuvant treatment[11], currently used in different medical fields. The interest in the application of PRP in dermatology has recently increased as being used in several different applications as tissue regeneration, wound healing, scar revision, skin rejuvenating effects, and alopecia[12]. PRP has the potential and capability to promote periodontal regeneration through various mechanisms. The effect of PRP in the localized acceleration of tooth movement is dependent on the concentration used. The use of injectable PRP at a different stage of orthodontic treatment can improve the quality of the treatment outcome by influencing the bone quality and enhancing the tooth movement rate[6].

PRP is defined as an autologous concentration of platelets in a small volume of plasma and is considered to be a rich source of autologous growth factors (GFs)[13]. GFs are natural biologic mediators that regulate key cellular events that are part of tissue repair and regeneration. After binding GFs to specific cell membrane receptors of target cells, intracellular signaling pathways are induced; this typically results in the activation of genes that may ultimately change cellular activity and phenotype. However, each GF's effect is regulated through a complex system of feedback loops, which involve other GFs, enzymes, and binding proteins. Recent advances in cellular and molecular biology have allowed a better understanding of the functions of GFs. In-vitro and in-vivo studies have confirmed that GFs can enhance the capacity of tissues to regenerate by regulating cell chemo-attraction, differentiation, and proliferation[10].

Patients and methods

This study was conducted in the Department of Pedodontics, Orthodontics, and Preventive dentistry/ College of the Dentistry/ University of Sulaimani, with the corporation of Dermo-Dento center and British smile design center.

Sample

Eighteen females with bimaxillary protrusion were selected from patients seeking orthodontic treatment (the protocol is presented as a diagram, shown in Figure 1) whose maxillary and mandibular first premolars were decided to be extracted after proper diagnosis using study models, digital cephalometry, and orthopantomography; all the cases were evaluated with a well-experienced orthodontist.

The Simplified Oral Hygiene Index (OHI-S)[14] was used to estimate the oral health of the selected patients, which differs from the original OHI (The Oral Hygiene Index) in the number of the tooth surfaces scored (6 rather than 12), the method of selecting the surfaces to be scored, and the scores, which can be obtained. The criteria used for assigning scores to the tooth surfaces are the same as those used for the OHI[14]. Zero oral hygiene indices were scored for all participants before beginning the sequences of treatment.

Design

An experimental study (split-mouth) was employed in this study.

Patients were considered eligible if they meet the following criteria:

1- They were aged between 18 and 26 years.

2- Bimaxillary protrusion.

3- Minimum crowding (less than 2mm) or minimum spacing (less than 4 mm).

4- Indication for extraction of upper and lower first premolars.

5- No previous orthodontic treatment.

6- No systemic diseases.

7- No smoking.

8- Good oral hygiene.

Exclusion Criteria

1- Patients with severe tooth displacement (e.g., ectopic canine)

2- Those were reporting the use of medications throughout the study.

The rights of patients were protected; the purpose and methods of the study were completely explained to the patients and parents; each of the participants signed informed consent.

Clinical procedure

Five to 7 days after first premolars extraction, fixed orthodontic appliances of MBT prescription 0.022-inch slot height was bonded. Then a 0.014-inch NiTi archwire was inserted and tied to each bracket using ligature wires.

Archwires sequence used was 0.014-inch NiTi, followed by 0.018 inch NiTi and 0.017× 0.025 inch NiTi finally 0.017×0.025 stainless steel. Before retraction, self-drilling temporary anchorage devices (TADs) of 10 mm length and 1.6 mm diameter were inserted with hand drill between the upper second premolar and upper first molar for both sides at the same level (mucogingival junction) as an anchorage for retraction force (Figure 2).

At this stage, upper canines were planned to be retracted using maximum anchorage (TADs). The Right side composed the study group, whereas the Left side served as the control group.

The retraction phase was initiated after PRP injection on the experimental side (right), using elastomeric chains with a force of 150gm; Translation movement, according to Kanuru et al. 2014[15] measured using stress and tension gauge Dial type (Dentaurum/ made in Germany).

For the control side, the retraction was started at the same time with the same mechanics. Patients were examined at two-week intervals, and the elastomeric chains were replaced at each appointment.

Sapphire bracket (MBT Slot 0.022-inch slot height) from DW Orthoworld Company (Italian origin), bonding was with OrthoFlow composite of the same company.

PRP preparation

Thirty cc (cubic centimeter) venous blood draw will yield (3-5 cc) of PRP[16]. In this study, we used the PRP method; an initial centrifuge was used to separate red blood cells (RBC) then followed by a second centrifuge to concentrate platelets, which are suspended in the smallest final plasma volume. Blood was initially collected in PRP tubes that contain Anticoagulant Citrate Dextrose (ACD). The first spin step is performed at constant acceleration to separate RBCs from the remaining blood volume for 9 minutes at about 2000 rpm. After the first spin step, blood was separated into three layers: an upper layer that contains mostly platelets and WBC, a thin intermediate layer that is known as the buffy coat, and that is rich in WBCs, and a bottom layer that consists mostly of RBCs. For pure PRP production, the upper layer and superficial buffy coat were transferred to an empty sterile tube; the second spin step was then performed for 10 minutes at 3870 rpm; thus, the lower 1/3rd will be the PRP. Therefore, the procedure in detail involves a 35 cc of venous blood draw using an aseptic technique from the medial Cubital vein of the patient. A butterfly needle was used to avoid causing any irritation and trauma to the platelets in a resting state. ROTIXA 500 RS Hettich floor standing Centrifuge, producing high concentration PRP (5 times the concentration in whole blood) as explained in Figure (3- A and B), an initial centrifuge to separate red blood cells (RBC) is followed by a second centrifuge to concentrate platelets, which are suspended in the smallest final plasma volume. Blood is initially collected in PRP tubes that contain Anticoagulant Citrate Dextrose (ACD).

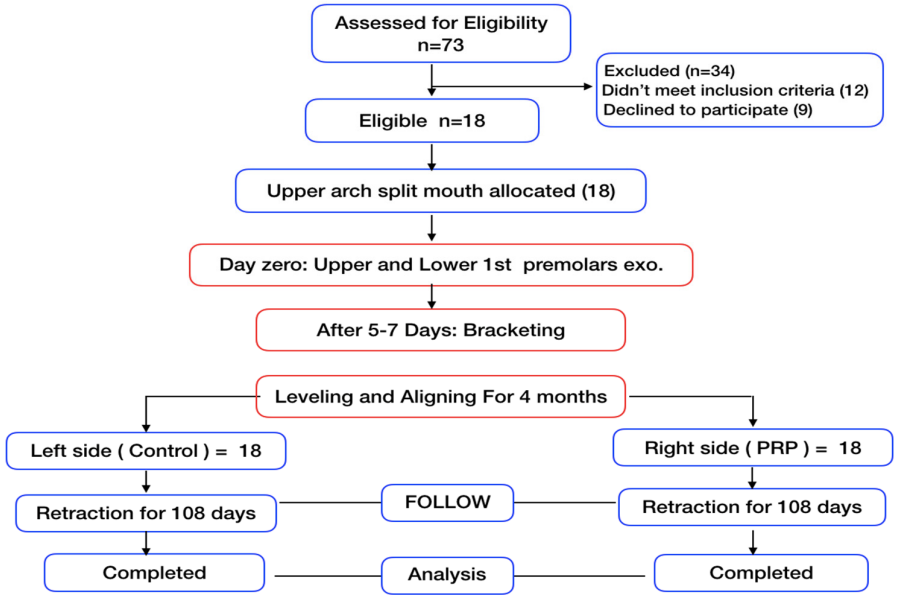

Figure 1: Flow diagram of the study.

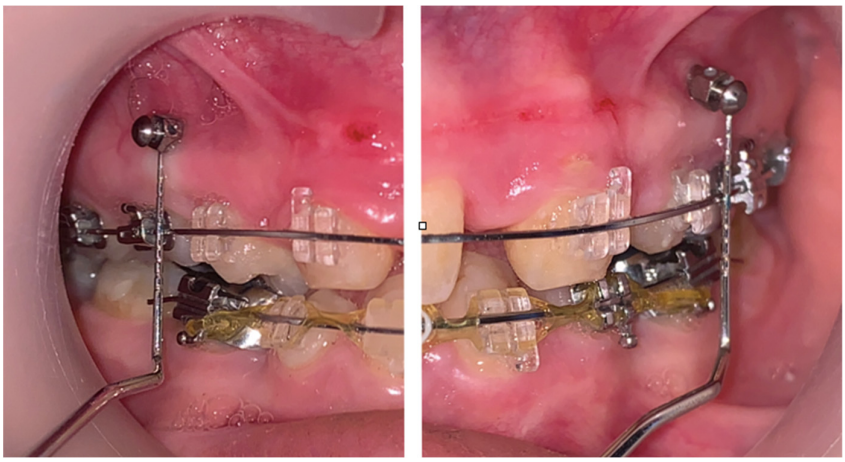

Figure 2: TAD's position.

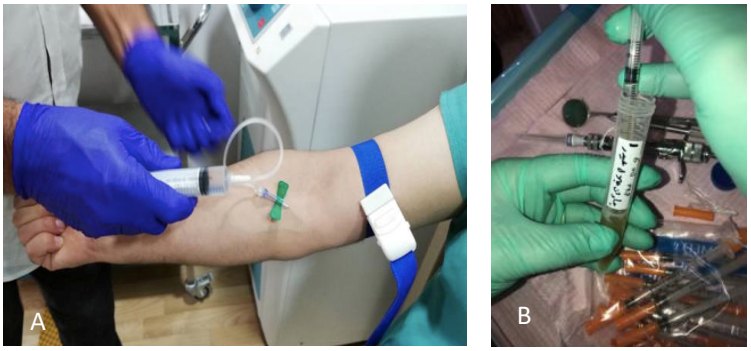

Figure 3: A- ROTIXA 500 RS Hettich floor standing Centrifuge was used, producing high concentration PRP (5 times the whole blood concentration). B- PRP Preparation.

Site of injection

Five cc of PRP was injected by means of a micro-syringe into the buccal vestibular mucosa and palatal attached gingiva distally to the root of the upper right canines of each patient under local anesthesia. All injections were volumetrically equivalent. Injections were done only once on day zero of retraction and not repeated (as illustrated in Figure 4).

Before the PRP injection, local anesthesia (Xylocaine) was used at the target sites for pain control. It is a submucosal injection rather than a sub-periosteal injection. It is similar to local anesthesia injection, and it has no certain injection pattern (six injections each one was 0.8 cc). Acetaminophen (500 mg) was prescribed for post-injection pain control[17].

Pre- and post-retraction intra-oral scanned images were constructed by CEREC Omnicam scanner and analyzed using inLab CAM 15.0 software 2015 at British smile design center by a well-trained technician, measuring the space between the upper canine and 2nd premolar for both sides.

Scanned image intervention

By the end of canine retraction, there were two scanned intraoral images obtained by direct oral scanning using the CEREC Omnicam scanner and analyzed using inLab CAM 15.0 software 2015. Each image was displayed on the screen and oriented (a line drawn passing through. The occlusal surfaces of the teeth so that the occlusal plane parallel to the floor and space was measured by sidebar measuring tool (mm), (Figure 5-A and B) so at the end, there were four values:

TR1 = space between the right canine's distal connector and the mesial connector of the right 2nd premolar before retraction.

TR2 =space between the right canine's distal connector and the mesial connector of the right 2nd premolar at the end of retraction.

TL1 = space between the left canine's distal connector and the mesial connector of the right 2nd premolar before retraction.

TL2 =space between the left canine's distal connector and the mesial connector of the right 2nd premolar at the end of retraction[18].

Statistical analysis

1. Values were analyzed using IMP SPSS statistical software (Statistical Package for Social Science, Version 24).
2. Shapiro-Wilk test was used to check for the normal distribution of the data obtained.
3. Intra- and inter-examiner calibrations to assess the accuracy of the measurements using independent t-tests were conducted.
4. Descriptive statistics: mean, standard deviation and minimum-maximum were calculated for each side considering the canine movement.
5. A paired sample test was used to detect the differences between the canine retraction rate between the sides at a 95% confidence level. A P-value of $\leq$.05 was considered significant.

Figure 4: Site of PRP injection and force application.

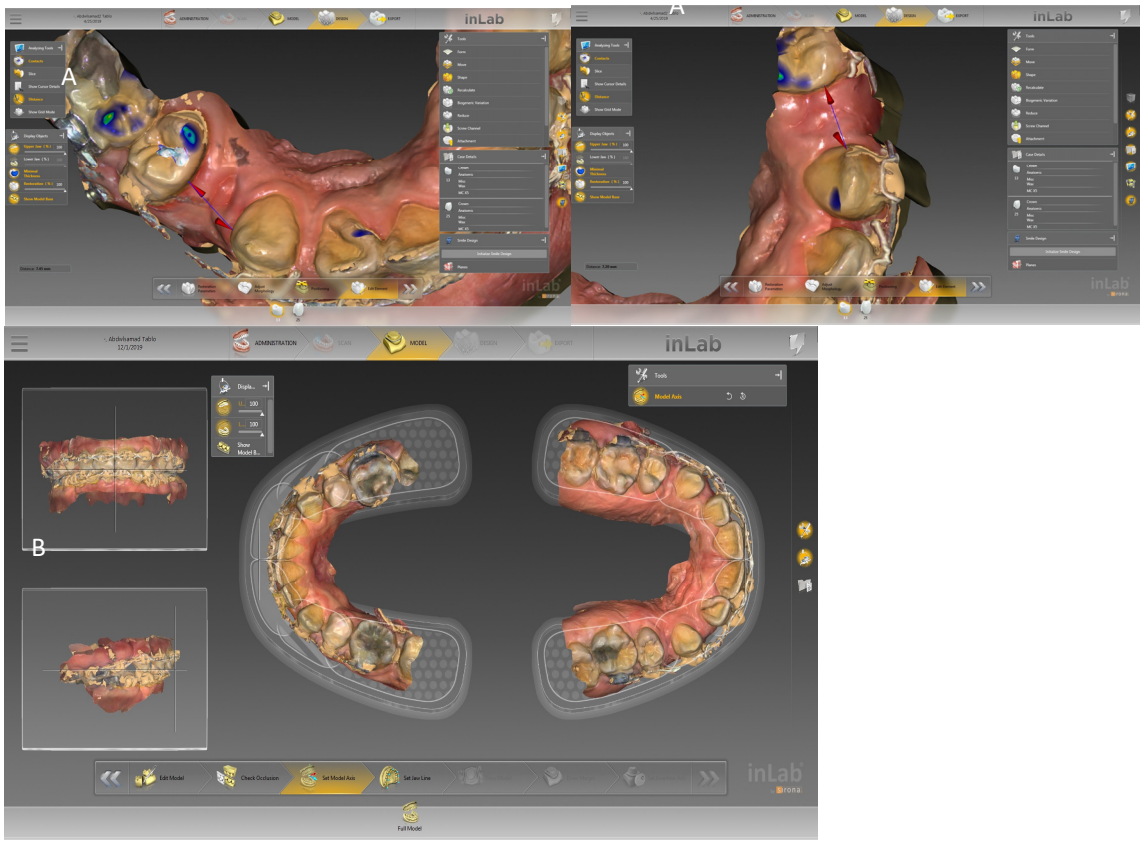

Figure 5: A-Scanned intra-oral images before retraction

B- Scanned intraoral images to measure the retraction of canine using inLab15 software

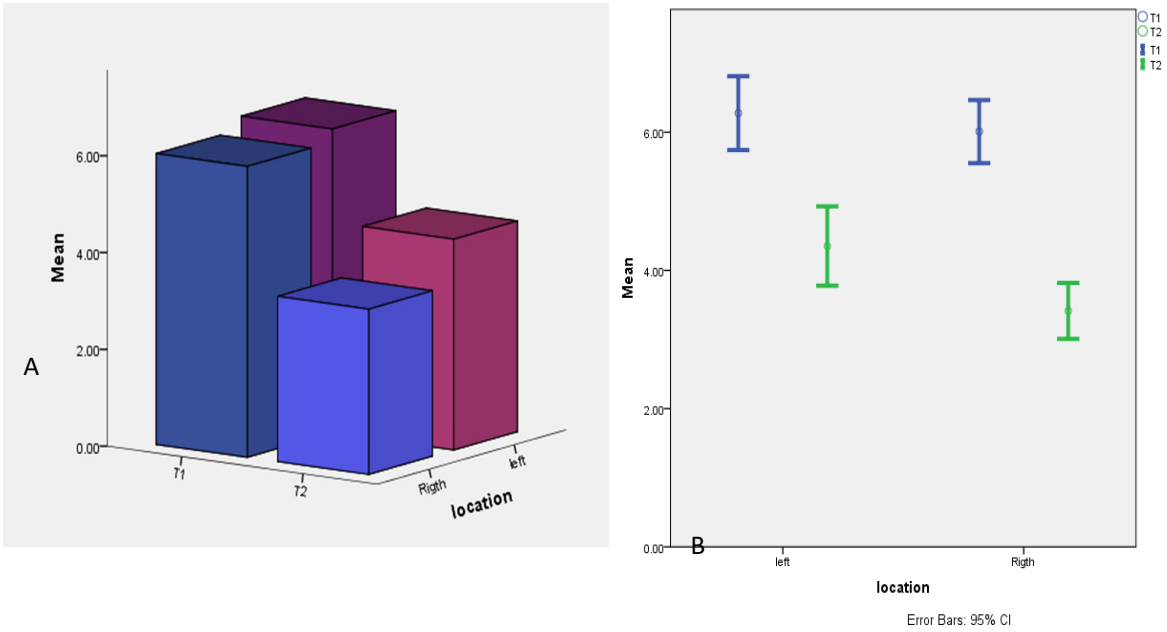

Figure 6: A-Bar chart explain the rate of canine movement (mm) for both sides.

B- Error bar shows the distribution of the results.

Results

Eighteen female patients were included in this study, ranging from 18 to 26 years (mean of 22.28 years).

Shapiro-Wilk test was non-significant at $p \geq .05$ for all of the measurements (distance between the canine and 2nd premolar), so all the parameters showed normal distribution (were *p*-value ranging from .09 to .3).

In addition, intra- and inter-examiner reliability was tested for each of the three measurements performed by the same examiner and a well expert orthodontist using independent t-tests at two weeks interval to compare the compatibility between these measurements.

Six random patients were chosen for each measurement, and the $P \geq .05$ was considered non-significant for statistical inferences.

Table 1 and Figure (6-A) show the descriptive statistics of the amount of canine retraction for both the right (PRP) and left (Control) side, including mean, minimum, maximum, and standard deviation; also error bar explained the distribution of the results as in Figure (6-B).

Table 2 reveals that there is a highly significant increase in the rate of canine retraction in the PRP side when compared with the control side at $p \geq .0001$ at a 95% confidence interval of the difference (lower 1.942 and upper 2.576).

Table 1: Descriptive Statistics for the amount of space between the 2nd premolar and canine for both the right and left side.

Measurement	Side	No.	Min	Max	Mean	St.dev.
TR1	PRP	18	4.46	7.20	6.00	.918
TR2		18	2.14	5.08	3.41	.814
TL1	Control	18	4.31	7.93	6.27	1.07
TL2		18	2.55	6.90	4.35	1.15
TR1-TR2	PRP	18	0.89	4.070	2.595	0.727
TL1-TL2	**Control**	18	0.42	2.84	1.923	1.017
	Total	36				

Table 2: Comparison between the control and intervention side using paired sample test.

95% confidence interval of the difference								
Variable	Mean	SD	Std.Error Mean	Lower	Upper	t	df	Sig. (2-tailed)
T1-T2	2.25	0.935	0.155	1.942	2.576	14.48	35	0.000*

Discussion

This was a paired observation and split-mouth design study, where the participants are serving as the control since both intervention and comparison treatments are applied to each patient. This design is more efficient since the sites that receive the interventions are more

similar, thus reducing variance and sample size requirements. The paired design can be effectively reduced to one sample design where the mean and the standard deviation of the difference between the two treatment arms are used for the sample calculation. According to Pandis et al. 2011, the split-mouth design is efficient since it reduces the sample size to half compared with the design that will randomize the two interventions to separate patients[19]. Moreover, sample size selection was within the range of previous studies[20-23].

On the other hand, by taking a larger sample size in the study, we put more population at risk of the intervention. It also results in wastage of precious resources and the researchers' time[24]. Subsequently, the patient's age was selected so that passing the growth spurt and in accordance with previous orthodontic clinical studies[20-25].

Only females were included in this study, as 65% of patients seeking orthodontic treatment were females according to a study done in Sulaimani city by Ahmed et al.[26], also in an attempt to reduce bias related to the biological response that differs between genders.

When studying single tooth movement, canine retraction can be considered as an ideal model as this is routinely done in clinical practice in maximum anchorage cases; it is the most significant part of orthodontic treatment in the therapeutic extraction of first premolar cases. Besides, this Force/Stress calculation based on root surface area is not cumbersome in this model[27].

The extraction of first permanent premolars for various malocclusions has become an integral part of the orthodontic treatment procedures[22]. The objectives may indicate extraction of the first premolar either for the relief of crowding, reduction of dentoalveolar protrusion and improving the facial esthetics, or correction of inter arch mal-relationships through dental camouflage. Since the canine retraction procedure takes the longest duration of the entire orthodontic treatment, the main goal of this stage is to achieve a rapid and controlled canine retraction[28].

A standardized protocol was used where the canines are retracted using a power chain representing the continuous mechanic's technique, as friction mechanics represent more distal movement than frictionless mechanics with controlled retraction force[22] and using TADs as the absolute anchorage is a recent method to control tooth retraction without loss of anchorage[29].

Since we are dealing with bimaxillary protrusion with four units extraction, the MBT system was used to express the tipping and necessary torque values for anterior teeth and reduce the anchorage loss in the posterior segments[30].

Since 1974, Boester and Johnston found that 150 gm of retraction force gives the highest canine retraction rate[31]. In addition, the force of 150gm employed in the present study followed the recommendations of many authors who applied forces between 100 gm and 200 gm for canine retraction[32].

One way to decrease the magnitude of the moment of force is to apply the force closer to the resistance center. In orthodontics, it is impractical to apply the force directly to the root, projected upward from the crown might use so that the force could be applied to the attachment such that its line of action passed near or through the center of resistance. Still, this procedure is not practical and liable to detachment between the visit interval leading to a loss for retraction force and loss of the study's standardization. In addition, it creates problems with oral hygiene. Therefore, direct traction is used from the TADs' head to the canine bracket with an optimum force for the two sides, eliminating the confounding factors[33].

The variation of platelet and other blood component concentrations between commercial PRP kits may affect clinical treatment outcomes[34]. Data from Seidel et al., 2019 study showed that higher platelet counts were obtained in the products that were prepared by the double-centrifugation protocol, as compared to the lower values that were obtained by the single centrifugation procedure[35].

Using the double centrifuge method, there is a risk of bacterial contamination during transfers between tubes; this can be efficiently reduced if transfers are conducted in a laminar flow hood under sterile conditions[36].

Throughout the retraction time, which was 108 days, the retraction rate was .024 mm/ day for the right side while .017mm/day for the left side. Thus, the canine retraction of the PRP side was faster at a rate of 0.007mm/ day than the control side. Using the submucosal injection of PRP can reduce the retraction time up to 29.1%, which means that it can reduce the two-year duration of orthodontic treatment to one and a half years.

It is an effective method for accelerating tooth movement, non-invasive, low cost, relatively no pain even after local anesthesia subsides, no post-operative swelling was found.

More than three months for canine retraction is a long duration, and this was because Sapphire brackets were used in which the resistance to sliding is more than that of metal braces[37].

Histomorphometric assessment of changes in alveolar bone quantification on rat study by Gulec et al. 2017[38], suggested that PRP injection caused the bone content of the intra-radicular space to decrease in PRP group compared with the control group. Thus the histologic finding of early and rapid bone resorption in the PRP group hypothesized that PRP injection created a regional acceleratory phenomenon-like effect, which forms the basis of rapid tooth movement compared with conventional orthodontic treatment. Besides, the exact mechanism of action of each GFs on tissue healing is complicated. For instance, TGFb (Tumor Necrosing Factor) is known to be critical for initiation or progression of tissue repair; however, TGFb actually may function to increase inflammation and retard wound healing. Moreover, PDGF (Platelet-Derived Growth Factor), which is known to be a stimulator for osteoprogenitor cells, also stimulates bone resorption by increasing the number of osteoclasts[22].

Human studies regarding the use of PRP as an intervention for accelerating tooth movements are scant; even the few published studies lack the international clinical trial registrations, which make the trails illegal, and the standardization for the selection of patients that limit the differences of biological response is not addressed, for example, A study done by El-Timamy *et al.* 2020[39] including 15 female patients concluded that despite the statistically significant increase in the rate of canine retraction during the early stages of tooth movement concomitant with PRP injections, PRP did not exhibit long-term acceleration effects, despite that measurements were done on scanned images from the stone cast, not from the direct intraoral images and TADs were used as an indirect anchorage which is not considered as an absolute anchorage.

Clinical significance of the study

Orthodontic treatment may take a long period of up to 2 to 3 years on average; osseous modulation can offer orthodontic patients an interesting finding related to treatment duration. The benefits of decreased treatment time far outweigh any of the costs or additional work required by the patient or the practitioner. Submucosal injection of PRP has been clinically shown to increase the rate of tooth movement. Completing treatment early, with fewer visits, a more predictable result, and reduced negative sequel are compelling reasons to introduce accelerated orthodontics into daily orthodontic practice.

Conclusions

Submucosal injection of PRP is a minimally-invasive and low-cost method that can be used for accelerating orthodontic tooth movement, thus reducing overall retraction time up to 29%. Repeated injections of PRP to accelerate tooth movement warrants further investigation.

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