Original Article

THE EFFECT OF SUBMUCOSAL INJECTION OF PLATELET-RICH PLASMA ON MAXILLARY CANINE RETRACTION: SPLIT-MOUTH RANDOMIZED CONTROLLED TRIAL

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ABSTRACT

Different methods have been introduced for acceleration of orthodontic tooth movement. Because it is less invasive with minimal side effects, Platelet rich plasma (PRP) is one of the most recently used techniques. To evaluate the effect of submucosal injection of PRP on maxillary canine retraction and to report any associated pain. Twenty patients were selected and randomly assigned in a split mouth trial designed to obtain PRP injection in one side (study side) while the other received no injection and served as a control. Before canine retraction, injection was done and not repeated again. After extraction, leveling and alignment; canine retraction was done on 0.017x0.025-inch stainless steel arch wire with closed coil spring from the canine hook to a mini-screw inserted between upper second premolar and first molar on both sides. The study duration was 4 months. Alginate impression was taken before canine retraction and every month for four months. Data was obtained from digitized models to measure the amount of canine retraction. Pain assessment was carried out by visual analogue scale (VAS). The canine retraction rate in the study side was faster than the control side with a statistically significant difference ($P<0.05$) in all the four months of the study duration. The total distance travelled by the canine was greater in the study side with a statistically significant difference ($P=0.022$). Higher pain levels were observed in the study side. Submucosal injection of PRP is a minimal invasive and safe approach for accelerating canine retraction and reducing overall treatment time.

Key words: Acceleration, Platelet rich plasma, Pharmacological approaches, Canine retraction.

Introduction

Orthodontic remediation is considered one of the most time-consuming dental treatments [1-4]. The estimated duration of the orthodontic treatment using conventional orthodontics is 24 months which may vary according to the severity of the case, individual characteristics, and treatment plan [2], prolonged treatment has a lot of adversities including caries [5, 6], external root resorption [7, 8], periodontal diseases and patient burnout [9]. Many attempts have been made to shorten the duration of orthodontic treatment including surgical, physical, and biological approaches [10], but there is still a lot of uncertainty and unanswered questions about most of these technologies. Several investigations have estimated the impact of many biological materials on the rate of orthodontic tooth movements (OTM) such as prostaglandin [11-13], vitamin D [14, 15], vitamin C [16], and parathyroid hormone [17] demonstrating favorable results. However, the use of supplementary hormones or other allogenic products require frequent injection and can cause undue systemic effect [18]. One of the lately applied biological agents to increase the rate of OTM is PRP. PRP is an autologous concentration of human platelets in a small volume of plasma [18]. PRP contains a lot of α granules which contain a lot of autologous growth factors and cytokines. These growth factors and cytokines play a very important role in osteoblastic and osteoclastic activity stimulating the alveolar bone remolding process [19, 20]. Liou [21] reported that PRP can accelerate different types of tooth movement clinically. Rashid *et al.* [22] and Gulec *et al.* [23] reported a positive relationship between local injection of PRP and acceleration of OTM in animal studies. On the contrary Akbulut *et al.* [24] reported that PRP was not beneficial in accelerating OTM. Also, Timamy *et al.* [25] reported the short-term acceleration effect of PRP. As the effect of PRP is still controversial, further clinical studies have to be conducted to confirm the effectiveness of PRP on OTM acceleration. The purpose of the present trial was to evaluate the influence of submucosal injection of PRP on maxillary canine retraction and to record any related pain.

Materials and Methods

This trial is a split-mouth randomized clinical study with 1:1 allocation. The trial was carried out at Mansoura University and approved by the faculty of dentistry ethical committee (No. M08070519).

The sample size was calculated using G power version 3.1.2.9 based on a type I error frequency of 5%. Taking into

consideration the vitro studies done by Rashid *et al.* [22] and Gulec *et al.* [23], we assumed the effect size difference between groups to be large (0.4). The calculation using repeated Anova had revealed that 17 patients were needed. The sample was increased by 10% to 20 patients to guard against any dropout during the trial. A simple randomization procedure drawing lots was used to allocate the side of the maxilla for the PRP injection, while the opposing side will serve as the split-mouth control. All patients were recruited from subjects attending the orthodontic department, faculty of dentistry, Mansoura University.

The following inclusion characteristics were applied: (1) Both male and female subjects with class II division 1 malocclusion that require therapeutic extraction of the upper first premolars, (2) Age ranging from 16 to 22 years, (3) Good general and oral health, (4) Maximum anchorage required using a mini implant. The exclusion characteristics were: (1) Systemic diseases or medication that are probable to influence bone biology, (2) Evidence of root resorption, (3) Poor oral hygiene, (4) Previous orthodontic treatment.

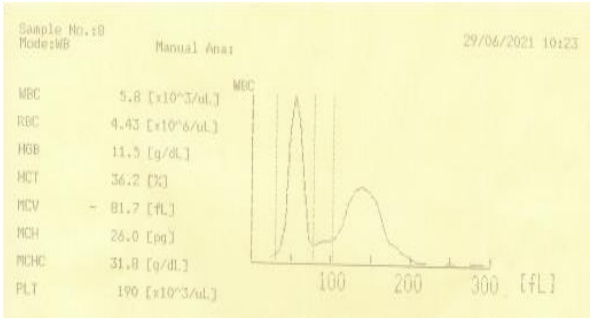
All patients were acquainted with the study and the injection procedures and then they were invited to sign a consent.

Methods

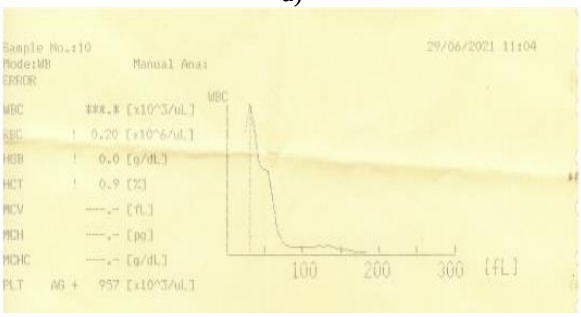
All patients were treated using fixed orthodontic brackets MBT prescription; 0.022-inch slot brackets (Dentaurum, Germany). After initial leveling and alignment, each patient received two mini-screws (3M-Unitek, 1.8x8 mm) buccally on both sides between the upper second premolar and the upper first molar, 5 mm from the alveolar crest to be used as direct anchorage. The patients were referred for extraction of the upper first premolars within the same week of the mini-screw insertion. After that, leveling and alignment were completed until reaching 0.017x0.025-inch stainless steel wire to minimize binding and friction during canine retraction. The canine retraction was started after 6 months to ensure complete healing of the extraction socket [26].

PRP preparation and injection

PRP preparation was done under aseptic processing procedures by double spin technique as described by Liou [21]. A thirty ml from whole blood was drawn from the patient and was put in 3 PRP tubes (Golden VAC), each contains sodium citrate as an anticoagulant. The tubes were turned 180^{o} upside down, shook, and mixed 6-8 times. The blood was first centrifuged under 1000 rpm for 12 minutes to separate red blood cells then pursued by a second centrifuge under 3000 rpm for 8 minutes to concentrate platelets. A high PRP concentration was obtained (5 times the concertation in whole blood) **(Figure 1)**.



a)



b)

Figure 1. AZZOTA USA B-LSC-6K Centrifuge was applied producing high concentration PRP (5 times the concentration in the entire blood)

Before PRP injection, local anesthesia was used in the study side for pain control. Then 30 units of PRP have injected submucosally in six injection sites into the buccal and palatal mucosa distal to the canine. There are three sites of injection on the buccal mucosa. The first one was at 3 mm distal to the canine. The second one was marked 3 mm from the first one. And the third point of injection was 3 mm from the second one. The same procedure was conducted on the palatal surface **(Figures 2 and 3)**. All the injections were volumetrically equal (5 units each area) and performed only before canine retraction and not repeated. No injection was done on the control side.

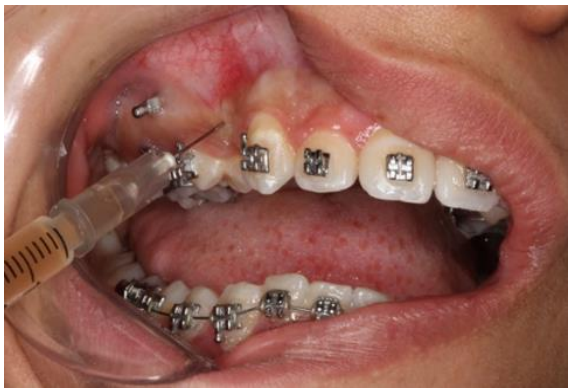


Figure 2. Submucosal injection of PRP buccally on the study side.

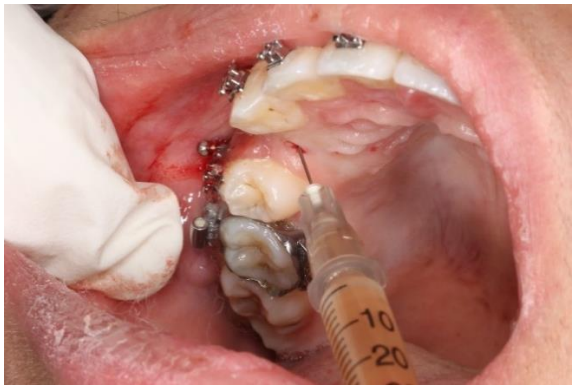


Figure 3. Submucosal injection of PRP palatally on the study side.

Following PRP injection, the canine retraction was initiated using a nickel-titanium closed coil spring delivering a retraction force of 150 gm per side and stretched between the mini-screws and the canine hook. Force level was adjusted using force gauge (Correx 100-500 gm, 040-712-Dentaurum, Pforzheim, Germany).

Alginate impressions were made just before canine retraction (T0) and monthly for four months (T1, T2, T3). Each stone cast from (T0-T4) was scanned using a 3D shape scanner (3 shapes, Copenhagen, Denmark). Superimposition of the five models was done using 3-shape analyzer software by using three points on the third rugae. The canine cusp tip was localized in each digital model. The space between the frontal plane and canine cusp tip was measured in each digital model. Then the amount of the monthly canine retraction was calculated from the variance in the canine cusp tip position in the five models. All the models were coded and shuffled before measurements to ensure blinding during data analysis.

Pain perception was recorded using a 10 mm visual analogue scale (VAS) at eight different time intervals (during injection, 1 hr later, 6 hr later, 12 hr later, one day later, three days later, five days later and seven days later). The questionnaire was 10 cm horizontal line and the patients were instructed to put a vertical mark to show the amount of pain they experienced, where (0-1) no pain, (2-4) mild pain, (5-7) moderate pain, (8-9) severe pain and 10 un-tolerable pain. All patients were instructed not to consume any analgesic to avoid any interference in comparison to pain perception. In the case of severe pain, analgesics were prescribed and those patients were excluded from pain assessment.

Statistical analysis

All the values were analyzed using the statistical package for social science (SPSS) version 24. Considering the canine movement, descriptive statistics (mean, standard deviation, minimum and maximum) were calculated for each side. Shapiro-Wilk test was applied to check the normal allocation of the obtained data. A paired t-test was applied to detect the statistical significance of variances through the canine retraction rate between the two sides at 95% confidence level, significance level was considered at $p < 0.05$

Results and Discussion

Twenty patients completed the study (4 months) with an age range of 16-22 years (mean 19±3.74 years). All the PRP injections were completed safely without any complications. The rate of the canine retraction and the associated pain were evaluated for all the 20 patients.

The canine retraction rate showed a statistically significant difference ($p < 0.05$) between both sides during the four months of the trial interval time with a mean value of 1.39, 1.16, 1.09, 0.87 mm in the first, second, third, and fourth month respectively for the control group and mean value of 1.66, 1.49, 1.10, 1.07 mm in the first, second, third and fourth month respectively in the study group **(Tables 1 and 2)** indicating acceleration of OTM with PRP injection.

Table 1. Mean values for the rate of canine retraction in two groups (mm)

Measurement	Group	Min.	Max.	Mean	SD
First Month (T0-T1)	Control	0.72	2.30	1.3980	0.62257
	Study	1.11	2.41	1.6680	0.48129
Second Month (T1-T2)	Control	0.67	1.88	1.1600	0.41644
	Study	0.95	2.62	1.4980	0.50749
Third Month (T2-T3)	Control	0.51	1.87	1.0900	0.44649
	Study	0.52	1.88	1.1050	0.44873
Fourth Month (T3-T4)	Control	0.06	1.99	0.8720	0.67115
	Study	0.42	2.18	1.0760	0.63353

Table 2. Comparison of the mean difference of the amount of canine retraction between groups for each month

Measurement	Group	Mean Difference	SD	SEM	95% Confidence Interval of the Difference		t	df	P-Value
					Lower	Upper			
First Month (T0-T1)	Control Study	-0.27000	0.31344	0.09912	-0.49422	-0.04578	-2.724	9	0.023*
Second Month (T1-T2)	Control Study	-0.33800	0.38381	0.12137	-0.61256	-0.06344	-2.785	9	0.021*
Third Month (T2-T3)	Control Study	-0.01500	0.01958	0.00619	-0.02901	-0.00099	-2.423	9	0.038*
Fourth Month (T3-T4)	Control Study	-0.20400	0.28159	0.08905	-0.40544	-0.00256	-2.291	9	0.048*

* Significant at P ≤ 0.05

The mean total distances traveled by the canine during the study period in the control and the study side were 4.273 mm and 4.331 mm respectively with statistically significant differences (P=0.022) **(Table 3)**.

Table 3. Comparison of the mean difference in the total amount of canine retraction between groups

Measurement	Group	Mean	SD	Mean Difference	SD	SEM	95% Confidence Interval of the Difference		t	df	P-Value
							Lower	Upper			
Total Retraction (T0-T4)	Control	4.2736	0.1385	-0.0574	0.0657	0.02079	-0.10443	-0.01037	-2.761	9	0.022
	Study	4.3310	0.1632								

The mean rate of canine retraction was 1.06 and 1.08 mm/month for the control and the study groups respectively.

All patients reported tolerable pain and none of them consume any analgesic, however, the data demonstrated higher pain in the study side as compared to the control side 1, 6, 12, and 24 hours later with statistically significant difference (p <0.05) **(Table 4)**. the highest pain scores were at 6 and 12 hours after injection. After 24 hours, no pain was reported on either side.

Table 4. Comparison of the mean difference in pain perception between groups at four different time intervals

Measurement	Group	Mean Difference	SD	SEM	95% Confidence Interval of the Difference		t	df	P-Value
					Lower	Upper			
1 hour	Control Study	0.500	0.707	0.224	-0.006	1.006	2.236	9	.050*
6 hours	Control Study	1.900	1.101	0.348	1.113	2.687	5.460	9	0.000*
12 hours	Control Study	2.100	0.738	0.233	1.572	2.628	9.000	9	0.000*
24 hours	Control Study	1.200	1.317	0.416	0.258	2.142	2.882	9	0.018*

* Significant at P ≤ 0.05

PRP has been considered to be a new technique in the acceleration of OTM because of its autogenous nature, healing abilities, and minimal side effects [27].

Several methods have been evolved to describe the method of PRP preparation [28]. PRP was first prepared by blending with calcium chloride and thrombin to coagulate the platelets into a gel form and activate the growth factor [29]. However, this gel form had a short duration of action. The PRP used in this study was prepared similar to PRP used by Liou without blending with calcium chloride and thrombin as long as that it could be preserved in liquid form, could be injected, and had a prolonged effect on the target tissue [21].

PRP used in this study was prepared by double centrifuge technique to obtain a high concentration of PRP (5 times concentration in whole blood). Seidel *et al.* [30] showed in

their study that a higher concentration of platelets was obtained when PRP was prepared by double centrifugation protocol, while a lower concentration of platelets was obtained by single centrifugation protocol.

Using a high concentration of PRP is more effective in the acceleration of OTM than moderate concentration as described by Gulec *et al.* [23] who stated that moderate concentration was effective but less so than a high concentration of PRP.

In contrast, Akbulut *et al.* [24] reported that a higher dose of PRP (4.5 fold) was not beneficial as an adjunct to orthodontic treatment.

PRP was injected submucosally not subperiosteally or intraligamentary following the proposal of the use of the PRP according to previous studies [21, 23, 24]. Also previous studies evaluating the effect of injection of the local pharmacologic agent in the acceleration of OTM reported submucosal injection [11, 23, 31, 32].

The injection technique submucosally into the buccal and palatal mucosa distal to the canine followed the same protocol reported by Liou [21]. This could be matched with the results of Akbulut *et al.* [24] who injected the PRP only at the buccal vestibular mucosa next to the distal root of the maxillary right first molar. Also, Gulec *et al.* [23] injected the PRP only at the buccal vestibular mucosa next to the mesial root of the maxillary right first molar. Conversely, Rashid *et al.* [22] and EL-Timamy *et al.* [25] assessed the effect of intraligamentary injection of PRP.

The injection was done before canine retraction and did not repeat as one injection of PRP last for five-Six months clinically with a higher rate of acceleration during the second to a fourth month after the injection as described by Liou [21].

The data of the present trial showed a higher rate of canine retraction in the study side in all the four months of the trial when compared to the control side with a total retraction of the canine 4.273 and 4.331 mm for the control and the study sides respectively. Although the results being statistically significant ($p = 0.022$) they were clinically non-significant. These results were in agreement with Gulec *et al.* [23] who recorded that PRP accelerates OTM by 1.4 to 1.7 times. Also, Rashid *et al.* [22], who observed greater significant acceleration in the PRP group.

Human studies using PRP in the acceleration of OTM are few. A study done by Ali *et al.* [33] reported that the canine retraction rate was faster on the study side as compared to the control side with a rate of 29.1%. On the other hand, El-Timamy *et al.* [25] concluded that PRP didn't exhibit a long-term acceleration effect however there was a statistically significant rise in the rate of cuspid retraction throughout the early phases of tooth movement.

Pain scores were higher in the study group than in the control group especially after 6 and 12 hours from the injection. These results were in agreement with the results described by Liou [21] who stated that 85 % of patients reported 6-12 hours of post-injection discomfort. This pain could be related to the higher PRP concentration as it has been clinically reported that the greater PRP concentration the greater the post-injection discomfort [21].

Conclusion

- Submucosal injection of PRP is a minimally invasive and cheap approach for accelerating canine retraction and reducing overall treatment time.
- Repeated injection of PRP during treatment time needs further investigation as to its effect decrease over time.

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Conflict of interest: None

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Ethics statement: The study protocol was approved by IRB (code no. M08070519).

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