

Original Article

Efficacy of platelet rich plasma (PRP) on mouth opening and pain after surgical extraction of mandibular third molars

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Abstract – Introduction: Surgery of mandibular third molar teeth for removal is one of the most common procedures undertaken in oral and maxillofacial surgery departments. The complications created by the post extraction wound healing and physiological consecution of third molar surgery can affect patients' quality of life. Platelet rich plasma (PRP) is an autologous concentrate of platelets suspended in the Plasma that accelerates healing by concentration of growth factors which lessen the inflammation and as a result pain and trismus. **Materials and methods:** A Study was conducted on 130 patients at the department of Oral and Maxillofacial Surgery, FUCD Islamabad, for a period of 6 months after ethical approval. The patients with impacted mandibular wisdom teeth were selected and divided equally into two equal groups. The pain score was measured using the visual analogue scale (VAS) and trismus was measured using Vernier caliper before the surgery, immediately after surgery, on 3rd and 7th follow up visits. **Results:** The mean postoperative pain on 7th day was significantly lower in the PRP group with statistically significant P -value <0.0001 . Trismus was also less reported in the PRP group with P -value <0.00065 . **Conclusion:** Platelet Rich Plasma is effective to lessen trismus and pain after surgical removal of mandibular third molar teeth.

Introduction

The removal of mandibular third molar teeth through surgery is one of the most prevalent procedures undertaken in oral and maxillofacial surgery departments. Most commonly the benefits of surgical removal of a third molar tooth include alleviation of the sign and symptoms of pericoronitis and its potential consequences. However, lower impacted wisdom tooth extraction through surgery usually causes post-surgical sequelae like pain, trismus and swelling as a result of postoperative inflammatory response. The problems created by the disturbances in the post extraction wound healing and physiological sequelae of third molar surgery can significantly affect the patient's quality of life [1,2].

Platelet rich plasma (PRP) is an autologous concentrate of platelets suspended in Plasma [3,4]. Its curative role is based on acceleration of healing by concentration of growth factors like Platelet derived growth factor (PDGF), Transforming growth factor- β (TGF β_1 & TGF β_2), vascular endothelial growth factor, 3 isomers of platelet-derived growth factors (PDGF- $\alpha\alpha$, PDGF- $\beta\beta$ & PDGF- $\alpha\beta$) and endothelial growth factors [3]. These growth

factors are considered to have the ability to facilitate chemotaxis, mitogenesis, angiogenesis, synthesis of collagen matrix and favors tissue repair when applied on bone wounds [5–7]. A study conducted in 2011 showed that the percentages of trismus in PRP and no-PRP groups were 33.6% and 55.5% respectively [1]. Similarly another study showed that mean pain score (VAS) in control and study groups were 1.05 ± 0.58 and 0.65 ± 0.65 respectively [8].

Mouth opening is the maximum distance between the maxillary central incisors and the mandibular central incisors using a millimeter caliper [1]. Inflammation of muscles of mastication is a frequent residuum to surgical removal of mandibular third molars (lower wisdom teeth). The condition is usually resolved on its own in 10–14 days, during this time eating and oral hygiene are compromised. PRP speeds-up healing by concentration of growth factors which can lessen the inflammation and decrease trismus and pain [9–13].

The purpose of this study is to determine the therapeutic effect of PRP in 3rd molar extraction socket in terms of pain and trismus (mouth opening) in post-operative period as compared to the extraction sockets of mandibular 3rd molars without PRP at 3rd and 7th post-operative day respectively.

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Materials and methods

Randomized controlled trial study was conducted at the department of Oral and Maxillofacial Surgery, Foundation University College of Dentistry, (FUCD) Islamabad, for a period of 6 months (from 21.07.2015 till 14.5.2016). Sample size was calculated by using WHO sample size calculator with the following calculations and non-probability consecutive sampling technique was used.

Level of significance:	5%
Power of test:	80%
Anticipated population proportion:	33.6% [1]
Anticipated population proportion:	55.5% [1]
Sample size:	65 patients in each group (i.e. Total 130 patients) 65 patients = control group (without PRP) 65 patients = study group (with PRP)

Initial hypothesis

Platelet rich plasma (PRP) is of difference in relieving trismus and pain when used topically after surgical removal of mandibular third molars by increasing the concentration of platelets in bone defects may lead to improved and faster healing and lessen the consequences associated with delayed healing as well as stimulate early new bone formation and decrease the pain and to enhance the mouth opening in immediate post operative period.

Inclusion criteria

- Age between 18–45 years
- Either gender
- Patients requiring extraction of mandibular 3rd molars
- ASA grade 1
- Nonsmokers and non alcoholics
- Not allergic to any medicines
- No pain before the extraction procedure
- No trismus, i.e. normal mouth opening before the extraction procedure

Exclusion criteria

- Systemic diseases
- Compromised immune system
- Platelet count less than 1.5 lacs/cmm

- Allergy to drugs
- Patients not willing to participate in the study
- Pregnant females / Lactating mothers
- Presence of pericoronitis, periapical infection or any associated lesion.

Data collection procedure was carried out after permission was taken from the Ethical Committee of Foundation University College of Dentistry (FUCD), for the study. Patients reporting to OMFS department of FUCD for surgical removal of mandibular 3rd molar teeth were selected after history, clinical examination and radiographic evaluation. Patients were distributed in two groups (PRP and no-PRP) through random lottery method. An informed written consent was obtained from all the patients participating in the study.

The pain (independent variable) score was recorded using Visual Analogue score (VSA) from 0–10, which is a horizontal line 10 cm long and was used with verbal cues on either ends with numbers given after every cm so in total of 10 nos (numbers). No 0 will be “no pain at all”, No 1 s “very very mild pain”, No 2 s “very mild pain”, No 3 s “mild pain”, No 5 s “moderate pain”, and No 10 “worst pain ever”. Patients will be instructed to point out a number that corresponds with the pain. The mark with the numerical value will be the pain score (pain was measured before the surgery, immediately after surgery, at 3rd and 7th post op day respectively). Trismus (dependent variable) was measured using Vernier-caliper from upper central incisor to lower central incisor teeth in mm (millimeter), and we have considered trismus in mouth opening less than 10 mm. Trismus was measured preoperatively, immediately after surgery, on 3rd and 7th post op day.

PRP preparation

The preparation of PRP was conducted at the department of Oral and Maxillofacial Surgery, Foundation University College of Dentistry, Islamabad. Prior to the start of the surgery, written and informed consent was taken, 8–10 ml blood was withdrawn intravenously from the patient (mostly from antecubital area, using median-cubital or cephalic vein) and collected in a sterile vacuum tube coated with anticoagulant (Na Citrate). The venous blood and anticoagulant were mixed by gently. Automated centrifuge machine was used to prepare PRP initially at a speed of 1200 rpm for 10 minutes, after which 3 layers formed. 1) An upper straw colored PPP (platelet poor plasma). 2) A middle buffy coat rich in platelets. 3) Lower layer rich in red blood cells. The straw colored upper layer was collected along with buffy coat and centrifuged again at 2000 rpm for 10 minutes. The PRP rich in leucocytes was formed at the bottom of the test tube; the upper PPP was discarded using pipette. For activation 1 ml of calcium chloride was added to Platelet Rich Plasma. This layer of approximately 2 ml of PRP was then taken into the residual bone cavity along with gelsponge to stimulate regeneration in wound healing after surgical extraction of mandibular third molar tooth followed by sutures.

At each follow up visit *i.e.* 3rd and 7th post op day pain and trismus were analyzed and the observations were documented in the data collection proforma. Final outcome will be measured at the 7th post-operative day. Both groups were given regular analgesics post-operatively for first three days every 8 hourly.

Data was entered and analyzed by using SPSS version 22.0. Mean and standard deviation will be calculated for Quantitative variables like age of patients and pain. Frequency and percentage will be calculated for Qualitative variables like gender of patients and trismus. Chi-square test was used to compare trismus in both the groups. Independent sample t-test was used to compare mean pain score in both the groups. *P*-value <0.05 will be considered significant.

Results

This study was based on comparison between Platelet Rich Plasma group (study group) and non-Platelet Rich Plasma group (control group) after surgical removal of mandibular third molars on mouth opening (trismus) and pain. A total of 130 patients with ages 18–45 years were divided into two equal groups of 65 patients each, in control (non PRP group) and study (PRP group). Out of total patients, 78 were females and 52 were males as shown in Table I. Out of 130 patients, the mean age was 27.95 with a standard deviation of 6.05. The minimum age limit was 19 years and maximum age limit was 43 years in our study as in Table II.

72 surgical extractions were carried out on Right sided mandibular third molar teeth whereas, 58 were on the Left side. Out of the 72 extractions on the Right sided third molars,

Table I. Frequency of Patients according to Gender.

	Frequency	Percent
Male	52	40.0
Female	78	60.0
Total	130	100.0

Table II. Age of the patients.

	N	Minimum Age	Maximum Age	Mean	Std. Deviation
Age of the patient	130	19	43	27.95	6.05

Table III. Trismus in PRP and non PRP groups.

Trismus	Two Groups		<i>P</i> -value
	PRP Group	Non-PRP Group	
Yes	21 (32.3%)	51 (78.5%)	<0.05
No	44 (67.7%)	14 (21.5%)	

38 were carried out in patients participating in the study group (PRP group) whereas 34 were carried out in the control group (non-PRP group). 27 patients from the study group (PRP group) had surgical extraction of mandibular third molar teeth on the Left Side from a total of 58 patients whereas, 31 patients belong to control group (non-PRP group). There was no statistically significant difference in age and gender in both the groups. (Tabs. I and II).

Out of 65 patients from PRP group (study group) 21 patients presented with trismus either on the 3rd or 7th post-operative day with 32.3% .whereas, 51 patients out of total 65 patients from the non PRP group (control group) presented with trismus on post-operative follow up visits with 78.5% as shown in Table III.

44 patients from PRP group (study group) had no trismus on any follow up visits either on 3rd or 7th post-operative day with 67.7%. Out of 65 total patients from non-PRP only 14 presented with no trismus on any follow up visits with 21.5%. The *p*-value is significant ≤0.00 as in Table II. None of the patient considered in the present study, had trismus initially before proceeding with the surgical removal of the mandibular third molar tooth as per inclusion criteria for patient selection.

Pain (independent variable) was measured by visual analogue scale (VAS) ranging from 1–10, where 1= no pain and 10= worst pain ever. From 130 total patient, the mean value for pain before surgery was 1.08 with standard deviation of ±0.27 (1.08 ± 0.27). Pain immediately after surgery, shown to have mean value of 2.91 with a standard deviation of ±0.83 (2.91 ± 2.91). On 3rd post-operative day the mean value was 4.76 with a standard deviation of ±1.77 (4.76 ± 1.77). Pain on 7th post-operative day had mean value of 1.45 with a standard deviation 0.70 (1.45 ± 0.70) as shown in Table IV.

Trismus (limited mouth opening) was more common in females as per our study after the surgical extraction of the mandibular third molars, 43 female patients presented with trismus on the follow up visits. Whereas, out of total 130 patients 35 females had no trismus on any follow up visit as shown in Table V. From total 130 patients only 29 male patients

Table IV. Mean value according to Visual Analogue Scale (VAS) for Pain.

Variable	N (in both the groups)	Minimum	Maximum	Mean	Std. Deviation
Pain (before surgery)	130	1	2	1.08	0.27
Pain (after surgery)	130	2	5	2.91	0.83
Pain (3rd day)	130	1	9	4.76	1.77
Pain (7th day)	130	1	4	1.45	0.70

Table V. Percentage values of Trismus according to gender.

Gender	Trismus (in both the groups)		P-value
	Yes	No	
Male (Total 52)	29 (40.3%)	23 (39.7%)	0.943
Female (Total 78)	43 (59.7%)	35 (60.3%)	

Table VI. Trismus according to Age groups.

Age (years)	Trismus (in both the groups)		P-value
	Yes	No	
18–30 years (Total 82)	49 (68.1%)	33 (56.9%)	0.190
31–45 years (Total 48)	23 (31.9%)	25 (43.1%)	

Table VII. Pain on 7th post-operative day in PRP and non PRP group.

PAIN	Two Groups	n	Mean	Std. Deviation	p-value
PAIN (7th day)	PRP Group	65	1.06	0.242	0.0001
	Non-PRP Group	65	1.85	0.795	

presented with trismus on follow up visits as compared to 23 males with no trismus on any follow up visits as shown in [Table V](#) with P -value of ≤ 0.943 . Trismus was more common in patients from age ranging 18–30 years, 49 patients presented with trismus out of 130 total patients. Whereas, 23 patients from age limit of 31–45 years presented with trismus with a total of 72 patients with trismus out of 130 total patients included in the study (PRP and no PRP group) shown in [Table VI](#). 33 patients from age group of 18–30 years, presented with no trismus on any follow up visit after surgical extraction of mandibular third molar whereas, 25 patients from the age group of 31–45 years had no trismus. From a total of 130 patients, 58 patients presented with no trismus. P -value is ≤ 0.190 as shown in [Table VI](#).

Pain was less in PRP group as compared to non PRP group according to Visual Analogue scale on the 7th follow up visit with a mean value of 1.06 ($SD \pm 0.242$) as compared to 1.85 ($SD \pm 0.795$) in non PRP group as shown in [Table VII](#) with a significant P -value ≤ 0.00 . According to statistics shown in

[Table VIII](#), On 7th post-operative day, 40 male patients experienced no pain on any follow up visit after surgical extraction of mandibular third molar teeth as compared to, 9 male patients with very very mild pain & 3 patients who experienced very mild pain. 46 female patients experienced no pain on 7th post-operative day. Whereas, 21 experienced very very mild pain, to 10 female patients with very mild pain. Only 1 female presented with mild pain on the 7th follow-up visit as shown in [Table VIII](#). A total of 86 (male and female patient) had no experienced of pain on 7th post-operative day as compared 30 patients with very very mild pain and 13 with very mild pain only. The P -value was ≤ 1.73 . Patients from age group of 18–30 years, 52 did not experience any pain. Whereas, 21 patient experienced very very mild pain on visual analogue scale (VAS), 8 patients experienced very mild pain and only 1 patient presented with mild pain on 7th day follow up visit as shown in [Table IX](#). 34 patients presented with no pain, 9 patients experienced very very mild pain whereas, 5 with very mild pain and none presented with mild pain on the 7th

Table VIII. Pain according to gender on 7th post-operative day.

Gender	PAIN (7TH DAY) (in total patients =130)				P-value
	no pain	very very mild pain	very mild pain	mild pain	
Male (52)	40 (46.5%)	9 (30.0%)	3 (23.1%)	0 (0.0%)	0.173
Female (78)	46 (53.5%)	21 (70.0%)	10 (76.9%)	1 (100.0%)	

Table IX. Pain according to Age on 7th post-operative day.

Age (years)	Pain (7TH day) (in total patients= 130)				P-value
	no pain	very very mild pain	very mild pain	mild pain	
18–30 years (82)	52 (60.5%)	21 (70.0%)	8 (61.5%)	01 (100.0%)	<0.05
31–45 years (48)	34 (39.5%)	9 (30.0%)	5 (38.5%)	0 (0.0%)	

postoperative day from the age group of 31–45 years shown in [Table IX](#). 86 patients from 18 years to 45 years after fulfilling the inclusion criteria presented with no pain on the 7th follow up visit. Whereas, 30 patients experienced very very mild pain and 13 presented with very mild pain. Only 01 patient experienced mild pain. The *P*-value was ≤ 0.05 .

Discussion

Impacted wisdom tooth extraction is one of the most common procedures, performed in the oral surgery department all around the world. Oral surgeons are constantly searching for ways and means to enhance wound healing, improve bone healing and lessen post-operative complication like pain, trismus and dry socket after surgical extraction of impacted third molars. One of the most recent and innovative technique is the use of platelet rich plasma (PRP) in the extraction socket immediately after extraction, particularly after surgical extraction of third molar teeth. This platelet rich in plasma has multiple functions starting from hemostasis to release of growth factors responsible in early wound healing, less pain and trismus with early bone formation. The concept behind this theory is increasing the concentration of platelets at the site of extraction socket for uneventful and enhanced wound healing which is desirable after third molar surgical extraction with less post-operative complications.

PRP is an autologous concentrate of platelets in plasma, that produce many growth factors which accelerate chemotaxis, mitogenesis, angiogenesis and synthesis of collagen matrix along with tissue repair. Growth factors like TGF b1 and b2 have shown not only to inhibit bone resorption, osteoclast activity and formation but also trigger rapid collagen maturation in early wounding. PDGF increase the wound healing capacity and recruits other angiogenic factors to wound site [14,15]. Research work has been carried out and is still going on to check the efficacy of platelet rich plasma on bone healing, mouth opening (trismus) and pain after surgical extraction of

mandibular third molars, although an increase of 3 to 5 times the whole blood baseline level is considered a clinical benchmark given by Marx *et al.* [5]. It is therefore a reasonable hypothesis that increasing the concentration of platelets in bone defects may lead to improved and faster healing and lessen the consequences associated with delayed healing as well as stimulate early new bone formation and decrease the pain and to enhance the mouth opening in immediate post operative period.

Platelet rich plasma with leucocytes accelerates wound maturity and epithelization. The activation of PRP causes degranulation of alpha granules present in the platelets, thus releasing the growth factors (PDGF, VEGF, IGF, bFGF, VEGF, SCDGF, EGF, FGF, Angioproteins) which are involved in angiogenesis [16]. Secreted growth factors directly stimulate local mesen-chymal and epithelial cells to migrate, divide and increase the synthesis of collagen and matrix with resulting formation of fibrous connective tissue many of the growth factors released in damaged tissue express combined action and interact between each other, providing the activation of different intracellular signaling pathways with enhanced tissue repair [17]. Leucocyte rich RPR at the wound site induce phagocytosis of debris, necrotic tissue and microbes by destroying extracellular matrix and allowing cellular migration through tissue, making the healing more efficient. Leukocytes are able to secrete many proteinases, including metallo-proteinases and serine which have important role in process of wound healing which will decrease the postoperative sequel of complications like pain and trismus after surgical extraction of mandibular teeth. Proteinases have ability to induce lymphocyte and platelet activation, activation of cytokines and formation of fibrin-platelet plug and are able to control the intensity of inflammatory process by deactivating the inflammatory cells [18].

In our study out of 130 patients 52 were male (40.0%) and 78 were female (60.0%). The mean age in our study was 27.95. Patients were considered from age between 18 to 45 years. Whereas, in one of the study it was 24.4 for PRP group and 24.9

for non-PRP group with a p -value of 0.677 [1]. In another study by Emeka V Obi and Seidu A Bello, the age range of the patients were 19 to 42 years with a mean age of 26.7 years [19].

In a study by Pushkar D. Gawande, mean value for pain on VAS on 3rd post-operative day was 1.05 with standard deviation of ± 0.58 and that on the 7th day was 0 with standard deviation ± 0 [8]. In another study Pain was significantly reduced to 1.1 ± 0.6 on day 3 and on day 7 as compared to 2.1 ± 0.7 on day 1 in the non PRP group. Pain had significantly reduced to 0.6 ± 0.6 on day 3 and to 0 on day 7 as compared to 1.8 ± 0.8 on day 1 in the PRP group using VAS [18]. Whereas in our study the mean value for pain on 3rd post-operative day was 4.76 with a standard deviation of 1.77 (4.76 ± 1.77) and that on 7th post-operative day was 1.45 with a standard deviation of 0.70 (1.45 ± 0.70). In comparison to another study the mean value for pain was 1.783 with a standard deviation of ± 1.22 (1.783 ± 1.22) on the 3rd postoperative day and a mean value of 0.945 with a standard deviation of 1.02 (0.945 ± 1.02) on the 7th post-operative day [19].

Following tooth extraction, a cascade of inflammatory reactions immediately begins, and the epithelial tissue is temporarily closed by clotting blood. Placement of PRP into the extraction socket, to promote the wound healing cascade by releasing therapeutic growth factors directly to the wound and hence patients experience less pain as compared to the control group [1].

In our study the pain on 7th post-operative in the non PRP group has a mean value of 1.85 with a standard deviation of ± 0.795 (1.85 ± 0.795) whereas, that in the PRP group on the 7th post-operative day has a mean value of 1.06 with a standard deviation of 0.242 (1.06 ± 0.242) with a p -value of ≤ 0.0001 . In a study by Pushkar D. Gawande, the mean value for pain on VAS on the 7th day was 0 with standard deviation ± 0 (0 ± 0) in the PRP as well as non PRP group [8]. The difference could be justified as only 20 patients were taken as sample size in the above mentioned study by Pushkar D. Gawande, whereas in our study 130 patients participated, more number of patients needs to be evaluated to justify the difference in both the groups. In our study pain was reported more in female patients, 1 patient (100%) reported mild pain, 10 patients (76.9%) with very mild pain and 21 (70.0%) reported very very mild pain.

We have divided the patients according to age into two subgroups, between 18–30 years and 30–45 years respectively after fulfilling the inclusion criteria in our study. Between the age group of 18–30 years, 52 patients reported no pain, 21 reported very very mild pain, 8 patients experienced very mild pain and only 1 patient reported with mild pain on the 7th follow up visit. Whereas patients between the age group of 31–45 years, 34 patients experienced no pain, 9 reported back with very very mild pain, 5 patients had very mild pain and none experienced mild pain on the 7th follow up visit. Whereas in a study by Olufemi K, the mean postoperative pain was lower for the PRP group at all the times when compared with the

control group with a p -value of ≤ 0.05 and mean value for post-operative pain on the 3rd post-operative day was 1.1 in the PRP group and 3.47 in the non PRP group whereas on the 7th post-operative day it was 0.46 in the PRP group and 1.23 in the non-PRP group [1].

In our study trismus was more common in female patients, 43 female patients experienced trismus and 29 male patients experienced trismus on 3rd or 7th follow up visit. The p -value was ≤ 0.943 . Trismus was more common in control group (non PRP group) in our study 78.5% reported trismus whereas, in study group (PRP group) it was significantly less, 32.3% reported trismus. The p -value was significant ≤ 0.00 . In a study by Olufemi K, the PRP group had trismus value 64.5% on 3rd day post-operative day and 33.6% on the 7th follow up visit as compared to 93.3% in the non-PRP group on the 3rd post-operative day and 55.5% on the 7th follow up visit. This difference was not statistically significant [1]. The reason could be less no of patients in the study (30 patients were considered) whereas, in our study sample size was 130 patients.

Conclusion

Platelet Rich Plasma (PRP) rich in leukocytes is able to enhance the healing process and stimulating growth factor release is effective in reducing trismus (decrease mouth opening) and pain after surgical removal of mandibular third molar teeth. It is highly efficient in resolving signs and symptoms associated with surgical extractions. PRP can be an effective treatment modality. It is not only cost effective but safe and reliable method for treating the patients requiring surgical extraction of their third molar teeth but more research work needs to be carried out to validate its efficacy.

Limitations

The limitation of this study includes, the sample size was only 130 patients with 65 in each group, large sample size should be included to comment more appropriate but even with this sample size prominent decrease in pain and mouth opening noticed in PRP group. Postoperative follow-up is of short duration to comment on the efficacy of PRP in complete soft tissue healing process but adequate enough to evaluate the effects of PRP in initiating and enhancing soft tissue healing. Longterm follow-up is required along with histological study of the bone for assessment of the efficacy of PRP on early bone formation.

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