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Addition of Demineralized Freeze-Dried Bone Graft to Platelet-Rich Fibrin is Superior to Platelet-Rich Fibrin Alone for Amelioration of Osseous Defect Following Impacted Mandibular Third Molar Removal: A Randomized Controlled Trial

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ABSTRACT

Background: Background: Extraction of impacted mandibular third molar causes osseous defect at the distal aspect of the adjacent second molar and the use of platelet rich fibrin (PRF) has shown to be a valid technique for promoting early bone regeneration at the osseous defect. Whether addition of demineralised freeze dried bone graft (DFDBA) to the PRF leads to better amelioration of the osseous bone defect has not been studied. We compared the application of DFDBA plus PRF versus PRF alone in the healing of post-extraction lower third molar osseous defect.

Materials and Methods: Patients with impacted mandibular third molars were randomly assigned into two groups: Group I: the extraction socket obliterated with DFDBA plus PRF;and Group II: the extraction socket obliterated with PRF alone. Post operative swelling, mouth opening, probing depth, bone density, and alveolar bone height were compared between the two groups.

Results: A total of 40 patients were included in the study and were randomized into the two groups of 20 patients each. Compared to Group II, the patients in Group I showed significantly more swelling at post-operative day-7.At post-operative 1-month and 6-months, patients in Group I showed lesser probing depth, higher bone density, and higher alveolar bone height compared to patients in Group II. Post-operative swelling at day-1 and mouth opening at day-1 and day-7 were comparable between the two groups.

Conclusion: Our study concludes that DFDBA mixed with PRF was more effective than PRF alone when used for obliteration of osseous defects following removal of impacted third molar. The mixture shows early evidence of new bone formation and better amelioration of osseous defect.

Keywords: Bone density; mandibular platelet-rich fibrin; demineralised freeze dried bone graft.

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INTRODUCTION

Mandibular third molar is reported to be the most frequently impacted tooth owing to the topography,phylogeny and ontogeny, and is directly or indirectly associated with various pathologies of maxillofacial region[1]. Therefore the surgical management of the impacted mandibular third molar for prophylactic or therapeutic reason is the most common surgical procedure performed byoral and maxillo-facial surgeons[1].Amongst the severalcomplications associated with surgical disimpaction of impactedmandibular third molars, dry socket and bony defects distal to the adjacent second molars remain very common. A number of augmentation procedures are performed today to stimulate regeneration or to enhance attachment of supporting structures in periodontal defect sites, such as scaling of contaminated cementum, application of acid conditioners, guided bone regeneration procedures and application of bone grafts.One of the most recent and innovative technique which is very successful is the use of platelet products such as platelet-rich fibrin (PRF)[2-4] and platelet-rich plasma (PRP)[5]. The use of PRF or PRP has shown to be an innovative technique for promoting bone regeneration at the distal surface of the mandibular second molar and to reduce post operative complication following extraction of impacted third molars.These platelet concentrates, especially when used in the surgical defect may stimulate osseous and soft tissue regeneration while also reducing inflammation, pain and unwanted side effects.[2-7]Many recent meta-analyses have supported the use of PRF for local application after lower third molar extraction for decreasing the incidence of alveolar osteitis and promoting early healing[2,3,7].

Another approach used in post-extraction osseous defect site is the use of various bone-grafting materials. The types of bone grafts are grossly classified as: autogenic bone, allogeneic bone, and xenogeneic bone. Autogenic bone has no antigenicity at all and is far superior in terms of quality as graft tissue. But because of the limited amount of bone that can be harvested, it is sometimes insufficient to fill large osseous defects and at such times allogeneic bone graft or artificial bone is indicated. Xenogeneic bone, derived from non-living bone of other species is highly antigenic and is now slowly becoming less popular.[8] Allograft bone is harvested from cadavers, and has both osteoinductive and osteoconductive properties, but lacks osteogenic properties because of the absence of viable cells.[9] The major advantage of harvesting allograft bone includes its ready availability in various shapes and size and no donor site morbidity. Thedemineralized bone allografts are used after appropriate irradiation.[10,11]

We have limited literature on the use allografts in surgical defects after mandibular third molar disimpaction; and whether addition of demineralisedfreeze dried bone graft (DFDBA) to the PRF leads to better amelioration of the osseous bone defect has not been studied. Therefore present study focuses to compare the results of healing of lower third molar sockets filled with DFDBA mixed with PRF versusPRF alone.

MATERIALS AND METHODS

Study design

In this randomized controlled trial patients who reported to the Department of Oral and Maxillo-Facial Surgery, People's Dental Academy, Bhopal, India, and who were willing for trans-alveolar extraction of impacted lower third molar, were recruited and randomly assigned into two groups:

- Group I: Extraction socket obliterated with PRF mixed with DFDBA.
- Group II: Extraction socket obliterated with PRF alone.

Aims and objectives

The objectives of the study were to assess post operative swelling, mouth opening, probing depth, bone density, and alveolar bone height in the two groups at various time points post operatively. Swelling and trismus were studied at post-operative day-1 and day-7, while probing depth, bone density, and alveolar bone height were studied at post-operative 1-month and 6-months.

Patients

Inclusion criteria

Patients within an age group of 25-50 years and those who were diagnosed with mandibular third molar impaction and who consented for trans-alveolar extraction of impacted lower third molar were included.

Exclusion criteria

Patients with significant medical co-morbidities and those who were on medications which could affect the bone density were excluded.

Fig 1: Freeze dried bone graft (Tata bone graft).

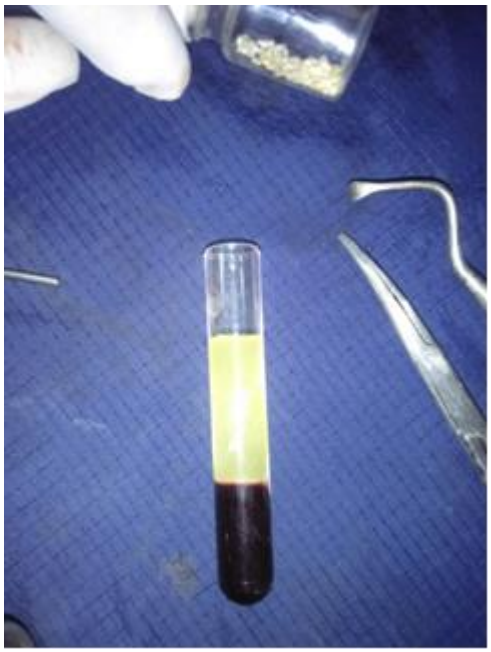

Fig 2: Platelet rich fibrin.

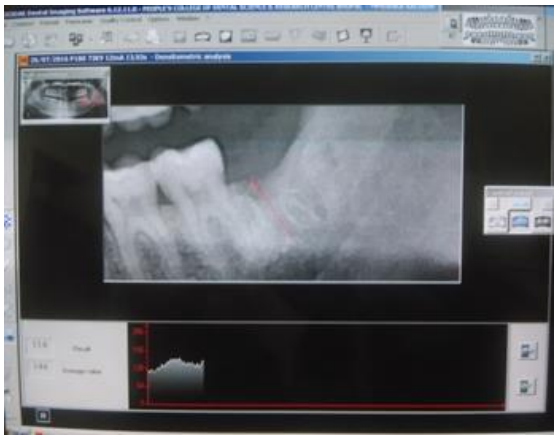

Fig 3: Densitometric analysis.

Table 1: Comparison of mean magnitude of swelling size among Group I and Group II at postoperatively 1st Day and 7th Day.

	Magnitude of swelling mean size(cm)			
	Postoperatively 1st Day		Postoperatively 7th Day	
Groups	Mean	SD	Mean	SD
Group I (DFDBAplus PRF)	0.79	0.22	0.26	0.08
Group II (PRF alone)	0.80	0.18	0.15	0.04
Student 't' Test Value	0.157		5.50	
Significance 'P' Value	0.875		0.001	

Table 2: Comparison of Mean Trismus (quantum of restriction) among Group I and Group II at postoperatively 1st Day and 7th Day.

	Trismus (cm)			
	Postoperatively 1st Day		**Postoperatively 7th Day**	
Groups	**Mean**	**SD**	**Mean**	**SD**
Group I (DFDBAplus PRF)	1.0	0.12	0.18	0.09
Group II (PRF alone)	1.06	0.15	0.20	0.11
Student 't' Test Value	**1.396**		**0.6293**	
Significance 'P' Value	**0.170**		**0.532**	

Materials and apparatus used

Materials:

- Demineralized freeze dried, irradiated bone allograft material (Figure 1) obtained fromTata Memorial Hospital Tissue Bank, Dr.Earnst Borges Marg, Parel, Mumbai, India.

- Platelet rich fibrin prepared according to techniques described by Choukroumet al[11] (Figure 2).

Particle size: <500 microns

Apparatus:

- Centrifugal machine with 3000 RPM
- Orthopantomogram (OPG) after extraction of mandibular third molars.
- Densitometric analysis software Kodak Dental Imaging Software version 6.7 with exposure parameters (73Kv; 10 mA; 13.3s) in the OPG program (Figure 3).

Surgical technique

Patients underwent surgical treatment in accordance with the rules of antisepsis and asepsis. The preoperative measurements of swelling and mouth opening were taken and all surgical extractions were performed by the same experienced surgeon. After administering local anaesthesia (1:200,000 Xylocaine with adrenaline), surgical removal of mandibular third molar was performed. Subsequently, the DFDBA mixed with PRF was placed in the extraction sockets of patients of Group I and the PRF aloe was administered to the extraction sockets of patients of Group II. The surgical incisions were primarily closed with 3.0 mersilk sutures. Post operatively all the patients were prescribed Amoxicillin 500 mg and Diclofenac sodium 50 mg three times a day for a period of five days. Sutures were removed on postoperative day 7 in all cases.

Measurements

Swelling (inflammation) was measured by using method described by Amin and Laskin.[12] Mouth opening was measured by inter-incisal distance pre-operatively and post- operatively. Probing depth measured using periodontal probe (Williams graduated periodontal probe) with Williams marking from 'free gingival margin(FGM)' to the bottom of the pocket in nearest mm. The average of probing depth was recorded at the three different position, i.e. Distobuccal(DB), Distal(D), and Distolingual(DL) on the distal surface of second molar taken 1st and 6th month postoperatively.

Table 3: Comparison of mean probing depth (mm) among Group I (Bone graft mixed with PRF) and Group II (only PRF) at postoperatively 1st month and 6th Month.

	Mean Probing depth(mm)			
	Postoperatively 1st month		Postoperatively 6th month	
Groups	Mean	SD	Mean	SD
Group I (DFDBAplus PRF)	1.5	0.35	0.9	0.12
Group II (PRF alone)	2.1	0.14	1.4	0.25
Student 't' Test Value	7.118		8.063	
Significance 'P' Value	0.001		0.001	

Table 4: Comparison of bone density analysis among Group I and Group II at 1stmonth and 6thmonth.

	Bone density Analysis			
	1st Month		6th Month	
Groups	Mean	SD	Mean	SD
Group I (DFDBAplus PRF)	144.50	5.11	151.60	4.90
Group II (PRF alone)	119.85	5.30	127.40	5.67
Student 't' Test Value	14.961		14.438	
Significance 'P' Value	0.001		0.001	

Table 5: Comparison of mean alveolar bone height among Group I and Group II at postoperatively 1st Day, 1st Month and 6th Month.

	Alveolar Bone Height(mm)					
	Postoperatively 1st Day		1st Month		6th Month	
Groups	Mean	SD	Mean	SD	Mean	SD
Group I (DFDBA plus PRF)	8.69	0.34	9.82	0.43	10.56	0.60
Group II (PRF alone)	8.76	0.25	9.082	0.25	9.59	0.20
Student 't' Test Value	0.795		6.620		6.792	
Significance 'P' Value	0.431		0.001		0.001	

Orthopantomogram (OPG) images were taken and bone density analysis was done at 1st month and 6th month post-operatively. Radiographic assessments were done using Densitometric analysis software of Kodak Dental Imaging Software version 6.7 with exposure parameters (73Kv; 10 mA; 13.3s). Densitometric analysis was done to compare the relative bone density at the extraction site at 1st

month and 6th month post-operatively. Alveolar bone height assessment was done from Point A (Alveolar crest distal to 2nd molar) to Point B (Apex of distal root of 2nd molar).

Statistical analysis

Data were entered into a spreadsheet (MS Excel 2007, Microsoft Inc., Redmond, Washington) and analyzed using a statistical software package (SPSS, version17.0, Chicago, Illinois). Quantitative data were expressed as means and standard deviation and compared using Student's T-test.

RESULTS

A total of 40 patients were included in the study and were randomized into the two groups of 20 patients each. Comparison of the clinical and radiographic parameters consisting of magnitude of swelling, trismus, probing depth, bone density and alveolar bone height among group I and group II were made.

Magnitude of swelling size

Table 1showsthe comparison of mean magnitude of swelling size between Group I and Group II patients at postoperative 1st day and 7th day. Postoperatively on 1st day the mean magnitude of swelling size was 0.79±0.22 cm among Group Iand 0.80±0.18 cm among Group II patients. Postoperatively on 7th day the mean magnitude of swelling size was reduced to 0.26±0.08 cm among Group I and 0.15±0.04 cm among Group II patients.At postoperative 1st day there was no statistically significant difference in magnitude of swelling size (P=0.875) while at postoperative 7th day the swelling reduced significantly in both the groups, however, the reduction was not equal, and swelling was significant leaaser in Group II than Group I (P=0.001).

Trismus (quantum of restriction)

Table 2displays the comparison of mean trismus (quantum of restriction) among Group I and Group II patientsat postoperative 1st day and 7th day. At postoperative 1stday,the mean trismus was similar between the two groups (Group I 1.0±0.12 cm versus Group II 1.06±0.15 cm; P>0.05). By 7th day postoperativedaythe mean trismus was significantly reduced in both the groups; and there was no statitiscally significant difference in trismus between the two groups (Group I 0.18±0.09 cm versusGroup II 0.20±0.11; P>0.05).

Probing depth

Table 3 reveals the comparison of mean probing depth among Group I and Group II at postoperative 1st month and 6th months. It was 1.5±0.35 mm and 2.1±0.14 mm among group I and II respectively at postoperatively 1st month while after 6 month it significantly reduced to 0.9±0.12 mm and 1.4±0.25 mm among groups I and II, respectively. Mean probing depth was significantly lower among group I as compared to group II both at postoperative 1st month and 6th month (P=0.001).

Bone density analysis

Table 4 reveals comparison of bone density analysis among Group I and Group II at postoperative 1st month and 6th month. Postoperatively at 1st month, the mean bone density analysis value was 144.50±5.11 and 119.85±5.30 among group I and II respectively. Postoperatively at 6th month mean bone density analysis value was 151.60±4.90 and 127.40±5.67 among group I and II respectively. Mean bone density analysis value was significantly higher in Group I as compared to group II, both at 1stmonth and 6th month (P=0.001).

Alveolar bone height

Table 5 reveals comparison of mean alveolar bone height among Group I and Group II at postoperative 1st day, 1st month and 6th month. Mean alveolar bone height was comparable in Groups I and II on postoperative 1st day. At postoperative 1st month and 6th month the mean alveolar bone height wassignificantly higher in group I as compared to group II.

DISCUSSION

We carried out a randomized controlled trial to compare the effectiveness and usefulness of PRF plus DFDBA versus PRF alone in the healing of lower third molar socket after extraction. This study evaluated the clinical and radiographic outcome and healing ability of both the approaches as a bone replacing material for treating osseous defects. We found that probing depth, bone density and alveolar bone height showed better results in test group at 6 months post-operatively compared to control group

thus proving that DFDBA mixed with PRF is superior to PRF alone for amelioration of osseous defect and shows early evidence of new bone formation.

The non-complicated surgical management of impacted mandibular third molar continues to challenge the clinicians[1]. After the removal mandibular 3rd molar an osseous defect is createdat the distal root of the second molar, which may lead to the loss of supporting structures distal to second molars. Also, the flap remains unsupported rendering the area distal to the second molar tooth more prone to the formation of periodontal defect after surgery. Bone augmentation procedures are considered to prevent formation of periodontal defects distal to second molar.There are various augmentation procedures reported in the literature, which are performed at the time of tooth extraction to maintain or enhance ridge form for prosthetic reconstruction and periodontal health[3,7]. Recently the use of platelet products such as platelet-rich fibrin (PRF)[2-4] and platelet-rich plasma (PRP)[5]have been found to be very successful for amelioration of osseous defects. Choukroun[11]and his associates were amongst the pioneers for using PRF protocol in oral and maxillofacial surgery to improve bone healing in implant dentistry. PRF is a second generation platelet concentrate and an autologous growth factor pool that contains a fibrin matrix incorporated with leukocytes, cytokines, platelets and circulating stem cells.[13,14]It has been used extensively to enhance wound healing, wound sealing, haemostasis, stabilization of graft and regeneration of bone.[13]Most of the studies conducted have found PRF application to be a valid method to control post operative signs and symptoms. A decrease in post-operative pain, swelling and trismus has been found after application of PRF following mandibular 3rd molar extraction.[13,15,16,17,18]Better soft healing and osseous regeneration was also noted.[18,19] PRF application has been found to be associated with significantly lower incidence of alveolar osteitis following 3rd molar extraction[13,15,20,21]and as a treatment modality for established dry socket[22]. Although a few studies and mata-analysis[23,24,25]have found contradictory results on the beneficial role of PRF, much evidence now supports the use of PRF.

It has also been shown that grafting of the surgical site results in faster regeneration of tissue. Graft materials can be broadly categorized as autografts, allografts or xenografts. Any bone graft can regenerate bone through three possible mechanisms: direct osteogenesis, osteo conduction, and osteoinduction[2]. The allograft, DFDBA has osteo inductive and osteoconductive properties.[26]

The hypothesis of our study was to tests whether addition of DFDBA to the PRF leads to better amelioration of the osseous bone defect. To the best of our knowledge the use of DFDBA mixed with PRF has never been reported in the mandibular 3rd molar area. The DFDBA was used because the very processes involved in its preparation i.e. decalcification exposes on its surface, the bone morphogenetic proteins which are osseoinductive[27,28] that is, they induce differentiation of mesenchymal cells into cartilage and bone and also acts as a scaffold for osteoconduction.[26]Not much literature could be found on usage of DFDBA with PRF and few studies which have been conducted are related to periodontal intra-bony and peri-implant defects. Few studies have found no significant difference between the regenerative capacity of DFDBA and PRF when used in the treatment of intra-bony defects.[29,30] A combination of DFDBA and PRF was found better than DFDBA alone in preservation of extraction sockets and treatment of intra-bony defects by few.[26,31,32] A study conducted by Simsek S et al[33] compared DFDBA plus saline, DFDBA plus PRF and DFDBA plus rifamycin in the treatment of artificially created peri-implant defects in rabbit tibias and found DFDBA plus PRF to show highest percentage of new bone formation.

In our study, swelling and trismus was compared between the study and control groups at 1st and 7th day postoperatively and probing depth, bone density and alveolar bone height were compared at 1st and 6th month postoperatively. Our study revealed that on 7th day postoperatively swelling was much lower in Group II as compared to Group I. The inter-incisal distance at postoperative 1st day and 7th day was comparable in group I and II and there was not much difference between the two groups. Our study was unique in this respect as no other study could be found related to magnitude of swelling and trismus after placement of graft in

mandibular third molar region. Other studies have found significant reduction in swelling and trismus after placement of PRF alone in third molar extraction sockets. Kumar N et al[16]conducted a study on 31 patients in which they placed PRF in socket after mandibular third molar extraction and found swelling and inter-incisal distance to be less on first post-operative day. Jeyaraj PE et al[18] conducted a similar study and found significant decrease in these post-operative sequelae on 3rd post-operative day.

A significant decrease in the probing depth was observed in both the groups at 1st and 6th month postoperatively, but the greatest decrease was present in Group I where the extraction sockets were treated with DFDBA plus PRF. This result was consistent with the results of various previous study conducted Bansal C et al[31] which was a split mouth study and they found greater probing depth reduction at 6 months in patients treated with DFDBA plus PRF as compared to DFDBA alone for the treatment of periodontal intra-bony defects. In a study, Agarwal A et al[32] found greater reduction in probing depth in patients where DFDBA plus PRF was used for intra-bony defects compared to DFDBA plus saline (4.15 ± 0.84 vs 3.60 ± 0.51 mm) at 12 months. In a similar study by Piemontese Mat al[34] they found that test group exhibited statistically significantly greater changes compared to the control group in probing depth reduction (4.3 +/- 1.7 mm versus 2.6 +/- 2.2 mm).However a study conducted by Shah et al[30] found no significant difference in probing depth reduction at 6 months when they used DFDBA in one group and PFR in another for periodontal intra-bony defects.

We also conclude from our study that Group I had higher bone density at the end of 1st month and 6th month. This initial rise in bone density simulates the results of Mulliken JB et al[35]who used demineralised allogenic bone implants for correction of maxillofacial deformities and found good osseous healing in about six months. This may be because healing by induced osteogenesis bypasses the resorption which is seen with healing of mineral-containing grafts[35]. Chadwick JK at al[29] found no difference in bone fill levels when he used DFBDA in one group and PRF in another for periodontal intra-bony defects(1.14 mm ± 0.88 versus 1.10 mm ± 1.01). Agarwal A et al[32] found greater bone fill in DFDBA plus PRF group as compared to DFDBA plus saline group when used for intra-bony defects.

The mean alveolar bone height at 1st month and 6th month was more in group I as compared to group II. We have measured alveolar bone height distal to 2nd molar as described by the Munhoz EA et al.[36]A study conducted by Thakkar DJ et al[26] found that 1.08 mm ridge height was lost in DFDBA plus PRF group and 1.36 mm of ridge height was lost in DFDBA group, thus concluding that DFDBA plus PRF was better in preserving bone height as compared to DFDBA alone. The above-mentioned observations was substantiated with radiographs (pre and post-operative) thereby providing enough evidence to prove definite osteogenic potential of demineralized freeze - dried bone allograft mixed with PRF.

We found no statistically significant difference in inflammatory response in both the groups. Minimum post-operative complication rate was observed in the study. DFDBA granules mixed with PRF assists in increasing the mean alveolar bone height of healing extraction socket and thus favour the osseous healing.Our study showed reduction in probing depth in cases were bone graft mixed with PRF was used as compared to PRF alone, which reveals better healing in the sockets. The mixture possibly promotes better wound healing and osseous regeneration through its osteoinductive and osteoconductive properties in the presence of growth factors.

Arenaz-Bua J et al[37]had compared allogeneic demineralized bone matrixin combination with PRP with autologous bone in combination with PRP and synthetic calcium hydroxyapatite (Novabone) in combination with PRP as a graft material after mandibular third molar extraction and foundautologous bone and demineralized bone matrix to have higher bone formation 6 months postoperatively. Composite xenogenic graft(Gent-tech) was used by Munhoz EA[36] et al at mandibular third molar region and they found no significant difference in bone density and crest healing among study and control groups. Baslarli et al[38]and Gurbuzer et al[39] have reported use of PRF alone in soft tissue impacted mandibular third molars and did not find significant enhancement in bone healing of extraction sockets.

One of the limitations of our study was that the 6 months postoperative follow-up is a short duration to comment on the significance of periodontal breakdown distal to second molar. However, this duration of time was adequate to evaluate the effects of DFDBA mixed with PRF and PRF alone in initiating and enhancing both hard and soft tissue healing. The other limitation of our study could be that to determine the quality of newly formed bone we did not perform histological analysis. Froum et al[40]conducted a histological comparison of healing extraction sockets implanted with bioactive glass and demineralized freeze dried bone allograft and found it to be a reliable method to assess surgical site after grafting procedures. Histological analysis was not undertaken in our study due to ethical considerations.

In conclusion, DFDBA mixed with PRF when used for the obliteration of osseous defects results in better soft and hard tissue healing of lower third molar post-extraction socket. The same combination can be used during the placement of implants or in the bony defects created after extractions for an orthodontic reason.

CONFLICTS OF INTEREST

The authors declare they have no potential conflict of interests regarding this article.

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