Original Article

Effect of "platelet rich" fibrin with bone marrow aspirate on the regenerative capacity of alveolar bone grafting with iliac bone graft: A randomized controlled trial

ABSTRACT

Objectives of the Study: (1) To evaluate the effect of platelet-rich fibrin (PRF) with bone marrow aspirate on regenerative capacity in patients undergoing iliac bone grafting for secondary alveolar bone grafting. (2) and to compare it with group 2 where only bone marrow aspirate was used along with iliac bone graft in secondary alveolar bone grafting.

Materials and Methods: A prospective study on patients with cleft alveolus, requiring bone grafting and reporting to our unit from October 2018 to October 2020 was included in this study. Group 1 (bone marrow aspirate with PRF along with cancellous iliac bone graft) and Group 2 (bone marrow aspirate concentrate and cancellous Iliac bone graft without PRF). Computerized tomography (CT) scan was done to assess the volume of defect at the following intervals: Pre-op, immediate post-op, and 12 months. The outcome is assessed using a CT scan by statistical analysis.

Results: Mean cleft alveolus volume measured preoperatively in group 1 was 2.5cc, post-operatively measured immediately was 3.2cc, and 12 months post-operative was 2.2cc. Mean volume defect in group 2 is 2.3cc, post-operatively measured immediately was 2.6 cc, and 12 months post-operative was 1.9cc. The average resorption rate at immediate post-op to 12 months interval in group 1 was 25% and in group 2 was 30%. The overall percentage of regenerated bone in group 1 was 75% whereas in group 2 it was 70%.

Conclusion: Platelet-rich fibrin in combination with bone marrow aspirate and autogenous bone was beneficial in improving the volume of newly formed bone in the reconstruction of the cleft defect and also results in greater osteogenic effect which increases new bone regeneration and better wound healing.

Keywords: Bone marrow aspiration, cleft alveolus, computed tomography, iliac bone graft, platelet-rich fibrin

INTRODUCTION

Successful and optimal reconstruction of cleft alveolus depends on the timing of grafting, appropriate selection of graft, preparation of defect site, and adequate volume of graft placement.[1] The timing of grafting though debatable has been well established wherein secondary alveolar bone grafting is the choice of the alveolar grafting, along with this the source of grafting includes autogenous, alloplastic, and allogenic bone graft. Preferred source and the first choice of bone grafting is autogenous iliac bone graft because of its abundance of cancellous bone, which transforms rapidly into the alveolar bone.[2] Bone marrow aspirates are rich in mesenchymal stem cells which are capable of producing potential osteogenic

Access this article online	
Website: www.njms.in	Quick Response Code
DOI: 10.4103/njms.njms_55_23	

Anil Kumar Desai, Akshay A. Byadgi, Niranjan Kumar[1], Anusha Janardhan, Tejaswini Manjunath

Departments of Oral and Maxillofacial Surgery, and [1]Plastic and Reconstuctive Surgery, SDM Craniofacial Unit, SDM College of Medical Sciences and Hospital, Dharwad, Karnataka, India

Address for correspondence: Dr. Anil Kumar Desai, Department of Oral and Maxillofacial Surgery, SDM Craniofacial Unit, SDM College of Dental Sciences and Hospital, Dharwad, Karnataka, India.
E-mail: anildes20006@yahoo.co.in

Received: 30 March 2023, **Revised:** 31 May 2023, **Accepted:** 14 June 2023, **Published:** 16 November 2024

How to cite this article: Desai AK, Byadgi AA, Kumar N, Janardhan A, Manjunath T. Effect of "platelet rich" fibrin with bone marrow aspirate on the regenerative capacity of alveolar bone grafting with iliac bone graft: A randomized controlled trial. Natl J Maxillofac Surg 2024;15:460-6.

cells.[3] Hence, bone marrow aspirates soaked in absorbable collagen sponge can be used in craniofacial regenerative and reconstructive procedures.[2] However, the donor site morbidity and bone resorption cannot be eliminated by alveolar bone grafting or bone marrow aspirate. Further individual factors such as age, sex, underlined systemic conditions, and the width and type of cleft alveolus can influence the clinical outcome.

To minimize bone resorption and to enhance osteoinductive, osteoconductive activity and non-immunogenic properties of graft various growth factors can be obtained from platelet-rich plasma (PRP) and platelet-rich fibrin (PRF).[2] Studies have reported PRF enhances bone integration process by accelerating bone and mucosal healing.[3] Studies have reported a marginal improvement in clinical outcome after alveolar bone grafting using iliac bone graft with bone marrow aspirate as well as the use of iliac bone graft with PRF. As the effect of PRF on bone marrow aspirate concentrate (BMAC) in secondary alveolar bone grafting is not reported in the literature. Hence, this study was aimed to assess the clinical and radiographic effect of PRF in secondary alveolar bone grafting with iliac bone graft and bone marrow aspirate among individuals with alveolar cleft. The null hypothesis was among the individuals with alveolar cleft there is minimal difference in the clinical and radiographic outcome of combined iliac bone graft and bone marrow aspirate with and without PRF.

MATERIALS AND METHODS

A randomized controlled trial was carried out on 30 patients with cleft alveolus in the department of oral and maxillofacial surgery. The ethical clearance for the study was obtained from institution review board with IRB NUMBER 2018/P/OS/59 on 15/11/2018. The consent for participation was obtained from the patient's parents or guardian prior to the onset of the study. The study was conducted between October 2018 and October 2020, and all surgeries were performed by a single operative surgeon to avoid operative bias.

Inclusion criteria

1. Patient with age group between 8 and 15 years.
2. Patient with unilateral cleft alveolus
3. Patients who have undergone orthodontic treatment preoperatively

Exclusion criteria

1. Presence of any systemic disorder that may influence wound healing like juvenile diabetes, immunocompromised conditions like AIDS, nutritional deficiency, and hereditary healing disorder.[4]
2. Previously operated for secondary cleft alveolar bone grafting.
3. Syndrome patient[5,6]
4. Patient with Bilateral cleft alveolus
5. Patients who have not undergone or completed orthodontic treatment preoperatively.

Patients were categorized into two groups:

Group 1- Iliac bone graft + BMAC with PRF
Group 2 - Iliac bone graft + BMAC without PRF

All the patients were randomly allocated to either groups using a lottery method.

Data on patients' age, sex, unilateral cleft side involved (right and left), amount of iliac bone graft harvested, pre-operative cleft alveolus defect volume and post-operative volume of bone harvested, height and width of the grafted bone were collected.

Bone grafting technique

Abylom's[7,8] technique was used for secondary alveolar bone grafting and the bone graft was harvested from the anterior iliac bone region using the trapdoor method. Harvested cancellous iliac bone was compressed mixed with BMAC plus PRF and placed in the alveolar defect. No scaffolds were used in the present study in the grafting procedure.

Bone marrow aspiration

Bone marrow was aspirated from the anterior iliac crest. The instrument used for this procedure was a 16-gauge fine spinal metal needle lever lock syringe. Around 5 cc to 10 cc of bone marrow was aspirated in our study[3] [Figure 1].

PRF preperation

Platelet-rich fribin was prepared according to Choukroun's[9,10] method. About 20 mL of fresh venous blood was drawn into two separate 10 ml "Becton Dickerson Inc." vacutainer tubes. The tubes were centrifuged at 2900 rpm for a period of

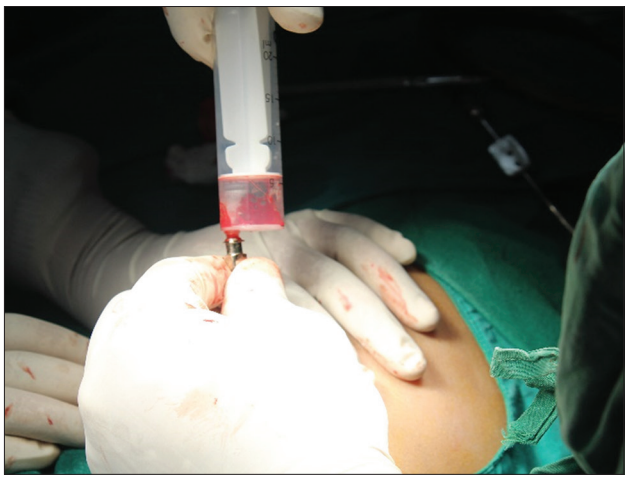

Figure 1: Harvesting of bone marrow aspiration concentrate from the anterior iliac crest

10 minutes with RCF (g-force) of 793.4 g4 (Sigma 3–3-ks; fixed angle rotor, Rotor diameter: 16 cm; Sigma laborzentrifugen Gmb H the tube showed three distinct layers, Middle zone: PRF was retrieved for harvesting [Figure 2].

For each patient in group 1 prior to the procedure around 6cc, BMAC was mixed with 2 ml of PRF gel. After alveolar bone grafting with iliac bone graft the mixture of BMAC and PRF was used as a membrane. For patients in group 2, alveolar bone grafting with iliac bone graft and bone marrow aspirate was placed. Mucoperiosteal flaps were approximated with mattress sutures and haemostasis was achieved.

Postoperatively, all patients were given paladai oral feed (clear fluids), 4 hours post-surgery for the first 24 hours and a semi-solid diet orally with paladai for the next one week. None of the patients were fed with nasogastric tube. Every patient was assessed clinically for wound healing, dehiscence, and pus discharge and radiologically for the volume of harvested bone and was evaluated for bone uptake immediately post-operative, and after 12 months. All the patients were subjected to computerized tomography (CT) scans (128 MULTISLICE CT SCANNER) for pre-operative, immediate post-operative, and 12 months, respectively, for volume assessment. The volume was assessed on CT using syngovia software (Siemens Healthcare GmbH, Version VB30) [Figure 3a preoperative defect, Figure 3b 12 months post-operative graft taken].

Statistical analysis

Data obtained is presented as mean and standard deviation. Analysis of variance was used for intergroup comparison. Paired *t*-test was used to compare the changes within the group over time. Statistical package for the social sciences version 26 was used for analysis. The $P < 0.05$ was considered significant

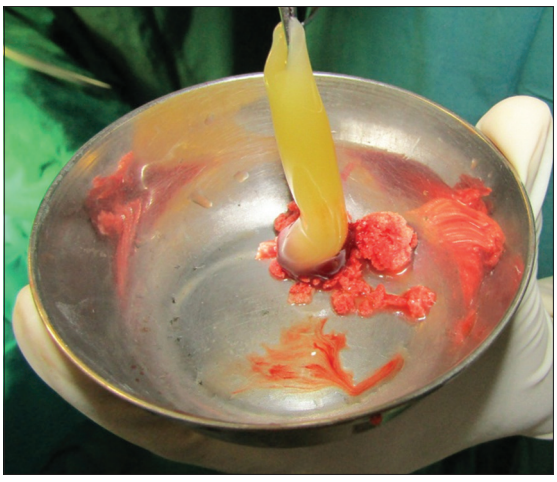

Figure 2: "Platelet rich" fibrin prepared using Choukroun's method

RESULTS

A total of 30 patients, between the ages of 8 years to 15 years were included in the study. There were 15 patients in each group with a mean age of 13 years in both the groups. In group 1, there were 5 females and 10 males, and in group 2, there were 7 females and 8 males. Among the unilateral cleft side involved (right and left), there were 8 cleft alveoli on the right side and 7 cleft alveolus on the left side in group 1 and 9 cleft alveolus on the right side and 6 cleft alveolus on the left side in group 2 [Table 1].

The mean volume of bone deposited in cleft alveolus defect was 2.5 cc in group 1 and 2.3 cc in group 2 and the mean of 5.6 cc of bone marrow was aspirated from each group and 20 ml of blood was taken for the preparation of PRF in group 1.

The CT volume of the cleft alveolus defect after secondary alveolar bone grafting was measured in both the groups, mean volume defect in group 1 was 2.5cc, post-operatively measured immediately was 3.2cc, and 12 months post-operative was 2.2cc. The mean volume defect in group 2 was 2.3cc immediately, post-operatively measured was 2.6cc, and 12 months post-operative was 1.9cc [Table 2].

DEFECT-With the help of high resolution computed tomography scan (HRCT) axial section (1mm cuts), the width and the height of the cleft alveolus defect were measured in both the groups. Mean vertical height and width pre-operatively measured in group 1 was 11.7 mm, 4.3mm, and in group 2 was 11.3 mm, 4.03 mm, respectively. The maximum defect of cleft alveolus measured (height and width) in group 1 was 14.4 mm, 4.8 mm, in group 2 was 12.8 mm, 4.0 mm.

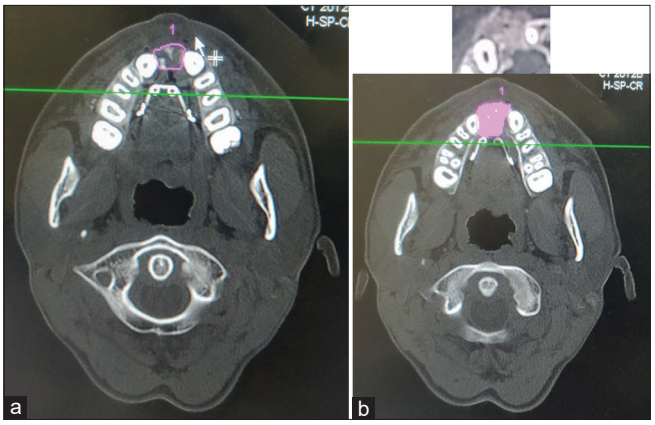

Figure 3: (a) CT scan using syngovia software- axial section showing Preoperative alveolar cleft defect volume. (b) CT scan using syngovia software- axial section showing Postoperative 12-month cleft alveolar graft taken volume

Table 1: Demographic profile of participants in Group 1 and Group 2

Profile	Group 1	%	Group 2	%	Total	Statistic	*P*
Age in yrs							
Mean±SD age	13.20±2.27		13.67±3.15		13.43±2.71	t=0.4648	0.6457
Gender							
Male	10	66.67	8	53.33	18	χ^2=0.5560	0.4560
Female	5	33.33	7	46.67	12		
Side of cleft alveolus							
Left side	7	46.67	6	40.00	13	χ^2=0.1360	0.7130
Right side	8	53.33	9	60.00	17		
Total	15	100.00	15	100.00	30		

Table 2: Comparison of Group 1 and Group 2 with pre-OP, immediate OP, and 12 months CT volume scores by independent *t*-test

Treatment times	Group 1		Group 2		Mean difference	*t*	*P*
	Mean	Std. Dev	Mean	Std. Dev			
Pre-OP	2.58	0.88	2.30	0.56	0.28	1.0210	0.3160
Immediate OP	3.02	1.10	2.66	0.54	0.36	1.1566	0.2572
12 months	2.28	0.78	1.95	0.52	0.32	1.3371	0.1919
Pre-Op Immediate OP	-0.44	0.22	-0.35	0.18	-0.09	-1.1915	0.2435
Pre-Op 12M	0.31	0.15	0.35	0.11	-0.05	-1.0137	0.3194
Immediate op 12M	0.75	0.34	0.70	0.15	0.04	0.4254	0.6738

Immediate post-operative height and width

With the help of the HRCT axial section (1 mm cuts), the width and the height of the grafted region were also measured in both the groups. Mean vertical height and width post-operatively measured immediately in group A were 12.63 mm, 4.7 mm, and in group B was 11.95 mm, 4.4 mm, respectively. The maximum height and width in group A was 15.0 mm, 5.7 mm, in group B was 13.5 mm, 4.8 mm.

AFTER 12 MONTHS-Similar measurements were carried out in both the groups after 12 months of grafting procedure. Mean bone height and width post-operatively 12 months, in group A was 11.2 mm, 4.0 mm, and in group B was 10.7 mm, 3.59 mm, respectively. The maximum height and width in group A was 14.4 mm, 5.3 mm, in group B was 13.0 mm, 4.4 mm. Comparison of height between the groups, a *P* value of less than 0.001 was noted and was not statistically significant. This was assessed with the mean and standard deviation values and the significance was analyzed using student's dependent paired *t*-test. It was observed that the resorption of bone was less in group 1 when compared to group 2 and post-operative bone retention after 12 months was seen more in group 1 when compared to group 2. Also, on comparison of width between the groups, a *P* value of less than 0.001 was noted and was not statistically significant. This was assessed with the mean and standard deviation values and the significance was analyzed using the student's independent paired *t*-test. Similar observations were noted as mentioned in the height assessment [Tables 3 and 4].

And immediate post-op to 12 months, the height and width of the grafted area was assessed between group 1 and group 2 and the results showed statistical significance suggesting group 1 showed better bone uptake with minimal bone resorption when compared to group 2.

DISCUSSION

The goal of reconstruction of the cleft alveolus is to improve the quality of the tissue, structural stability, and to increase the volume of bone.[3,7,8] The current study observed a significant improvement in the clinical and radiographic outcomes in the group which used BMAC with PRF.

A standard treatment protocol for bone graft harvesting was carried out throughout the study. Secondary alveolar bone grafting was selected because it provides a predictable mature viable stock of bone, provides adequate width for tooth eruption, facilitates orthodontic movement and implant placement. In our study, all the patients underwent presurgical orthodontic palatal expansion prior to bone grafting to facilitate tooth eruption. Hall and Posnick *et al.*[11] in 1983 Al-ashmawy *et al.*[3] had performed orthodontic palatal expansion prior to alveolar grafting procedure. Orthodontic treatment [dental appliance] facilitates surgery by correcting the collapse of arch and malopposed teeth.

Autogenous cancellous bone grafts are considered as the gold standard because of their osteoconductive, osteoinductive, and non-immunogenic properties. Anterior iliac graft was most commonly used because of ease of access and availability

Table 3: Comparison of pre-OP, immediate OP, and 12 months height (mm) in Group 1 and Group 2 by dependent and repeated measures of ANOVA test

Groups	Treatment times	Mean	SD	Mean Diff.	SD Diff.	*t*	*P*	Effect size
Group 1	Pre-OP	11.74	1.93					
	Immediate OP	12.63	2.13	-0.89	0.30	-11.4589	<0.001	0.9040
	Pre-OP	11.74	1.93					
	12 months	11.21	1.76	0.53	0.30	6.9593	<0.001	
	Immediate OP	12.63	2.13					
	12 months	11.21	1.76	1.42	0.52	10.5230	<0.001	
Group 2	Pre-OP	11.33	1.33					0.9200
	Immediate OP	11.95	1.17	-0.63	0.26	-9.4202	<0.001	
	Pre-OP	11.33	1.33					
	12 months	10.74	1.37	0.59	0.25	8.9748	<0.001	
	Immediate OP	11.95	1.17					
	12 months	10.74	1.37	1.21	0.37	12.6982	<0.001	

ANOVA: Analysis of variance

Table 4: Comparison of pre-OP, immediate OP, and 12 months width (mm) in Group 1 and Group 2 by dependent t and repeated measures of ANOVA test

Groups	Treatment times	Mean	SD	Mean Diff.	SD Diff.	*t*	*P*	Effect size
Group 1	Pre-OP	4.31	0.89					0.9060
	Immediate OP	4.73	1.04	-0.41	0.18	-8.6696	<0.001	
	Pre-OP	4.31	0.89					
	12 months	4.00	0.82	0.31	0.11	10.7825	<0.001	
	Immediate OP	4.73	1.04					
	12 months	4.00	0.82	0.73	0.25	11.0429	<0.001	
Group 2	Pre-OP	4.03	0.55					0.9470
	Immediate OP	4.42	0.56	-0.39	0.25	-6.1849	<0.001	
	Pre-OP	4.03	0.55					
	12 months	3.59	0.55	0.44	0.15	11.0000	<0.001	
	Immediate OP	4.42	0.56					
	12 months	3.59	0.55	0.83	0.24	13.7324	<0.001	

ANOVA: Analysis of variance

of sufficient amount of bone when compared to other sites as reported by Eufinger and Leppanen.[12] They provide Scaffold for osteointegration, growth factors for osteoconduction, and progenitor cells for osteoinduction. Eufinger H and Leppänen H 2000 showed that autogenous bone graft harvested from the anterior iliac crest has a high success rate.[12] Kang *et al.*[13] in 2017 supported the use of particulate cancellous iliac grafts since they produced a favourable result. In our study particulate iliac bone grafts were used as they readily incorporate, remodel the defect, and revascularize rapidly.[14]

To enhance the bone regeneration, growth factors such as bone morphogenic proteins, platelet-derived growth factors in combination with different types of bone substitutes are studied. Several studies were done in combination with platelet derived growth factor and autogenous bone graft to enhance the regeneration of bone. Various platelet-derived growth factors such as PRF and PRP have been used to facilitate increased bone formation. It promotes soft tissue healing and acts as a biological connector when its fragments are mixed with graft material and promotes neoangiogenesis and carries the osteoprogenitor cells to the center of graft.[15]

Sauerbier *et al.*,[16] in 2010 suggested that the combination of mesenchymal stem cells (MSCs) with biomaterial can improve bone formation. Gimbel *et al.*[17] in 2007 used bone marrow aspirate soaked in an absorbable collagen sponge was used to close alveolar clefts. Kumar A *et al.*[18] in 2004 suggested that bone marrow stem cells incorporated onto resorbable collagen matrix sponge have satisfactory results in terms of healing of alveolar bone.

Combination of growth factors such as platelet-derived growth factors with human-derived bone marrow aspirate may enhance the regeneration of bone. A study by Behnia *et al.*[11] reported platelet derived growth factors has a augmenting effect on the capacity of stem cells for regeneration of bone.

In our study, the bone resorption rate immediate post-operative to 12 months interval in groups 1 and 2 was 25% and 30%,

respectively. The overall percentage of regenerated bone in group 1 was 75% whereas in group 2, it was 70%. In the study done by Al Ashmawy, *et al*. in 2017,[3] the newly formed in bone marrow aspirate with PRF ranged from 62.23% to 90.68% whereas in BMAC, only group ranged from 32.6% to 60.87%. In our study, mean density of bone in group 1 at 12 months interval was greater than in group 2, which was similar to the study conducted by Al-Ashwamy *et al.*[2]

In the present study, none of the patients had any major problems at both the cleft alveolar site and donor site. Wound healing was enhanced faster in group 1 when compared to group 2 where it took 26 days for complete healing. The delay in the wound healing in group 2 is attributed to the dehiscence in 3 patients, which was managed conservatively by local dressing. This observation can be attributed to the factor that the role of BMAC and PRF in group 1. Dohan *et al.*[19] proved in his study that there is slow release of growth factors in PRF and there was better-organized fibrin matrix, which helped in direct migration of stem cells and satisfactory wound healing. According to Marukawa *et al.*[20] in 2011, in their study, have mentioned that the addition of PRF helps in speeding up of soft tissue wound healing. Miron RJ, *et al*.[21] in his randomized clinical trial concluded that the addition of PRF membrane provides an additional gain in mucosal thickening, when compared to the conventional method.

Siddiqui MA, *et al*.[22] in their study found significant bone regeneration due to the addwition of PRF with mesenchymal stem cells because of their osteoconductive properties with high bone density. In Feichtinger *et al*'s.[23] study, the mean graft survival was 49% over a period of one year. Kumar A *et al*.[18] in 2004 there are limitations regarding the ideal time to measure the survival graft volume of cleft defect.

Caplan AI and Dennis JE[24] in their study have found that human MSCs produce ectopic bone in mice, but are reluctant to produce bone in human jaw defects. They concluded that the following prerequisites for MSCs to be able to generate new bone: firstly, sufficient number of cells with high osteogenic capacity. Secondly, an appropriate scaffold to seed the cell. Thirdly, the combination of factors to stimulate cells in-vivo. Caplan AI and Dennis JE[24] also mentioned that the fourth demand for success is dependent on patients' factors, namely, sufficient vascular supply, also age factor plays an important role, as the patients get old, their red bone marrow (rich in stem cells) decreases and is replaced by yellow bone marrow.

Also, gender-related volumetric difference was not stated in our study due to a limited number of samples, which showed nil or marginal significance. Thus, a higher number sample of cases should be considered to obtain the statistical significance between the genders and the groups. Similar study was conducted Saruhan N and Ertaş Ü.[25] on 22 patients, where the effect of gender on the post-operative newly formed bone volume in alveolar cleft treatment with iliac bone grafting and PRF was examined with volumetric analysis in the patients. Minimal statistical significance difference was noted between the female and male for postoperative newly formed bone ($P > 0.05$). One of the limitations of this study was also a small sample size which failed to correlate the statistical significance between the gender and the volumetric difference in cleft alveolus cases.

Hence, our study reports that iliac bone graft alone may not give significant bone regeneration capacity and wound healing but can treat larger alveolar cleft. The addition of PRF to BMAC had superior effects in terms of wound healing, less resorption, and better bone regeneration capacity. However, resorption might occur to a smaller extent because the alveolar process depends upon the teeth present. Absence of teeth could be one of the reason, another is tension of mucoperiosteal flap covering bone graft, and size of defect which usually have less rapid revascularization.

CONCLUSION

Bone marrow aspiration and PRF have added advantage in the outcome of alveolar bone grafting. The addition helped us in achieving better bone regeneration and faster healing in cleft alveolar bone grafting patients when compared to bone marrow aspiration alone group. With less amount of iliac bone graft harvest, we could achieve better results with addition of bone marrow aspiration and PRF. Further studies can be carried out to assess the role of stem cells and PRF especially in ABG.

Declaration and acknowledgment

The above study has been conducted without any financial support or grant from the personnel or the institution. The necessary approval and clearances for the study were obtained from the institutional review board (ethical committee board)—IRB No. 2018/S/OS/56. The study was conducted with all informed consents of the patients. And I would like to acknowledge DR NIRANJAN KUMAR Vice Chancellor SDM University, Dharwad and Principal, SDM Dental College, Dharwad for their support and guidance to conduct the study.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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