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Bone Morphogenic Protein in Demineralized Freeze Dried Bone Allograft - A Possible Reason for its Osteoinduction

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Abstract: ***Introduction: Augmentation of bone healing by bone grafts at the recipient site occurs through one or more of the following mechanisms: osteoconduction, osteoinduction and osteogenesis. The objective of the present study was to assess the osteoinductive potential of decalcified freeze dried bone allograft material in bony defects of jaws. Materials and Methods: 35 patients with non-infected jaw lesions measuring 3cm to 5cm in size were enrolled in the study. The first group includes 25 patients in which decalcified freeze dried bone allograft was placed and a second group included 10 patients in which the bony defect was left empty and served as a control group. The presence of new bone formation in defects was judged measuring the bone density of the radiographs taken at 1st, 3rd and 6th month follow ups using Gray Scale Histogram on subsequent follow-ups. Results: The difference between the means of post-operative bone density in group 1(1st month-21.75, 3rd month-9.71 and 6th month-5.74) and group 2 (1st month-62.61,3rd month-39.75 and 6th month-33.86) respectively was found to be statistically significant with a P value of 0.00001 when compared using a t test. Both the groups showed new bone formation in the defect space with a greater increase in bone density and new bone formation in the demineralised freeze dried bone allograft group. Conclusion: Demineralised freeze dried bone allograft maybe osteoinductive and leads to new bone formation within a 4weeks period.***

Keywords: Allografts, DFDBA, Freeze Dried Demineralized Bone.

1. Introduction

The increased use of dental implants have made it necessary for oral and maxillofacial surgeons to retain or regain the bone lost secondary to oral surgical procedures or atrophy. Various bone replacement materials are available 2 but none of them are without significant limitations. The field of tissue engineering has been evolving over last 10 years. Bone morphogenetic proteins (BMP) 3 is one key component in bone tissue engineering. Although autogenous bone grafting remains the gold standard, allogenic bone grafting is gaining wider attention in scientific and clinical communities, and some promising results have been achieved in terms of generating excellent quantity and quality of bone for subsequent placement of dental implants and other prosthesis which will otherwise affect the psychology of the patient. Since the osteoinductive source of allogenic bone grafts is BMP according to literature3,4 we designed the present study using demineralised freeze dried bone allograft to fill the bony defects of jaws. The purpose of the present study was to analyse radiographically the osteoinductive potential of demineralised freeze dried bone allograft on the healing of bony defects created after removal of lesions by comparing pre-operative radiograph with radiographs taken on the 1st,3rd and 6th month post operatively with the use of Gray Scale Histogram.5

2. Materials and Methods

Thirty five patients with bony defects caused secondary to removal of the lesions measuring in between 1cm to 5cm in size (photo-1) were included in the study. Each patient was operated under local anaesthesia with standard aseptic protocol. Healthy patients willing to participate in the study, having benign, non-infected lesions or any surgery causing bone defects such as enucleation of odontogenic and non-odontogenic cyst, excision of benign tumours and granulomas of jaws, surgical removal of impacted canine and 3rd molars were included in the study. Medically compromised patients, children below 14 years, chronic tobacco chewers, chronic smokers, patients on anti-coagulant therapy were excluded from the study. These patients were divided in two groups. The study group included 25 patients in whom DFDBA with particle size 500 to 1040 microns was placed (photo-2). The control group included 10 patients in which the empty bony defects were left to heal spontaneously. In all patients radiographs were taken including periapical radiographs, occlusal radiographs and orthopantamograms both preoperatively and at the 1st, 3rd and 6th month postoperatively.

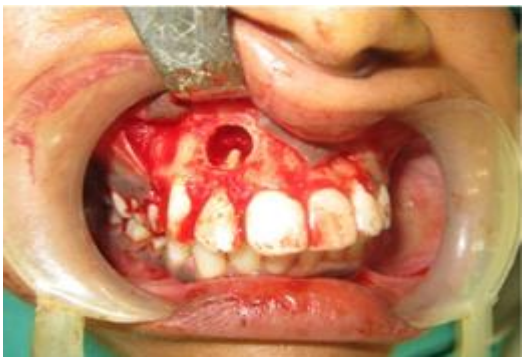

Photo 1: Bone defect

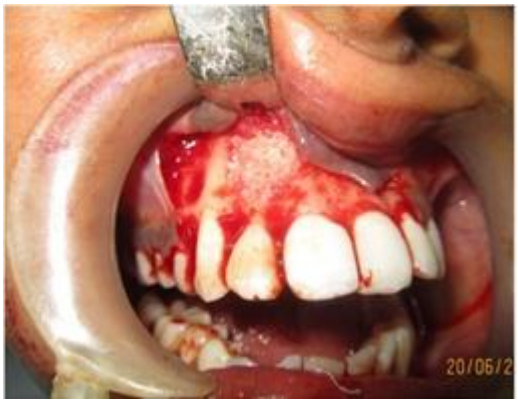

Photo 2: Bone graft

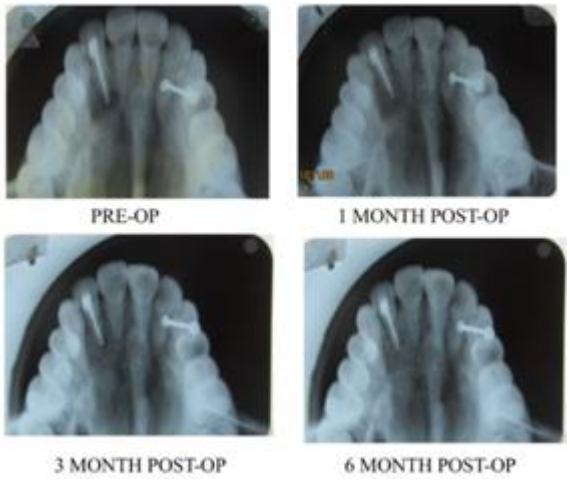

Photo 3: Occlusal radiographs

3. Bone Density Estimation[6, 7]

The defects were evaluated by comparing pre-operative radiographs (photo-3) with 1st, 3rd and 6th month post-operative radiographs (photo 4, 5, 6). The bone density was estimated based on 3 criteria 1] increase in bone density of defect area in pre-operative radiographs compared to post-operative radiographs in order to analyze the new bone formed 2]difference in density of healthy bone and 3] comparison of healthy bone density to defect bone density. Using anatomical landmarks specific sites were chosen for both healthy and defect bone on pre-operative radiographs and compared to post-operative radiographs using these same landmarks as references so as to estimate the bone formed in that specific area. The radiographic findings were analyzed both subjectively and by using a digital technique to minimize the bias derived from the subjective evaluations. The computer analysis was performed using a PC (Pentium 4, 3.0 ghz, Intel Corporation) with Adobe Photoshop Software to transfer the areas on the radiograph into pixels. A digital camera (Canon power shot A3300 IS) was used to photograph the panoramic radiographs on the negatoscope. Bone density was measured on the radiographs using a gray scale with 256 tonalities. Areas with 2828 pixels were selected both on the lesion and healthy bone from the symmetrical regions of the cysts. Denser parts had higher tonality values.

4. Results

The 35 patients were divided into two groups, Group-1 in which DFDBA was placed and group-2 in which no bone graft was placed. When the entire population of 35 patients in the present study were compared radicular cysts (65.71%), impaction cases (31.43%) and submerged teeth case (2.86%) made cases all together. In group-1 twenty five patients were included out of which most common being radicular cysts representing 72% of total patients in group-1 followed by impaction cases (24%) and submerged teeth case (1%). In group-2 out of total ten patients radicular cysts (50%) and impaction cases (50%) were included. In overall study females predominated with 57.14% compared with males being 42.86%.

Table-1 and table-2 shows analysis of the new bone formed which depicts increase in bone density of defect area in post-operative radiographs compared to pre-operative radiographs. Table-3 shows difference in density of healthy bone and defect bone to see percentage increase in defect bone density with respect to healthy bone. Using the anatomical landmarks specific site was chosen on pre-operative radiographs and post-operative radiographs which were compared to estimate the bone formed in that specific area for comparison of healthy bone density to defect bone density in group 1 and group 2 as shown in table-4 and table-5 respectively.

The difference between healthy bone and pathologic bone tonalities were calculated on the radiographs taken at different times. The decrease in this difference at 1 month, 3 months and 6 months after surgery shows the increase in bone density in the healing operation site, decrease in this value meant that there is a tonal increase in the operated region (increased opacity in the radiograph).

Mean pre-operative bone density in group 1 was 52.54 (SD=31.10) while that in group 2 was 64.76(SD=17.00). When both values were compared using t test the difference was found to be statistically insignificant (P=0.2514).

Mean 1month post-operative bone density in group 1 was 21.75 (SD=12.06) while that in group 2 was 62.61 (SD=14.55). Mean 3 month post-operative bone density in group 1 was found to be 9.71 (SD=8.38) while that in group 2 was 39.75 (SD=12.08). The difference between both values was found to be statistically highly significant with P value of 0.0000 when compared with t test.

None of the patients in the present study reported with any sign of infection. There were no reported cases for allergic reactions in study group where DFDBA was placed. Soft tissue healing was uneventful.

5. Tables

Table 1: Comparison of Pre-operative, 1 month post-operative, 3 months post-operative and 6 month post-operative with respect to bone densities in defect bone scores in group 1 by paired t test

Time points	Mean	Std.Dv.	% of change	Paired t	P-value
Pre-OP	45.02	28.03	-69.76	-5.5597	0.0000*
1 month post OP	76.43	28.34			
Pre-OP	45.02	28.03	-80.67	-4.6675	0.0001*
3 months post OP	81.34	28.52			
Pre-OP	45.02	28.03	-105.24	-6.5862	0.0000*
6 month post OP	92.40	26.41			
1 month post OP	76.43	28.34	-6.42	-0.6139	0.5450
3 months post OP	81.34	28.52			
1 month post OP	76.43	28.34	-20.90	-2.3043	0.0302*
6 month post OP	92.40	26.41			
3 months post OP	81.34	28.52	-13.60	-1.4210	0.1682
6 month post OP	92.40	26.41			

Table 2: Comparison of Pre-operative, 1st, 3rd and 6th month post-operative with respect to bone densities in defect bone scores in group 2 by paired t test

Time points	Mean	Std.Dv.	% of change	Paired t	P-value
Pre-OP	59.99	25.56	16.25	0.8075	0.4402
1 month post OP	50.24	17.29			
Pre-OP	59.99	25.56	-3.75	-0.1933	0.8510
3 months post OP	62.24	24.14			
Pre-OP	59.99	25.56	-15.89	-0.7445	0.4756
6 month post OP	69.52	25.23			

Table 3: Comparison of group 1 and group 2 with respect to difference of bone densities in healthy bone and defects scores by t test

Variable	Group	Mean	SD	t-value	p-value
Pre-operative	Group 1	52.5476	31.1040	-1.1673	0.2514
	Group 2	64.7650	17.0002		
1 month post-operative	Group 1	21.7512	12.0622	-8.5393	0.0000*
	Group 2	62.6160	14.5531		
3 months post-operative	Group 1	9.7124	8.3897	-8.4170	0.0000*
	Group 2	39.7570	12.0833		
6 month post-operative	Group 1	5.7484	2.8934	-10.4336	0.0000*
	Group 2	33.8690	12.9587		

Table 4: Comparison of healthy and defect bone densities in group 1 by t test

Time	Densities	Mean	SD	t-value	P-value
Pre OP	Healthy bone	124.7600	26.4398	1.1665	0.2586
	Defect bone	112.8500	18.5307		
1 month	Healthy bone	102.0100	19.2110	-0.1605	0.8742
	Defect bone	103.3800	18.9502		
3 months	Healthy bone	59.9900	25.5601	0.9991	0.3310
	Defect bone	50.2400	17.2947		
6 months	Healthy bone	62.2400	24.1413	-0.6593	0.5180
	Defect bone	69.5200	25.2281		

Table 5: Comparison of healthy and defect bone densities in group 2 by t test

Time	Densities	Mean	SD	t-value	P-value
Pre OP	Healthy bone	97.7320	40.7139	5.3318	0.0000*
	Defect bone	45.0200	28.0337		
1 month	Healthy bone	98.1760	30.6520	2.6046	0.0122*
	Defect bone	76.4280	28.3445		
3 months	Healthy bone	90.8360	30.5335	1.1368	0.2613
	Defect bone	81.3360	28.5234		
6 months	Healthy bone	98.1960	26.8362	0.7697	0.4452
	Defect bone	92.4000	26.4083		

6. Discussion

Exploitation of regenerative capacity of bone has spawned a diverse spectrum of modalities to correct these bony defects. Bone is unique in connective tissue healing because it heals entirely by cellular regeneration and the production of a mineral matrix rather than just collagen deposition known as scar10. Bone is fundamentally composed of cells, osteoblasts, osteoclasts and osteocytes11. The stem cells are hematopoietic (blood forming) and non-hematopoietic (non-blood forming) in nature present in the bone marrow, osteoblasts are either endosteal osteoblasts or cambium osteoblasts of which endosteal osteoblasts line the trabecular bone between the cortices and inner or cambium osteoblasts line the inner surface of each cortex.10,11,12 Osteocytes are mature osteoblasts encased in a mineral matrix. Osteoclasts are those ells which resorb bone upon stimulation and begin the bone renewal process often termed as “bone turnover” or “bone remodelling”.10, 13, 14 The basic organic component is type 1 collagen, which embrace 98.5% of the non-cellular organic matrix. The inorganic matrix is nearly all hydroxyapatite. Essentially, bone matrix is mostly type 1 collagen laced with crystals of hydroxyapatite. However, there are several important non collagen proteins in bone, namely BMP (Bone Morphogenic Protein), insulin like growth factors-1 and 2(IGF-1 and IGF-2), sialoprotein and osteopontin10, 11, 12. Bone is normally inhibited from resorption by osteoprotegerin (OPG), which is a protein secreted by osteoblasts to regulate the rate of resorption as an inhibitory signal to the osteoclast10. The osteoclast only begins active bone resorption in response to the overriding signal of circulating parathyroid hormone and locally secreted Receptor Activator Nuclear Kappa-B Ligand (RANKL) 15, 16. RANKL binds to RANK receptors on the osteoclast cell membrane to initiate resorption. Although RANKL 10, 14, 17 once known to be secreted by cancer cells to create pathologic cavities in bone, is also secreted by normal osteoblasts to increase bone resorption. This osteoclast mediated normal bone resorption begins the bone renewal/bone turnover process10. A bone graft is any implanted material that promotes bone healing, whether alone or in combination with other material.18 The fate of a bone graft depends on the source it has been taken or the type of configuration of bone structure like cortical bone or cancellous bone19. Cortical bone 18 is taken from outer compact bone of the graft source which has fewer osteoblasts and osteocytes, less surface area per unit weight, and contributes a barrier to vascular ingrowth and remodelling, thus the initial remodelling response to cortical bone is resorptive as osteoclastic activity predominates. Conversely, cancellous graft is taken from the softer trabecular bone which becomes progressively stronger because of its ability to induce early, rapid, new bone formation8. The ideal bone graft should be: 1) osteogenic, osteoinductive and osteoconductive 2) biomechanically stable 3) disease free and 4) containing minimal antigenic factors8. The only osteogenic graft is autogenous bone graft that transfers osteocompetent cells that begin the bone forming process. Osteoinductive graft transfers proteins present in the graft, which begin a signalling cascade for the host to form new bone, whereas osteoconductive5, 20 graft simply provides scaffolding for host to create new bone and has no biological influence on the host. As acknowledged by

the literature, the gold standard of bone grafting materials is autogenous bone21, 22. This material forms bone by all processes of osteogenesis, osteinduction and osteoconduction1, 23. The advantage of autogenous bone is that it maintains bone structures such as minerals and collagen, as well as viable osteoblasts and Bone Morphogenic Proteins(BMPs)9, 24, 25 but there are some shortcomings of autografts which include the need for a separate incision for harvesting, increased operating time and blood loss, the risk of donor site complications, and frequent insufficient quantity of bone graft.8 Allografts has been reported in literature as a best alternative to autografts, allografts are tissues taken from individuals of the same species as the hosts1,9,26. There are three main divisions of allografts:1) Frozen, 2) Freeze-Dried, and 3)Freeze Dried Demineralised, the most common being Demineralized Freeze-Dried Bone Allograft (DFDBA). Demineralized bone allograft matrix is commonly used as a bone graft substitute, either alone or to supplement an osteoconductive material, because of its osteoinductive properties25, 27. The DFDBA was used because the very processes involved n its preparation i.e. decalcification exposes on its surface, the Bone Morphogenetic Proteins(BMPs) which are osteoinductive that is, they induce differentiation of mesenchymal cells into cartilage and bone9, 24, 25. BMPs are natural proteins which play important role during embryogenesis and mediate in specific aspects of skeletal growth and development during later adult life20. The BMPs are members of The Transforming Growth Factor-b (TGF-b) family of growth and differentiation factors. Bone Morphogenetic Protein members interact with the extracellular domain of a family of cell surface type I and type II receptors to signal across the cell membrane and elicit a cellular response3. A complex of a type I receptor and a type II receptor assembles through interaction with a BMP ligand. Formation of this ligand-receptor complex results in the phosphorylation of the type I receptor's kinase domain by the serine/threonine kinase domain of the type II receptor. This activation of the type I receptor's serine /threonine kinase results in the downstream phosphorylation of target substrates, including the smad family of signal transducing proteins3.

Narang et al1 and Deok-Won Lee et al27 concluded from their study that decalcified allogenic grafts shows new bone formation within 4 weeks period. Our results are correspondent to the results showing new bone formation in 1st month in study group where in control group this was achieved only after 3rd month post-operatively. We also observed from our study that there was reduction in bone density in 1st month postoperative radiographs in control group suggesting of initial bone resorption of defect bone in absence of graft, while there was consistent increase in bone density with maximum increase observed in 1st month post-operative radiographs in study group. This initial raise in bone density of defect bone stimulates the results of Mulliken et al 15 who concluded from his study that allogenic demineralised bone grafts triggers bone healing by induced osteogenesis bypassing the resorption seen with healing of other grafts or non-grafted defects15. The above results proved the osteoinductive potential of decalcified freeze dried bone allograft material which was source of new bone formation in study group accelerating the healing process of bone and making it radiographically evident. As in our study chronic smokers and children were excluded from study the uneventful healing due to nicotine and spontaneous bone healing in children are more or less evened out preventing any possible bias.

7. Conclusion

The present study affirms that the demineralised freeze dried bone allograft is befit bone replacing agent for future prosthetic rehabilitation aspect as it abets the evidence of fresh bone formation within 4 weeks radiographically. The possible reason for neo-osteogenesis being BMP of the decalcified graft material thus proving demineralised grafts to be better opton than mineralised grafts. Further long-term studies should be directed towards the use of decalcified freeze dried bone allograft in the treatment of large osseous defects in the field of oral and maxillofacial surgery. DFDBA can be a prophylactic grafting material for early prosthetic intervention of implant and other prosthetic rehabilitation procedure.

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