Original Article

Evaluation of bone formation using recombinant human bone morphogenetic proteins-7 in small maxillofacial bony defects

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Abstract

Context: Bone morphogenetic proteins (BMP) are multifunctional molecules of transforming growth factor-β superfamily that induces the differentiation of fibroblasts into osteoblasts to form bone.

Aims: This study was undertaken to evaluate the effects of recombinant human BMP-7 (rhBMP-7) in bone healing of small maxillofacial bone defects and assess the serum levels of osteopontin (OPN) and receptor activator of nuclear factor kappa-B ligand (RANKL) biomarkers for bone remodeling.

Materials and Methods: Twenty patients with small maxillofacial bony defects were enrolled in this study and randomly allocated to two groups; wherein after apicoectomy of the involved teeth, the control group had defect filled with collagen sponge only while the experimental group had rhBMP-7 impregnated collagen sponge placed in the defect.

Results: The clinical parameters showed no significant difference between the two groups ($P > 0.05$). The radiographic parameters showed a significantly slower rate of reduction in bone defect volume ($P < 0.01$) in control group than the experimental group when followed at 2, 4 and 24 postoperative weeks. RANKL and OPN serum levels showed no significant changes in pre- and post-operative stage.

Conclusion: This study confirms that rhBMP-7 in collagen definitely accelerates bone healing in maxillofacial bone defects and minimizes postoperative complications. RANKL and OPN biomarkers in serum may not show bone remodeling, hence tissue samples may be used to assess their levels.

Keywords: Cysts, maxillofacial bony defects, recombinant human bone morphogenetic protein-7

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INTRODUCTION

Any congenital malformation, trauma or pathology in the maxillofacial region can lead to a defect, and reconstruction of these bony defects is important to restore normal function and esthetics. An optimal construct should be able to replicate the lost structure, restore function, be biodegradable and easily replaceable through the body's physiologic processes, be harmless to the patient and reliable in defects.[1-3]

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Bone morphogenetic protein (BMP) has immense potential for tissue engineering.[4] It was first described by Urist in 1965.[5-11] Since then, several scientific studies have been reported. BMPs are multifunctional and may be used for growth and differentiation of many tissue types in the body. They are members of the transformation growth factor-β (TGF-β) superfamily except for BMP-1 that lacks the C-terminal sequence of the TGF-β family. BMPs are misleadingly named, as they are involved in the development of many tissues other than bone such as heart, gut and kidney.[12] BMPs induce the differentiation of fibroblasts into osteoblasts to form bone. The aim of this study was to evaluate the effects of recombinant human BMP-7 (rhBMP-7) in bone healing of small maxillofacial bone defects and assess the serum levels of osteopontin (OPN) and receptor activator of nuclear factor kappa-B ligand (RANKL) biomarkers for bone remodeling.

OPN is phosphorylated sialic acid-rich noncollagenous bone matrix protein and is found in several biological fluids such as plasma, serum, breast milk and urine. OPN was named for its function as a bridge between cells and minerals, and implicated as an important factor in bone remodeling. It is expressed by both osteoclasts and osteoblasts, and its role is in anchoring the osteoclasts to mineral matrix of bone. OPN is believed to initiate the process by which osteoclast cells develops their ruffled borders to begin bone resorption. It is also found in urine, where it inhibits kidney stone formation.

RANKL is a membrane protein and a member of the tumor necrosis factor superfamily. It has been identified to affect the immune system and control bone regeneration and remodeling. It is expressed in several tissues and organs such as osteoblast, skeletal muscle, liver, pancreas, thymus, adrenal gland, mammary gland and prostate; but its concentration levels vary through different organs. This surface-bound molecule found on osteoblast serves to activate osteoclasts.

Several studies have been done to evaluate the clinical significance of RANKL and OPN levels in human diseases characterized by local or systemic changes in bone remodeling. However, the clinical usefulness of circulating OPN and RANKL levels in bone remodeling process of cystic bony defects is still unknown.

This study was undertaken to evaluate the effects of rhBMP-7 in bone healing of small maxillofacial bone defects and assess the serum levels of OPN and RANKL biomarkers for bone remodeling.

MATERIALS AND METHODS

The study comprised of 20 patients with small maxillofacial bony defects of 1–2 cm size, who visited our outpatients department. It was a prospective randomized experimental study conducted after the institutional ethical approval. Irrespective of sex, caste and creed, 17–60-year-old patients with periapical pathology of 1–2 cm in size were enrolled in the study after obtaining their informed consent. Patients having any bone disease like Pagets, cherubism, etc., previous irradiation, any known metabolic disease and smokers were excluded from the study.

Detailed medical history was recorded on a set pro forma designed for the study. Patients were diagnosed on the basis of clinical as well as radiographic examination and were randomly allocated to two groups: control group (Group I, n = 10) where collagen sponge was used to fill the bony defect after apicoectomy of involved teeth and experimental group (Group II, n = 10) where rhBMP-7 impregnated collagen sponge was used to fill the bony defect [Figure 1a-c].

The clinical evaluation involved assessment of pain (visual analog scale Score 1–10), swelling, ulceration, pus discharge, gape and extrusion of graft. Postoperative clinical assessment was done on 1st day, 1, 2, 4 and 24 weeks whereas radiographic assessment followed at 2, 4 and 24 weeks [Figure 1d-f] to check for bone defect volume reduction. The radiographic evaluation involved computed tomography (CT) scan to check for postoperative reduction of bone defect volume. One image is selected from coronal/sagittal/axial and three-dimensional reconstruction view of CT which showing maximum dimension of the defect and then defect volume was measured 3 times in millimeters^3 using dolphin software, to prevent selection and measurement error and then converted the qualitative data into most accurate quantitative data.

Enzyme-linked immunosorbent assay for OPN and RANKL, as both are expressed by osteoblasts and osteoclasts are markers for bone healing, was done preoperatively and at 4 weeks postoperative using 1 ml of venous blood collected in plain vial and centrifuged, separating serum and stored at −20°C till evaluation.

Surgical technique

Access opening of root canals, biomechanical preparation and obturation were performed in respect to the offending teeth, before surgery. Patients were operated under local anesthesia. Part preparation and surgical draping were done to ensure sterile field. A crevicular

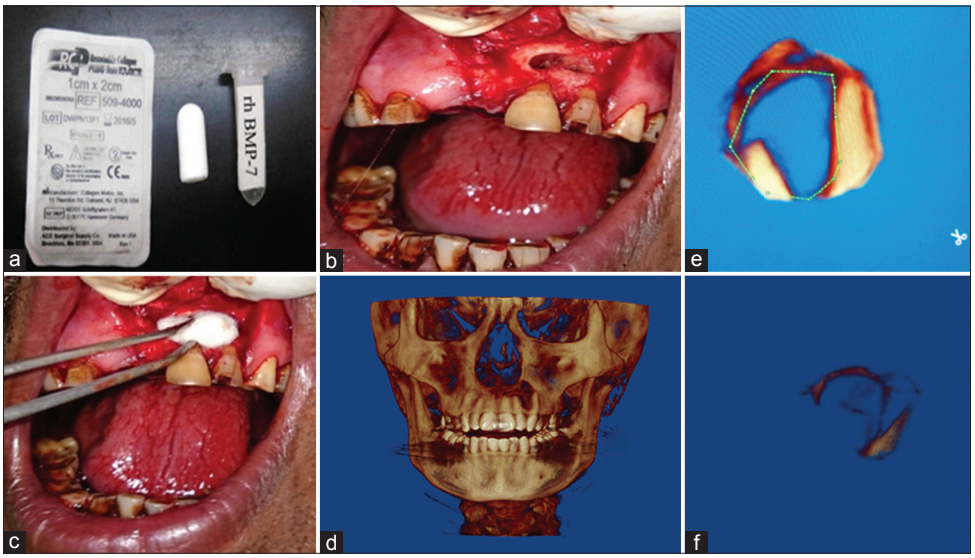

Figure 1: (a) Absorbable collagen sponge and recombinant human bone morphogenetic proteins-7. (b) Bone cavity after enucleation of cyst. (c) Bone cavity filled with collagen sponge. (d-f) Cystic bone defect volume measurement in Dolphin software using DICOM format computed tomography images

incision was made in the gingiva, with two releasing incisions extending up to attached gingiva to raise a trapezoidal flap and the cystic lesion was exposed and enucleated. Apicoectomy was performed if needed, and the defect was irrigated and dried before filling it either with collagen sponge [Figure 2] or with collagen sponge soaked with rhBMP-7 [Figure 3] as per their allocated group protocol. Closure of flap was done, and patients were advised frequent oral rinses.

RESULTS

Results were assessed on the basis of clinical and radiological findings. The comparison of clinical parameters between the groups showed no statistically significant difference between the two groups ($P > 0.05$). Reduction in bone defect volume was significantly ($P < 0.001$) lower in control group than the experimental group in the initial weeks [Table 1]. There were no complications in terms of gape, pus discharge and extrusion of graft in any group.

There was no significant difference in the values of OPN and RANKL ($P > 0.05$) in the two groups at preoperative as well as week 4 [Table 2].

DISCUSSION

Osteoconduction, vascularization, pooling of signaling molecules and committed cells for osteogenesis are an advantage with the autologous bone grafts; however, complications and nonunions are frequently reported.[1,2]

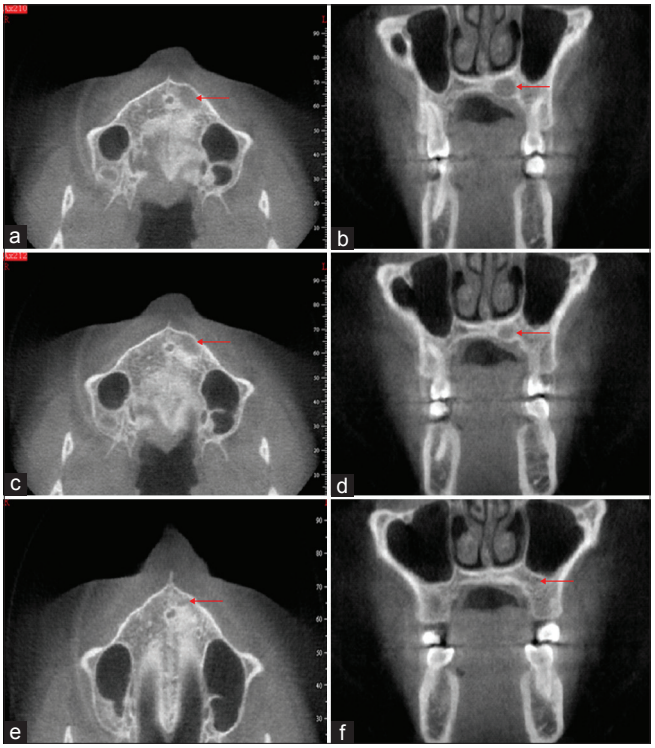

Figure 2: Computed tomography scan (Group I: collagen sponge only). (a) axial section at 2 weeks' postoperative. (b) coronal section at 2 weeks' postoperative. (c) axial section at 4 weeks' postoperative. (d) coronal section at 4 weeks' postoperative. (e) axial section at 24 weeks' postoperative. (f) coronal section at 24 weeks' postoperative

The introduction of biomaterials for tissue regeneration is an attempt to overcome this problem.

BMPs are necessary for the development of tissues and organs in embryogenesis and thereafter.[13-16] Studies have

Table 1: Bone defect volume in the groups at different time intervals

Bone defect volume (mm^3)	Group I			Group II			*t*	*P*
	Mean±SD	Minimum	Maximum	Mean±SD	Minimum	Maximum		
2 weeks	231.74±43.75	161.00	299.27	287.47±80.83	194.07	457.06	−1.92	0.071
4 weeks	151.26±38.24	84.12	201.60	227.56±69.20	150.87	384.96	−3.05	0.007
24 weeks	64.66±19.58	38.24	97.88	10.97±5.87	5.88	26.11	−8.30	<0.001

SD: Standard deviation

Table 2: Osteopontin and receptor activator of nuclear factor kappa-B ligand at different time intervals

Bone healing markers	Time intervals	Group I	Group II	*P*
OPN	Preoperative	5.24±2.30	5.14±2.44	0.92
	Postoperative week 4	5.55±0.74	5.47±2.45	0.91
RANKL	Preoperative	1.91±1.62	1.81±1.84	0.90
	Postoperative week 4	2.26±1.74	2.34±2.35	0.93

Unpaired *t*-test, insignificant. RANKL: Receptor activator of nuclear factor kappa-B ligand, OPN: Osteopontin

shown that the addition of rhBMP-7 to the culture of bone cells stimulates cell proliferation, collagen synthesis, induces alkaline phosphatase and osteocalcin synthesis.[17-19] rhBMP with absorbable collagen sponge has been successfully used to augment floor of maxillary sinus in 11 patients, in a dose from 1.77 to 3.40 mg/patient.[20] It has also been observed to stimulate periodontal wound healing even in Class III furcation defects.[21]

Warnke transplanted a construct of titanium mesh in a postradiotherapy mandibular defect in a 56-year-old man. This mesh cage was filled with hydroxyapatite blocks coated with rhBMP-7 an d bone marrow aspirate; grafted into the latissimus dorsi and harvested 7 weeks later as a myo-osseous free flap. He recorded evidence of successful remodeling of bone using bone scintigraphy.[22] However, there is still much to discover about growth signaling molecules in tissue, both in animal and human models.[23]

In our study, OPN and RANKL biochemical markers were studied. We observed no significant change in their values. It may be because we used blood samples to study their values. Ideally, local tissue sample would have represented real measure of their values but obtaining local tissue might be considered unethical.

When assessed bone healing using CT scan preoperatively and postoperatively, we observed accelerated bone healing with the use of rhBMP-7 with collagen sponge. This shows early and faster healing of bone defects in the experimental group. However, our study relates only with the experiences of one center and one set of patients. A properly designed randomized prospective study with a larger sample size and longer follow-up is required to arrive at a definitive conclusion.

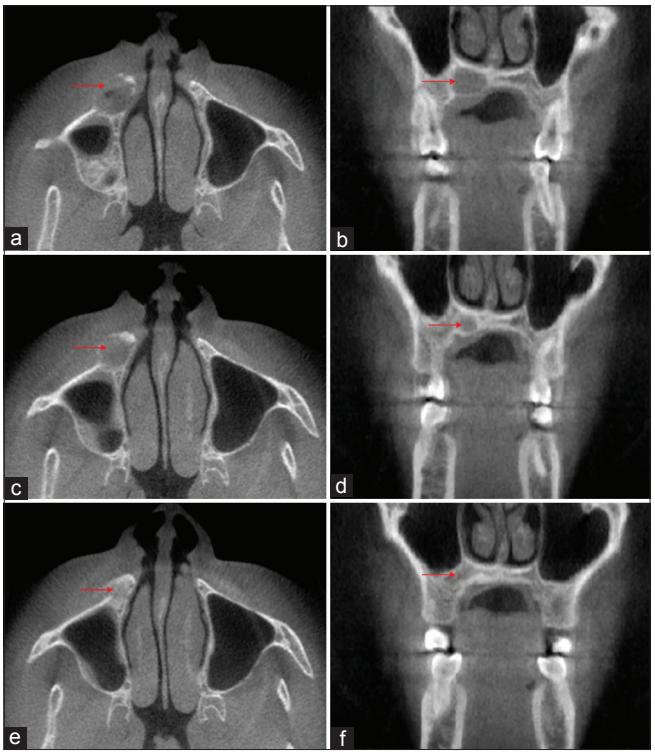

Figure 3: Computed tomography scan (Group 2: collagen sponge soaked with recombinant human bone morphogenetic proteins-7). (a) axial section at 2 weeks' postoperative. (b) coronal section at 2 weeks' postoperative. (c) axial section at 4 weeks' postoperative. (d) coronal section at 4 weeks' postoperative. (e) axial section at 24 weeks' postoperative. (f) coronal section at 24 weeks' postoperative

CONCLUSION

This study confirms that rhBMP-7 in collagen accelerates bone healing in maxillofacial bone defects and minimizes postoperative complications. RANKL and OPN biomarkers in serum may not show bone remodeling, hence tissue samples may be used to assess their levels.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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