Hydroxyapatite and B-Tricalcium Phosphate for Bone Regeneration in Large Cystic Cavities

Sunil Vasudev¹, Chinmay Dilip Vakade², Roshan Cherian Paramesh³, Prerna R⁴, Deepak S⁵, Anjaleena Elizabeth Mathew⁶

INTRODUCTION

Maxillofacial surgery involves treatment of a wide spectrum of diseases of the head and neck which include infectious diseases, carcinoma, as well as congenital and acquired malformations. Although cystic lesions of the oral and maxillofacial region are relatively rare as compared to other pathologies affecting the general population, these lesions are quite common in the practice of maxillofacial surgeons and remain a major cause of bone destruction in the maxillary and/or mandibular arches. Cysts constitute about 17% of all the tissue specimens submitted for histopathological examination.¹ Radicular cysts are the most common and comprise between 42 to 44% of all apical lesions,² while dentigerous cysts are the second most frequently occurring at a rate of 1.44 cysts for every 100 unerupted teeth.² Although not as common as the radicular and dentigerous cysts, odontogenic keratocyst represent about 5.4-17.4% of all jaw cysts.¹

Management of such cystic lesions depends on factors like age of the patient, size of the lesion, its location in the arch and the histopathologic nature and behavior of the lesion. Multiple surgical techniques have been advocated which include marsupialization, enucleation, curettage or resection. Carl Partsch in 1910 introduced marsupialization as a method for the surgical treatment of large mandibular cyst. In marsupialization, the cyst is converted into a pouch and allowed to regress over time. This technique is named after the author as Partsch I. Later Partsch himself introduced another treatment modality for the management of cysts less than 2.5cm called Enucleation or Partsch II. In enucleation, the cyst lining is separated from its inner bony attachment, and the cavity left to the organization of a blood clot. Although marsupialization preserves important anatomical structures, it requires a longer healing time, and a second surgical procedure for the complete removal of cyst by enucleation at a later date. Therefore, complete enucleation in the first attempt is the preferred treatment of choice for cystic lesions of the jaw.³ But larger defects can predispose to complications such as clot breakdown, infection and pathologic fracture of the involved bone. In order to prevent such complications, a good technique is to obliterate the dead space of a sizable defect by packing the cavity with bone or a bone substitute. Use of bone substitutes prevents the collapse of soft tissue into the cavity and also minimizes the extent of the defect. It also allows the healing process to occur rapidly leading to the good quality bone deposition which is necessary for dental/prosthetic rehabilitation.

Traditionally a bone graft is applied for bone regenerative procedures. The variety of grafts used are autogenous, allogeneous, xenografts or alloplastic materials. Although autogenous bone graft is said to be the gold standard that all alternatives must meet or exceed, autograft has limitations of donor site morbidity, inadequate amount, and inappropriate form.⁴ These limitations have prompted an increasing interest in alternatives to bone grafts. Any ideal bone substitute should be biocompatible, and not

Aims and Objectives: The purpose of this study was to assess the usefulness of a combination of Hydroxyapatite and β-Tricalcium Phosphate as a graft material to fill large cystic cavities post enucleation. Objectives: To evaluate-1) Bone density during immediate post-operative period 2) Bone density at 6 months post-operative period 3) Bone density at 1 year follow up. Materials and Methods: Ten patients with cystic lesions less than 5cm confined within the cortical margins of the maxilla and the mandible were included in the study. Following enucleation, the cystic cavities were filled with 20% Hydroxyapatite and 80% β-Tricalcium Phosphate. Difference in bone density in pre-operative period, immediate post-operative period, during 6 month follow-up and at 1 year follow-up was recorded and evaluated. Results: The mean bone density at 1 year follow-up was significantly greater than pre-op control bone density (P<0.005). Conclusion: Combination of Hydroxyapatite and β-Tricalcium Phosphate is an effective graft material for bone regeneration in large cystic lesions and results in significant bone density radiographically post-operatively.

KEYWORDS: Alloplasts, Hydroxyapatite, β-Tricalcium phosphate, Cyst, enucleation, Bone regeneration

induce an antigenic response. Also, they must be osteoinductive and osteoconductive. Due to advances in the fields of biotechnology and material sciences, we now have a plethora of materials mimicking bone to choose from. These include bioactive glasses, glass ionomers, aluminum oxide, calcium sulfate, calcium phosphates, α and β-tricalcium phosphate (β-TCP), and synthetic hydroxyapatite (HA). These materials bring about an early migration of osteoprogenitor cells which in turn help in enhancing new bone deposition and in the stabilization of blood clot by acting as a scaffold.

Beta tricalcium phosphate is a bone substitute that has high biocompatibility, favorable resorption properties, and osteoconductivity. Hydroxyapatite is known as a slowly biodegradable material with high osteocompatibility and bone binding capability. The resorption rate of Hydroxyapatite is relatively slow compared to the rate of new bone formation. Thus it helps in stabilizing the clot for longer. Both these materials have been extensively studied, and encouraging results have been obtained for filling of osseous defects.

Hence, in this study, we evaluated the healing of large defects of bone post-enucleation using a combination of Beta Tricalcium Phosphate and Hydroxyapatite as the alloplastic materials of choice.

**MATERIALS AND METHODS**

This study was a prospective clinical study conducted on a total of 10 patients (8 males and 2 females) who came to the Department of Oral and Maxillofacial Surgery at DAPM RV Dental College, Bangalore (Table 1) Routine

<table>
<thead>
<tr>
<th>SL</th>
<th>Name</th>
<th>Age / Sex</th>
<th>Site</th>
<th>Final Diagnosis</th>
<th>Treatment</th>
<th>Date of Surgery</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Someshkhar</td>
<td>44/ M</td>
<td>Lower Right Mandible</td>
<td>Dentigerous Cyst</td>
<td>Enucleation And Grafting</td>
<td>15/5/13</td>
</tr>
<tr>
<td>2</td>
<td>Kalash</td>
<td>22/ M</td>
<td>Anterior Maxilla</td>
<td>Radicular Cyst</td>
<td>Enucleation And Grafting</td>
<td>13/9/1</td>
</tr>
<tr>
<td>3</td>
<td>Niranjani</td>
<td>33/ M</td>
<td>Lower Left Mandible</td>
<td>Dentigerous Cyst</td>
<td>Enucleation And Grafting</td>
<td>29/9/1</td>
</tr>
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<td>43/ M</td>
<td>Lower Left Mandible</td>
<td>Dentigerous Cyst</td>
<td>Enucleation And Grafting</td>
<td>9/10/1</td>
</tr>
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<td>5</td>
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<td>10/ M</td>
<td>Anterior Maxilla</td>
<td>Dentigerous Cyst</td>
<td>Enucleation And Grafting</td>
<td>18/11/14</td>
</tr>
<tr>
<td>6</td>
<td>Ruchi</td>
<td>29/ F</td>
<td>Lower Left Mandible</td>
<td>Radicular Cyst</td>
<td>Curettage</td>
<td>13/11/14</td>
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<tr>
<td>7</td>
<td>Vigan</td>
<td>27/ M</td>
<td>Right Mandible</td>
<td>Odontogenic Keratocyst</td>
<td>Enucleation And Grafting</td>
<td>12/12/14</td>
</tr>
<tr>
<td>8</td>
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<td>38/ F</td>
<td>Lower Left Mandible</td>
<td>Dentigerous Cyst</td>
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<td>2/2/15</td>
</tr>
<tr>
<td>9</td>
<td>Ayaan Ahmed</td>
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<td>Enucleation And Grafting</td>
<td>30/1/1</td>
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<tr>
<td>10</td>
<td>Advik</td>
<td>11/ M</td>
<td>Lower Left Mandible</td>
<td>Dentigerous Cyst</td>
<td>Enucleation And Grafting</td>
<td>8/5/15</td>
</tr>
</tbody>
</table>

Table 1

investigations were carried out and those patients diagnosed with cystic lesion of maxilla or mandible were enrolled in the study. The period of the study was from November 2013 to May 2015. The investigator discussed the aims and details of the study with the patients and Informed Consent were obtained. In the case of the 3 minor patients (aged 10, 11 and 15), the parents/guardians were explained about the procedure and consent obtained from them. Cystic lesions less than 5 cm in diameter and those confined within the cortical margins of maxilla and mandible were included in the study. Large bony lesions measuring more than 5 cm in diameter, eroding the cortical plate and spreading to adjacent soft tissues and those involving vital structures like the floor of the nasal cavity or maxillary sinus in the maxilla or the inferior alveolar nerve in the mandible were excluded from the study.

**Method:** The preoperative panoramic view was taken in all cases. Routine blood investigations were performed, and written consent for surgery was obtained from all patients prior to the procedure. In all cases, first, a biopsy was performed to confirm the presence of a cystic lesion and to determine the type of cyst involved. Then the cyst was histopathologically classified in accordance with the World Health Organisation classification system. All procedures were carried out under local and field anesthesia using 2% Lignocaine with 1:80000 adrenaline. An intraoral mucosal incision was placed in the sulcus or vestibule, depending on the location of the cyst. Vertical releasing incisions were added to the first incision as required. A full thickness mucoperiosteal flap was raised using a No.9 Molt periosteal elevator. Next, a bony window was created using a Round Tungsten Carbide bur ensuring minimum damage to the cyst wall. Then the cyst was gently separated from the surrounding bone using a Freer elevator and recovered in toto. Once the cyst had been removed, the underlying bone was inspected for remnants of tissue and any residual tissue was removed with curettes. Hemostasis achieved. In all the cases where the biopsy report had revealed the cyst to be an Odontogenic keratocyst, a cotton pellet imbibed in Carnoy’s solution was applied along the cystic walls to promote chemical necrosis so as to reduce recurrence of the lesion. The cystic cavity was then filled with granules of the alloplastic material (20% HA+80% β TCP) of size 1000-2000μ. The full thickness mucoperiosteal flap was repositioned in a tension free manner, and water tight closure achieved using 3-0 vicryl suture material.

Post surgery, all patients were put on empirical antibiotic therapy for 5 days. The patients were instructed to follow a soft diet for 2 weeks and to maintain their oral hygiene. Follow up examinations were conducted at one and two weeks; one, three, and six months; and one year after surgery, following which the patient visited the outpatient department on an annual basis.

**Clinical and Radiographic Assessment:** Clinical assessment was carried out for all patients with regular follow up in the first month, at 3 months, 6 months and at 1-year post-op. In the first month, the patients were assessed for swelling, pain, infection, pus discharge and wound dehiscence.
Radiographic assessment was done immediately following cystectomy and at 6 months and 1 year postoperatively using ImageJ software. Pre-operatively, the radiographic extent of the lesion was noted. 3 points were taken as reference points on the radiolucency of the cystic lesion on the pre-operative X-ray and on each follow-up X-ray. These 3 points were in the same horizontal plane and 1.5cm apart from each other. The relative bone density of the pixels of the reference points was recorded and analyzed at 6 months and at 1-year post-op. The bone density on the adjacent unaffected site in each pre-op X-ray was also recorded and used as a control.

RESULTS

- Table 2 reveals the gray units of the area of interest in the preoperative radiograph, immediate postoperative radio-graph, at 6 months follow up and at 1 year calculated using ImageJ software.

<table>
<thead>
<tr>
<th>SL No</th>
<th>FEMALE</th>
<th>PRE-OP VALUE</th>
<th>BONE DENSITY OF AFFECTED SITE (GREY)</th>
<th>BONE DENSITY IMMEDIATE POST-OP (GREY)</th>
<th>BONE DENSITY AFTER 6 MONTHS (GREY)</th>
<th>BONE DENSITY AFTER 1 YEAR (GREY)</th>
</tr>
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<tr>
<td>1.</td>
<td>Somaiah</td>
<td>45</td>
<td>88</td>
<td>99</td>
<td>90</td>
<td>108</td>
</tr>
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<td>2.</td>
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<td>3.</td>
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<td>90</td>
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<td>Rajul</td>
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<td>Richa</td>
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<td>Nikashree</td>
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<td>Joya Ahmed</td>
<td>45</td>
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<td>100</td>
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<tr>
<td></td>
<td>MEAN</td>
<td>41.5</td>
<td>82.7</td>
<td>103.6</td>
<td>88.1</td>
<td>94.6</td>
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</tbody>
</table>

- A Friedman test was conducted to evaluate differences in the bone density at different time intervals at:
  - control (mean=82.70 ± 10.07)
  - immediate post-op (mean=103.60 ± 22.65)
  - 6 months follow-up (mean=89.10 ± 14.49)
  - 1 year follow-up (mean=98.60 ± 16.52). (Table 3)

- The mean at Immediate Post-op was significantly greater than pre-op control bone density at P<0.02
- Similarly, the mean at 1 year follow up was significantly greater than pre-op control bone density at P<0.005.
- However, the mean bone density at pre-op did not significantly differ from 6 months post follow-up period at P=0.386.

There was an evident decrease in the size of the radiolucency and a gradual increase in the radiodensity of bone in the cystic defect. The bone density of the affected site was comparable and even relatively greater than the adjacent unaffected site at 1-year post-op period. (Figure 1)

DISCUSSION

Odontogenic cysts account for 7-13% of the pathologies diagnosed in the oral cavity. The massive destruction of bone surrounding a cystic lesion cannot be explained by intracystic pressure alone. Researchers have found that the cyst wall cells release IL-1α which in turn stimulates production of PGE_2 which is one of the major factor responsible for the bone resorption associated with odontogenic cysts. Other cytokines involved in resorption of bone in cystic defects are IL-1β and IL-6. The objective of the treatment is to eliminate the cystic pathology completely, prevent recurrence and to restore the defect if any and hence re-establish form and function. There are two basic surgical procedures, namely marsupialization and enucleation in treating any cystic lesion. Marsupialization, a relatively simple procedure, consists of making a window or an access to the cystic wall with an objective to drain out the cystic contents. Then the defect is packed with Iodoform Gauze in order to prevent infection and to achieve closure of the edges of the opening. The gauze is left in place for 1-2 days. Three to six months later, when the defect has considerably regressed due to the release of intracystic pressure, enucleation is performed. On the other hand, enucleation is a one stage procedure and is therefore the treatment of choice.
The re-establishment of the integrity of any tissue depends on the type of tissue involved and the nature of tissue damage. Tissue healing takes place by the action of more than 40 growth factors which has effects on many general and specific cell types. Once the cyst is enucleated, there starts an Inflammatory phase, where the blood clot will serve as a biological scaffold for migration and proliferation of inflammatory cells initially, and subsequently form bone through various growth factors such as interleukin-1, platelet-derived growth factor, tumour necrosis factor (TNF), and TGF-β (transforming growth factor β), among others. The Proliferative phase is characterized by angiogenesis from pre-existing vessels into the center of the clot. This angiogenesis is stimulated by early wound hypoxia, which with the help of growth factors induce a balance between mitosis and apoptosis of endothelial cells, giving rise to outbreaks of new blood vessels. When the delicate balance between mitosis and apoptosis is disturbed, clot breakdown occurs, leaving an unaesthetic and functional defect. Bone reconstruction is therefore an essential requirement for the complete functional rehabilitation after jaw surgery.

In our study, we included patients of age group ranging from 10 to 44 years. Six out of the ten cases were found to be Dentigerous Cyst and of the remaining four, there were two cases of Odontogenic Keratocyst and two of Radicular Cyst. All cases were grafted post-enucleation with a combination of 20% Hydroxyapatite and 80% Beta Tricalcium Phosphate. The literature has vast data on bone healing at the site of enucleation and simple closure of jaw cysts without using bone grafts, even in cases of large bone defects. Chiapasco et al., in 2000 evaluated the spontaneous bone healing after enucleation of large mandibular cysts with a computer analysis of postoperative panoramic radiographs. They concluded that spontaneous bone regeneration can occur even in case of large cysts without the aid of any grafting materials and also has the added advantage that the surgical procedure is simplified. It also decreases the economic and biologic costs; and reduces the risk of postoperative complications.

The role of Hydroxyapatite in the form of nanoparticles for regeneration of bony defects has been extensively studied. Thorwarth et al., in 2005 evaluated the efficacy of resorbable nanoparticle Hydroxyapatite OSTIM in filling of bone defect as compared to autogenous bone grafts. OSTIM was found to form bone that was microradiographically and histologically similar to bone formed by autogenous graft. Bezrukov et al., in 1998 reported the effectiveness of Lincomycin with Hydroxyapatite (33% OSTIM-100 paste), for filling the bone cavity formed after cyst enucleation and compared it with non-filling cases. They reported that the above preparation decreased the incidence of postoperative complications and created an optimal condition for bone repair at the site of the defect.

TCPs are bone substitute materials that are marked out by their high biocompatibility, osteoconductivity and favourable resorption properties. Horch et al., in 2006 studied the long-term effect of the ceramic β-TCP (CERASORB) at different sites of alveolar reconstruction and evaluated its properties. They found that β-TCP has low complication rate and good long-term results; and hence was a suitable material for the filling of bone defects following cyst enucleation. Palm et al., also reported satisfactory healing and reossification of cysts, even in larger sized defects (<2.5cm) using β-TCP.

Many animal studies have examined the osteoconductivity, biodegradability and safety of a combination of HA and βTCP as a scaffold for bone regeneration in areas of bone defect. It has been suggested that the ratio of HA to βTCP is an important factor affecting graft degradation and bone regeneration. TCP provides a higher amount of Calcium and Phosphate necessary for bone formation but a high proportion exhibits long term volume instability. HA, on the other hand, stabilises the graft for longer owing to its slow biodegradability; and it also has better bone binding capacity as compared to TCP. Jenson et al., in 2009 conducted a study with different ratios of HA/TCP in treating mandibular defects of minipigs and came to the conclusion that HA/TCP in the ratio of 20:80 showed similarities to autografts in terms of biodegradation and bone formation capacity. Hence in our study we have used HA and TCP in the ratio of 20:80 as the alloplastic material of choice in regeneration of cystic defects of the jaw. Using this combination of HA and TCP in our study, we found that the bone density post enucleation at the area of cystic defect was comparable to the unaffected adjacent site.

**CONCLUSION**

Evidence regarding grafting versus non-grafting for regeneration of bone in cystic defect is divided. The advantages of bone grafting are its ease of availability, osteoconductive properties, excellent biocompatibility and natural cementing mechanism to the adjacent natural bone. They also cause less patient discomfort and no donor site morbidity as compared to autografts. The drawbacks are the cost of the material itself, the lengthening of the operative time and like any foreign body, its predilection for infection. In our study we noted that using bone graft to fill enucleation defects increases clot stabilization and provides better support to the overlying soft tissue. We observed that the bone density at the site of cystic defect after using a combination of 20% Hydroxyapatite and 80% Beta-Tricalcium phosphate as graft material resulted in a comparable bone density at 1 year follow up as compared to the adjacent unaffected bone.

Our study had limited sample size being a pilot study. We would like to further continue the research in the same study by doing a bone biopsy in the grafted sites and thereafter establishing the quality of bone formed.

**ACKNOWLEDGEMENT**

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REFERENCES


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