Original Article

Histologic and histomorphometric evaluation of two grafting materials Cenobone and ITB-MBA in open sinus lift surgery

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Abstract

Aims and Objectives: Alveolar ridge reduction caused after tooth extraction can be minimized through ridge preservation and application of graft materials. The aim of this study was to compare the histologic and histomorphometric aspects of bone particulated allografts, Cenobone and ITB-MBA, in the reconstruction of vertical alveolar ridge after maxillary sinus augmentation. **Materials and Methods:** This clinical trial was performed among 20 patients. The participants were randomly divided into two groups of 10 participants. The first group received Cenobone and the second group received ITB-MBA. Tissue samples were prepared 6 months later at the time of implant installation and after successful maxillary sinus floor augmentation. Tissue sections were examined under a light microscope. The data were analyzed by Chi-square and *t*-test. **Results:** The mean trabecular thickness of the samples in the Cenobone group was 13.61 ± 7.47 μm compared to 13.73 ± 7.37 μm in the ITB-MBA group (P = 0.93). A mild inflammation process (Grade 1) was detected in both the groups. The amount of remaining biomaterial in the Cenobone group was estimated to be 8 ± 19% vs. 7 ± 12% in the ITB-MBA group (P = 0.30). Bone formation was reported 49.71% in the Cenobone group vs. 40.76% in the ITB-MBA group (P = 0.68). The mean newly formed vessel in the Cenobone group was 0.64 ± 0.7 vs. 1.5 ± 2.3 in the ITB-MBA group (P = 0.14). **Conclusions:** There was no significant difference between the two groups of patients regarding trabecular thickness, remaining biomaterial allograft, and the density of blood vessels after sinus floor elevation; hence, there was no difference between the two groups regarding implant outcome. More designed studies as randomized controlled trials and controlled clinical trials, which evaluate the long-term implant outcome; comparing the different bone graft materials is also required to improve evidence on survival and success rate.

Key words: *Allografts, biomaterials, bone substitutes, sinus floor augmentation*

INTRODUCTION

Reduction of alveolar ridge is considered to be the inevitable consequence of tooth extraction and change in alveolar physiological status.[1] The reduction in the height of the ridge is estimated to be between 0.2 and 3.25 mm after tooth extraction in the first three months.[2]

Loss of teeth results in reduced bone volume as well as trauma caused by removable dentures or

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different pathologies.[3] Limited bone height of the posterior maxilla, due to maxillary sinus anatomical place, is another limitation that could impair implant installation.[4]

Implant treatment is an excellent alternative to replace a missing tooth. Brånemark *et al*.[5] were the first to describe bone to implant contact called osseointegration. Albrektsson *et al*.[6] defined the term direct contact between living bone and implant at the light microscope level. Supporting alveolar ridge minimizes the reduction process of the remaining ridge upon tooth extraction and determines maximum function of the prosthesis.[1] Bone preservation not only supports fixed and removable prostheses but also ensures proper and successful osteointegration of implanted prostheses.[7] Changes in alveolar ridge dimensions results in difficulties in the placement of a conventional bridge or implant prostheses, and in cases of serious degeneration, implant placement will face problems and requires complex treatment such as bone graft procedures, which increases the cost of treatment.[8] Autogenous bone graft has been considered the gold standard for bone graft applications for a long time. In recent decades, research on bone substitutes has increased due to lack of autograft bone in some patients, need for additional surgery procedure on graft donor, and limitation of available bone amount.[9] The ideal bone grafting material should have both osteoinductive and osteoconductive properties and should also be able to osseointegrate to the implant surface. These properties vary in different bone grafting materials.[9] Osteoinduction is defined as primitive, undifferentiated, and pluripotent cells that are stimulated by inductive means in order to induce bone-forming cells and osteogenesis process. Osteoconduction is defined as the bone growth on a surface which is called as an osteoconductive surface allowing the osteogenesis process on itself, down into the pores.[10] Allografts are bone tissues that are derived from the same species and are treated with various techniques, e.g., freeze dried or exposed to radiation.[9] It is expected that the grafting materials that are utilized in floor augmentation could provide the bone formation process by replacing the bone materials due to capillary infiltration and supporting the implants.[11] The most frequently used allograft are known as freeze-dried bone-allogeneic grafts (FDBA) and Decalcified freeze-dried bone allogeneic (DFDBA), the latter includes more osteogenic potential due to target bone morphogenic protein graft.[12] Previous researches have been performed regarding the benefits of bone allografts for the reconstruction of alveolar ridge, which have proved to have positive results, although they are mostly limited to animal models.[13-15] Cenobone is a biocompatible bioimplant with allograft origin. This bioimplant maintains the extracellular matrix, which acts as a scaffold and causes the accumulation of fibroblasts and blood vessels. In a study, the biocompatibility of Cenobone was investigated as graft material, which showed positive results.[16]

A preliminary study by Amouian indicated that the use of Cenobone can stimulate bone formation with a notable increase in the clinical width at 2 and 5 millimeter of alveolar crest for implant placement.[17]

Rokn *et al*. reported no statistically significant differences in bone generation among ITB, CenoBone, and Grafton, and concluded that allografts manufactured in Iran can be suitable alternatives to Grafton with similar good properties.[15]

In this study, we aimed to evaluate the histological and histomorphometric aspects of bone particulated allograft (Cenobone) use versus ITB-MBA allograft in the reconstruction of vertical alveolar ridge after maxillary sinus augmentation.

PATIENTS AND METHODS

In this semi-experimental clinical trial, 20 patients were enrolled and treated between 2012 and 2013; 20 males and females (mean age: 49 ± 4.32 years) were enrolled in this study after taking medical history and performing dental examinations, which included standard radiographs at the department of periodontology in Babol University of Medical Sciences.

Based on the formula: $n = \frac{\left(Z_{1-\alpha/2} + Z_{1-\beta}\right)^2 \left(\sigma_1^2 + \sigma_2^2\right)}{\left(\overline{X_1} - \overline{X_2}\right)^2}$

with confidence interval of 95% and power of 80%, and based on the previous study, the number of samples was calculated to be 10.[18]

This study was reviewed and approved by the ethics committee of Babol University of Medical Sciences. The inclusion criteria were determined as patients with good general health who presented with at least a single area of unilateral posterior maxillary atrophy, with sinus pneumatization with ≤5 mm of residual floor thickness of the maxillary sinus in the planned implant insertion site. Participants with maxillary sinus problems such as acute sinusitis, heavy smoking,

and uncontrolled systemic diseases such as diabetes or history of surgery/chemotherapy or radiotherapy during the past 12 months were excluded. All participants filled a written informed consent, which was approved by the ethics committee for human research of the dental faculty of Babol University.

Statistical analysis

The data were collected using the Statistical Package for Social Sciences version 20 software (IBM Corp, Released 2011) and analyzed by Chi-square test and *t*-test. $P < 0.05$ was considered to be statistically significant.

Maxillary sinus augmentation procedure

For randomization and avoiding patient selection bias, patients who met the inclusion criteria were treated with two study materials: Cenobone (Tissue Regeneration Co., Kish, Iran) in one group and ITB-MBA (Tehran University, Iran) in another group. The sinus lift technique was performed after applying local anesthesia using 2% xylocaine with 1:80000 adrenaline (Darupakhsh, Tehran, Iran); the flap was prepared by a horizontal dissection in alveolar crest using a no. 15 scalpel such that it was shifted approximately 2 mm to the palatal side. Then, two vertical dissections were made in the mesial and distal area. Thereafter, the full thickness flap was pushed upward by a Prichard periosteal flap elevator by a blunt dissection method, such that the sinus area and a bone window were fully available. Using a round burr, a window was made in the wall of the maxillary sinus, and the sinus membrane was then released and elevated and the collagen membrane (ITB) (Iranian Tissue Bank Research and Preparation Center, Tehran) was placed under the sinus membrane. Then, the sinus was partially filled with Cenobone (Tissue Regeneration Co., Kish, Iran) in one group and with ITB-MBA (Tehran University, Iran) in another group, which are two types of mineralized allograft bone powders (1000–2000 μm). Subsequently, the windows area was covered with a collagen membrane and sutured by Vicryl 05 after removing tension from the flap [Figure 1]. After the surgery, the patients received post-surgical recommendations.

The implant surgery was performed 6 months after sinus bone graft. Core biopsies were prepared on the day of the surgery (after preparing the flap) by using a trephine (3 mm in diameter and at least 10 mm height) from the implant hole [Figure 1]. After coding

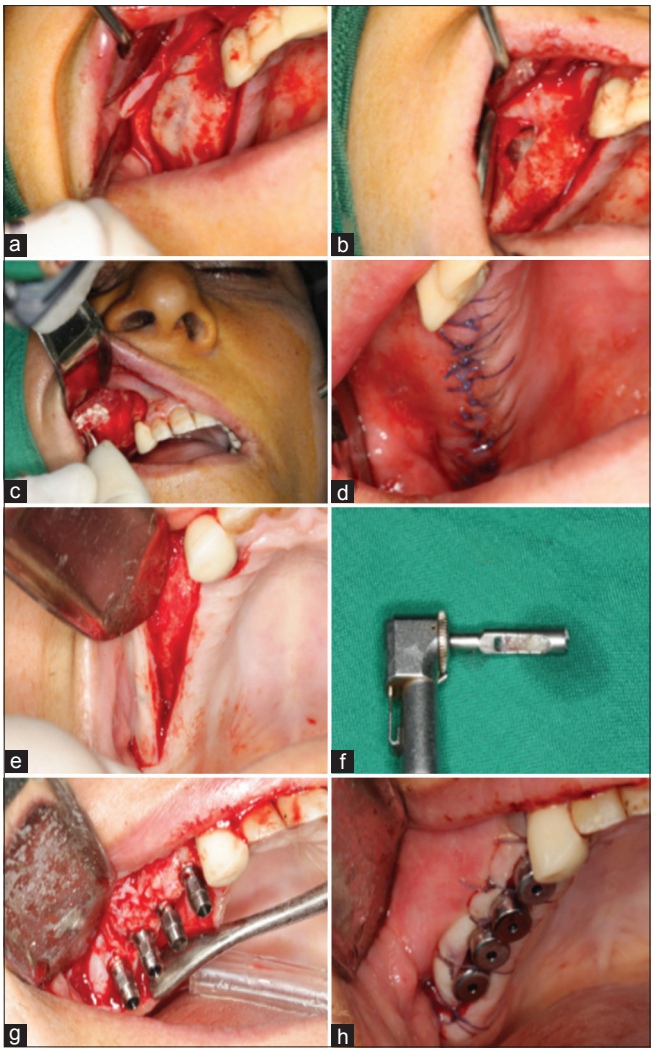

Figure 1: Intraoral photographs of surgical procedure: (a) Reflection of the soft tissue; (b) window preparation at the surgery site; (c) particulate allograft placed; (d) recipient site sutured; (e) the second flap surgery 6 months later; (f) core biopsy using a trephine bur for getting specimen for the histological analysis; (g) placement of implant; (h) turning the flap and suture

the samples to identify the crestal and apical points, the samples were sent to the pathology laboratory for histological examination.

Histology sample preparation

The block biopsies were harvested and fixed in 10% formalin for 10 days, and decalcified in 10% formic acid for 1 week. The samples were daily observed to measure the decalcification extent. In the next step, the samples were transferred into 20% lithium bicarbonate solution for buffering for 5 minutes. Each sample was coded by a number, and finally bone samples were cut vertically into two parts. The cut edges, which represented the middle part of the bone, was marked

by Indian ink and an identification code. The samples were then placed in alcohol with different degrees of purity for serial dehydration, and then were paraffin embedded. Seven cuts with a thickness of 5 microns were obtained from each paraffin blocks. These selected sections were stained with hematoxylin and eosin and evaluated by light microscopy (Olympus BX41, Japan). In the present study, the samples were evaluated both histologically and histomorphometrically to assess the extent and severity of inflammation, the thickness of trabecular bone, as well as the presence or absence of connective tissue between the biomaterial and bone fragments. The number of blood vessels was also calculated in each microscopic field at a ×40 magnification and classified according to the study by Karring.[19]

Samples were categorized into 5 groups according to the level of inflammation:
Grade 0: Absence of inflammatory cells
Grade 1: Few scattered inflammatory cells (Mild)
Grade 2: Number of inflammatory cells 5 to 10 in HPF (Mild to moderate)
Grade 3: Presence of inflammatory cells up to 10 to 50 in HPF (Moderate)
Grade 4: Inflammatory cells >50 in HPF (Severe inflammation).

A computerized image analysis system consisting of a light microscope (Olympus BX41) at a ×40 magnification and digital camera (DP12) connected to an image analysis software (SIS LS Starter) was used to obtain area measurements.

The thickness of bone trabeculas was also evaluated histomorphometrically, which was categorized based on the study by Nyman.[20]

The slides were coded such that the pathologist was blind to the treatment group and biopsy content to avoid interpretation bias. To ensure the accuracy of the study, 7 sections were obtained from each biopsy specimen and the average of each studied variable was reported.

RESULTS

The study was performed on 20 patients (10 males and 10 females) with a mean age of 49 ± 4.32 years who volunteered for implant. All prepared biopsies from the patients in both groups contained vital bone. The mean trabecular thickness of the samples in Cenobone group was 13.61 ± 7.74 μm, (range: 3.29–34.44 μm), whereas in the other group (ITB-MBA), the mean measured trabecular thickness was reported 13.72 ± 7.37 μm (range: 2.67–43.70 μm); in both the groups, average trabecular thickness did not show a significant difference ($P = 0.93$) [Table 1; Chart 1]. No foreign body reaction/severe inflammation was observed in the field of surgery in both the groups, however, a mild inflammation process (Grade 1) was detected [Figure 2]. The amount of remaining biomaterial in the Cenobone group was estimated to be 8 ± 19% vs. 7 ± 12% in the ITB-MBA group, which showed no significant difference ($P = 0.30$). Furthermore, the biomaterial contact with newly formed bone was reported to be direct in all cases. The mean newly formed vessel in the Cenobone group was 0.64 ± 0.7 vs. 1.5 ± 2.3 in the ITB-MBA group, which was not statistically significant ($P = 0.14$). In addition, the amount of bone formation was 49.71% in Cenobone group vs. 40.76% in ITB-MBA group, which did not show significant difference ($P = 0.68$) [Table 1].

DISCUSSION

In the present study, we evaluated both histologically and histomorphometrically Cenobone and ITB-MBA in maxillary sinus floor augmentation; the results from our study demonstrated that there was no significant difference between the two groups of patients regarding trabecular thickness, remaining biomaterial allograft, and the density of blood vessels after sinus floor elevation.

The survival and failure rates of implant placement performed with sinus augmentation and bone graft material is associated with many different variables

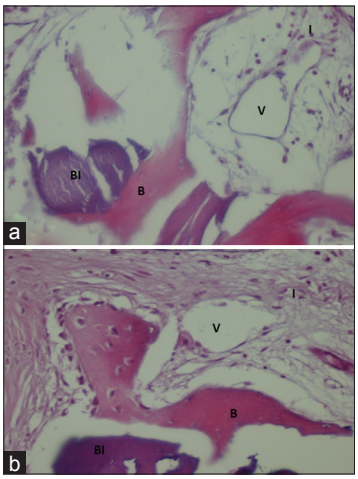

Figure 2: Histological view of the (a) Cenobone and (b) ITB-MBA material (Hematoxylin and eosin; magnification ×40). (BI: biomaterial, B: bone, v: vessel, I: inflammatory cells)

Table 1: Evaluation of the characteristics between two groups

Biomaterial group	Mean trabecular thickness (μm)	Remaining biomaterial (%)	Bone formation (%)	Newly formed vessels
Cenobone	13.61±7.74 μm	8±19%	49.71%	0.64±0.7 Grade 0=100% Grade 1=0% Grade 2=0%
ITB-MBA	13.72±7.37 μm	7±12%	40.76%	1.5±2.3 Grade 0=92.9% Grade 1=3.6% Grade 2=3.6%
P value	0.93	0.30	0.68	0.14

There is no significant difference between the two groups of patients

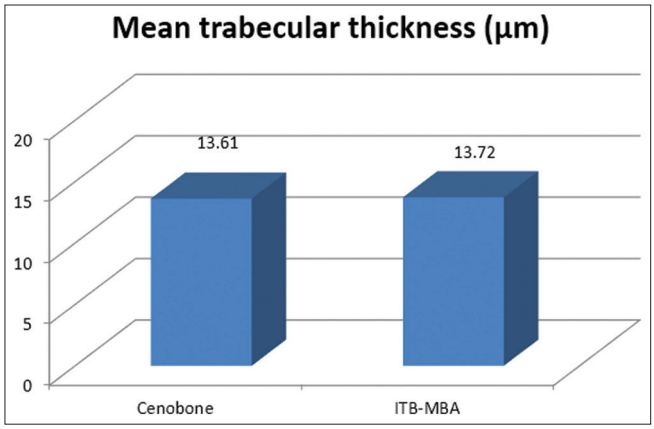

Chart 1: Mean trabecular thickness after biomaterial allograft application

such as bone graft material origin, grafted bone volume, residual bone volume, implants surface and design, patients' age, smoking habits, bone graft, implant healing time, etc.[21] Wide variety of FDBA products exist in the market which have different inductive capabilities. Sarkarat *et al*.[16] compared Cenobone and OSSEO+ and reported that both types of allografts showed relatively similar effects in maintaining the height and width of the alveolar ridge. This result confirms our results that Cenobone stimulates bone formation. It has been proven that utilization of allogeneic graft materials can substitute autografts in the surgical treatment of bone reconstructive surgery. Harvesting autograft bone can cause additional and unwanted trauma to the patients, and using allografts can be a suitable alternative link that has the same healing effects.[9] In a histologic analysis by Stentz *et al*.[22] using demineralized FDBA plus barrier membrane therapy, the authors indicated that, in large size defects, this combination treatment modality could obtain optimum osseointegration. In contrast, De Vicente *et al*.[4] reported that the implants and bone defects around them were filled with FDBA showing similar BIC to the implants that their defects had just covered with collagen membranes. These differences may be related to the origin and methods of preparation of FDBA, and if the preparation methods were the same in different bone banks, this would be due to individual donors' ages and gender, disease and injury, medical treatment, or genetic variability. In addition, shape and size differences of FDBA particles could affect the inductive ability. On the other hand, the time difference between death and bone extraction may negatively alter the bone inductive ability. Surface roughness of the implant is another factor that may have an impact on the phenotypic expression characteristics of cells *in vivo*.[23]

Therefore, adding FDBA can have advantages over membrane, which is currently a matter of controversy. In the present study, bone formation was measured to be 49.71% in the Cenobone group vs. 40.76% in the ITB-MBA group, which was not a statistically significant difference ($P = 0.68$). Results from previous studies reported bone volumes between 20% and 37% after 5–8 months of healing.[24]

In a similar study Hakimi investigated the osteoconductive potential of bovine-derived porous hydroxyapatite plus demineralized freeze-dried bone allograft in the maxillary sinus engraftment, the author reported that the amount of new bone formation was measured to be 5.36% (after six months) and 43.68% (after twelve months). They concluded that histomorphometric and histologic evaluation may play determinant roles in the evaluation future implant site status.[25]

In another study, Strietzel[26] compared the tissue composition of augmented sites after the use of a nanocrystalline hydroxyapatite (ncHA) bone substitution material and after a 2-year follow up; in a bone core histomorphometric study, he found that the mean percentage area of bone colonizing the defect was 52.3%. He also reported that the amount of percentage

area of the ncHA decreased from the peripheral (23.4%) to the central zones (15.1%), however, the differences were not significant ($P = 0.262$). It appears that the application of allografts has a significant induction on bone formation effect. However, it has been documented that repeated trauma to the implant or the neighboring surface in the process of healing could negatively affect the survival of the implant.[27]

Lumetti *et al*. has shown that, if the implant is installed after 6 months of bone healing, the degree of osseointegration is increased; most implant failures occurred during the healing period and the second stage surgery.[28] In our study, volumetric assessment of bone volume, such as trabecular thickness, did not show significant differences between the two groups. However, trabecular thickness in patients with ITB-MBA bioimplant was higher than the Cenobone group. The better efficacy of ITB-MBA may be associated with higher osteoconductivity than Cenobone. However, Cenobone showed effective osteoconductivity in some studies.[15,17,29]

This study demonstrates that the use of Cenobone and ITB-MBA as a graft material for sinus floor augmentation resulted in bone mass gain in both the groups. Although the graft materials were biocompatible, both materials were not completely resorbed after 6 months, and the remains were integrated into the bone.

Of the study limitation were difficulties in case selection, impossibility of follow-up of more than 6 months, and uncooperative patients. According to the results obtained from our study, we recommend using allografts in alveolar ridge restoration in order to improve clinical outcomes of implant treatment. More large-scale studies designed as randomized controlled trials and controlled clinical trials with larger study population and long-term follow up are needed to improve evidence on the survival and success rate.

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Conflicts of interest

There are no conflicts of interest.

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