Effect of hyperbaric oxygen on bone healing after enucleation of mandibular cysts: a modified case-control study

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Abstract

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Introduction: Mandibular cysts may require enucleation, resulting in large cavities that compromise mandibular strength and functions. We investigated the effects of hyperbaric oxygen therapy (HBOT) on healing after enucleation of mandibular cysts.

Patients and methods: Fourteen healthy individuals in whom no modifiers of wound healing were present received a median of 20 post-operative HBOT. The rate of filling of the defect was derived from the number of pixels in the residual cavity after transformation of the area of the lesion in orthopantomograms taken immediately after surgery and at six months post operation. Modifications in bone density, detected on panoramic radiographs, were defined through a gray scale of 256 tonalities. The radiolucency of a healthy tooth was used as a reference to control for differences between radiographs taken at different times in the same patient. Both the rate of filling and changes in bone density were compared with corresponding data from a previous study of 27 healthy subjects who were allowed to heal spontaneously without HBOT.

Results: At six months post operation, the HBOT group showed 55 ± 9% reduction in the size of the residual cavity and 55 ± 17% increase in bone density compared to immediate postoperative values. Corresponding values in the control study were 12 ± 4% and 37 ± 23%, respectively. These differences were significant (t = -16.95; P = 1.21E-11 [x 10^{-11}] for the reduction in cavity size and t = -2.39; P = 0.029 for bone density).

Conclusion: HBOT merits a place as a useful adjunct in the surgical management of defects of the mandible.

Key words

Hyperbaric oxygen therapy, dental, radiological imaging, clinical audit, outcome

Introduction

The mandible is one of the important bones of the facial skeleton. It provides a framework for many of the structures involved in mastication, phonation, deglutination, facial expression and aesthetics. Several lesions, such as cysts of the mandible, may need to be enucleated as a part of treatment, resulting in large cavities that compromise its strength and functions. Additionally, this may lead to a pathological fracture and result in protracted morbidity, treatment and sometimes hospitalisation. The natural healing process takes up to twelve months for 43% of bone filling to occur with a 48% increase in bone density.[1]

Accelerating the rate of filling of these mandibular defects without compromising the quality of the regenerated bone is a much desired innovation. Various methods used include bone grafts (autogenous, allograft or xenograft) alone or in combination with alloplastic materials (e.g., bovine-derived collagen paste), hydroxyapatite granules (proposed with the aim of providing a scaffold for enhanced bone tissue repair) and guided bone regeneration with the use of semi-permeable barriers. However, all these methods may present some limitations, and are reviewed elsewhere.[1]

In the last few decades, hyperbaric oxygen therapy (HBOT) has been found to be an important adjuvant in the healing of wounds and fractures. A substantial literature is available to support the benefit of HBOT in cases such as osteoradionecrosis and osteomyelitis.[2–4] Even in mandibles which neither have been irradiated nor are infected, HBOT has been found to be of value in restoring bone defects.[5–7]

Nonetheless, the inferences of the latter cannot be translated to mandibular defects arising after enucleation of cysts because most of such studies are confined to animals. Healing in a human mandible is conceived to be different from that in rat or rabbit due to variation in structural morphology which includes distribution as well as local modifications of compact bone and arrangement and development of spongy bone trabeculae.[8] Some recent studies and reviews have raised doubts on the usefulness of HBOT in osteoradionecrosis or delayed union/non-union of bony fractures.[9–12]

The present study investigated the effects of HBOT on the healing process and rate of bone regeneration subsequent to enucleation of mandibular cysts in a group of individuals in whom no other modifier of wound healing was found to be present. The above objective was achieved by quantifying the rate of filling and changes in the bone density of the lesion in these patients over a period of six months and then comparing these variables with the data reported in a previous study of 27 subjects who were allowed to heal spontaneously (i.e., without HBOT).[1]

Table 1
Patient details and summary of presenting pathology and surgical management of mandibular cysts

Case	Age (yr)	Sex	Diagnosis	Site	Treatment
1	11	M	Radicular cyst	Lt body, from canine to 1st molar	Enucleation
2	16	F	Odontogenic keratocyst	Rt ramus	Enucleation and chemical cauterization
3	12	M	Odontogenic keratocyst	Rt parasymphysis, from canine to Lt ramus	Enucleation and chemical cauterization
4	56	M	Odontogenic keratocyst	Rt angle and ramus	Enucleation and chemical cauterization
5	58	M	Multiple radicular cysts	Lt 1st molar, Rt lateral incisor and premolar	Enucleation and curettage
6	29	M	Odontogenic keratocyst	Lt body to ramus	Enucleation and chemical cauterization
7	14	M	Dentigerous cyst	Rt body	Enucleation
8	31	M	Odontogenic keratocyst	Rt body to Lt parasymphysis	Enucleation
9	18	M	Odontogenic keratocyst	Lt angle and ramus	Enucleation and chemical cauterization
10	26	M	Dentigerous cyst	Lt angle	Enucleation
11	12	M	Radicular cyst	Lt body	Enucleation
12	72	F	Radicular cyst	Rt body	Enucleation
13	34	M	Multiple odontogenic keratocyst	Rami bilaterally	Enucleation and chemical cauterization
14	36	F	Radicular cyst	Symphysis	Enucleation and curettage
15*	13	M	Dentigerous cyst	Lt body	Enucleation
16**	32	F	Odontogenic keratocyst	Rt angle	Enucleation and chemical cauterization
17**	48	F	Radicular cyst	Lt body	Enucleation

* Refused HBOT
** Lost to follow up

Methods

PATIENT SELECTION

In all, we received 17 patients in whom the inclusion and exclusion criteria (*vide infra*) were met. Of these, one (case 15) refused to undergo treatment with HBOT and two (cases 16 and 17) were lost to follow up. The remaining 14 patients ranged between 11 and 72 years old. Such a varied age group was admitted to the study because cysts and cystic lesions of the mandible are uncommon (confinement of subjects to a narrower age group, e.g., 20–40 years old, would have entailed rejection of nine patients, precluding a statistically viable sample size). Also the 'control' group study to which we compared our data varied between 6 and 63 years in age.[1] Age, sex, diagnosis and the site of lesion(s) and the surgical treatment given for individual subjects are shown in Table 1. All the subjects were of middle socioeconomic status and apparently well nourished.

Inclusion criteria included 1) cysts and cystic lesions of the mandible treated surgically and 2) defects not less than 30 mm in a single lesion and not adding up to 30 mm in multiple lesions. These inclusion criteria were to make the subjects comparable to those studied by Chiapasco et al.[1] Exclusion criteria included patients with any intercurrent illness and/or medication that would have affected healing and patients with bone grafts or osteopromotive membrane.

The patients were informed about the nature of the study and all possible risks involved, and consent was obtained. The protocol was approved by the Ethics Committee of the Institute of Aerospace Medicine (IAM), Bangalore, India. All the patients were subjected to thorough pre-operative and pre-anaesthetic evaluation. The investigations included routine haematology, urinalysis, bleeding and clotting times, fasting and postprandial blood sugar, serum urea, a resting ECG, pulmonary function tests and a chest X-ray. The last was especially to identify Gorlin-Goltz syndrome associated with odontogenic keratocyst. Pre-operative orthopantomograms (OPG) were taken to calculate baseline values of the area of bony defect and density.

SURGERY

All the surgical procedures were performed by an intraoral approach under local and general anaesthesia. Appropriate antibiotics were administered at induction. Intra-operative corticosteroids were administered to reduce oedema. For cysts involving the dento-alveolar region, a sulcular approach was used in most cases. Whenever tooth preservation was possible, a crevicular incision was used. The cyst wall was enucleated. In cases of odontogenic keratocyst, peripheral ostectomy was performed to destroy the daughter cysts.

Patients with odontogenic keratocysts also underwent, after enucleation, chemical cauterization with Carnoy's solution (6 parts ethyl alcohol (absolute or 95%), 3 parts chloroform and 1 part glacial acetic acid). This kills epithelial remnants and dental lamina in the osseous margin. The cystic cavity was irrigated with saline and hydrogen peroxide and haemostasis was secured. In all cases, the wound was closed primarily with 3-0 Vicryl. All patients received perioperative antibiotic cover. In-patients received intravenous antibiotics whereas patients treated on an outpatient basis were administered oral antibiotics.

HYPERBARIC TREATMENT

Within a week of surgery, HBOT was commenced daily, five days per week, in a multiplace chamber at a pressure of 253 kPa with the patients breathing 100% O_2 via an oronasal mask and demand regulator (British MK 17E). Duration of each treatment session was about 90 min, inclusive of compression and decompression, the rate of which was approximately 10 kPa min^{-1}. The median number of HBOT sessions was 20 (18 and 21 being the lower and upper quartiles, respectively). Before HBOT, mobility of tympanic membranes and the ability to ventilate middle ears was ascertained by Valsalva procedures.

FOLLOW UP AND RADIOGRAPHIC ANALYSIS

Patients were followed up clinically and radiologically. This included postoperative assessment, after HBOT and then on a monthly basis for a period of six months. Clinical evaluation included an examination of the operation site for inflammation, wound dehiscence, evidence of infection, the amount of healthy granulation tissue, pathologic fractures and the general condition of the patient.

OPGs were taken immediately after surgery and at six months postoperatively to evaluate the reduction in size of the residual cavity and changes in bone density. Radiographs were also assessed for trabecular pattern, amount of radiological bone filling and evidence of infection and fracture. The size of the residual surgical defect and the bone density were calculated by the technique described by Chiapasco et al.[1] Accordingly, the area of the residual defect was transformed into pixels using Corel Photo-Paint 5™ (Corel Corporation, Canada). The number of pixels in the residual cavity was calculated and the variation in the number of pixels in the remaining surgical defect across time was interpreted as bone filling in the area of the lesion.

Similarly, modifications in bone density, detected on the panoramic radiographs, were defined through a gray scale of 256 tonalities. Put simply, it was an average of all the 256 tonalities weighted for their numbers. The grayscale value thus obtained was normalised to the corresponding value derived from a healthy tooth, the radiolucency of which served as a reference to control for differences between the radiographs taken at different times in the same patient. MATLAB®, version 7.2.0.232 (Release 2006a), was used for this analysis. The selection of the area of the lesion at six months was kept corresponding to that seen immediately after surgery. For bone density, a smaller number of cases (n = 8) was available as bone density estimates could not be made in the others due to artefact shadows from tongue or cervical vertebrae.

To avoid any bias and consequent imprecision in the above analysis, the two computations were performed by a resident from the Department of Oral and Maxillofacial Surgery, RV Dental College and Hospital, Bangalore, who was blinded to identity of panoramic radiographs and was not associated with the study. Such assessments of size of the residual surgical defect and of bone density are reported to have good agreement with the corresponding measurements derived from computed tomography.[1] A similar/comparable procedure has been used by others.[13,14]

STATISTICAL ANALYSIS

An unpaired Student t test, as applicable to data samples with unequal variance, was employed to examine the significance of differences in the reduction in the size of the residual cavity and increase in bone density in the two groups (viz, one which healed spontaneously, and the other with HBOT). Such a comparison of cases with rather unrelated (but not un-matched) controls which have been studied by others, is regarded as an acceptable procedure employed by others.[15,16] It was also employed to see if the healing was different after chemical cauterization of the defect, as part of the standard treatment, in a subset of patients in the present study. Single factor ANOVA was used to examine variation in healing in cystic defects arising after enucleation of different types of cysts because it was, essentially, a multi-sample hypothesis. Pearsonian product moment correlations were calculated (between age and rate of filling/increase in bone density) to find out if bone healing was influenced by age. The level of significance was kept at $P < 0.05$. However, exact significance with the associated degrees of freedom is annotated in the results.

Results

Cases in the present study were comparable in age with the 'controls' studied by Chiapasco et al[1] (cases: 30 ± 19 yr versus 'controls': 35 ± 13 yr; t = 0.96, P = 0.341 for df = 39). Except for two patients, who developed wound dehiscence, all experienced uneventful primary healing. These two cases were allowed to granulate and heal secondarily. One of them developed infection at the operated site that resolved with antibiotic therapy. Clinically, the degree of bone regeneration after six months was sufficient for the dental surgeons to consider implant rehabilitation of the patients in the following few months. In fact, the healing was so good that, in two of the initial cases, implants were placed and osseous integration occurred without any problem.

Table 2
Reduction in size of residual cavities (expressed as number of pixels) and increase in bone density (expressed as normalised grayscale values) in cases (*n* = 14 or 7; present study) and 'controls' (*n* = 27; derived from Chiapasco et al, 2000[1])

	Present study			'Control' study			
	Immed. post-op	6 months post-op	% change	Immed. post-op	6 months post-op	% change	*P*-value
No. of pixels	137,143 ± 84,252	60,303 ± 40,215	55 ± 9	14,832 ± 6,238	12,999 ± 5,589	12 ± 4	1.21E-11 for df = 15.65
Bone density	39 ± 12	60 ± 17	55 ± 17	82 ± 59	103 ± 61	37 ± 23	0.029 for df = 15.95

Note: Degrees of freedom (df) are for unpaired Student t tests for data samples with unequal variance and, in the case of bone density, also fewer cases (*n* = 7) as density estimates could not be made in all patients because of artefact shadows from tongue or cervical vertebrae.

Table 2 shows the reduction in size of the residual cavities (expressed as number of pixels) and increase in bone density (expressed as normalised grayscale values) in cases in the present study and in the 'controls' study.

As can be seen from Table 2, the number of pixels amongst the patients in our study was 137,143 ± 84,252 in the immediate post-operative period. This reduced to 60,303 ± 40,215 at six months. Corresponding values in the control study were 14,832 ± 6,238 and 12,999 ± 5,589. Therefore, in normalised terms, a reduction in the size of the defect of 55 ± 9% was seen in the HBOT group (present study) at six months, compared to 12 ± 4% in the 'control' study. The difference in the reduction in the size of defect was highly significant (t = -16.95, *P* = 1.21 E-11 [x 10^{-11}], df = 15.65). The above values of 't' and degrees of freedom are calculated considering unequal variance in the two groups, hence degrees of freedom are not integers (see Table 2).

It is to be appreciated that the large difference in the values in the number of the pixels/grayscale values between the two studies is because the images were enlarged in the present study to facilitate easy computation. However, this does not affect the analysis because it influenced the number of pixels/grayscale values in the films taken immediately and at six months post-operatively in a similar manner.

Weighted grayscale histograms of the area of interest (cavity), along with those of the reference (healthy) tooth are given in Figure 1. Figure 2 shows serial orthopantomograms in a representative case (Case 3).

Bone density, expressed as the average grayscale value of 256 tonalities and normalised to a healthy tooth, increased from 39 ± 12 in the immediate post-operative period to 60 ± 17 at six months in our subjects. Corresponding values in the 'control' study were 82 ± 59 and 103 ± 61. Thus, an increase of 55 ± 17% in bone density was seen in the HBOT group (present study) at six months; whereas the increase in the 'control' study was 37 ± 23%. The above difference was, again, statistically significant (t = -2.39, *P* = 0.029, df = 15.95). Both the reduction in the size of defects and the increase in bone density correlated poorly with age (r = 0.026, t = 0.09, *P* = 0.928 for reduction in size of defect and r = 0.554, t = 1.629, *P* = 0.154 for increase in bone density).

In the present study, chemical cauterization (employed as part of the treatment of odontogenic keratocyst after enucleation to prevent recurrence) did not modify healing. Reduction in size of defect was 57 ± 8% in the group treated with chemical cauterization and 52 ± 10% in the group not so treated. The two values were not significantly different (t = 1.05, *P* = 0.314, df = 9.31). Similarly, increase in bone density (50 ± 19% in the group treated with chemical cauterization and 63 ± 8% in the group not so treated) was statistically comparable (t = -1.12, *P* = 0.306, df = 5.70).

No significant variation in healing of the defect arising after enucleation of different type of cysts was observed, both in terms of reduction in the size of defect (56 ± 6% for radicular cysts, 52 ± 9% for odontogenic keratocysts and 60 ± 12% for dentigerous cysts; F = 1.01, *P* = 0.395) and an increase in bone density (45 ± 26% for radicular cysts, 59 ± 11% for odontogenic keratocysts and 57 ± 24% for dentigerous cysts; F = 0.42, *P* = 0.680).

Discussion

Bone healing occurs in three distinct but overlapping stages: an early inflammatory stage, the repair stage and the late remodelling stage. In the inflammatory stage, a haematoma develops within the defect during the first few hours and days. Inflammatory cells (macrophages, monocytes, lymphocytes, and polymorphonuclear (PMN) cells) and fibroblasts infiltrate the bone under prostaglandin mediation. This results in the formation of granulation tissue, in-growth of vascular tissue, and migration of mesenchymal cells. Once a haematoma forms at a fracture site, it must be invaded by a vascular spindle. A continuously occurring state of bone deposition, resorption, and remodelling facilitates the healing process.

Control of angiogenesis in wound healing is shown to be

Figure 1
'x' axis represents pixels with grayscale values from 0 to 256. Each bar singularly represents a grayscale tonality weighted for its number averaged across subjects. Weighted values, represented on 'y' axis, have no units.

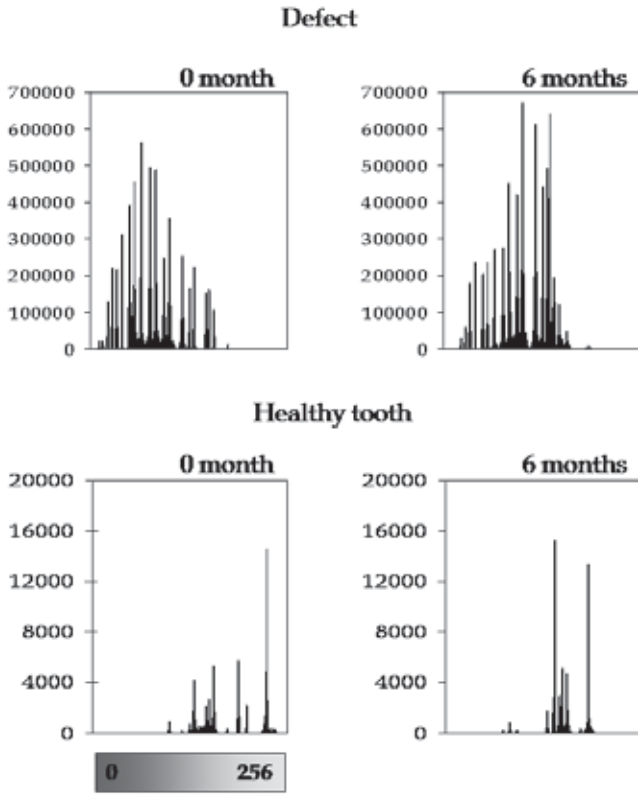
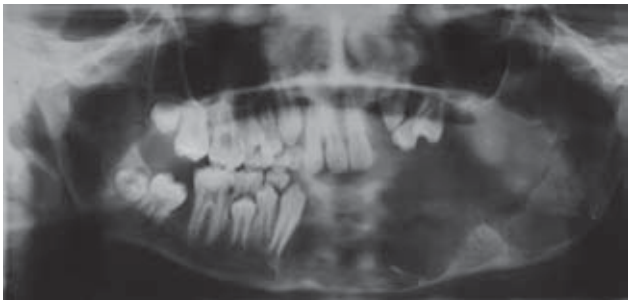
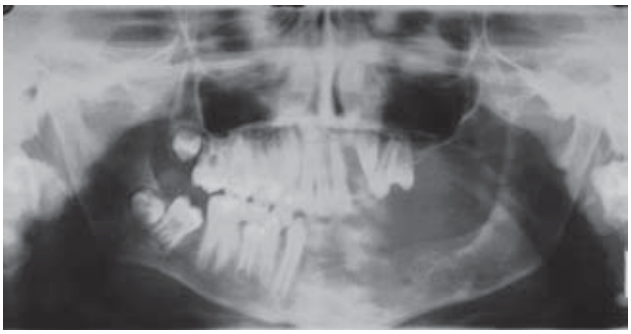


Figure 2
Serial orthopantomograms from Case 3
immediate post-op



6 months post-op



the result of macrophages responding to tissue oxygen (O_2) tension without the necessity of interacting with other cell types or biochemical signals.[17] Hyperbaric oxygen has been shown to increase the expression of vascular endothelial growth factor, a potent stimulus for endothelial cell activity, in calvarial critical-sized defects in rabbit.[18] With the progression of vascular in-growth, a collagen matrix is laid down, while osteoid is secreted and mineralised, and leads to formation of a soft callus. O_2 is essential to promote fibroblast proliferation and collagen production. Hydroxylation and cross-bonding of the collagen precursors also require O_2. Collagen cannot be synthesised by the fibroblast unless adequate amounts of both proline and lysine are hydroxylated with O_2. Synthesis requires one atom of O_2 for every three amino acids in sequence. O_2 is also required in increased amounts during the repair process to provide energy for protein synthesis.[19] A high rate of energy metabolism, evident from adenosine triphosphate content of callus is observed in the early phase of fracture healing, which persists until the callus is corticalised and remodelling starts.[20] Several animal studies have shown that hyperbaric O_2 speeds up callus formation.[21,22]

Resistance to infection is extremely dependent on local O_2 tension. The bactericidal action of polymorphonuclear leukocytes uses O_2. Thus, HBOT acts as an adjuvant to antibiotic therapy.[23] PMN cells require O_2 to kill organisms by producing superoxide, hydrogen peroxide, singlet O_2, and other products via the respiratory burst phenomenon.[24] The PMN is protected by detoxifying free radicals with superoxide dismutase, catalase, and glutathione. It has been shown that the degree of PMN cell function in killing of bacteria is directly dependent on O_2 tension.[25] There is also O_2-independent killing with lysozymes, acidic vacuoles, and lactoferrins.[26] However, they are less efficient and vary significantly according to the organism.

Subsequent bone formation is also O_2 dependent, and variations in O_2 supply may affect the type of tissue that differentiates in a culture of multipotential cells.[27,28] Hyperoxia causes differentiation to osseous tissues, whereas hypoxia resulted in cartilage formation. HBOT has been shown to increase the mineralisation and density of bone.[29] Finally, hyperbaric O_2 also affects osteoclastic activity.[30] Thus, HBOT influences all the stages of bone healing.

Results of the present study also suggest that, in the absence of a pathological condition which can adversely affect tissue regeneration and healing, the latter is not affected by age. This is apparent from the poor correlation between age and both reduction in the size of defects and the increase in bone density.

Since histopathological characteristics of the three cystic lesions (radicular cysts, odontogenic keratocysts and

dentigerous cysts) examined in the present study are different, it was of interest to explore whether the rate of healing was different across the three groups. It was especially applicable to odontogenic keratocysts which are well circumscribed by a thin shell rich in calcium. We did not find any significant difference in healing across the three groups. Nonetheless, this inference is to be viewed with caution because of the small sample size (four radicular cysts, seven odontogenic keratocysts and three dentigerous cysts).

As mentioned earlier, some recent studies and reviews have lent support to scepticism regarding the usefulness of HBOT in osteoradionecrosis of mandible or healing of bony fractures.[9–12] In sharp contradistinction to these studies, our results have demonstrated spectacular improvement in bone healing with HBOT, given after mandibular cyst surgery. We believe there are several reasons for this. The study of Annane et al. was under-powered (as admitted in the accompanying editorial) and was terminated prematurely with a less than required number of subjects.[9,31] It is also questionable if employment of 'intention-to-treat' analysis was appropriate in such a study with a large Type-II error. The other study, by Maier et al. employed a different treatment strategy that was overtly out of phase.[10] In any case, these two studies investigated the effect of HBOT in a pathological condition and are not comparable to the healing of cystic defects after enucleation of mandibular cyst(s) in healthy humans. On the other hand, two systematic reviews, per se, do not refute the appropriateness of HBOT in fracture healing.[11,12] What these reviews stress is the necessity of well-controlled trials to endorse the usefulness of HBOT in such instances. Non-availability of controls is the most commonly felt deficiency in the research related to HBOT involving human subjects. Ours is an investigation which overcomes this, albeit imperfectly, comparing cases with unrelated controls. Thus, in real terms, our results do not contradict these reviews.

The present study demonstrates that the rate of filling of the residual cavity and increase in bone density in surgical defects of the mandible were significantly greater in subjects given HBOT than in subjects in a previous study who healed spontaneously. The present study, with certain limitations, is a case-control study. Nonetheless, the 'controls' did not form part of our study. Even though the two groups were comparable in age, it could not be ascertained whether the 'control' patients were comparable to the our patients with respect to nutritional status and absence of other modifiers of healing.[1] However, we have no reason to presume that the two groups are not comparable, even though other factors which influence tissue regeneration and wound healing were not reported by Chiapasco et al.[1] Their subjects were from a well-developed Western country so it is unlikely that they were undernourished, receiving drugs or had less time for rest and recuperation, or were suffering from illness which could have influenced healing.

Conclusion

We conclude that HBOT significantly improved bone healing and merits a place as an effective adjuvant therapy in the post-operative management of bony defects of the mandible arising from enucleation of large cysts.

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Conflict of interest: none

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Editor's note:
The M file used to calculate the grayscale values is available from the corresponding author or from the journal office. It can be read in MSWORD™ or NOTEPAD, and provides a method to import a file in MATLAB®, select a portion of the image and calculate the grayscale values.