Heliyon 9 (2023) e19955

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Serum BMP-2 and osteocalcin levels, and CT Hounsfield unit post hyperbaric oxygen therapy in patients with cleft lip and palate post alveolar bone graft: A case study

Mendy Hatibie Oley [a,b,c,*], Maximillian Christian Oley [c,d,e], Fima Lanra Fredrik G. Langi [f], Walter Flapper [g], Andi Asadul Islam [h], Mochammad Hatta [i], Sachraswaty R. Laidding [j], Nidia Limarga [k], Muhammad Faruk [l,m]

[a] Division of Plastic Reconstructive and Aesthetic Surgery, Department of Surgery, Faculty of Medicine, Sam Ratulangi University, Manado, Indonesia
[b] Division of Plastic Reconstructive and Aesthetic Surgery, Department of Surgery, R. D. Kandou Hospital, Manado, Indonesia
[c] Hyperbaric Centre Siloam Hospital, Manado, Indonesia
[d] Division of Neurosurgery, Department of Surgery, Faculty of Medicine, Sam Ratulangi University, Manado, Indonesia
[e] Division of Neurosurgery, Department of Surgery, R. D. Kandou Hospital, Manado, Indonesia
[f] Department Epidemiology and Biostatistics, Public Health Faculty, Sam Ratulangi University, Manado, Indonesia
[g] Cleft and Craniofacial South Australia, University of Adelaide, Australia
[h] Department of Neurosurgery, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia
[i] Department of Clinical Microbiology, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia
[j] Division of Plastic Reconstructive and Aesthetic Surgery, Department of Surgery, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia
[k] Department of Surgery, Faculty of Medicine, Sam Ratulangi University, Manado, Indonesia
[l] Department of Surgery, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia
[m] Institute for Research and Community Services, Hasanuddin University, Makassar, Indonesia

ARTICLE INFO

Keywords:
Cleft lip
Cleft palate
Alveolar bone grafting
Hyperbaric oxygen therapy
Osteocalcin
Hounsfield unit
Bone morphogenetic protein 2

ABSTRACT

Background: This study investigated the levels of bone morphogenetic protein 2 (BMP-2), osteocalcin, and 3D CT Hounsfield units following hyperbaric oxygen therapy (HBOT) in patients with cleft lip and palate (CLP) undergoing alveolar bone grafts to provide a pilot evaluation of the role of HBOT in osteogenesis.

Methods: This prospective, quasi-experimental, pre–post-intervention study evaluated seven patients with CLP receiving HBOT after single-stage reconstructions with alveolar bone grafts. The outcomes included the serum levels of BMP-2 and osteocalcin and the 3D CT Hounsfield units obtained before and after the surgery, and after the five HBOT sessions, to a total of 12 measurements. The data were analyzed with linear mixed-effects models using the intervention stage (pre-surgery, pre-HBOT, first to fifth HBOT sessions) as covariates and adjusting for several baseline factors.

Results: A significant difference was found in outcome measures across time (ANOVA $p < 0.001$ for BMP-2 and osteocalcin, $p = 0.01$ for Hounsfield units), with mean values appearing to steadily

* Corresponding author. Division of Plastic Reconstructive and Aesthetic Surgery, Department of Surgery, Faculty of Medicine, Sam Ratulangi University, Jalan Raya Tanawangko No.56, Malalayang Satu Barat, Malalayang, Manado, Sulawesi Utara, 95162, Indonesia.

E-mail addresses: mendy.hatibie@unsrat.ac.id (M.H. Oley), max_oley@unsrat.ac.id (M.C. Oley), flangi2@unsrat.ac.id (F.L.F.G. Langi), wjflapper@gmail.com (W. Flapper), andiasadul@yahoo.com (A.A. Islam), hattaram@yahoo.com (M. Hatta), sachras1276@gmail.com (S.R. Laidding), agnesnidia@gmail.com (N. Limarga), muhammadfaruk@unhas.ac.id (M. Faruk).

https://doi.org/10.1016/j.heliyon.2023.e19955
Received 22 February 2023; Received in revised form 29 August 2023; Accepted 6 September 2023
Available online 9 September 2023

increase once HBOT began. Regression analyses indicated that the effect of HBOT was evident in serum osteocalcin after the 1st HBOT session (adjusted b = 1.32; 95% CI 0.39, 2.25) and in serum BMP-2 after the third session (adjusted b = 6.61; 95% CI 1.93, 11.28). After the fifth session, the HBOT effect was fairly pronounced on the two outcomes: the adjusted increase compared to the baseline was 28.06 ng/mL for BMP-2 and 6.27 ng/mL for osteocalcin. Our mixed-effect models also showed a post-HBOT increase in Hounsfield units.

Conclusion: We found an increase of BMP-2, osteocalcin, and Hounsfield units following the HBOT intervention. These may suggest an effect of HBOT on osteogenesis.

1. Introduction

Cleft lip and palate (CLP) is a congenital malformation that is often treated by plastic surgeons. Its proper management requires a multi-disciplinary team, including plastic surgeons, orthodontists, otolaryngology specialists, and speech therapists [1]. In patients with CLP, tooth growth is disrupted due to bone defects in the area of the cleft lip and palate; the effects include impacted teeth, agenesis, supernumerary teeth (excessive tooth growth), and changes in the tooth crown to root ratio (overall the minimum crown-to-root ratio necessary is 1:1) [2]. The goals of alveolar bone graft include the closure of the vestibular and palatal nasal oral fistula, adequate bone for tooth eruption, sufficient skeletal nasal floor, adequate bone for implant placement, functional airways, bone reconstruction, and sufficient muscle/soft tissue architecture [3].

Hyperbaric oxygen therapy (HBOT) is a non-invasive adjunct therapy for the management of chronic nonhealing wounds [4] and the standard of care in some countries [5]. It is often recommended to patients before and after surgical procedures to expedite the recovery process, accelerate scar healing, and improve the osteogenesis process after bone graft surgery [6,7]. HBOT enhances the body's natural defenses and maximizes the benefits of oxygen to enhance the wound healing process [4]. Previous studies have found that HBOT reducing swelling, accelerates neovascularization, and reduces inflammation. This leads to improved tissue perfusion, reduced edema, and improved blood circulation [8,9]. In a surgical process known as bone grafting, diseased or damaged bones are repaired and rebuilt using transplanted bone. The bone graft can be natural, taken from the patient's body, or synthetic. The aim of bone grafting is to promote osseointegration and facilitate healing of the implant in cases where insufficient bone exists in the targeted area. HBOT works with other therapies to promote healing after bone grafting; it has been shown to accelerate the healing process [10]. Therefore, we decided to investigate the osteogenesis process in patients who received HBOT following alveolar bone graft surgery.

2. Methods

2.1. Research design

We designed this research as a prospective, quasi-experimental, pre–post-intervention study. The setting was R.D Kandou General Hospital (KGH), a central referral hospital in Manado, North Sulawesi, Indonesia and Siloam Hospital, Manado, Indonesia. The study protocol was reviewed and approved by the KGH ethical board (IRB approval no. 077/EC/KEPK-KANDOU/IX/2020) and registered with the Thai Clinical Trials Registry (no. TCTR20220926001). This study is reported in line with the Strengthening the Reporting of Cohort Studies in Surgery (STROCSS) Guidelines [11].

2.2. Study participants

Patients with CLP eligible for alveolar bone graft were recruited after they or their legal guardian provided written informed consent. All patients were aged 12 years or older. The exclusion criteria were (1) poor oral hygiene (for example, one excluded patient had periodontitis); (2) suboptimal nutritional status, marked by a body index under the 5th percentile in the CDC development curve; and (3) contraindications to HBOT (such as untreated pneumothorax, COPD, barotrauma, asthma, epilepsy, and Eustachian tube dysfunction).

All patients underwent an initial evaluation and standard, age-based management throughout the procedures. Surgical treatment consisted of single-stage reconstruction with alveolar bone grafts. Around five days after the operation, each patient received five sessions of adjuvant HBOT on alternating days. Data were collected at the baseline (pre-operative), after the surgery, and before and after each HBOT session, to a total of 12 measurements. Due to ethical and practical considerations, we decided it unfeasible to form a control group because most CLP patients in our setting were geographically dispersed and financially challenged; following them up for serial laboratory examinations after the surgery would be difficult, unless they stayed in or close to the hospital, and this was deemed very complicated to deal with, especially when the patients did not receive any meaningful post-surgery treatment (such as HBOT). We thus used the individual baseline, pre-surgery values of variables as the control measures. Since it became a single-arm study, random allocation was not performed. Given the nature of the intervention, blinding the patients was impossible.

2.3. HBOT procedure

The patients underwent a 100% oxygen therapy session in a 2.4 ATA pressure chamber for 60 min immediately after alveolar bone graft surgery (on the five days after the surgery, the patients received successive HBOT treatments). Before surgery, all patients underwent bone morphogenetic protein 2 (BMP-2) examinations, osteocalcin readings, and 3D CT scans for Hounsfield analyses. The same evaluation tools were administered on the same day post-surgery and at the end of the 100% oxygen therapy sessions.

2.4. Surgical procedure

In alveolar bone graft surgery, local anesthesia is administered to the labio-buccal alveolar soft tissues on the smaller and medial segments for hemostasis. The procedure involves either a vertical incision over the alveolar cleft or a marginal incision with vertical release away from the cleft. Laterally along the gingival edge, the incision is prolonged around the necks of the teeth posterior to the anterior section of the permanent first molar tooth. The alveolar cleft and nose floor are subsequently exposed by raising the two mucoperiosteal flaps. Separating the oral and nasal mucosa is important, which is done by scalpel. The tissue in the alveolar cleft is then removed. Resorbable sutures are used to repair the mucosa of the nasal floor. The alveolar defect is subsequently filled with cancellous bone obtained from the anterior iliac crest. Soft bone fragments that have accumulated in the defect over time are removed. Oral tissues in the labium are closed.

2.5. Radiological evaluation

Occlusal view X-rays were performed using the Ortho Stage (Auto Zero III CM, Asahi, Japan). The Bergland scale was used to determine bone graft integration before and after surgery; for this scale, the grades depend on the degree of integration, as measured against the height of the interalveolar septum. In particular, the Bergland scale is divided into the following grades (Fig. 1) [12].

All patients underwent 3D CT scans before and after surgery using an Ingenuity Core 128 (Philips Healthcare Co., Ltd., Suzhou, China). BMP-2 and osteocalcin serum levels were measured according to the protocols in the Human BMP-2 and osteocalcin ELISA Kit from Assay Genie, cat. no. HUES01298 and HUFI02679, respectively (Dublin, Ireland) [13–15].

2.6. Variables

The outcome variables were the levels of BMP-2, osteocalcin, and 3D CT Hounsfield units. The concentrations of BMP-2 and osteocalcin in the conditioned medium were measured using an ELISA assay in an accredited laboratory. The primary independent variable was the time of measurement, represented as 1–12 in the descriptive tabulation and the intervention stage (baseline/pre-surgery, pre-HBOT, and post-HBOT sessions 1–5) in the regression analyses. The latter simplified the pre- and post-HBOT measurements in each session as one time category depending on the session (hence only 7 instead of 12 categories) to ease modeling and interpretation. In addition, age, sex, and the levels of hemoglobin, leukocytes, thrombocytes, blood urea nitrogen, creatinine, non-fasting glucose, potassium, sodium, chloride, serum glutamic oxaloacetic transaminase (SGOT), serum glutamate-pyruvate transaminase (SGPT), albumin, and vitamin D were covariates in the analysis.

2.7. Statistical analysis

Descriptive statistics were presented according to the variable type. The mean differences across time of measurement following HBOT for BMP-2, osteocalcin, and Hounsfield units were tested using repeated-measure ANOVA. The effect of the intervention stage on

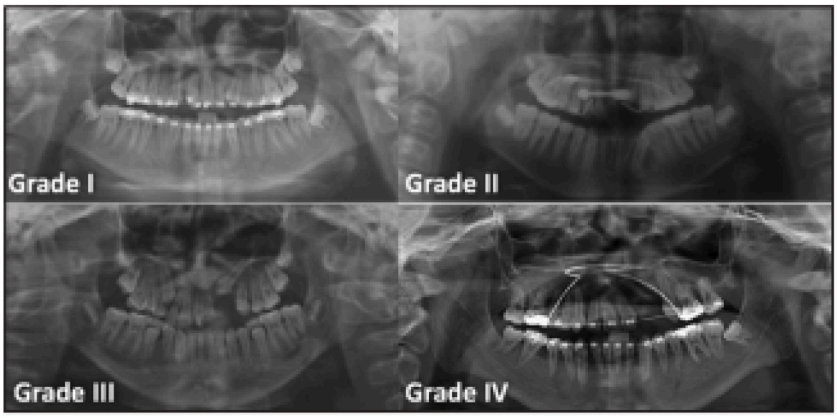

Fig. 1. Orthopantomography Grades. Grade I: Ossification equals the height of the interalveolar septum, Grade II: Ossification is up to at least 75% of the interalveolar septum's height, Grade III: Ossification is <75% of the interalveolar septum's height, Grade IV: No evidence of bone integration.

each outcome was modeled through linear mixed-effects regression with a random intercept. Covariates of the final models were selected in a stepwise forward fashion based on the Akaike information criterion (AIC). The estimates, along with the 95% confidence intervals and p-values, were tabulated for each final model of the outcomes. All data management and statistical analyses were performed using R software version 4.1.3. The R package "nlme" was used for the analysis of linear mixed-effects models, and "ggplot2" was used for graphical presentation [16].

3. Results

Seven CLP patients with alveolar bone grafts participated in the study (Table 1). Two were boys and five were girls, with mean age of 17 years (SD 5 years). Table 2 shows that none of the patients had anemia, infection, or any sign of blood clotting disorders before surgery. Kidney function markers, blood sugar, and liver function tests were all within normal limits. The median vitamin D level was 27.9 IU (IQR 19.7 IU, 29.3 IU).

Table 3 displays the serum levels of BMP-2 and osteocalcin across 12 measurements, and Hounsfield unit quantities pre surgery, before the first session, and after the fifth session of HBOT. This last study outcome was only measured 3 instead of 12 times to minimize the radiation hazard. Repeated-measure ANOVA confirmed the mean difference across time for all outcomes. The BMP-2 values increased slowly up to the 10th measurement (fourth HBOT session), after which the values increased more rapidly. In the fifth and final HBOT session, the BMP-2 levels were approximately 14 ng/ml higher than in the previous measurements and more than double the initial values overall. In addition to a drastic increase in the average level, the 10th to 12th measurements exhibited larger variations; i.e., variability nearly doubled from that of previous measurements.

Periodic increases in osteocalcin were also evident after the first HBOT session (Time 5). However, the changes were not as striking as those for BMP-2. The variations in osteocalcin readings were relatively consistent. Despite the small sample size, the distributions of BMP-2 and osteocalcin values over the 12 measurement times were relatively normal; hence, means and SDs were calculated for these variables. In contrast, the CT Hounsfield unit values after HBOT were generally distributed skewed to the right, especially for the second and third measurements. Hence, the median was used. In addition, the overall increases in these values indicated progress after HBOT. Fig. 2 provides a graphical representation of the outcome distribution at each time of measurement, distinguishing the stages before and after HBOT. For BMP-2, the variation tended to be wider in the last measurements. This was not the case for the osteocalcin and Hounsfield unit distributions.

A formal analysis of the effect of post-alveolar bone graft–adjuvant HBOT on BMP-2, osteocalcin, and CT Hounsfield unit values was performed using random-intercept mixed-effects models, as shown in Table 4. For the outcomes BMP-2 and osteocalcin, the final multivariable model adjusted the effects of sex, hemoglobin, leukocytes, platelet, and albumin levels at the baseline before surgery. The multiple regression for CT Hounsfield units controlled the same variables, excluding sex. The other variables presented in Table 2 did not pass the covariate selection for multivariate models. For the first outcome of Table 4, the effect of HBOT on BMP-2 became significant by the third session. The adjusted difference was around 6.61 ng/mL (95% CI 1.93,11.28 ng/mL; $p = 0.006$) compared to the baseline. By the fifth session, the adjusted difference from the baseline measurement reached 28.06 ng/mL (95% CI 22.74, 33.38 ng/mL; $p < 0.001$). On the other hand, for osteocalcin, the increase was noticed since the first session and continued over the remaining HBOT sessions. Controlling for other variables, the difference between osteocalcin level in the last HBOT session and that at the baseline was 6.27 ng/mL (95% CI 5.18, 7.36 ng/mL; $p < 0.001$). Lastly, for Hounsfield units, the adjusted difference at the end of the HBOT sessions compared to the baseline measurement was around 305.8 (95% CI 139.13, 472.46; $p = 0.002$). Importantly, the quantity of regression estimates at least for BMP-2 and osteocalcin tended to increase for the additional HBOT sessions. This seemed to indicate a dose–response relationship.

3.1. Case presentation

A female patient, aged 12 years, complained of asymmetrical nose and lips, accompanied by a nasal sound when speaking; she did not complain of any problems when eating or drinking (Table 1). When laboratory tests were performed, the values were within normal limits. As part of the preparations for bone graft surgery for an alveolar defect, radiological examinations were performed in the form of occlusal view X-ray (Fig. 3A) and facial 3D CT scans (Fig. 4A). After HBOT on Day 5 post-surgery, the patient continued with HBOT on Days 6, 7, 8, and 9. Each session ran for 60 min at 2.4 ATA. After the patient concluded HBOT treatment, an assessment was

Table 1
Patients' age and chief complaint.

Patient No.	Age (years)	Chief complaint
1	12	Asymmetrical nose and lips, accompanied by a nasal sound when speaking.
2	14	Abnormalities in her face, especially clefts in the lips. She also felt that there was an abnormality in her nose, accompanied by a nasal sound when speaking.
3	12	Asymmetrical nose and lips, accompanied by a nasal sound when speaking.
4	13	Asymmetrical nose and lips, accompanied by a nasal sound when speaking.
5	23	Asymmetrical nose and lips, accompanied by a nasal sound when speaking.
6	12	Asymmetrical nose and lips, accompanied by a nasal sound when speaking.
7	18	Asymmetrical nose and lips, accompanied by a nasal sound when speaking.

Table 2
Characteristics of patients in the study (N = 7).

Characteristics	n (%)	Mean ± SD	Median (Q1–Q3)
Sex			
Male	2 (29)	–	–
Female	5 (71)	–	–
Hemoglobin (g/dL)	–	13.2 ± 1.8	–
Leukocytes (x$10^3/\mu$l)	–	8.8 ± 2.4	–
Thrombocytes (x$10^3/\mu$l)	–	283.0 ± 40.0	–
Blood urea nitrogen (mg/dL)	–	–	18.0 (17.0–23.0)
Creatinine (mg/dL)	–	0.8 ± 0.1	–
Non-fasting blood glucose (mg/dL)	–	89.6 ± 14.7	–
Potassium (mg/dL)	–	3.8 ± 0.3	–
Sodium (mg/dL)	–	138.4 ± 3.4	–
Chloride (mg/dL)	–	102.6 ± 2.4	–
SGOT (mg/dL)	–	–	18.0 (14.5–33.5)
SGPT (mg/dL)	–	–	19.0 (15.0–69.5)
Albumin (g/dL)	–	4.9 ± 0.2	–
Vitamin D (IU)	–	–	27.9 (19.7–29.3)

Note: SD = standard deviation, Q1 = 1st Quartile, Q3 = 3rd Quartile, SGOT = serum glutamic oxaloacetic transaminase, SGPT = serum glutamate-pyruvate transaminase.

Table 3
Serum BMP-2 levels, osteocalcin levels, and CT hounsfield unit values across time of measurement (N = 7).

Outcome	Mean ± SD	Median (Q1–Q3)
BMP-2 (ng/ml) ($F_{11,60}$ $p < 0.001$)		
Baseline	24.1 ± 1.2	–
Post-surgery	24.8 ± 0.8	–
Pre-HBOT I	25.4 ± 0.7	–
Post HBOT I	26.8 ± 1.4	–
Pre-HBOT II	27.4 ± 1.6	–
Post HBOT II	28.0 ± 1.7	–
Pre-HBOT III	29.3 ± 1.6	–
Post HBOT III	30.0 ± 1.8	–
Pre-HBOT IV	32.1 ± 1.8	–
Post HBOT IV	35.4 ± 4.6	–
Pre-HBOT V	38.6 ± 7.9	–
Post HBOT V	52.5 ± 14.6	–
Osteocalcin (ng/ml) ($F_{11,60}$ $p < 0.001$)		
Baseline	3.1 ± 1.7	–
Post-surgery	3.9 ± 2.0	–
Pre-HBOT I	3.6 ± 2.3	–
Post HBOT I	3.7 ± 2.4	–
Pre-HBOT II	3.9 ± 2.3	–
Post HBOT II	5.7 ± 1.8	–
Pre-HBOT III	6.0 ± 1.8	–
Post HBOT III	5.7 ± 2.6	–
Pre-HBOT IV	6.2 ± 2.5	–
Post HBOT IV	7.6 ± 1.8	–
Pre-HBOT V	7.5 ± 3.2	–
Post HBOT V	9.4 ± 2.6	–
CT Hounsfield Unit ($F_{2,11}$ $p = 0.010$)		
Pre-surgery	624.7 ± 108.3	–
Pre-HBOT I	–	677.0 (662.0–764.5)
Post HBOT V	–	788.5 (775.8–951.2)

NOTE: SD = standard deviation, Q1 = 1st Quartile, Q3 = 3rd Quartile; p-value for repeated-measure ANOVA.

performed using occlusal view X-ray (assessed via the Bergland scale) (Fig. 3B) and a facial 3D CT scan (assessed by Houndsfield unit measurements) (Fig. 4B). The results showed bone growth in the alveolar defect that had been given a bone graft from the patient's own iliac bone; significant improvements were also observed in the postoperative wound and postoperative laboratory results.

4. Discussion

Cases of CLP are often referred to plastic surgeons in Eastern Indonesia. This study aimed to evaluate the osteogenesis process in

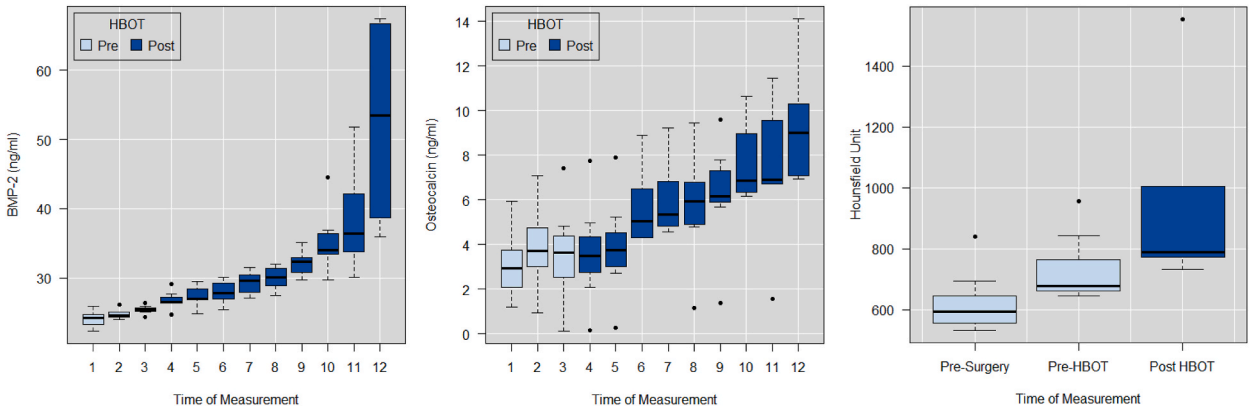

Fig. 2. BMP-2, osteocalcin, and Hounsfield unit values across time of measurement: (1) Pre surgery (baseline), (2) Post surgery, (3) Pre HBOT session I, (4) Post HBOT session I, (5) pre HBOT session II, (6) Post HBOT session II, (7) pre HBOT session III, (8) post HBOT session III, (9) pre HBOT session IV, (10) Post HBOT session IV, (11) Pre HBOT session V, (12) Post HBOT session V.

Table 4
Mixed-effects models for changes in BMP-2, osteocalcin, and CT hounsfield unit values postoperative and post HBOT.

Outcome	Model Univariate		Model Multivariate	
Fixed Effect	β (95% CI)	*p*	β (95% CI)	*p*
BMP-2 rowhead				
Post-surgery	0.89 (−3.85; 5.63)	0.709	0.81 (−3.90; 5.53)	0.731
Post HBOT 1	2.80 (−1.90; 7.49)	0.239	2.65 (−2.02; 7.33)	0.261
Post HBOT 2	4.32 (−0.37; 9.02)	0.071	4.18 (−0.49; 8.85)	0.079
Post HBOT 3	6.75 (2.05; 11.45)	0.006	6.61 (1.93; 11.28)	0.006
Post HBOT 4	12.70 (8.01; 17.40)	<0.001	12.56 (7.89; 17.24)	<0.001
Post HBOT 5	28.20 (22.85; 33.55)	<0.001	28.06 (22.74; 33.38)	<0.001
Osteocalcin rowhead				
Post-surgery	0.94 (0.01; 1.88)	0.048	0.95 (0.02; 1.89)	0.046
Post HBOT 1	1.30 (0.37; 2.23)	0.007	1.32 (0.39; 2.25)	0.006
Post HBOT 2	2.71 (1.76; 3.65)	<0.001	2.71 (1.76; 3.65)	<0.001
Post HBOT 3	3.50 (2.57; 4.43)	<0.001	3.52 (2.59; 4.45)	<0.001
Post HBOT 4	4.79 (3.85; 5.72)	<0.001	4.80 (3.86; 5.73)	<0.001
Post HBOT 5	6.27 (5.18; 7.36)	<0.001	6.27 (5.18; 7.36)	<0.001
CT Hounsfield Unit rowhead				
Post-surgery	108.29 (−62.36; 278.93)	0.190	108.29 (−50.30; 266.87)	0.161
Post HBOT	309.82 (130.12; 489.52)	0.003	305.80 (139.13; 472.46)	0.002

Note: CI = confidence interval. Values at all measurement times compared to the baseline value as a reference.

CLP patients who received alveolar bone grafts and continued with HBOT [17]. HBOT is used to correct anoxia by increasing the amount of dissolved oxygen, thereby increasing collagen synthesis, capillary growth, neovascularization, and osteogenesis [18]. HBOT may be able to reduce the bone resorption caused by hypovascularization through the administration of oxygen at high pressure, thus overcoming a hypoxic bone state resulting from poor vascularization. Physiologically, the amount of hemoglobin in the blood is fixed, and the oxygen supply is therefore limited [7]. However, due to the high solubility of oxygen in the high-pressure state, it can be directly transferred to the plasma. Theoretically, plasma oxygen at 100% oxygen delivery at 2.5 ATA may be approximately 17 times greater than plasma oxygen at 21% oxygen delivery at 1 ATA [19]. This level can meet the oxygen demand in the bone. Hypoxia is necessary for the initiation of angiogenesis, but a prolonged state of hypoxia affects the healing process [20]. Persistent hypoxia inhibits fibroblast differentiation, collagen synthesis, and granulation of the tissue [21]. HBOT contributes to the healing process by eliminating hypoxia. Moreover, HBOT increases alkaline phosphatase activity during bone regeneration and also contributes to osteoblast activity and angiogenesis in bone disorders [22].

An autogenous bone graft is the gold standard in terms of bone graft material because it contains all the properties required for bone formation: osteoinduction (BMP and other growth factors), osteogenesis (osteo-progenitor cells), and osteoconduction. An iliac crest bone graft (ICBG) is the most commonly used graft [17]. As it is taken from the patient's own body, autogenous bone has histocompatibility and is nonimmunogenic, thereby reducing the chances of infection and immunoreaction [23]. A bone graft should be osteoconductive, osteoinductive, and osteogenic [24]. There are several other goals and advantages associated with alveolar bone grafting: maintaining the continuity of the maxillary arch, maximizing bone support for tooth growth, stabilizing the maxillary segment after orthodontic therapy, eliminating an oronasal fistula, supporting the nasal alar cartilage, shaping the alveolar morphology ideally, and ensuring the availability of bone with attached soft tissue for endosteal implant placement should it prove necessary [3].

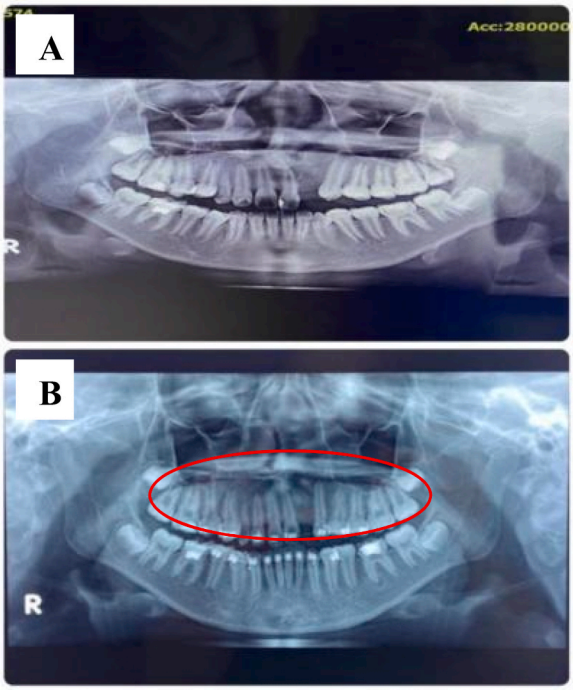

Fig. 3. Occlusal view X-ray compared with the Bergland Scale Method (A) Before Surgery and (B) 12 Months After Surgery.

Bone graft evaluation after surgery can be performed via radiographic study. Volumetric tomography is the current gold standard due to its low radiation effect, affordability, excellent clinical radiological reliability, and capacity for bone gap recognition and bone graft integration. Several authors from various countries have reported an 80% integration rate in alveolar bone graft surgery. This new bone formation can be evaluated using various methods. For instance, the Bergland scale is a validated instrument that allows this evaluation to be performed easily and directly [25]. Bergland Grades I, II, and III are considered to be indicative of good bone graft integration.

Oral hygiene is an important determining factor in the emergence of postoperative complications; patients with poor oral hygiene face a greater risk of infection in the wound, which could eventually lead to the emergence of a palatal fistula [12]. However, such problems can be prevented with the use of adequate anti-septic mouthwash, additional antibiotics, and local silver nitrate wound care aimed at suppressing the granulation of tissue [26].

A limitation of this study is that the HBOT procedure was carried out in 2 different facilities, KGH and Siloam Hospital, Manado, Indonesia, where KGH uses a monoplace chamber while Siloam Hospital uses a multiplace chamber, however, the pressure and duration of the HBOT procedure were the same in both places.

5. Conclusion

This pilot study demonstrates an increase of BMP-2, osteocalcin, and Hounsfield unit following HBOT intervention. Such increase was particularly noticed with the additional sessions of HBOT, seemingly indicating a dose-response relationship. Overall, this may suggest the effect of HBOT on osteogenesis process by increasing bone mineral density. Further studies are therefore needed to support the roles of HBOT in accelerating bone repair and osteogenesis at defect sites.

Author contribution statement

Mendy Hatibie Oley and Maximillian Christian Oley: conceived and designed the experiments; performed the experiments; contributed reagents, materials, analysis tools or data; wrote the paper.

Fima Lanra Fredrik G. Langi, Walter Flapper and Muhammad Faruk: conceived and designed the experiments; analyzed and interpreted the data; wrote the paper.

Andi Asadul Islam and Sachraswaty R. Laidding: conceived and designed the experiments; wrote the paper.

Mochammad Hatta: conceived and designed the experiments; contributed reagents, materials, analysis tools or data; wrote the paper.

Nidia Limarga: conceived and designed the experiments; performed the experiments; analyzed and interpreted the data; wrote the paper.

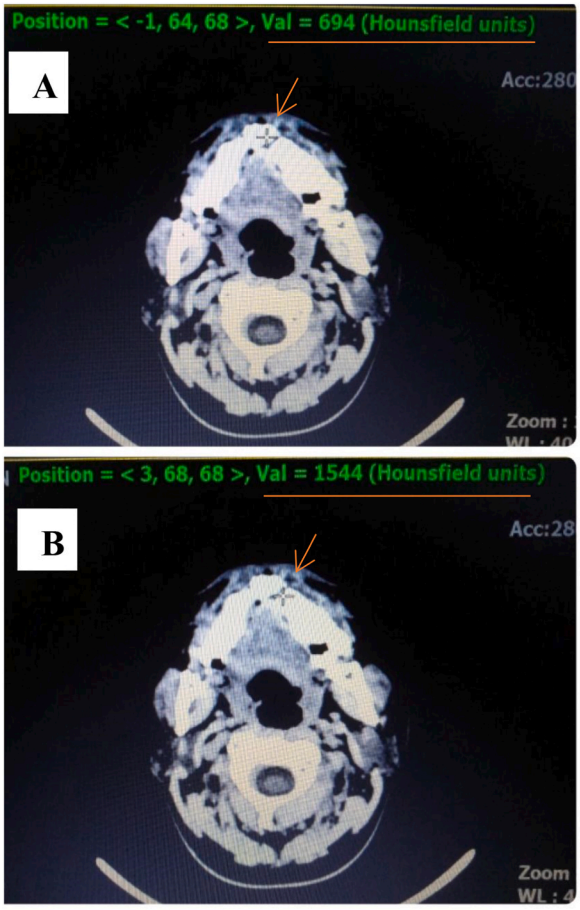

Fig. 4. 3D CT scans (A) Before Surgery (A) and (B) One Year After Surgery for the Calculation of the Houndsfield Units.

Data availability statement

The data supporting this study's findings are available on request from the corresponding author (Mendy Hatibie Oley).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

None.

References

[1] C.I. Alois, R.A. Ruotolo, An overview of cleft lip and palate, JAAPA 33 (12) (2020) 17–20.
[2] E. Li Yen Tan, M. Yow, Dental development and anomalies in cleft lip and palate, in: A. Gülşen (Ed.), Current Treatment of Cleft Lip and Palate, IntechOpen, 2020.
[3] M. Vuletić, P. Knežević, D. Jokić, J. Rebić, D. Žabarović, D. Macan, Alveolar bone grafting in cleft patients from bone defect to dental implants, Acta Stomatol. Croat. 48 (4) (2014) 250–257.
[4] M. Klakeel, K. Kowalske, The role of hyperbaric oxygen therapy for the treatment of wounds, Phys Med Rehabil Clin N Am 33 (4) (2022) 823–832.
[5] B. Hajhosseini, B.A. Kuehlmann, C.A. Bonham, K.J. Kamperman, G.C. Gurtner, Hyperbaric oxygen therapy: descriptive review of the technology and current application in chronic wounds, Plast Reconstr surgery Glob open 8 (9) (2020), e3136.
[6] C. Gardin, G. Bosco, L. Ferroni, et al., Hyperbaric oxygen therapy improves the osteogenic and vasculogenic properties of mesenchymal stem cells in the presence of inflammation in vitro, Int. J. Mol. Sci. 21 (4) (2020) 1452.
[7] H. Al Hadi, G.R. Smerdon, S.W. Fox, Hyperbaric oxygen therapy accelerates osteoblast differentiation and promotes bone formation, J. Dent. 43 (3) (2015) 382–388.
[8] N. Schottlender, I. Gottfried, U. Ashery, Hyperbaric oxygen treatment: effects on mitochondrial function and oxidative stress, Biomolecules 11 (12) (2021) 1827.

[9] C. Armand, K. Grzegorz, S. Agata, et al., Thermal imaging and TC oximetry measurements of hyperbaric oxygen therapy (HBO) effects on trophic ulceration of the crura, J. Therm. Anal. Calorim. 108 (1) (2012) 25–31.
[10] K. Re, S. Patel, J. Gandhi, et al., Clinical utility of hyperbaric oxygen therapy in dentistry, Med. Gas Res. 9 (2) (2019) 93–100.
[11] G. Mathew, R. Agha, STROCSS Group, Strocss 2021: Strengthening the reporting of cohort, cross-sectional and case-control studies in surgery, Int. J. Surg. 96 (2021), 106165.
[12] C.A. Buckner, R.M. Lafrenie, J.A. Dénommée, J.M. Caswell, D.A. Want, Complementary and alternative medicine use in patients before and after a cancer diagnosis, Curr. Oncol. 25 (4) (2018) 275–281.
[13] M.H. Oley, M.C. Oley, B.J. Kepel, et al., ICAM-1 levels in patients with covid-19 with diabetic foot ulcers: a prospective study in southeast asia, Ann Med Surg 63 (2021), 102171.
[14] Islam AA. Wahyudi, M. Hatta, et al., The role of MLC901 in reducing VEGF as a vascular permeability marker in rats with spinal cord injury, Ann Med Surg 75 (2022), 103344.
[15] P. Prihantono, W. Rahardjo, S.A. Syamsu, N. Smaradhania, Profile of anterior gradient 3 (AGR3) mRNA expression and serum levels in benign and malignant breast tumors, Breast Dis. 40 (S1) (2021) S39–S43.
[16] R Core Team. R: a language and environment for statistical ## computing, R Foundation for Statistical Computing, Vienna, Austria, 2022. https://www.R-project.org/.
[17] M. D'Souza, N.A. Macdonald, J.L. Gendreau, P.J. Duddleston, A.Y. Feng, A.L. Ho, Graft materials and biologics for spinal interbody fusion, Biomedicines 7 (4) (2019) 1–15.
[18] N. Prameswari, I.R. Sunaryo, D.W. Damaiyanti, A. Febrina, The influence of hyperbaric oxygen therapy (HBOT) to intercausal relationship between blood vessels, osteoblast, and new bone formation during maxillary suture expansion, Padjadjaran J Dent 32 (1) (2020) 14.
[19] M.D. Rollins, J.J. Gibson, T.K. Hunt, H.W. Hopf, Wound oxygen levels during hyperbaric oxygen treatment in healing wounds, Undersea Hyperb. Med. 33 (1) (2006) 17–25.
[20] A. Zimna, M. Kurpisz, Hypoxia-inducible factor-1 in physiological and pathophysiological angiogenesis: applications and therapies, BioMed Res. Int. 2015 (2015), 549412.
[21] H.-M. Shih, W.C. Lin, C.-H. Wang, L.-C. Lin, Hypertensive patients using thiazide diuretics as primary stroke prevention make better functional outcome after ischemic stroke, J. Stroke Cerebrovasc. Dis. 23 (9) (2014) 2414–2418.
[22] H. Al Hadi, G.R. Smerdon, S.W. Fox, Hyperbaric oxygen therapy suppresses osteoclast formation and bone resorption, J. Orthop. Res. 31 (11) (2013) 1839–1844.
[23] H. Shegarfi, O. Reikeras, Review article: bone transplantation and immune response, J. Orthop. Surg. 17 (2) (2009) 206–211.
[24] M.D. Markel, Bone grafts and bone substitutes, in: Equine Fracture Repair, Wiley, 2019, pp. 163–172.
[25] N. Gericke, J. Boeve-de Pauw, T. Berglund, D. Olsson, The Sustainability Consciousness Questionnaire: the theoretical development and empirical validation of an evaluation instrument for stakeholders working with sustainable development, Sustain. Dev. 27 (1) (2019) 35–49.
[26] C. Bassetti, A. Kallenberger, Influence of chlorhexidine rinsing on the healing of oral mucosa and osseous lesions. A histomorphometric study on experimental animals, J. Clin. Periodontol. 7 (6) (1980) 443–456.