EGYPTIAN DENTAL JOURNAL
VOL. 64, 1137:1146, APRIL, 2018
I.S.S.N 0070-9484
ORAL SURGERY
www.eda-egypt.org • Codex : 246/1801

BONE MARROW ASPIRATE VERSUS TWO BONE AUTOGRAFT TYPES IN RECONSTRUCTION OF ALVEOLAR CLEFT: A HISTOMORPHOMETRIC ANALYSIS

Hassan Abdelghany* *and* Hesham Fattouh*

ABSTRACT

Objectives: The aim of this study was to compare histomorphometrically between bone marrow aspirate on a collagen carrier with autogenous bone grafts harvested from two different donor sites in alveolar cleft defect repair.

Materials and Methods: eighteen patients with alveoalar cleft defect were included in the study. Twelve out of eighteen patients were reconstructed with auotogenous bone graft; six patients grafted with illium bone graft and six patients grafted with chin bone graft. The remaining patients out of eighteen were reconstructed using bone marrow aspirate implanted on a collagen carrier. Six months after grafting, bone specimens from augmented alveolar ridge sites were retrieved by trephine burs for histomorphometric analysis.

Results: Good consolidation of the grafts have been observed, this was demonstrated by intense osteogenesis indicating an active remodeling process. In all groups, the improvement in bone quality of the receptor site was clear with no statistical significance between the groups, however, the autografts presented better bone quality.

Conclusions: From this study it was possible to conclude that quality of bone repair using autogenous bone graft is superior to bone marrow aspirate. The time needed for bone maturation is less when chin or iliac autogenous grafts have been used in comparison to the bone marrow aspirate.

KEYWORDS: Alveolar cleft - stem cells - autogenous grafts - bone marrow aspirate.

INTRODUCTION

Alveolar cleft is a common congenital malformation due to abnormal primary palate formation during weeks 4 to 12 of gestation [(1)]. Secondary bone graft in mixed dentition stage is the most adequate technique utilized for repairing alveolar defects in cleft patients. Integration of alveolar ridge as one piece, closure of oronasal fistula and providing sufficient bone foundation for eruption, orthodontic repositioning of teeth and dental implant placement are the main objectives of alveolar repair [(2)].

* Lecturer of Oral and Maxillofacial Surgery, Faculty of Oral and Dental Medicine, Cairo University.

The management of alveolar clefts has been surrounded with controversy regarding the timing of treatment and the best donor material. Controversies regarding the most suitable donor sites will proceed as will further research into less morbid therapies[3]. Due to their osteoconductive and osteoinductive properties, autogenous tissues have been utilized extensively and remained till now the gold standard for alveolar cleft repair. The iliac bone is the most common donor site in the repair of alveolar cleft, it is simple technique which provides huge volume of spongy bone, but it has some disadvantages including endochondral origin with high resorption rate, less cortical bone, expanded operation time, prolonged bleeding, donor site morbidity including risk of hematoma, scaring, infection, discomfort, pain and motor or sensory disorders, especially in children [4-5].

The mandibular symphysis is an attractive donor site with less morbidity compared to the ilium and accepted success rate for filling clefts. Its advantages include restriction to one intraoral site of operation, similar embryonic origin with the receptor area (intramembranous) with low resorption rate, greater quantity of cortical bone with minimal pain or discomfort and a hidden scar in the lower labial sulcus [6]. However, the limited accessible volume especially in children with unerupted tooth buds and the confinement to one intraoral operating site that does not permit two teams to work at the same time were the main disadvantages of this donor site [3].

In prospective of the mentioned restrictions of the autogenous grafts and the expanding demand for bone grafting procedures, surgeons are searching for a superior approach as tissue engineering which is simply defined as the regeneration of new tissues through the combined utilization of biomaterials and biologic mediators like the stem cells [7]. Bone tissue engineering comprises of harvesting bone marrow from a patient, then seeding them onto a suitable synthetic scaffold former to implantation into the same patient. It has been indicated that from a little volume (0.1–3 ml) aspirate, alveolar bone marrow stromal units expanded by 70% [8].

The aspirated bone marrow is a rich hotspot of stem cells and so it is an effective osteogenic graft that is likely underused in present clinical practice. Bone marrow aspirates gained from the iliac crest contains an average of about 1000 osteoblastic progenitor for every milliliter, in addition to other cells rich in cytokines. They also contain fibrin that may facilitate rapid revascularization. Osteogenic precursor cells which are able of generating bone have been shown among the stromal and endosteal cells of bone marrow, which are the main key elements in the process of bone formation and fracture healing [9].

The aim of this study is to compare histomorphometrically the bone marrow aspirate on a collagen carrier with autogenous bone grafts harvested from two different donor sites in alveolar cleft defect repair.

PATIENTS AND METHODS

Eighteen patients with alveolar cleft defect were included in this study which was carried in accordance with international standards of quality for clinical trials, the Declaration of Helsinki in its revised version (Seoul, Korea, 2008). Selection of the patients was based on the following criteria: absence of any systemic disorders that may influence the course of wound healing, absence of any blood disorders which might cause complications after dental implant placement procedures, any syndromic cases were excluded from this study. All the patients received information about the surgical procedures and gave written informed consent.

The patients were divided into three equal groups (6 patients each) according to the used graft type in the repair of the alveolar cleft defect. Group A (iliac crest bone graft group) were grafted using autogenous anterior iliac crest bone graft, Group B

(chin bone graft group) were grafted by autogenous chin bone graft, while Group C (bone marrow aspirate graft group) were grafted with autogenous bone marrow aspirate gained from the posterior iliac crest seeded on a collagen sponge carrier.

Preoperative procedures included: history taking, extraoral and intraoral examination, Cone beam computed tomography (CBCT) and orthodontic palatal expansion for cases presented with collapsed arch. Clinical examinations were done at the graft reconstructed site. The vertical height and horizontal width of the augmented bone were analyzed clinically. Radiographic assessment was done by CBCT postoperatively with the same parameters used in the pre-grafting state recording the healing process in term of: bone formation bridging the alveolar cleft sides, volume, density and the height of the newly formed bone.

Operative procedures:

Phase I: alveolar cleft grafting:

Alveolar bone grafting was done by the same surgeons with the same standardized surgical approach. In all cases the preferred method was a vestibular gingival marginal incision with elevation of a wide mucoperiosteal flap including at least one tooth lateral and mesial to the cleft. In the area of the cleft itself, the incision was led vertically into the vestibular sulcus. If necessary, the mucoperiosteum was reflected on the palatal aspect by a marginal incision. In clefted alveoli with perforation to the nasal cavity, the nasally directed mucosa was elevated allowing for a tension-free repair of the nasal mucosal layer. In group A and B: The cancellous bone either from the iliac crest or chin were pressed between the alveolar segments. In addition, amounts of cancellous chips were positioned over the actual cleft both on the vestibular and palatal sides so that they extended over the cortical part of the bordering alveolar process with a certain degree of overcorrection to compensate for postoperative physiological resorption, while in group C the bone marrow on a collagen sponge was held alone at the cleft site. Finally, the grafted alveolus was covered by readapting the mucoperiosteal flaps on the vestibular and palatal aspect before water-tight closure of the wound was achieved using synthetic suture material. The wounds were never dressed.

Group A: (Iliac Bone Grafting):

After local anesthesia with vasoconstrictor injection, a 3-cm incision was placed obliquely below the anterior superior iliac spine. Subcutaneous dissection was performed to the iliac crest periosteum with care taken to avoid any harm to the lateral femoral cutaneous nerve. An osteotome was used on three sides of the iliac crest to create a ''book flap.'' The periosteum and cortical bone was opened lateral to medial to expose the cancellous bone. The osteotome was then used to remove a wedge of cancellous bone to be used for nasal alar support. Variable-sized curettes were used to acquire 20 to 30 cc of iliac crest cancellous bone. Gelfoam (Pfizer, Inc., New York, NY) soaked with bupivacaine and epinephrine was put in the defect for hemostasis and pain reduction. The cortical ''book flap'' was closed with 2-0 Vicryl sutures. Finally, closure with deep dermal 4-0 Vicryl (Ethicon, Somerville, NJ), subcuticular 4-0 Monocryl (Ethicon, Somerville, NJ), and Steri-Stripsi (3M, St. Paul, MN) was performed. Corticocancellous bone was placed in the alveolar defect **(Fig. 1)**.

Group B: (Chin bone Grafting group):

After infiltration of local anesthetic with vasoconstrictor into the anterior vestibular sulcus, a marginal incision into the gingival sulcus along the lower incisors with two vertical releasing incisions in the canine region was made. This was followed by exposure of the anterior surface of the mandibular symphysis and a rectangle is outlined (between the developing canine teeth) with a minimum distance of 5 mm from the apices of the lower incisors and

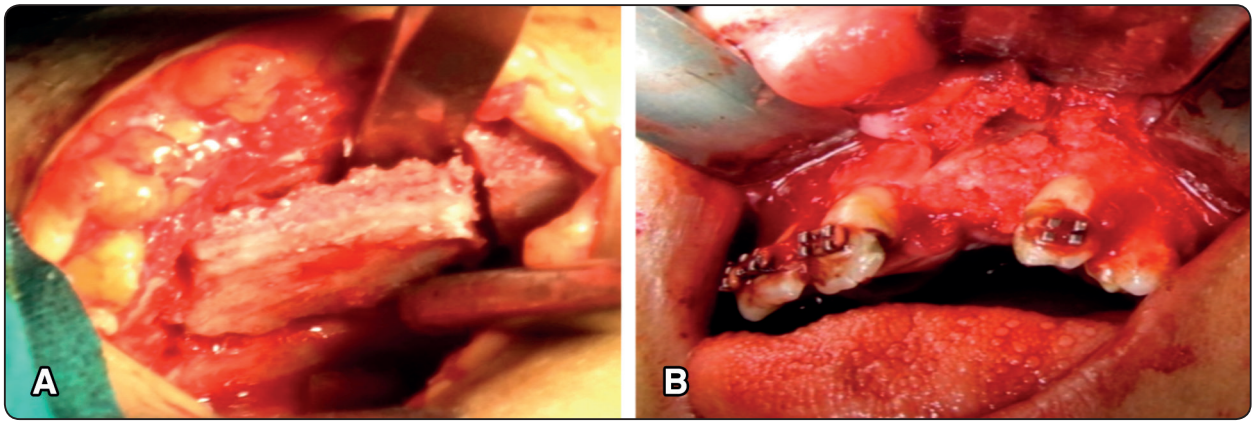

Fig. 1 (A): cutting and removing cortical bone from the ilium, (B): filling defect site with the iliac bone.

keeping the lower chin border intact. The chin bone graft is then delivered via freeing it from the lingual muscles. The buccal cortex with underlaying spongiosa were removed in one piece. Suturing was done with interdental interrupted stitches (3-0 vicryl). Extraorally, a submental elastic tape covering the chin area is put as a pressure bandage for four days. The chin bone graft is remodeled and firmly inserted into the recipient site **(Fig. 2)**.

Group C: (Bone marrow aspirate graft group)

The patient was put on his side, hips and knees flexed, there was no need for any incision to obtain the bone marrow aspirate, only the skin was stretched over puncture site, after exact point for aspiration was determined (the most prominent part of the posterior iliac crest) forty milliliters of bone marrow aspirate was obtained. Slight repositioning of the trocar was done for each 10 ml to access different areas of cancellous bone marrow utilizing the same cortical access hole. Bone marrow stem cells were then seeded onto a resorbable collagen sponge* by pulling them through a heparin-treated column, 2 collagen matrix with implanted cells were used. One was used to fill the defect of the alveolar cleft and the other piece was placed under the alar base on the maxilla for support. The hip trocar site was covered with a dressing **(Fig. 3)**.

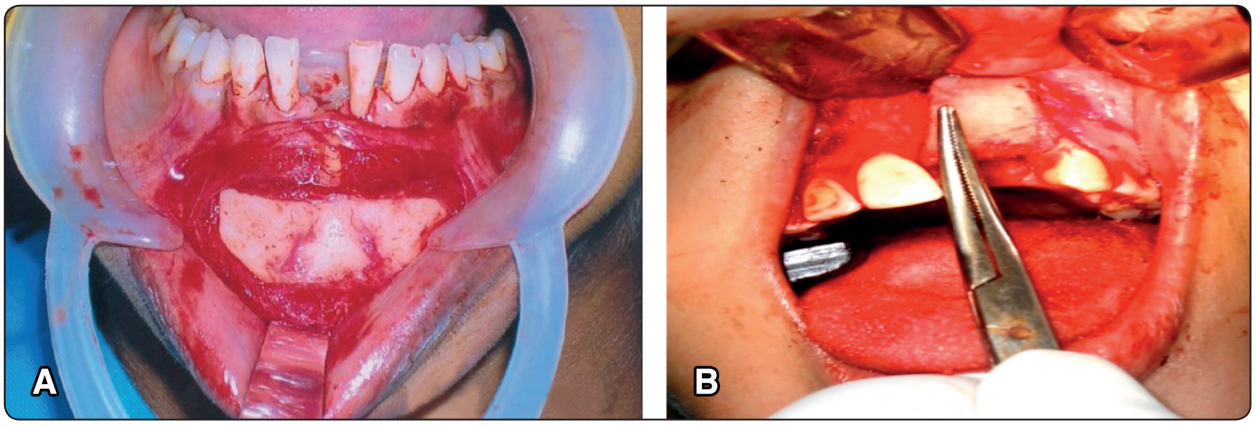

Fig. 2: (A): chin bone graft harvesting, (B): the bone graft put into the defect

* Helistat absorbable collagen (1/2 inch x 1 inch x 7.0 mm) manufactured by Integra life sciences corporatin (FDA approved).

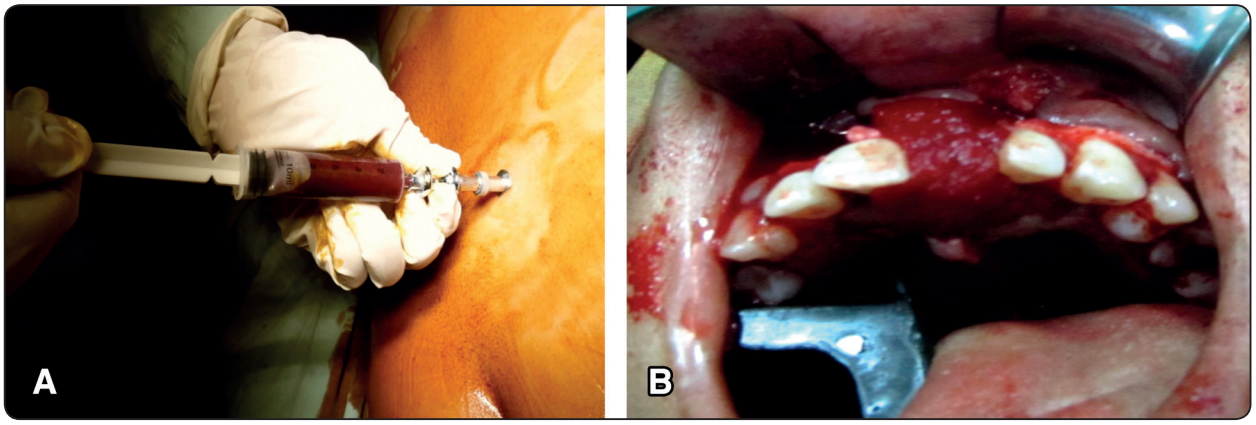

Fig. 3 (A): advancement of the trocar and aspiration of the bone marrow. (B): collagen soaked with the aspirate at the cleft site.

Postoperative assessment

A graft was considered successful when fulfilling certain criteria both clinically and radiographically at 6 months postoperatively: Evidence of establishment of bony continuity between the two alveolar segments with acceptable alveolar bone hight. Mucosal closure of the alveolus without any signs of presistant oronasal fistula. Comparing the bone height in all groups using Bergland scale (described by **Bergland et al.** in 1986), with this scale the occlusal level of the bony infill at the cleft site is compared with the normal side and reported as: type I: septal height approximately normal, type II: septal height at least three-quarters of normal, type III: septal height less than three quarters of normal and type IV: absence of a continuous bony bridge [10].

Phase II:

Surgical Procedure for core biopsy and dental implant placement :

All patients received titanium dental implants after 6 months from alveolar cleft grafting. An incision was started slightly palatal to the crest of the ridge at the site of implant placement and extended on crestal bone enough to ensure proper access to the area of interest. Then a releasing incision for superior reflection of the mucoperiosteal flap exposing the alveolar ridge was made. Core bone biopsy specimens were obtained from all patients using a trephine bur (2mm in diameter) on a contra angled hand piece under copious irrigation, the drilling depth was planned from the CBCT and with the help of a surgical stent to ensure that the biopsy contains newly formed bone and native bone. Then implant drills were used in a sequential pattern till reaching the final drill for the corresponding implant size, implants were placed under copious irrigation and covered by the cover screws **(Fig.4)**. The flap was readapted and sutured in place using 4-0 black silk sutures in an interrupted fashion.

Specimen Processing:

Every core bone biopsy held both the grafted area and the local native alveolar bone were fixed using 10% buffered formalin. When submitted for histologic examination, decalcification of the specimen was attained by suspension for fourteen days in EDTA 10% solution with regular rechanging of the solution every day. Dehydration of the specimen was then achieved using alcohol, followed by clearing in xylol. Afterward it was inserted in paraffin wax to be in a block form. The paraffin block was segmented longitudinally utilizing a microtome into thin paraffin sections,

each of approximately 5 microns thick. The sections were stained with Hematoxylin and Eosin. Stained sections were examined in a blind fashion in order to estimate the bone quality in the graft in the three different groups. **(Fig. 5)**.

Statistical analysis of our data was performed using SPSS® (Statistical package for the social sciences- IBM Corp., Armonk, NY). The data were represented as mean ± standard deviation. Mann–Whitney U-test was used to compare the variables between the groups. The results were considered statistically significant if the p value was less than 0.05.

RESULTS

For the entire patients, the alveolar cleft healing process went straight forward without any major complication; Statistical analysis revealed that the difference in the healing process between the 3 groups was not statistically significant. No abnormal bleeding was observed in any patient. All patients were discharged from the hospital on the same day of the surgery, minimal edema and no evidence of infection was observed in patients of all groups at either the donor or the recipient sites. Radiographic results after 6 months, showed new bone formation bridging the alveolar cleft gap in all cases.

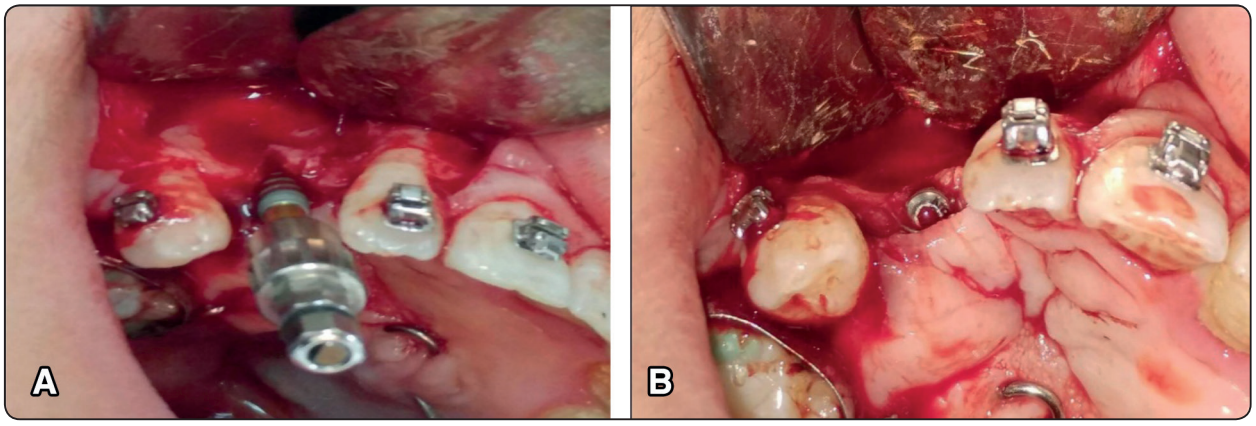

Fig. (4) A-B: placement of dental implant after harvesting 2 mm core biopsy.

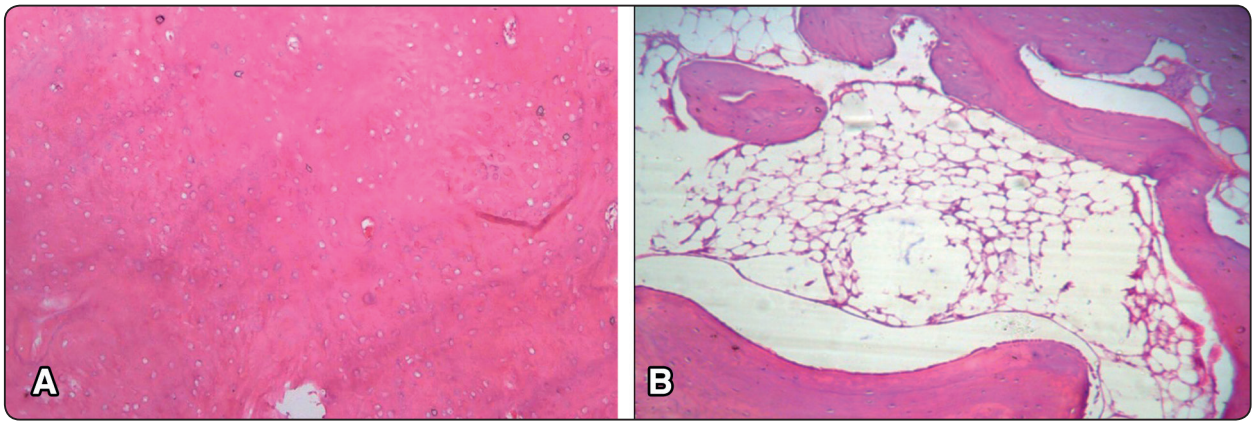

Fig. 5: (A) core biopsy of iliac crest group showing more mature compact bone, (B): core biopsy of bone marrow group showing less mature spongy bone

The histomorphometric analysis showed variable percentage of bone formation between the 3 groups, it ranged from 58 % to 76% with mean % of 67%± 6.93 in the iliac crest group, 44 % to 66% with mean % of 57%± 8.39 in the chin group and from 27 % to 62 % with mean % of 42± 22.6 in the bone marrow group. Although the mean density of the grafted cleft site in the bone marrow group was less than both autogenous graft groups, statistical analysis revealed that the difference between all groups was not statistically significant.

Comparing the bone height in all groups using the Bergland scale on the reformatted panoramic image obtained from CBCT, 11 patients of the autogenous groups were assigned to the successful groups I and II, while 1 patient was assigned to the insufficient group IV. For the bone marrow group, 4 patients were assigned to the successful groups I and II, while 1 patient was assigned to the unfavorable group III and 1 patient for the insufficient group IV, the 2 patients assigned to the group IV were re-operated for osteoplasty at a later date.

Sixteen implants were put in 16 patients and were successfully integrated: there was no mobility of the implants and no pain, swelling, or inflammation around the peri-implant tissue. Clinically, all implants have good initial stability however a long-term follow-up of all cases is recommended. Implant length ranged from 10 to 16 mm; the most frequently used length was 13 mm.

DISCUSSION

Although treatment of cleft lip and palate is now a standardized process, treatment of the alveolar cleft remains a controversial issue or rather it can be said that each step of the treatment is addressed in a very distinctive manner. Surgical techniques currently use various types of bone grafts that include autogenous bone as iliac crest and mandibular symphysis, allogeneic bone and bone substitution materials. However, there are a lot of disadvantages related to these strategies. Thus, improving surgical techniques and searching for novel bone substitute materials are needed for the improvement of clinical results.

Many reports suggest that autogenous bone from the iliac crest is the gold standard by which other types of alveolar grafts should be compared [3]. **Sadove et al.** mentioned that the iliac crest bone is easy to access and can supply huge amounts of cancellous bone with osteogenic precursor cells that support osteogenesis in the early period after the grafting procedure [11], however complications and post-operative morbidity has led to attempts to describe a minimally invasive harvesting technique for iliac crest bone graft harvest. Despite the multiple available donor sites, the ideal would be to eliminate completely the need for bone grafting and therefore donor site morbidity [12].

Williams A. et al. mentioned that the success of iliac crest bone grafting to close the cleft alveolar defects in patients 7 to 15 years of age is very high. Any new procedure for grafting must at least meet or improve this success [13]. Consequently with these meaning **O'Hara C. et al**. concluded that tissue engineering procedure with bone marrow derived stem cells seeded on a resorbable collagen matrix sponge does have comparable outcomes with iliac crest bone in the grafting procedure of the alveolar cleft [14].

Adult bone marrow has stem cells that can be induced to undergo differentiation into a lot of other cells capable of forming hematopoietic and mesenchymal progenitors. Therefore, the bone marrow can not only generate blood cells but also other undifferentiated cells capable of forming tissues, like cartilage and bone [15].

In 2006, **Smiler and Soltan** first mentioned a technique for chair-side cellular graft preparation using fresh aspirated bone marrow from the ilium that was blended with a resorbable matrix, they also demonstrated bone marrow aspirate that was

transplanted with biocompatible scaffolds or even allograft bone blocks could successfully regenerate bone [16]. Later, another technique by **Kadiyala et al.** was used by isolating stem cells and cultivating them in vitro [9].

In our studies we used approach to stem-cell-based bone regeneration relying on the direct use of a patient-derived fresh cellular graft prepared at the chair-side, these procedures are relatively convenient for clinicians because they do not require any laboratory support or extensive training. Also an interesting point of the chair-side method is the absence of any clinical or histologic inflammatory response after the operation. The cells in freshly processed grafts are not completely homogeneous and may contain several cell types, such as mesenchymal cells (MSCs), osteogenic cells, angiogenic cells and stromal cells. Therefore, the freshly prepared cellular grafting material may act somewhat similarly to a primitive bone niche providing easier acceptance by the host environment without an unfavorable local inflammatory reaction. Furthermore, recent studies have demonstrated that MSCs have a great anti-inflammatory effect when administered directly or intravenously to harmed tissue [17].

We concluded that new sufficient bone was formed at the cleft site in most of the cases of the bone marrow aspirate (five out of six cases), when comparing the quality of the formed bone to that formed in the iliac crest and chin group after 6 months of doing the operation, the aspirate group has a lower bone maturation quality, mean volume and density values, but with dramatic decrease in the donor site morbidity.

Consistent with these results **Nakade et al.** concluded that new bone was formed in ectopic places where bone marrow MSCs were implanted, suggesting a possible role of MSCs in the formation of different osteoinductive molecules [18]. In another study, **Kawaguchi et al.** used autologous MSCs isolated from a bone marrow aspirate of the iliac crest to promote periodontal regeneration. The treatment helped in the regeneration of alveolar bone, cementum and periodontal ligament [19].

Our results came consistent with the results of **Michael Gimbel et al.** who claimed that the best technique for alveolar cleft grafting regarding the donor site morbidity for the school aged alveolar cleft patients was a bone marrow aspirate with a resorbable collagen. He considered its result has an aesthetically acceptable donor site scar, which was rated highly by patients; also patients of the aspirate bone marrow were able to walk normally and returned to normal activities in less time compared to the traditional autogenous bone graft group [20].

Unlike the results we get, **Caplan** found that human MSCs produce ectopic bone in mice, but are reluctant to produce bone in human jaw defects, he concluded that, for human MSCs to be able to generate new bone, there should be the following prerequisites: sufficient numbers of cells with high osteogenic capacity, an appropriate scaffold to seed the cells on it in combination of factors to stimulate the cells 'in vivo'. All these 3 items can be fulfilled by engineering. However, the fourth demand for success is dependent on patient factors, namely sufficient vascular supply [21]. Also a potential cause of variation in the results gained when using stem cells may be due to age factor of the patient. As patients get old, their red marrow (rich in stem cells) decreases and is replaced by yellow marrow (poor in stem cells). Consequently, techniques for concentrating the stem cells for older patients are now being evaluated.

Chin symphysis bone graft was reported with successful results and somehow low morbidity rate when used for alveolar bone grafting in some studies. We encountered transient paresthesia reported by 2 of our patients, but no major morbidities, as tooth buds injury or sensory complications were observed in the 6 months follow-ups. However, almost in

all of the chin graft studies, the two-dimensional plain radiographs are used for evaluation. Very little previous investigations have measured the volume of the regenerated bone following chin bone graft harvesting [22].

Complex strategies of bone graft analysis was proposed to determine success related to providing bone support for teeth adjacent to the cleft. Over the years, the success of bone-grafting procedures is evaluated using histomorphometric techniques which we relied on in this study. Depending only on a radiograph to evaluate alveolar bone graft success has been doubted. Difficulties in quality, standardization and interpretation remain problematic, also high radiation administered to the patient and its expensiveness remains a huge drawback [23].

CONCLUSION

MSCs from bone marrow could make a good bone formation in various bone surgeries, such as oral and maxillofacial surgery, plastic surgery, craniofacial anomalies, and orthopedics, although the degree of bone maturation and formation was not great enough as the autogenous grafts groups, but the lack of morbidity at the donor site with the decrease in the hospitalization time, and good soft tissue healing is a good criteria for future comprehensive research on MSCs based bone engineering.

REFERENCES

1. Zhang D, Chu F, Yang Y, Xia L, Zeng D, Uludağ H, Zhang X, Qian Y, Jiang X: Orthodontic Tooth Movement in Alveolar Cleft Repaired with a Tissue Engineering Bone: An Experimental Study in Dogs. Tissue Eng Part A 17:1313-1325, 2011.
2. Tai CE, Sutherland IS, McFadden L: Prospective analysis of secondary alveolar bone grafting using computed tomography. J oral Maxillofac Surg 58: 1241, 2000.
3. Eppley BL, Sadove AM. Alveolar cleft management. In: Achauer BM (ed). Plastic Surgery: Indications, Operations, and Outcomes. St. Louis: Mosby 809–817, 2000.
4. Gimbel M, Ashley RK, Sisodia M, Gabbay LS, Wasson KL, Heller J, et al. Repair of Alveolar Cleft Defects: Reduced Morbidity With Bone Marrow Stem Cells in a Resorbable Matrix. J Craniofac Surg 18:895-901, 2007.
5. Mulliken JB, Glowacki J. Induced osteogenesis for repair and construction in the craniofacial region. Plast Reconstr Surg 65:553-6, 1980.
6. Enemark H, Jensen J, Bosch C. Mandibular bone graft material for reconstruction of alveolar cleft defects: long-term results. Cleft Palate Craniofac J 38:155–63, 2001.
7. Petite H, Viateau V, Bensaid W. et al. Tissue-engineered bone regeneration. Nat Biotechnol. 18: 959–63, 2000.
8. Derubeis AR, Cancedda R. Bone marrow stromal cells (BMSCs) in bone engineering: limitations and recent advances. Ann Biomed Eng. 32: 160–5, 2004.
9. Khan Y, Yaszemski MJ, Mikos AG, et al. Tissue engineering of bone: material and matrix consideration. J Bone Joint Surg Am. 90: 36–42, 2008.
10. Bergland O, Semb G, Abyholm F: Elimination of the residual alveolar cleft by secondary bone grafting and subsequent orthodontic treatment. Cleft Palate J 23: 175–205, 1986.
11. Sadove AM, Nelson CL, Eppley BL, Nguyen B. An evaluation of calvarial and iliac donor sites in alveolar cleft grafting. Cleft Palate J: 27:225–9, 1990.
12. Aoi Matsuo, MD, Yasuharu Yamazaki, DDS, Chikara Takase MD, Kazuya Aoyagi, MD, Eiju Uchinuma, MD Osteogenic Potential of Cryopreserved Human Bone Marrow Derived Mesenchymal Stem Cells Cultured With Autologous Serum J Med Dent Sci: 50:63Y69, 2006.
13. Williams A, Semb G, Bearn D, et al. Prediction of outcomes of secondary alveolar bone grafting in children born with unilateral cleft lip and palate. Eur J Orthod: 25:205Y211, 2003.
14. O'Hara C, Sisodia M, Kawamoto H-K, et al. Bone marrow stem cells and resorbable collagen matrix heals alveolar cleft defects with reduced morbidity. 73rd Annual Meeting of the American Society of Plastic Surgeons, Philadelphia, PA, October 9, 2004.
15. Schliephake, H., Knebek, J., Aufderheide, M. & Tauscher, M. Use of cultivated osteoprogenitor cells to increase bone formation in segmental mandibular defects: an experimental pilot study in sheep. The International Journal of Oral & Maxillofacial Surgery 30: 531–537, 2001.

16. Smiler D, Soltan M. Bone marrow aspiration: technique, grafts, and reports. Implant Dent: 15:229–35, 2006.

17. Rickert D, Sauerbier S, Nagursky H, Menne D, Vissink A, Raghoebar GM. Maxillary sinus floor elevation with bovine bone mineral combined with either autogenous bone or autogenous stem cells: a prospective randomized clinical trial. Clin Oral Implants Res; 22:251–8, 2011.

18. O. Nakade, K. Takahashi, T. Takuma, T. Aoki, T. Kaku, Effect of extracellular calcium on the gene expression of bone morphogenetic protein-2 and -4 of normal human bone cells, J. Bone Miner. Metab. 19: 13–19, 2001.

19. H. Kawaguchi, A. Hirachi, N. Hasegawa, T. Iwata, H. Hamaguchi, H. Shiba, T. Takata, Y. Kato, H. Kurihara, Enhancement of periodontal tissue regeneration by transplantation of bone marrow mesenchymal stem cells, J. Periodontol. 75 : 1281–1287, 2004.

20. Michael Gimbel, MD, Rebekah K. Ashley, BS, Manisha Sisodia, DDS, Joubin S. Gabbay, MD, Repair of Alveolar Cleft Defects: Reduced Morbidity With Bone Marrow Stem Cells in a Resorbable Matrix J Craniomaxillofac Surg ; 19: 7Y14, 2008.

21. Caplan AI. Mesenchymal stem cells. J Orthop Res; 9: 641–50, 1991.

22. Borstlap WA, Heidbuchel KLWM, Freihofer HPM, Kuijpers-Jagtman AM: Early secondary bone grafting of alveolar cleft defects: a comparison between chin and rib grafts. J Cranio-Maxillofacial Surg 18: 201, 1990.

23. Waitzman AA, Posnick JC, Armstrong DC, Pron GE. Craniofacial skeletal measurements based on computed tomography: Part I. Accuracy and reproducibility. Cleft Palate Craniofac J. 29:112–7, 1992.