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Evaluation of Autogenous Bone Graft with or without Intra-Marrow Penetration in Treating One Osseous Wall Defects: An experimental study

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ABSTRACT

Purpose: The study was designed to assess the autogenous bone grafting (ABG) alone or in combination with intra-marrow penetration (IMP) in treating one osseous wall defects in dog model. **Material and methods**: This study was conducted using 8 Beagle dogs that were divided randomly into two groups: Group I**:** received open flap debridement with autogenous bone graft**,** Group Π: received open flap debridement with autogenous bone graft and IMP. Each animal received two treatment modalities, one for each jaw. Each jaw was divided into two halves (right and left). The surgery intervention was performed at base line in the left side. The right side received the same treatment after 6 weeks. All experimental defects were followed up through histological analysis. **Results:** Histomorphometric study presented significantly better bone neogenesis within both groups at 14, 60 days. The total area percent of new bone formed in group I was 38.46±7.71% while it was 33.17±7.3% in group II with non-significant variance between test groups throughout the study intervals. **Conclusion**: The regenerative therapy using either autogenous bone graft alone or with IMP showed an improvement in the management outcome of one osseous wall defects.

KEYWORDS

Periodontitis,
Autogenous Bone Graft,
Intra-Marrow Penetration.

INTRODUCTION

Periodontitis is a compound disease with several causal factors that all together play a part with three chief causal risk factors: microbiology (subgingival bacterial biofilm), genetics, and lifestyle. Subgingival plaque microbiota is necessary to initiate the periodontal disease;

- A paper extracted from Master thesis titled "Evaluation of Autogenous Bone Graft and Intra-Marrow Penetration Combined Therapy either alone or Augmented with Melatonin in Treating One Osseous Wall Defects: An experimental study"

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as well as the genetic predisposition which modified host inflammatory immune reaction to pathogenic bacteria, Which leads to more tissue destruction [1,2].

Infrabony pockets are categorized on the basis of the number of osseous walls. Angular defects may have one, two, or three walls. Hemi septal defects i.e., vertical defects with the existence of adjacent roots and where part of a septum left over on the tooth, represents an exceptional situation of one-wall defects and the treatment is always a challenge despite the various periodontal regenerative therapies[3].

The main objective for periodontal management is the regeneration of the missing periodontal tissues, but remains unpredictable and challenging. Given that the main etiologic agent in periodontitis is plaque and calculus, scaling and root planning are basically performed to eradicate these irritants. Open flap debridement displayed better elimination of calculus allowing healing by long junctional epithelium. In comparison, reformative therapy permits reconstruction of constituent tissues and function by regeneration of the attachment apparatus on the affected root surface[4].

Many varieties of bone grafting materials used in regenerative periodontal treatment depend on their capability to assist the restoration of the missed supportive apparatus. Most bone grafts used in dentistry include autogenous bone grafts, allografts, xenografts and alloplastic. Ultimately, on reviewing the literature, autogenous bone grafts have been known to be the gold standard grafts because of their osteogenic ability and cell viability[5]. The use of intramarrow penetration (IMP) in bone regenerative procedures has been mentioned; it accelerates rate of bone formation, increases bone fill and better bone density[6].

Generally, the primary methods used for evaluation of any regenerative technology include histology, surgical Re-entry, periodontal probing and radiographic analysis. Histology is the definitive standard to measure the extent of periodontal regeneration. When the possibility of a new treatment to restore alveolar bone, cementum and a correctly oriented periodontal ligament has been proven by histological analysis, the usage of clinical and radiographic measurements are enough to evaluate the lasting result of the reformative treatment [7].

Since limited studies have investigated the impact of using the autogenous bone graft alone versus its combination with IMP, therefore, this current trial was performed to evaluate this therapeutic approach in a dog model.

MATERIAL AND METHOD

Study design

This current study was an Experimental study on 8 Beagle dogs weighing up to (12–15 kg) supplied from Animal Laboratory in Cairo University. The animals used in this experiment were treated according to protocols assessed and permitted by the Experimental Animal Ethical Committee of Al-Azhar University.

Sample Size

According to different regenerative materials used, a total of 32 defects in eight animals were distributed unsystematically into two groups as follows

Group I: 16 defects received autogenous bone graft.

Group II: 16 defects received autogenous bone graft +IMP.

Surgical procedures

Each animal received two treatment modalities, one for each jaw. Each jaw was divided into two halves. The surgery intervention was performed at base line in the left side. The right side received the same treatment after 6 weeks. Thus, at time of sacrificing (after 60 days) (end point), there were defects that received treatment of fourteen days duration and other defects received treatment of sixty days duration.

Anesthesia was prompted by giving atropine (Atropine®, Aguettant, France; 0.05 mg/kg intramuscular)and tiletamine-zolazepam (Zoletil® 100,Virbac, , France; 5–10 mg/kg intramuscular). Then, a dose of 10 to 15 mg/kg thiopental sodium was given intravenous (Nesdonal®, Merial,Lyon, France) then retained on 1 to 4% O2–N2O isoflurane mix [8]. After anesthesia, Buccal and lingual intra-sulcular incisions were performed from the mesial of first premolar P1 to the mesial of the fourth mandibular premolar P4 then a full thickness Muco-periosteal flap was reflected[9]. Surgically induced periodontal defects were created in the form of box shaped (5*5) (figure 1).

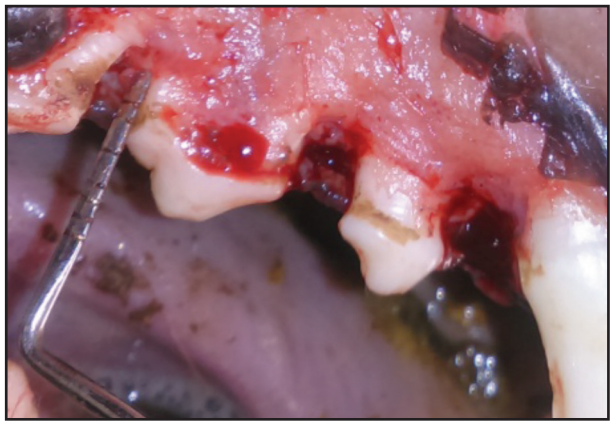

Figure (1): box shaped induced defect (5*5)

These defects were created with trephine burs size 4 under copious amounts of saline solution irrigation. The bone obtained with intrabone marrow penetration was used in treatment as the source of autogenous bone graft [10]. Experimental periodontitis associated with one wall osseous defect was induced on the premolars of each quadrant of both jaw[11].The four defects in each arch were managed as follow: the defects in group I received autogenous bone graft that was harvested and refrigerated for ten days before surgery. In group II the defects received autogenous bone graft freshly harvested. Lastly flaps were repositioned and held with interrupted sutures of absorbable poly glycolic acid 000suture.

After operation, the post-operative regimen directed was: Antibiotic prophylaxis: spiramycine 750,000 IU and metronidazole 125 mg/day for 6 days (Stomorgyl®, Merial, Lyon, France), Anti-inflammatory: Profene 50 mg/day for 7 days (Rimadyl® Pfizer Santé Animale, Orsay,France). and butorphanol (0.3 mg/kg) (TorbuGesic®, Fort Dodge Animal Health, Southampton, UK) [12]. The dogs were fed a soft diet the day of the surgery and the next day.

The animals were euthanized at 2 months of treatment period. After anesthesia with an intramuscular dose of Zoletil® (50 mg/kg), heparin was injected by intravenously (100 IU/kg). Followed by a toxic dose shot of Dolethal® (pentobarbital sodique, Vetoquinol), then formalin injection. Fixation was done by a dose nearly 300 mL of 10% formalin in the common carotid artery. Then the mandibles were cut up after the first molar and resected. Each sample was divided and saved in 10% solution of buffered formalin [12].

Histological evaluation:

Samples were placed in containers and labeled by animal number. Fixation of the tissue was done by 10% formalin for 3 weeks.[13] Decalcifications of the samples were done using 20% formic acid and 10% Soduim citrate for 8 weeks. The samples were then washed in running water to remove any excess of the decalcifying agent and cut into small blocks. The specimens were then dehydrated in arising grades of ethyl alcohol beginning by 70% till 100% absolute alcohol then methyl benzoate for a day followed by paraffin benzol for two hours. To eliminate the alcohol remains, the specimens were immersed in paraffin wax in three changes; 1, 2 and 3 and then mounted in wax blocks of appropriate bulk to be cut. Using Leitz Wetzlar microtome, sequential sections of 5-8 um thick on rotary microtome were cut[14], then mounted on slides and stained with Hematoxylin and Eosin (H&E) for histological analysis with valuation of new bone establishing [15].

RESULTS

Healing was without incident in all test locates in all animals.

Histological Findings

For both test groups, at day 14 the bone graft particles in the defects exhibited a variation in size and shape. It hardly showed signs of vitality; osteocytes were hardly seen inside lacunae .Graft displayed several crack-lines. Osteoclast-like cells could be seen near the autograft particles in some samples proposing a dynamic resorption procedure.

For both groups, at day 60, recently formed bone marrows spaces with large trabeculae, was observed. The bone graft particles were surrounded by newly formed bone, osteoid tissue, and connective tissue. The most superficially located particles appeared partially surrounded by epithelium. The greatest percentage bone fill was achieved in the trial sites at 60 days of healing.

Histomorphometric Evaluation

Comparison between groups at each observation time

At 2 weeks: The maximum mean value was noted in group II (44.47±12.27), while the least value was recorded in group I (38.99±10.54). Independent t test shown that the alteration within groups was statistically not significant (p=0.558) (table1).

Table (1) *Descriptive statistics and comparison of bone density area percent between groups at 14 days (independent t test)*

		Group I	Group II
2 weeks	**Mean**	38.99	44.47
	SD	10.54	12.27
t		0.601	
P		0.558ns	

Significance level p≤0.05, ns=non-significant

At 2 months: The maximum mean value was noted in group I (38.46±7.71), while the least value was recorded in group II (33.17±7.3). Independent t test shown that the alteration between groups was statistically not significant (p=0.155) (table 2).

Table (2) *Descriptive statistics and comparison of bone density area percent between groups at 2 months (independent t test)*

		Group I	Group II
2 month	**Mean**	38.46	33.17
	SD	7.71	7.3
t		1.49	
P		0.155ns	

Significance level p≤0.05, ns=non-significant

Comparison of both observation times within the same group

In group I, a higher mean value was recorded at 2 weeks (38.99±10.54), in comparison to 2 months (38.46±7.71). This variance was not statistically significant (p=0.9044).

In group II, a higher mean value was recorded at 2 weeks (44.47±12.27), in comparison to 2 months (33.17±7.3). This variance was statistically significant (p=0.0304).

DISCUSSION

The current experiment was done as an effort to assess healing of intrabony periodontal defects induced in dog model after using autogenous bone grafting (ABG) alone or in combination with Intra-Marrow Penetration (IMP) histologically.

As histological assessment is the only trustworthy technique to decide the effectiveness of periodontal treatments [16], so, the current study evaluated healing of intrabony surgically created periodontal defects in dog model histologically. The box-form of one-wall intrabony defect in dog is a

well-established model and has been used to assess the outcome of certain biomaterials on periodontal treatment[17,18]. Thus, in this paper, one-wall defects which had minimal self-healing abilities were used. Reference notches were prepared at the apical end of defect separately to help in histologic assessment.

Autogenous bone grafts come from the patient's own body and have osteoconductive and osteoinductive, osteogenesis and osseointegration properties (binding capability to the adjacent bone without fibrous tissue, permitting integration of the graft at the defect site that found only in autogenous bone graft). They completely lack immunogenicity, have no risk of viral transmission and maintain their viability immediately after transplantation. [21,22].

Various explanations are presented as explanation for the improved osseous regeneration attained with IMP in bone augmentation procedures by improving angiogenesis, bone morphogenic proteins increased locally and some growth factors from the hurt cortical surface, and the injury to the cortical bone initiating a regional acceleratory phenomenon (RAP) [23,24].

The results demonstrated that experimental sites with IMP showed earlier osteoneogenesis when compared to autogenous bone graft alone, the same bone neogenesis at end of evaluation intervals. At 14 days, a mean total bone density of 44.47±12.27% of new bone was observed in group II, while group I showed a density of 38.99±10.54% at the same interval. At 60 days, bone density observed was 38.46±7.71% 23.83 within the group I, while the corresponding figures in the group II were 33.17±7.3%.

Results show non-significant difference between two groups at the end of study. The study in two groups shows higher bone formation this supporting that autogenous bone graft remains the gold stander. This could be attributed to its osteoconductive and osteoinductive, osteogenesis and osseointegration properties.

CONCLUSION

This experimental study has shown that IMP in combination with autougenous bone graft have no advantages over autogenous bone graft alone in intrabony periodontal defects induced in dog. Clinical context, results of this study needs more investigation.

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