Austin Journal of Dentistry

Open Access

Austin Publishing Group

Research Article

Early Loading of Dental Implant Grafted with Autogenous Bone Alone or Combined with Melatonin Gel: A Randomized Clinical Trial

Hazzaa HH*[1], Shawki NA[2], Abdelaziz LM[3] and Shoshan HS[4,5]

[1]Department of Oral Medicine, Periodontology, Diagnosis and Oral Radiology, Al-Azhar University for Girls, Egypt
[2]Department of Oral Medicine, Periodontology, Diagnosis and Oral Radiology, Al-Azhar University for Girls, Egypt
[3]Department of Oral Medicine, Periodontology, Diagnosis and Oral Radiology, Al-Azhar University for Girls, Egypt
[4]Department of Oral and Maxillofacial Surgery, Cairo University, Egypt
[5]Department of Oral and Maxillofacial Surgery, Alfarabi Private College for Dentistry and Nursing, Jeddah, Saudi Arabia

***Corresponding author:** Hazzaa HH, Department of Oral Medicine, Periodontology, Diagnosis and Oral Radiology, Faculty of Oral & Dental Medicine, Al-Azhar University for Girls, Nasr City, Cairo, Egypt

Received: May 25, 2020; **Accepted:** June 23, 2020; **Published:** June 30, 2020

Abstract

Purpose: Evaluation of early loaded dental implants augmented with Autogenous Bone Graft (ABG) either alone or combined with melatonin (MLN) gel.

Material & Methods: This split mouth study included 23 patients with 46 sites indicated for dental implants.

Participants were equally divided into: Control group (Gp I) for patients received dental implant and ABG, and test group (Gp II) for patients received dental implant and ABG/MLN (1.2 mg) combination. Clinical (Pre-Implant Probing Depth-PPD and Pre-Implant Soft Tissue Thickness-PIST) and radiographic evaluations (Marginal Bone Loss-MBL and Bone Density-BD) were assessed pre- and 6 months postoperatively. The crevicular expression of Nitric Oxide (NO) was monitored at baseline, 2 weeks and 2 months post-implant insertion.

Results: At the study end, a significant difference was shown in the mean PPD between GP I 2.60 mm ± 0.46 and GP II 1.95 mm ± 0.44 (p=0.005). GP II showed a significant gain in PIST (2.60 mm ± 0.41), versus 2.15 mm ± 0.53 in GP I (p=0.0001). There was a significant difference in MBL between GP I (0.67 ± 0.14) and GP II (1.66 ± 0.28) at p=0.000. GP II showed better gain in BD, 649.33 ± 63.06, versus 582.80 ± 31.20 in GP I (p=0.061). The expression of NO was significantly reduced in GP II (0.001 ± 0.000; p=0.000), at 2 month post-implant.

Conclusions: Early loaded implants grafted with ABG/MLN seems to be a promising clinical choice based on better gain in MBL, PIST, and more reduction in NO-crevicular expression.

Keywords: Early loaded dental implant; Autogenous bone graft; Melatonin; Nitric oxide

Abbreviations

Autogenous Bone Graft (ABG); Melatonin (MLN); Control group (Gp I); Test group (Gp II); Pre-Implant Probing Depth (PPD); Pre-Implant Soft Tissue Thickness (PIST); Nitric Oxide (NO); Bone Density (BD); Marginal Bone Loss (MBL)

Introduction

Dental implants are now considered the main procedure to replace the missing teeth [1] through a biologic procedure called osseointegration, where the materials of fixture make close cling to bone. However, in the presence of movement, a soft tissue interface may encapsulate the implant [2] causing its failure. To minimize the risk of soft tissue encapsulation, it has been recommended that implants be kept load-free during the healing period (3-4 months in mandibles and 6-8 months in maxilla) [3].

In general, removable prostheses are used during the healing period; however, many patients find these provisional prostheses rather uncomfortable. Therefore, it would be beneficial if the healing period could be shortened. Fortunately, due to improvements in the implant surface treatment process, there is a trend of early loading within two months in the mandible and within four months in the maxilla [4].

The efficacy of early loading dental implants has been studied in animals. A number of authors have reported that early loaded implants show a greater percentage of bone-to-implant contact and more mature cortical bone than delayed-loaded controls. Although the long-term clinical reports appear to support the application of early loading of dental implants [5, 6], immediately and early loaded implants may be at a greater future risk of failure than conventionally loaded ones [7]. Therefore, it would be more useful to know whether there are certain clinical modifications able to promote faster bone healing and minimize the Marginal Bone Loss (MBL) over time to minimize the rate of implant failure [8].

Autogenous Bone Graft (ABG) is harvested from donor sites in the patient's own body and have reliable osseointegration properties [9]. From a biological point, ABG is the best material available, its osteogenic properties and its immunological inertness [10]. Nevertheless, the resorption rate of the ABG is usually high; a fact

Citation: Hazzaa HH, Shawki NA, Abdelaziz LM, Shoshan HS. Early Loading of Dental Implant Grafted with Autogenous Bone Alone or Combined with Melatonin Gel: A Randomized Clinical Trial. Austin J Dent. 2020; 7(2): 1137.

that is attributed to the less graft exposure to the recipient bone vasculature, leading to decreased bone remodeling. Moreover, ABG is exposed to forces during harvesting leading to more osteoclastic resorption [11]. The reduced Reactive Oxygen And Nitrogen Species (RONS) was regarded as a responsible factor for the preservation of ABG with better remodeling and proliferation of osseous cells [12].

In this aspect, melatonin (MLN) (N-acetyl-5-methoxytryptamine) is a powerful hormone derived from an essential amino acid tryptophan [13] that is considered as an efficient scavenger of free-radicals and a broad-spectrum antioxidant [14]. Increased reactive oxygen species scavenging by MLN and its metabolites in the inflamed area would help reduce the degree of tissue damage. Additionally, MLN acts on osteoclasts to reduce resorption of osseous matrix in different forms, as MLN being an antioxidant, is able to affect the process detoxifying free radicals, which are produced during osteoclastogenesis [15]. Furthermore, it was reported that the local application of MLN stimulates differentiation in preosteoblast lines into osteoblasts after a period of 12 days around dental implants, instead of 21 days [16].

Taken together; the combined use of MLN with ABG around early loaded dental implants seems to be of clinical significance regarding MBL. In this respect, this study was applied to evaluate the early loading protocol of dental implants, based on the predominance of ABG/MLN graft regarding better hard and soft tissue clinical outcomes compared to ABG alone.

Materials and Methods

Study design

This trial was applied on 23 patients with 46 numbers of edentulous sites. The design of this trial was accepted by the Ethics Committee of Al-Azhar University for girls (OMPDR-103-2c). This treatment protocol was applied in accord with the ethical fundamentals of the Declaration of Helsinki and good clinical practice.

Participants

Inclusion criteria:

1) Participants had good oral hygiene.

2) Participants were periodontally healthy and free of systemic diseases [17].

3) Presence of at least a single-mandibular edentulous area in the premolar-molar region.

4) The edentulous area is completely healed; at least 8 weeks after extraction [18].

Exclusion criteria included patients who had parafunctional habits, smokers, former smokers, pregnant, lactating women and patients who were not able to follow the treatment protocol.

The aim of this study was clarified for all the participants. Every patient then signed an informed consent that was obtained before the start of the study.

Study setting: The participants were recruited from the Oral Medicine clinic, Faculty of Oral and Dental Medicine for girls, Al-Azhar University, Cairo, Egypt. This study was performed between May 2017 and September 2018.

Randomization: A computer-generated table was used to provide a random and equal distribution of sites into two groups. Only one person randomly enrolled the patients to groups using a single allocation ratio (1:1) and a computer program (Excel 2013 v15.0, Microsoft Windows, RAND function).

Grouping: In this study, the selected sites were randomly and equally (n=23) assigned (Figure 1) by a blind investigator into: a) Control group: which incorporated those patients enrolled for dental implant with utilization of ABG. b) Test group: which included patients who underwent dental implant placement after the use of ABG blended with (1.2mg) MLN gel (Shaanxi Sangherb BioInc-Melatonin pure powder). Radiographic examination was preoperatively done including: CBCT for each patient to allow evaluation of fixture position.

Preparation of MLN gel: Methylcellulose solution (1.5% w/v) was prepared by gradually adding the calculated amounts of the polymer (1.5 g methylcellulose, high viscosity 4000) while stirring to one third of the required amount (33 mL out of total 100 mL) of freshly prepared distilled water at 80°. The final volume was made by adding the remaining volume of water (about 67 mL) in which 150 mg of MLN was dispersed while stirring. The preparation was placed in a vacuum to remove entrapped air, prior to storage at 4°C until required [19].

The surgical protocol:

Stage I: Every patient received Augmentin 1g twice/day immediately before surgery. Infiltration anesthesia was done using lidocaine 2% (1:80,000 concentration of adrenaline). The perioral site was decontaminated utilizing betadine, crestal flap was performed on edentulous ridge and a full thickness mucoperiosteal flap was reflected.

Low speed engine (2000 rpm) was utilized in clock/anti clock

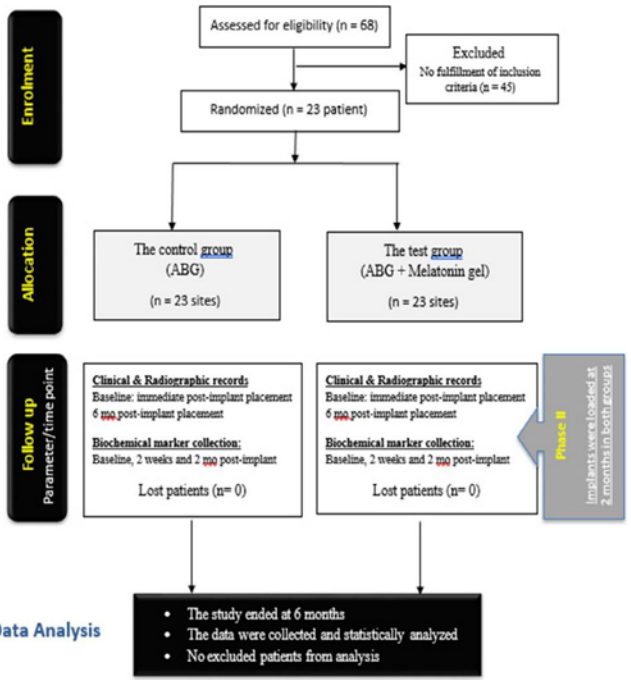

Figure 1: The flow chart showing the study design and time line.

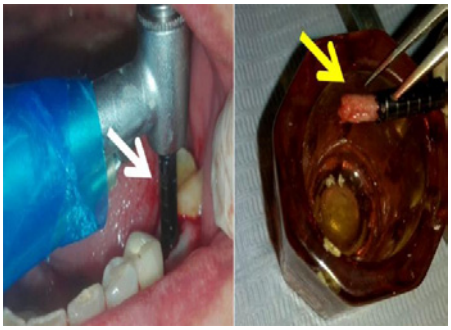

Figure 2: Low speed engine (2000 rpm) (white arrows) was utilized in clock anti clock manner to allow autogenous bone block (yellow arrows) removal using trephine bur from the osteotomy site.

manner to allow bone block removal utilizing trephine bur. Bone block was crushedinto small pieces utilizing rongeur to enable its application around fixture after its insertion.Each patient received two pieces of dental implant (NuCloss: 10018sokak No:7 itoborganizeSanayiBolgesi 35477 Tekeli, Menderes/Izmir/Turkiye) with inner hexagon connection in one stage. The implant site was prepared by sequential drilling according to surgical kit,with reduced low speed (2000 rpm), under copious irrigation with normal saline, till reaching the desired dimensions. The implant was inserted using manual ratchet torque handle until the full length covered with bone.

In the test group, 1.2 mg MLN gel was mixed with ABG (Figure 2) to be applied around implant fixture. In the control group, ABG chips were applied without MLN gel. Healing abutment was immediately inserted to allow opening of socket for Peri-Implant Sulcular Fluid Collection (PISF) collection. Flap was closed using interrupted non-resorbable suture which were removed 14 days after implant surgery.

After surgical procedures, all patients were educated to apply extra oral ice packs (10-20 minutes) over implant site to avoid hematoma development. Patients received Augmentin (625 Amoxicillin Trihydrate, 125mg clavulanc corrosive. GSK Glaxo Smith kline, Egypt) for 7days postoperatively twice/day. Ibuprofen (Brufen kahira pharma& CHEM.IND.CO. Cairo-Egypt), (600 mg twice/day for 1 to 3 days postoperatively) was prescribed as an anti-inflammatory and analgesic effects. Chlorhexidine mouthwash (Antiseptol Kahira CO. for pharm. what's more, Chem., IND organization, Cairo, Egypt) was utilized twice/day for 3 weeks post operatively.

Stage II: The healing cap was replaced by the abutment after 2 months. A direct impression was then made for fixed prosthodontic construction. The oral hygiene measures were given, and the patients were followed up (by a non-study periodontist investigator) till the end of the follow-up period.

Outcomes assessment: Each patient was clinically and radiographically evaluated immediately after implant placement; baseline (T0), then at 6 months (T1) after implant placement.

Biomarker analysis: PISF was collected at baseline (T0), 2weeks (T1) and 2months (T2) after implant insertion to monitor the level of nitric oxide (NO) as a biomarker for oxidative stress. Paper points were applied at (buccal, mesial, lingual and distal aspects) to collect PISF that was frozen at –80oc for analysis. The same non-study calibrated investigator did all the measurements.

Figure 3: A photograph showing preoperative view representing the placement of virtual implant in relation to adjacent teeth and vital structure.

Study parameters

Clinically:

a) The Pre-implant Probing Depth (PPD) was measured using the periodontal probe with Williams's graduation at 4 points (buccal, lingual, mesial, and distal) around each implant.

b) Peri-implant soft tissue thickness (PIST) was also measured by using an endodontic file with a stopper, with the aid of a millimeter ruler the thickness was measured at the mid-point between the cervical limit of the free peri-implant margin and the apical limit of the attached peri-implant margin.

Radiographically: The Cone Beam Computed Tomography (CBCT) imaging, utilizing Planameca machine (Promax 3DxM mid. Planameca Finland), was used (Figure 3). The orientation beam was utilized to adjust the jaw bone parallel to the reference surface. The tube voltage was 90kvp, the current was 12mAs and the exposure time was 4-12s as indicated by Field of view (FOV) of pulsed exposure.

1-Linear measurements: On the multiplanar screen, navigation was done until an accurate view of the implant was seen on the reformatted panorama and sagittal cut. Using the tools from the machine software, a line was drawn on the mesial, distal, buccal, and palatal form on the collar margin of the implant to the alveolar crest (Figure 4).

2-Densitometric analysis: lines were drawn tangential to the implant from mesial, distal, buccal, and lingual on the chosen images. Using the density area tool, the density of 3 points was demarcated on each line; their mean was then calculated.

Biomarker analysis: Bio diagnostic Nitrite Assay Kit (Nitric oxide Assay Colorimetric Determination of Nitrite for research Biodignostic and research reagent; Dokki, Giza, Egypt) was used for measurement of endogenous nitrite concentration as indicator of NO production in biological fluids (20). The PISF was extracted from the frozen paper point after sterilization using Vortx and centrifuge; each sample was then divided into test sample and blank sample (0.1 ml). 1.0 ml from (reagent1 standard sodium nitrite 50 µmol/L) was applied to standard sample and standard blank then 1.0 from (reagent 2

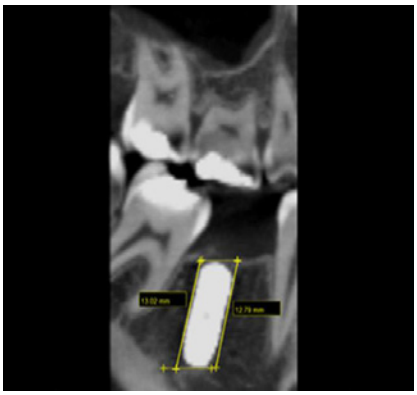

Figure 4: Radiographic CBCT showing linear bone measurement.

Sulphanil amide 10µmol/L) was applied to test sample, sample blank, standard sample and standard blank was mixed well to stand for 5 min and 0.1 from (regant3 N-(1-naphthyl)-ethlene dimine (NEDA) was applied to sample and standard mixed well and allowed to stand for 5min until color appear. Finally, absorbance of test sample against sample blank and of standard sample against standard blank was red at 540 nm using Chem7 photometer.

Sample size calculation: The sample size was calculated using G*Power program (University of D¨usseldorf, D¨usseldorf, Germany). Student's t-test was used to do comparisons between the two groups in the crest bone loss and degree of osseointegration. The primary outcome variable (crestal bone loss) within each group was normally distributed with an average standard deviation of 0.7. The true difference between the means of the two groups was 0.48, a total sample size of 42 was sufficient to detect an 80% power and a significance level of 5%. A 10% increase in the total sample size to allow for those lost to follow-up, to reach a total sample size of 46 (23per group).

Statistical analysis: All data were collected, revised, coded and entered to the Statistical Package for Social Science (IBM SPSS Chicago, IL, USA) version'23. The quantitative information was displayed as mean, standard deviations and extents when their appropriation found parametric. Likewise subjective factors were introduced as number and percentages. The examination between two free gatherings with quantitative information and parametric conveyance were finished by utilizing Independent t-test. The correlation between two matched gatherings with quantitative information and parametric dispersion was finished by utilizing combined t-test. The examination between two increasingly combined gatherings with quantitative information and parametric conveyance was finished by utilizing Repeated ANOVA test. The outcomes were spoken to in tables and graphs. The certainty interim was set to 95%and the safety buffer acknowledged was set to 5%.

Results

The present trial included 23 patients (11 males/12 females) with a mean age (42.54 ± 3.82 years) (Table 1). The two treatment modalities were well tolerated by all participates with no complications. During the study, all patients proceeded with their clinical follow up visits, and showed stable and good oral hygiene standards.

Table 1: The patients' demographic Data.

Total number of Implant Sites	Age (years) Mean ± SD	Sex (M/F)
46		
23/Control 23/Test	42.54 ± 3.82	11-12

SD: Standard deviation, no: number, M: Male, F: Female.

Table 2: Comparing mean changes (± SD) of Pre-implant Probing Depth in the control and test groups.

Total number of Implant Sites	Control group Mean ± SD (mm)	Test group	*P-value*
Baseline	1.90 + 0.21	2.10 ± 0.39	0.174
6 months	2.60 ± 0.46	1.95 ± 0.44	0.005*
P-value	0.000*	0.394	

*Significant difference (p-value<0.05), mm: millimeter.

Table 3: Comparing mean changes (± SD) of Pre-Implant Soft Tissue in the control and test groups.

	Control group	Test group	P-value
	Mean ± SD (mm)		
Baseline	2.55 ± 0.42	2.10 ± 0.38	0.115
6 months	2.15 ± 0.53	2.60 ± 0.41	0.021*
P-value	0.065	0.005*	

*Significant difference (p-value<0.05), mm: millimeter.

Table 4: Comparing the mean radiographic changes (± SD) in the control and test groups during the study.

		Control group	Test group	*P-* value
MBL	6 month	1.66 ± 0.28	0.67 ± 0.14	0.000*
BD	Baseline	477.73 ± 47	460.35 ± 34.38	0.074
	6 month	582.80 ± 31.20	649.33 ± 63.06	0.061
	P-value	0.057	0.051	

*Significant difference (p-value<0.05), mm: millimeter; MBL: Marginal Bone Loss; BD: Bone Density

Table 5: Comparing mean changes (± SD) in the crevicular fluid expression levels of Nitric Oxide in the control and test groups during the study period.

	Control group	Test group	Test value	*P-value*
	Mean ± SD			
Base line	0.003 ± 0.001	0.003 ± 0.001	-0.567	0.578
15 days	0.005 ± 0.001	0.001 ± 0.001	8.083	0.000*
2 month	0.007 ± 0.003	0.001 ± 0.000	7.164	0.000*
Repeated measures ANOVA	19.675	36.247		
	0.001*	<0.001*		

*Significant difference (p-value<0.05).

Concerning PPD changes, the control group reflected a statistically significant reduction in the mean PPD at 6 months (2.60 ± 0.46), (p-value=0.001). On 6 months, a statistically significant difference was revealed between the two groups in favor of the test group, (p-value=0.005) Table 2. The changes in PIST throughout the study were shown in Table 3, there was a statistically significant gain in PIST within the test group at 6months, (p-value=0.005), as well as a statistically significant difference between the two groups at the end of the study in the test group side(p-value = 0.021). Table 4 reflected the radiographic changes during the study course. Regarding the mean changes in MBL, a statistically significant difference was shown between the two groups in favor of the test group (p-value=0.000) at 6 months. A non-significant difference was revealed in BD, at the end

of the study, either between or within the two groups (p-value≥ 0.05).

In Table 5, the mean changes in the crevicular expression levels of NO are present. At baseline, there was no statistically significant difference between the two groups, (p-value=0.578).On the 15th day samples, the mean changes of NO biomarker was (0.005 ± 0.001) for control group and (0.001 ± 0.001) for test group, (p-value=0.0001). Concerning the 2 months-samples, the control group was (0.007 ± 0.003) and (0.001 ± 0.000) for test group with a statistically significant difference has been noted between the two groups (p-value=0.0001).

Discussion

It is worth noting that this was the first clinical trial to supply data about the use of ABG alone or combined with MLN gel in the early loading of dental implant. In this study, the test group resulted in a significant reduction in PPD, a statistically significant gain in PIST, and a statistically significant reduction in MBL; compared to the control group at the study's end. These results were supported by the preliminary findings of Hazzaa et al. [21] who used ABG/MLN composite graft around immediate implants compared with ABG. The authors reported that ABG/MLN is a valuable alternative to augment the immediate in terms of significant better hard and soft tissue parameters.

In our model, the statistically significant lowest mean PPD was shown in the test group at the end of the study. This finding may be related to the larger amount of bone tissue that is in direct contact with the implants and the minimal amount of marginal bone resoption. It may also reflect greater synthesis of bone matrix in the peri-implant area. In this concern, it was reported that MLN increases pre-osteoblast/osteoblast/osteoblast-like cell proliferation, promotes the expression of type I collagen and bone marker proteins (e.g., alkaline phosphatase, osteopontin, bone sialoprotein and osteocalcin), and stimulates the formation of a mineralized matrix in these cells [22].

Regarding the PIST, there was a statistically significant difference within test group at the end of the study. A significant improvement was also revealed between the two groups at 6 months in favor of the test group. This finding is supported by the results of El-Gammal et al. [23], who reported that MLN could keep and pick up the integrity of gingival tissues through increasing collagen, decorin, and interlukin-10 (IL-10) expression and decreasing the matrix metalloproteinase-1/tissue inhibitor of metalloproteinases-1 ratio, through its antioxidant properties.

Considering that RONS were regarded as risk factors for the resorption of ABG, its recorded reduction in our test model would be a responsible factor for decreasing the rate of ABG resorption with better preservation of osseous cells (12). In this respect, our results was compatible with Ladizesky et al., [24] who found that MLN administration may be beneficial in suppressing the effects of free oxygen radicals and regulating antioxidant enzyme (SOD) activity, thereby accelerating bone formation in the fracture-healing. Moreover, Cutando et al., [25] reported that after two weeks of MLN application, a significant increase resulted in the perimeter of bone that was in direct contact with the treated implants, bone density, new bone formation, and inter-thread bone compared with the control group. They also observed an increase in osteoblast proliferation brought about by MLN in the peri-implant zone. They reported that the estimated dose of MLN required to enhance ossointegration of dental implant and minimize the marginal bone resorption is 1.2 mg of melatonin powder for each implant that was in line with the selected dose in this current study.

On comparing both groups in all the time points, the mean changes in the crevicular expression levels of (NO) revealed a statistically significant reduction with respect to the test group. These results are agreed by the recent findings of Huang et al. [26] who concluded that MLN treatment effectively prevented the hypoxia-induced increases of NO production in the hippocampus. Other studies have also shown that MLN can enhance endogenous anti-oxidative defense systems to protect from oxidative injuries [27,28].

NO appears to have biphasic effects on osteoblast activity. In this aspect, in vitro studies have indicated that small amounts of NO that are produced constitutively by osteoblasts may act as an autocrine stimulator of osteoblast growth and cytokine production. Whilst some investigators have shown that slow release of NO donors stimulate osteoblast growth and differentiation [29]. The last mentioned speculation can explain our radiographic results in terms the statistically significant reduction in MBL shown in the test group at the end of the study. This result is also supported by the fact that MLN acts by inhibiting the differentiation of osteoclasts [30] its influence on the Receptor Activator for Nuclear Factor kB Ligand (RANKL) system, suppressing its activity and favoring the formation of new bone. MLN has an additional positive role in new bone formation around implants via the differentiation of new preosteoblasts, which are transported from bone marrow to the alveolar bed [24].

Eventually, although non-significant outcome, the maximum increase in BD was shown in favor of the test group at the end of the study. This finding was in accordance with other researchers [21] who reported that the local administration of MLN around immediate implants results in remarkable improvement in BD when used in combination with ABG. Recently, an experimental report was delivered denoting that the fluorecent light exposure inhibits the endogenous MLN synthesis in rats which can be prevented by MLN administration [31]. Therefore, we can speculate that MLN supplementation to ABG strongly promoted better regenerative events due to its previously mentioned activities.

In ultimate, the current study has the following shortcoming: it is a 6-month clinical trial. Indeed, longitudinal studies are still needed to further understand the effectiveness of the proposed treatment option over time.

Conclusion

In the present study, the test group demonstrated superior clinical, radiographic and biochemical changes. These findings illustrate that the combined use of ABG with MLN is a promising alternative in augmenting the early loaded dental implants for future clinical success.

Acknowledgement

The authors acknowledge the financial support of the Faculty of Oral and Dental Medicine, Al-Azhar University by providing us with the necessary materials used in this research in addition to the technical support.

References

1. Thoma DS, Haas R, Tutak M, Garcia A, Schincaglia GP, H€ammerle CHF. Randomized controlled multicentre study comparing short dental implants versus longer dental implants in combination with sinus floor elevation procedures. Demographics and patient-reported outcomes at year of loading. J ClinPeriodontol. 2015; 42: 72-80.

2. Brunski JB, Moccia AFJ, Pollack SR, Korostoff E, Trachtenberg DI. The influence of functional use of endosseous dental implants on the tissue-implant interface. Histological aspects. J Dent Res. 1979; 58: 1953-1969.

3. Brånemark PI, Hansson BO, Adell R, Breine U, Lindström J, Hallén O , et al. Osseointegrated implants in the treatment of the edentulous jaw. Stockholm Almqvist & Wiksell International. 1977; 16: 1-132.

4. Cochran DL, Morton D, Weber HP. The Early Loading of RBM and SLA Surface Implants Maxillofac Plast ReconstrSurg and recommended clinical procedures regarding loading protocols for endosseous dental implants. J Oral Maxillofac Implants. 2004; 19: 109-13.

5. Vidyasagar L, Apse P. Biological Response to Dental Implant Loading Overloading. Implant Overloading. Empiricism or Science Dental and Maxillofacial Journal. 2003; 5: 83-9.

6. Esposito M, Grusovin MG, Willings M, Coulthard P, Worthington H. The Effectiveness of Immediate, Early, and Conventional Loading of Dental Implants. A Cochrane Systematic Review of Randomized Controlled Clinical Trials. J Oral Maxillofac Implants. 2007; 22: 893-904.

7. Ottoni JM, Oliveira ZF, Mansini R, Cabral AM. Correlation between placement torque and survival of single-tooth implants. J Oral Maxillofac Implants. 2005; 20: 769-776.

8. Smith DE, Zarb G. Criteria for success of osseointegratedendosseous implants. J Prosthet Dent. 1989; 62: 567-572.

9. Moshiri A, Oryan A. Role of tissue engineering in tendon reconstructive surgery and regenerative medicine current concepts, approaches and concerns. Hard Tissue. 2012; 1: 11.

10. Khan SN, Cammisa FP, Sandhu HS, Diwan AD, Girardi FP, Lane JM. The biology ofbone grafting. J Am AcadOrthop Surg. 2005; 13: 77e86.

11. Majzoub Z, Berengo M, Giardino R, Aldini NN, Cordioli G. Role of Intramarrow Penetration in Osseous Repair. A Pilot Study in the Rabbit Calvaria . J of Periodontology. 1999; 70: 1501-10.

12. Koerdt S, Siebers J, Bloch W, Ristow O, Kuebler A, Reuther T. Role of oxidative and nitrosative stress in autogenous bone grafts to the mandible using guided bone regeneration and deproteinized bovine bone material. J CraniomaxillofacSurg. 2014; 42: 560-567.

13. Kalsbeek A, Buijs RM. Output pathways of the mammalian suprachiasmatic nucleus coding circadian time by transmitter selection and specific targeting. Cell Tissue Res. 2002; 309: 109-118.

14. Allegra M, Reiter RJ, Tan DX, Gentile C, Tesoriere L, Livrea M. The chemistry of melatonin's interaction with reactive species. J Pineal Res. 2003; 34: 1-10.

15. Cutando A, Arana C, Gómez-Moreno G, Escames G, López A, Ferrera MJ, et al. Local application of melatonin into alveolarsockets of beagle dogs reduces tooth removal-induced oxidativestress. J Periodontol. 2007; 78: 576-83.

16. Hosseini SH, Kazemian M, Ghorbanzadeh S. A brief overview of cellular and molecular mechanisms of osseointegration. J Contemp Dent Med Rev. 2015; 010415.

17. Abramson J. The Cornel Medical index as an epidemiological tool. Am J Pub Health. 1996; 56: 287-98.

18. Bormann K, Gellrich N, Kniha H, Schild S, Weingart D, Gahlert M. A prospective clinical study to evaluate the performance of zirconium dioxide dental implants in single-tooth edentulous area. BMC Oral Health. 2018; 18: 181.

19. Simonpieri A, Choukroun J, Del Corso M, Sammartino G, David M, Ehrenfest D. Simultaneous sinus lift and implantation using micro threaded implants and leukocyte- and platelet rich fibrin as sole grafting material. Implant Dent. 2011; 20: 2-12.

20. Montgoymery HAC, Dymok JF. The determination of nitrite in water. Analyst. 1961; 86: 414-416.

21. Hazzaa HA, El-Kilani NSh, Elsayed SAB, Abd El MassiehPh M. Valuation of Immediate Implants Augmented with Autogenous Bone/Melatonin Composite Graft in the Esthetic Zone. A Randomized Controlled Trial. J of Prostho. 2017; 28: 637-42.

22. Sethi S, Radio NM, Kotlarczyk MP, Chen CT, Wei YH, Jockers R, et al. Determination of the minimal melatonin exposure required to induce osteoblast differentiation from human mesenchymal stem cells and these effects on downstream signaling pathways. J Pineal Res. 2010; 49: 222-38.

23. El-Gammal MY, Salem AS, Anees MM, Tawfik MA. Clinical and Radiographic Evaluation of Immediate Loaded Dental Implants With Local Application of Melatonin. A Preliminary Randomized Controlled Clinical Trial. Journal of Oral Implantology. 2016; 42: 119-25.

24. Ladizesky MG, Cutrera RA, Boggio V, Somoza J, Centrella JM, Mautalen C. Effect of melatonin on bone metabolism in ovariectomized rats. Life Sci. 2001; 70: 557-65.

25. Cutando A, Gomez-Moreno G, Aran C, Munoz F, Lopez-Pena M, Stephenson J, et al. Melatonin stimulates osteointegration of dental implants. J Pineal Res. 2008; 45: 174-9.

26. Huang C, Lai C, Tsai M, Wu Y, Chen K, Jou M, et al. Effects of melatonin on the nitric oxide system and protein nitration in the hypobaric hypoxic rat hippocampus. BMC Neuroscience. 2015; 16: 61-72.

27. Chang HM, Huang YL, Lan CT, Wu UI, Hu ME, Youn SC. Melatonin preserves superoxide dismutase activity in hypoglossal motoneurons of adult rats following peripheral nerve injury. J Pineal Res. 2008; 44: 172-80.

28. Wu UI, Mai FD, Sheu JN, Chen LY, Liu YT, Huang HC, et al. Melatonin inhibits microglial activation reduces pro-inflammatory cytokine levels, and rescues hippocampal neurons of adult rats with acute Klebsiellapneumoniae meningitis. J Pineal Res. 2011; 50: 159-70.

29. Riancho JA, Salas E, Zarrabeitia MT, Olmos JM, Amado JA, Fernandez-Luna JL. Expression and functional role of nitric oxide synthase in osteoblast-like cells. J Bone Miner Res. 1995; 10: 439-46.

30. Koyama H, Nakade O, Takada Y, Kaku T, Lau KH. Melatonin at pharmacologic doses increases bone mass by suppressing resorption through down-regulation of the RANKL mediated osteoclast formation and activation. J Bone Miner Res. 2002; 17: 1219-1229.

31. Oryan A, Monazzah S, Bigham-Sadegh A. The Effects of Melatonin in Bone Healing. Vet Sci Res. 2018; 3: 000155.