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Clinical and Radiographic Evaluation of Immediate Loaded Dental Implants with Local Application of Melatonin: A Preliminary Randomized Controlled Clinical Trial --Manuscript Draft--

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Abstract:	<p>Introduction:immediate loading of dental implants in situations where low bone density exist such as the posterior maxillary region became possible recently after the introduction of biomimetic agents. This 1-year preliminary clinical trial was carried out to clinically and radiographically evaluate immediate-loaded one-piece implants with local application of melatonin in the osteotomy site as a biomimetic material. Materials and Methods:14 patients with missing maxillary premolars were randomized to receive 14 implants of one-piece type that were subjected to immediate loading after 2 weeks of initial placement. Group I included 7 implants with acid etched surface while group II included 7 implants with acid-etched surface combined with local application of melatonin gel at the osteotomy site. Patients were recalled for follow up at 1, 3, 6, and 12 months after loading. Results:all implants were considered successful after 12 months of follow up.Significant difference ($P < 0.05$) was found between both groups at 1 month of implant loading when considering the implant stability. At one and three months there were significant differences in the marginal bone level between the two groups.Conclusions:These results suggest that the local application of melatonin at the osteotomy site is associated with good stability and minimal bone resorption. However, more studies for longer follow up periods are required to confirm the effect of melatonin hormone on osseointegration of dental implants.</p>
Response to Reviewers:	

**Clinical and Radiographic Evaluation of Immediate Loaded Dental Implants
with Local Application of Melatonin: A Preliminary Randomized Controlled
Clinical Trial**

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ABSTRACT

Introduction: immediate loading of dental implants in situations where low bone density exist such as the posterior maxillary region became possible recently after the introduction of biomimetic agents. This 1-year preliminary clinical trial was carried out to clinically and radiographically evaluate immediate-loaded one-piece implants with local application of melatonin in the osteotomy site as a biomimetic material. **Materials and Methods:** 14 patients with missing maxillary premolars were randomized to receive 14 implants of one-piece type that were subjected to immediate loading after 2 weeks of initial placement. Group I included 7 implants with acid etched surface while group II included 7 implants with acid-etched surface combined with local application of melatonin gel at the osteotomy site. Patients were recalled for follow up at 1, 3, 6, and 12 months after loading. **Results:** all implants were considered successful after 12 months of follow up. Significant difference ($P<0.05$) was found between both groups at 1 month of implant loading when considering the implant stability. At one and three months there were significant differences in the marginal bone level between the two groups. **Conclusions:** These results suggest that the local application of melatonin at the osteotomy site is associated with good stability and minimal bone resorption. However, more studies for longer follow up periods are required to confirm the effect of melatonin hormone on osseointegration of dental implants.

Keywords: One-Piece Implant; Melatonin; Acid-Etched; immediate Loading

INTRODUCTION

Good osseointegration is a prerequisite for immediate loading of dental implants. Optimal osseointegration depends on the formation of new bone around implants, which may be stimulated by the application of biomimetic agents.¹

Considering the bone metabolism, melatonin acts directly on the osteoclast, a multinucleated cell, which resorbs the extracellular matrix through various mechanisms, including the production of free radicals.² Also, melatonin, may interfere with the function of the osteoclast and thereby inhibit bone resorption³.

These actions of melatonin on bone tissue are of interest as it may be possible to apply melatonin during endosseous dental implant surgery as a biomimetic agent. As a result, the healing process may be more precise, initial conditions of receptor tissues may be enhanced, the period of osteointegration and settling of the implant maybe shortened⁴.

Cutando et al,⁵ conducted an experimental study using melatonin with dental implants in dogs. Two weeks after implant insertion, melatonin significantly increased all parameters of osteointegration.⁵

Considering the next step of the application of melatonin in clinical dentistry, the present study is designed to clinically and radiographically evaluate the effect of Melatonin when locally applied in the osteotomy site before one piece implant insertion.

MATERIALS & METHODS

The study followed the Declaration of Helsinki on medical protocol and ethics and was approved by the Regional Ethical Review Board of Mansoura University. This study was performed at the Oral and Maxillofacial Surgery Department, Faculty of Dentistry, Mansoura University from January 2013 to April 2014. Patients with missing maxillary premolars were asked to participate in the study after signing an informed consent.. The selected cases should have adequate amount of bone vertically and horizontally in the edentulous area as well as adequate occlusion. For patient demographic data, please refer to Table (1)

Fourteen single- piece sandblasted-acid etched (SLA) endossous implants (Dentium Co. Ltd Dang-dong, Gunpo-si, Gyeonggi-do, Korea) were used to restore missing upper premolars. Implant length was 10 mm and the width was 3.5 mm. Patients were randomly divided into two groups: group I (study group): patients received implants with local application of melatonin gel, group II (control group): patient received implants without local application of melatonin. Melatonin gel was prepared by mixing the melatonin powder with propylene glycol (1.2/ml) to act as a carrier. According to the study reported by Cutando A et al⁵ the estimated dose of Melatonin required to enhance ossointegration of dental implant and minimize the marginal bone resorption is 1.2 mg of melatonin powder for each implant. In our study, we used 1 ml of 1.2 mg/ ml of Melatonin gel for each implant.

For proper implant placement, surgical stents and preoperative radiographs were obtained .All patients were instructed to use Chlorhexidene 0.12% mouth wash and Amoxycillin 500 mg q.d.s (Emox, Egyptian Int. Pharmaceutical Industries Co., E.I.P.C.O., A.R.E.) or erythromycin500 q.d.s (Erythromycin, Sedico. Egypt.) in case of sensitivity to penicillin for five days starting from the day before the operation.

After local anesthetic infiltration, a mucoperiosteal flap was reflected. Manufacture instructions were followed for the preparation of the implant osteotomy site. A serious of drills used included: 1.8 , 2 and 2.6 mm. For group I, 1 ml of 1.2 mg/ml of melatonin gel was injected in each osteotomy site before the insertion of the implant. For group II, no melatonin gel was injected and implant were inserted directly after the irrigation of the prepared implant site. Figures 1&2

Sutures were placed after flap replacement, immediate preoperative radiographs were obtained and immediate temporary composite crowns were inserted. Patients were instructed to use soft diet and proper oral hygiene measures. Permanent metal fused to porcelain crowns were cemented within two weeks of implant insertion.

Patients were followed up regularly at 3, 6 and 12 months. Periapical standard parallel radiographs were taken and converted to a soft copy. A digital radiographic analysis

(ImageJ software, 1.42q. Wayne Rasband, National Institutes of Health, USA) was done to measure the marginal bone loss (MBL)

The clinical evaluation comprised A. Implant stability using Periotest. B. Gingival health according to the Angulated Bleeding Index (AngBI). A periodontal probe was passed along the buccal margin at 60 degrees angulation in the gingival sulcus. The resultant bleeding was recorded as present (+) or absent (-).⁶

C. Peri-implant propping depth was measured mesially and distally using a graduated periodontal probe.

D. Esthetics: digital photographs were used for evaluation of the pink esthetic score (PES). Charts containing the seven variables were designed. These include: mesial papilla, distal papilla, soft-tissue level, soft-tissue contour, alveolar process deficiency, soft-tissue color and texture. Each variable was recorded with a 2-1-0 score, where 2 is the best and 0 is the poorest score.

The collected data was statistically analyzed in the form of mean \pm Standard deviation (SD). Student's *t*-test: -Used to compare between mean of two groups of numerical (parametric) data. Inter-group comparison of categorical data was performed by using chi square test.

RESULTS

At the end of the one year follow up period, all were implants considered successful

Adequate implant stability was obtained at regular follow up periods for both groups. However, better values of the periotest measurements (BTM) were observed in the study group. There was significantly higher implant stability for group I at one month ($p=0.01$). At subsequent follow up periods, there was no significant statistical difference in the BTM between the two groups in table (1).

The (PES) at the time of permanent crown placement was good for both groups. The mean values of PES for the study and control groups were 10.6 ± 1.13 and 10.14 ± 0.69

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4 respectively. There was no significant statistical difference between the two groups
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6 (p=0.4). Figure(3)
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9 The gingival health was accepted for both groups despite of the recorded two cases with
10 positive (AgBI) values at one month among the control group (p=0.35).
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13 The probing depth measurements for both groups lied within normal limits and they were
14 less than 2 mm which is considered one of the successful criteria of dental implants. The
15 data about mesial and distal probing depth throughout the one year of follow up were
16 showed in table (2). There was no significant difference when comparing the probing
17 depth of the study and control groups.
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23 MBL was measured at 1, 3, 6 and 12 months and all values were within the accepted
24 limit for dental implants. The mean MBL at one month for the study group was
25 0.34 ± 0.12 and 0.98 ± 0.29 for the control group. At three months the mean MBL for the
26 study group was 0.60 ± 0.16 and 1.1 ± 0.41 for the control group. There were significant
27 statistical differences between the two groups at one and three months (p= 0.001 and
28 0.01) respectively. Table (2)
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35 Different evaluation criteria are compared to each other in both study and control groups
36 in table (3). There was a significant difference between the MBL and the depth of the
37 distal pocket in the study group. Also, significant difference was found between the distal
38 pocket depth and the periotest measurements in the control group.
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43 **DISCUSSION**

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45 While several randomized controlled trials and reviews have demonstrated clinical
46 efficiency in shortening the time of loading for edentulous patients,⁸⁻¹¹ it is critical for
47 providing rigid fixation of a dental implant within the alveolar bone and promoting the
48 long-term success of dental implants^{12,13}. One of the most widely investigated topics is
49 searching for novel biomaterials to improve the host/implant tissue response^{14,15}.
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58 Melatonin, by the regulation of RANKL and osteoprotegerin synthesis and release from
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osteoblasts, can induce osteoblastogenesis and at the same time inhibit osteoclastogenesis. In addition, melatonin's free-radical scavenging and antioxidant properties are additional mechanism by which melatonin can maintain the bone health..

Recently, melatonin is used in bone-grafting procedures, in reversing bone loss due to osteopenia and osteoporosis, and in treating periodontal diseases.¹⁶

The present study compared the topical application of melatonin gel at the implant osteotomy site (group I) with those without local application of melatonin (group II).

At one month of follow up, the PTM in group I in the present study were significantly more than the PTM in group II. It is well known that the implant stability is directly related to the percentage of the bone to implant contact area (BIC).¹⁷ Cutando et al.⁵ showed that after a two-week treatment period, melatonin significantly increased the perimeter of bone that was in direct contact with the treated implants, bone density, new bone formation and inter- thread bone.

The same outcome also was demonstrated by Guardia et al.¹⁸ They found that after five- and eight- week treatment periods, melatonin significantly increased the inter-thread bone and new bone formation in comparison to control implants in both weeks. Moreover, in a further study performed by Guirado et al.¹⁹, the results revealed that melatonin plus porcine bone significantly increased the perimeter of bone that was in direct contact with the treated implants.

Our results can be explained by the biological effect of melatonin on bone such as the modulation of bone formation and resorption.²⁰ This could be explained by three principle actions. Firstly, melatonin 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 mineralized matrix formation in these cells ²¹⁻²³.

In addition, melatonin prevents the differentiation of osteoclasts by reducing the expression of RANK mRNA and increases in both the mRNA and protein levels of osteoprotegerin ²⁴. Secondly, melatonin can indirectly regulate bone metabolism via the interaction with systemic hormones (e.g., PTH, calcitonin, and estrogen) or other moleculars. Ladizesky et al. ²⁵ showed that estradiol treatment might prolong the effect of melatonin to enhance bone remodeling in ovariectomized rats. The third mechanism is that osteoclasts create high levels of superoxide anions during bone resorption that is linked to the degradative process. Melatonin can remove these free radicals and protect bone cells from oxidative attacks ²⁶.

An interesting study by Witt-Enderby et al, ²⁷ showed that hormone replacement therapy (HRT) alone increased surface bone, reduced trabecular space, and decreased the number of osteoclasts without affecting osteoblasts. Chronic HRT + melatonin therapy did not significantly increase bone density, although this combination significantly increased Bglap mRNA levels. HRT with or without nocturnal melatonin in cycling mice showed unique effects on bone markers and bone density. The effects of estrogen/progesterone- or melatonin-based therapies alone and combined may enhance the bone health in women in perimenopause and with low nocturnal melatonin levels as a result of too little sleep, too much light, or age. However, in the present study all the female patients were premenopausal and their implants were successful when combined with melatonin application. Further studies are required to compare the effect of melatonin in both perimenopausal, perimenopausal or postmenopausal females.

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7 Our results showed that in both groups, the PTM were decreased at three months of
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9 follow up and then elevated at 6 months to values very close to those observed at one
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11 month. This result suggests that the roughness caused by surface treatment
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13 increases the friction between the implant surface and bone, influencing primary
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15 stability.^{28,29}
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21 Moreover, in preosteoblast cultures from rats, melatonin, in a dose-dependent manner,
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23 promoted the development of bone sialoprotein and other protein bone markers, including
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25 alkaline phosphatase, osteopontin, and osteocalcin, and speeds up their period of
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27 differentiation into osteoblasts from the normal rate which is 21 days to 12 days. This
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29 reaction is mediated by the membrane receptors for the indole²
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33 Another study²¹ showed that melatonin stimulates certain genes which control the
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35 presence of determined proteins at the osteoid matrix. Accordingly, it has been shown
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37 that melatonin is capable, after a period of 5 and 9 days, of stimulating the presence of
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39 osteocalcin, sialoprotein, and alkaline phosphatase. Also, this could be explained by a
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41 greater percentage of inter-thread bone and new bone formation around the implants
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43 treated with topical melatonin.
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48 Interthread bone is an intermediate situation of new bone in contact with the implant by
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50 stimulation of osteoblasts and the old bone in the total periimplant area by osteoclast
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52 inhibition, and melatonin continues its activity passing on from the second to the fifth
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54 week and to the eighth week.^{21,30}
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At one and three months of follow up, the implants in the study group showed a significant less marginal bone loss. This was in agreement with the findings of Guardia et al. 2009³¹. They stated that melatonin acts by inhibiting the action of osteoclasts. This is a direct action lasting for a very short time, as there is no bone remodelling but only the existence of inter-thread bone and total peri-implant bone area. For this reason, melatonin continues to act on a part of the bone that has suffered aggressive placement of the implant, necessitating remodelling by osseous matrix production which requires 5 to 8 weeks.³²

The enhanced ossointegration and minimal bone changes at the first follow up assessment in our study was in the same line with Clafshenkel et al³³ who showed that CA-Mel scaffolds, when compared with CA scaffolds, improved the adhesion, viability, and proliferation of normal human osteoblasts cells but not that of NIH3T3 fibroblasts. Moreover, human adult mesenchymal stem cells grown on CA or CA-Mel scaffolds have a time-dependent induction into osteoblasts over 14 days. The improved MBL at three and six months in the study group continued was also in concomitance with the studies of Clafshenkel et al³³ who showed that the implantation of CA-Mel scaffolds into critical size calvarial defects in female, ovariectomized rats have the ability to allow for tissue infiltration, and scaffold biodegradation by 3 and 6 months.

Regarding the gingival health, no patients in group I showed a positive AngBI while only 2 patients showed a positive AngBI in group II. This was in agreements with Gómez-Florit et al. whom suggested that melatonin could protect and recover the integrity of gingival tissues as it increases collagen, decorin and IL10 expression,

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4 reduces the matrix metalloproteinase-1/tissue inhibitor of metalloproteinases-
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6 1(MMP1/TIMP1) ratio and promotes wound healing without scars, in addition to
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8 its antioxidant properties.³⁴
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11 There was no statistical significant difference between both groups when comparing
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13 the PES at the time of placement of permanent restorations. Both groups showed an
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15 acceptable PES and this may be related to the one stage immediate loaded protocol which
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17 allow undisturbed healing of the soft tissue as well as the immediate provisionalization
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19 which helps the surrounding soft tissue to take the emergence profile around the crown
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21 resampling the appearance of natural gingiva.
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26 Considering the pre-implant pocket depth, there was no significant statistical
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28 difference between both groups at mesial or distal pockets at 1,3,6 and 12 months of
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30 follow up. This can be explained by the one piece implant design and the single stage
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32 surgical protocol. This was in agreement with Garcia et al,³⁵ whom found that there was
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34 no significant difference considering the pocket depth, gingival health and plaque
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36 retention between single stage and two stage implants, there was an advantage of single
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38 stage implants protocol over the other surgical protocol which is the retaining of a band
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40 of keratinized mucosa.
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46 However, the slight decrease in the pocket depth and the significant difference
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48 between the distal pocket depth and marginal bone level that were observed in group I
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50 could be related to the anti-inflammatory and anti osteoclastic activity of melatonin.
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52 Melatonin has anti-inflammatory effect, free radical scavenger and stimulator of
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54 antioxidant enzymes .Also, melatonin levels are correlated with the periodontal health
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56 status indicating that it may have a protective role in periodontal disease. Moreover, it
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has antibacterial effects against gram-positive and gram-negative bacteria by decreasing the availability of intracellular substrates, such as free iron and fatty acids.³⁶

Also the decrease in pocket depth in our study group can be explained according to Srinath R et al whom revealed that melatonin was expressed in the gingival crevicular fluid (GCF). Salivary and GCF melatonin levels decreased in patients with periodontitis compared to their levels in healthy patients suggesting that melatonin may protect from periodontal diseases.^{36, 37}

CONCLUSION

Within limitation of this study as the small sample size and early follow up we can conclude that the topical application of melatonin at the osteotomy site at the time of implant placement is associated with good implant stability and minimal marginal bone loss which are indicators of a good oseointegration.

The topical melatonin application could be a good treatment option for immediate loaded dental implants especially in posterior maxilla. We recommend further studies in the future with long time follow up and in a wide scale.

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Figure captions list:

Fig. 1. Series of photographs for OPI placement with local application of melatonin gel and follow-up of digital standard periapical radiographs. A, preoperative panoramic radiograph B, A photograph shows a raised flap at the implant premolar site. C-F shows the implant placement and local melatonin application. G, immediate post insertion radiograph. H, photograph shows final prosthetic restoration in place and in occlusion, 2 weeks after initial implant placement. I-L, Periapical radiographs show levels of MBL around the implant after 3, 6, and 12 months, respectively.

Fig. 2. Series of photographs for OPI placement without local melatonin application and follow-up of digital standard periapical radiographs. A and B, A perioperative photograph and flap reflection. C-H, photographs show the steps of implant placement. I& J photograph and a radiograph show the implant in place after initial placement. K, A photograph shows final prosthetic restoration in place and in occlusion, 2 weeks after initial implant placement. L-O, Periapical radiographs show levels of MBL around the implant after 3, 6, and 12 months, respectively.

Fig.3. The pink esthetic score for Study and control groups.

Table (1):

	Age		Gender		Menopausal		Smoking		Systemic disease	
	<30Y	>30Y	Male	Female	Pre	Post	smoke	non	with	without
	7	10	5	9	9	0	0	14	0	14
Received Melatonin	2	5	3	4	4			7		7

Table (2)

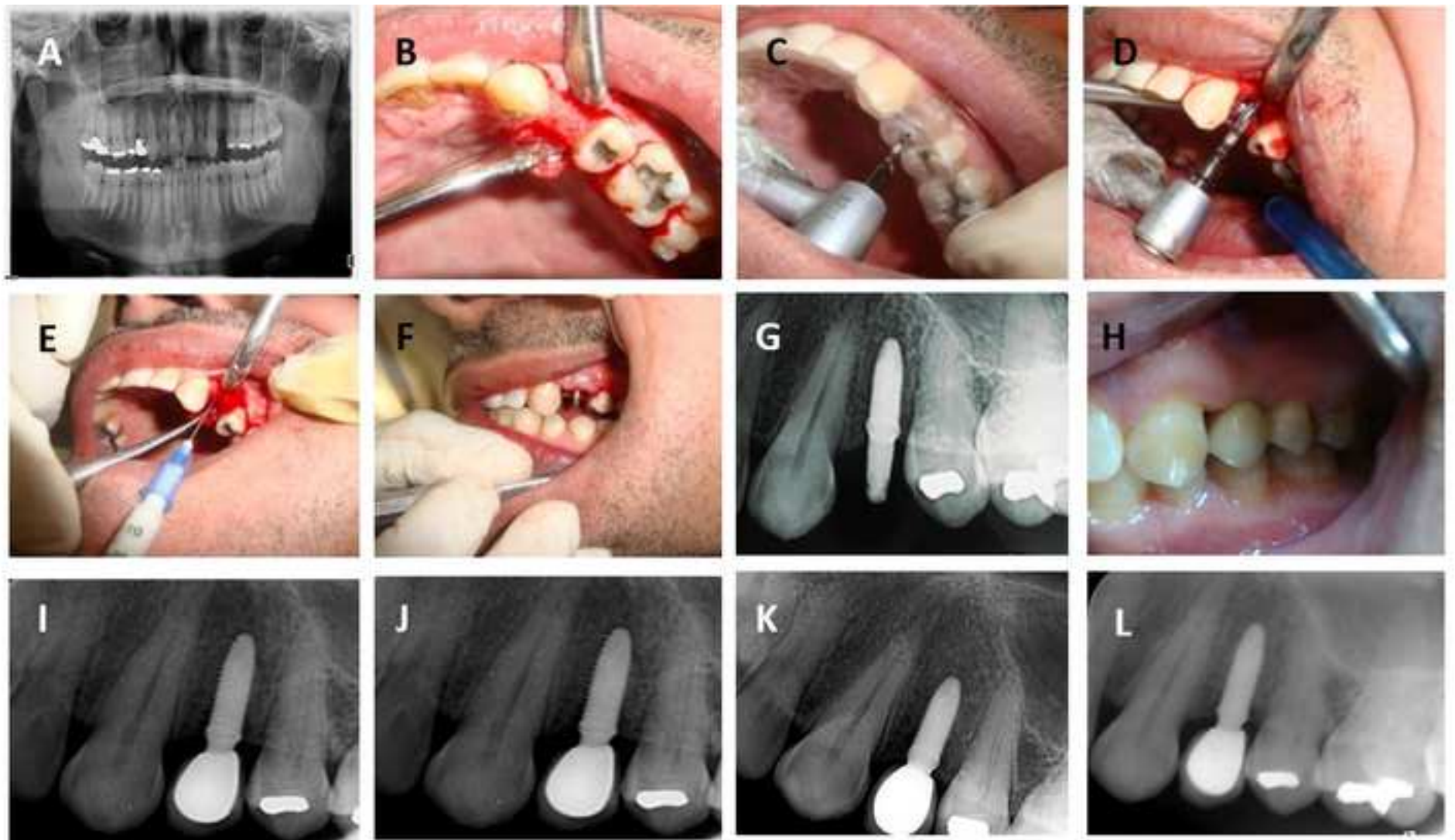
	Periotest measurements (PTM)			Mesial probing depth			Distal probing depth			Marginal bone level		
	Study group (n=7)	Control group (n=7)		Study group (n=7)	Control group (n=7)		Study group (n=7)	Control group (n=7)		Study group (n=7)	Control group (n=7)	
	Mean \pm SD	Mean \pm SD	P	Mean \pm SD	Mean \pm SD	P	Mean \pm SD	Mean \pm SD	P	Mean \pm SD	Mean \pm SD	P
1 month	- 1.28 \pm 0.76	0.85 \pm 1.77	0.01	0.90 \pm 0.19	1.00 \pm 0.31	0.4	1.1 \pm 0.19	0.85 \pm 0.24	0.054	0.34 \pm 0.12	0.98 \pm 0.29	0.001
3 months	0 \pm 0.58	0.42 \pm 0.78	0.2	1.35 \pm 0.24	1.50 \pm 0.41	0.4	1.14 \pm 0.24	0.94 \pm 0.09	0.054	0.60 \pm 0.16	1.1 \pm 0.41	0.01
6 months	- 1.71 \pm 1.25	-1.5 \pm 0.77	0.38	1.54 \pm 0.31	1.57 \pm 0.35	0.88	1.5 \pm 0.17	1.35 \pm 0.24	0.26	1.05 \pm 0.33	1.35 \pm 0.56	0.2
12 months	- 2.14 \pm 1.57	-2 \pm 0.58	0.45	1.67 \pm 0.32	1.78 \pm 0.39	0.4	1.75 \pm 0.25	1.64 \pm 0.19	0.36	1.38 \pm 0.43	1.57 \pm 0.57	0.27

Table (3)

Groups				Mesial pocket depth	Distal pocket depth	Marginal bone level
	Study group	Periotest	P	0.945	0.057	0.563
		Mesial pocket depth	P		0.116	0.424
		Distal pocket depth	P			0
	Control group	Periotest	P	0.972	0	0.095
		Mesial pocket depth	P		0.505	0.098
		Distal pocket depth	P			0.292

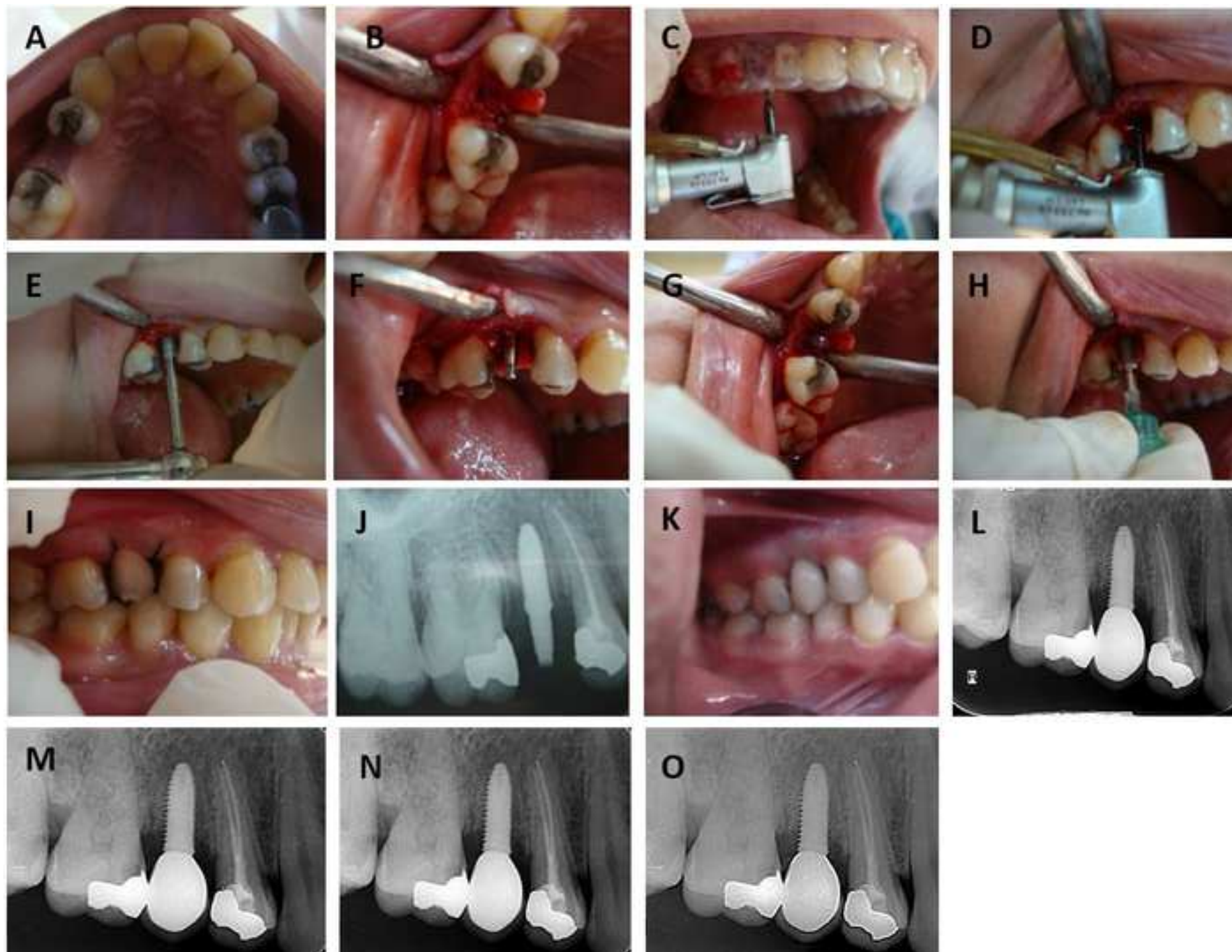
Figure

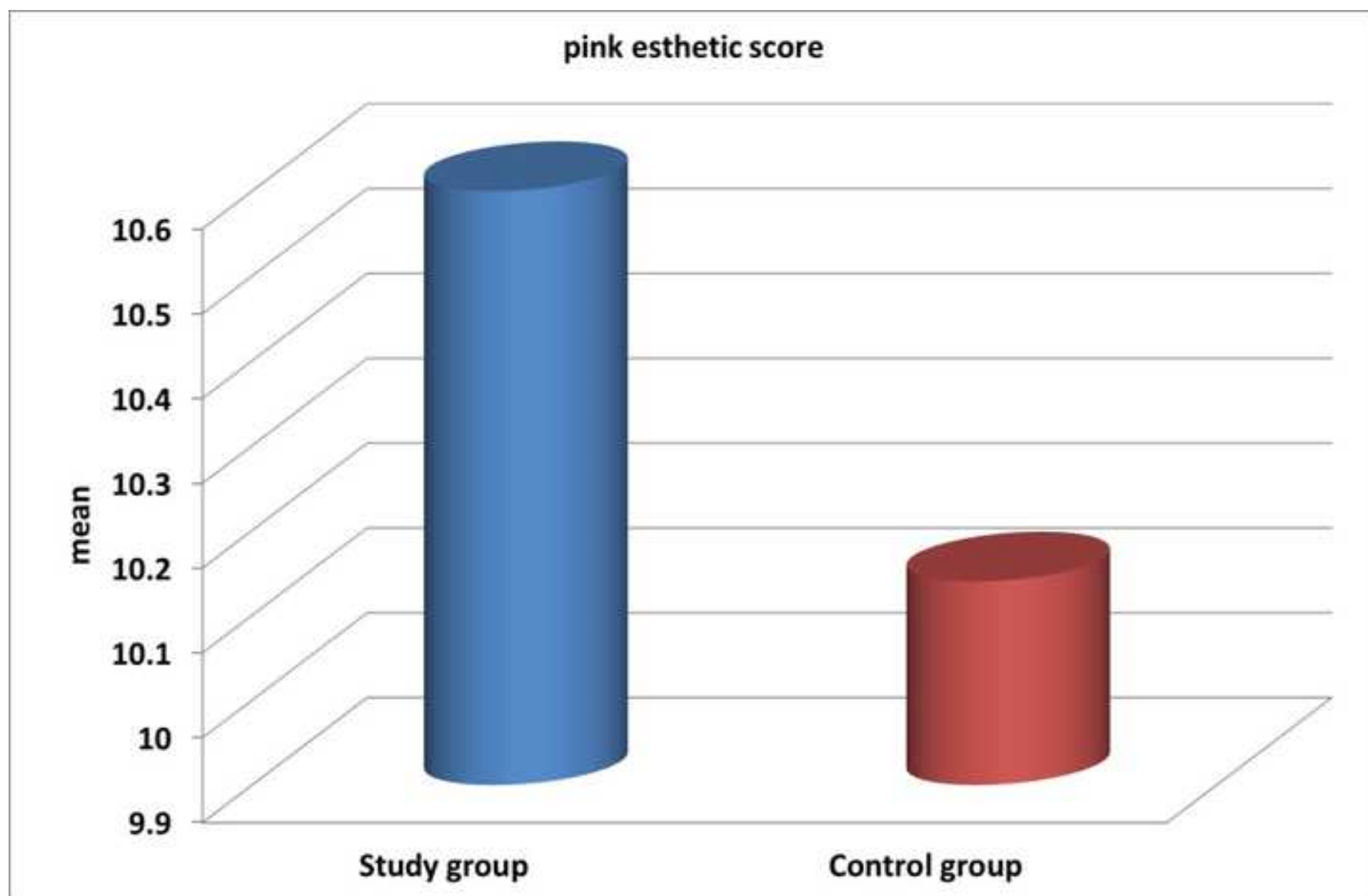
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Figure

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