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Effect of Laser Irradiation on Bony Implant Sites in Diabetic Patients: A Preliminary Study.

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ABSTRACT

The aim of this work was to evaluate the effect of the low intensity laser radiation on bone density around dental implants in diabetic patients. Sixteen implants were placed in the maxilla of eight controlled diabetic patients. Each patient received two implants, one on the right side in which the implants were left to heal without any intervention [control], and the other on the left side, in which the patients received Gallium arsenide soft laser. A core bone biopsy was also taken for histopathological analysis. Postoperative digital panoramas were taken after two weeks, three and six months postoperatively. Changes in bone density along the zones of bone-implant interface [zone 1] and the bone surrounding zone 1 [zone 2] were assessed using the IDRISI Kilimanjaro software. Bone area percentage and bone cells' count were measured using the image analyzer. The bone density along zone 1 was higher than that of zone 2 irrespective of the laser effect with no statistical significance. A statistically significant increased difference with regard to the rate of bone density was observed between zone I and zone II favoring the laser group. Conclusion: The low intensity laser irradiation promoted bone healing and enhanced osseointegration emphasizing the laser's biostimulatory effect.

Keywords: laser, implants, bone, osseointegration, diabetes

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INTRODUCTION

Dental implants have become an ideal means for replacing tooth loss, where osseointegration is becoming the most accepted phenomenon for success in implants' procedures. However, many patients who need dental implants may have one or more systemic disease that can often lead to failure of the implant prosthesis. Diabetes mellitus (DM) is one of those diseases that can influence the success rate of dental implants [1].

DM is a group of chronic metabolic disorders characterized by persistent hyperglycemia. Type 2 DM is characterized by variable degrees of insulin resistance, impaired insulin secretion and increased glucose production. Many systemic complications associated with DM can influence the osseointegration process [2]. The chronic hyperglycemia associated with DM enhances the oxidative stress and amplifies the inflammatory events thus affecting the response of bone and connective tissue to injury [3]. DM negatively affect bone metabolism as a result of imbalance between bone formation and resorption causing decreased number of osteoblasts and loss of bone density [4]. The negative impact of DM on bone repair has encouraged the search for alternative therapy to encounter the effects of DM on bone metabolism [5].

DM is no longer considered to be a contraindication for implant-supported prostheses, provided that the patient's blood sugar is under control, and that there is motivation for oral hygiene procedures [6]. The clinical outcome of dental implants in well – controlled type 2 diabetic patients was found to be satisfying and encouraging [7]. However when compared with the general population, a higher failure rate is seen in diabetic patients during the first year of functional loading ,owing to the microvascular complications of this condition . When placing implant in diabetic patients, patient selection should be exceptionally strict and include only well-controlled patients who demonstrate excellent compliance. Preoperative prophylaxis, aseptic technique, a traumatic tissue handling, and frequent close follow-ups are mandatory [8].

The effect of Low level laser therapy (LLLT) on bone regeneration has become a focus of recent research. It may act as an inducer factor that improves vascularization, anti-inflammatory effect and enhanced collagen synthesis and cell proliferation, which in turn would accelerate bone healing [9]. Previous studies investigated the beneficial action of LLLT in enhancing the regeneration of alveolar bone and soft tissues thus promoting the bone peri-implant hard & soft tissue healing. LLLT demonstrated also a positive effect on the percentage of newly formed bone in rabbit mandibles that underwent distraction osteogenesis [10, 11].

The aim of this study is to assess radiographically the effect of low level laser therapy on the Stability of delayed implant placement in diabetic patients.

MATERIALS AND METHODS

Patient selection

Eight controlled type II diabetic patients with age ranging from 35-55 years old were recruited in this study. They were selected from the outpatient clinic of Oral and Maxillofacial Surgery Department, faculty of Oral and Dental Medicine, Cairo University. All patients needed fixed restoration in anterior or premolar maxillary region. The study has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans. All patients signed an informed consent before enrollment and the protocol was approved by the Ethical Committee of the National Research Centre and each patient signed a consent form before enrollment. Inclusion criteria were absence of any other systemic diseases other than type II diabetes, Missing teeth in the maxillary anterior or premolar area with the adjacent teeth free from periapical pathology, with sufficient bone volume in the receptor site to accommodate for implant length and diameter. Patients were non- smokers, with good oral hygiene or at least the ability to improve it.

Preoperative preparation

A complete medical and dental history together with a preoperative panoramic X-ray was taken for each patient and blood glucose level was measured to assure that all patients were controlled diabetics. A detailed oral and general examinations and a thorough scaling and root planning was done for all selected patients. Patients were instructed to use hexitol (Cholorohexidine HCL, Arab Drug Company, Cairo, A.R.E.) mouth wash twice daily and take Augmentin (Amoxicillin 875 mg and clavulanic acid 125 mg, GlaxoSmithKline, Cairo, A.R.E) 1gm tablet one hour preoperatively.

Study design

Sixteen endosseous root form dental implants (Dentium, Gwanggyo-ro, Yeoungtong-gu, Suwon-si, Gyeonggi-do, Korea) were placed in eight controlled diabetic patients. Each patient received two implants, one on the right side (Non-laser or control group) and one on the left side (Laser or study group). The implants used were of a submerged type with a tapered design, double threaded, platform switching , sandblasted with large grit and acid etching and all implants used were of length ranged from 12-15 mm and diameter ranged from 3.5-4.5 mm. In the control group, the healing phase was left to progress spontaneously without any intervention, while in the laser group healing phase was augmented with LLLT.

Surgical procedure

A gingival incision was performed through inter dental papillae of the teeth neighboring the edentulous area and connected by crestal incision deep to the alveolar bone over the edentulous area. The flap was then elevated buccally and slightly palatally and trephine bur was used to penetrate the alveolar crest. Bone core biopsy was taken before dental implant placement for histopathological examination. Drilling was accompanied with copious external irrigation, and enlarged sequentially by a series of gradually increasing drills, to a dimension that precisely conforms to the dimensions of the required implant size. The implant was then inserted in the bone by hand driven screw tightened with ratchet wrench. Insertion of the covering screw was performed after copious saline irrigation. The screw was adapted in the implant by using screw driver. After the implant was placed to the final position, the flap was closed by a tension free closure using 3-0 black silk suture to prevent wound dehiscence.

Patients were instructed to use extraoral cold application to the surgical area (10minutes/30minutes) for (4-6 hours) to reduce the postoperative edema, and take Augmentin 1gm capsule every 8 hours, Amrizole 500 mg tablet (Metronidazole, Amrya Pharm. IND., Alexandria - Egypt.) every 8 hours and Brufen 400 mg tablet (Ibuprofen, Kahira Pharm. & Chem IND. Co, Cairo, egypt) every 8 hours for one week postoperatively. Starting from the second postoperative day, the patient will apply extraoral hot application to the surgical site and use warm saline and Hexitol mouth washes 5 times daily till recall after 7-10 day for suture removal. Group II patients were instructed to attend the low intensity laser application sessions 3 months and 6 months postoperatively). Radiographic follow up was done 2 weeks, 3 months and 6 months postoperatively. After six months the patients were recalled again to expose the implant by crestal incision and prosthetic part was fabricated

Laser application

Ga A1As diode laser device (Optodan, Saratovskaja provinces, Saratov, Russia) was used in the present study with a wavelength of 904 nm in contact mode, continuous wave and spot diameter of 4 mm. The laser device was adjusted to deliver an output power of 0.02 watt and exposure time 30 seconds at dose 4.7 J/cm^2

During the pre-adjusted time (five minutes), the buccal, the palatal as well crestal surfaces were allowed to receive the laser beam. The laser beam was continuously delivered from the laser probe exposing the target surface with the laser probe touching the tissues gently and directed towards the implant site. The laser probe was moving in a continuous slow circular motion to assure full exposure of the target surface to the laser beam.

Four laser sessions were performed two weeks before implant placement (two sessions per day with one hour rest period in-between on two consecutive days) and the four sessions were repeated every two weeks during the six months follow up period, starting 14 days after implant placement.

Histopathologic assessment:

Specimen preparation for microscopic examination:

Bone core biopsy was immediately fixed in 10% formalin, and then decalcified in acid (EDTA) for 4 days. After decalcification, tissue blocks were routinely processed and embedded in paraffin. Five µm sections were perpendicular to the long axis, mounted on glass slides, deparaffinized, hydrated and stained with Hematoxylin and eosin stain for histological evaluation and histomorphometric analysis.

Measuring of the area percent of newly formed bone and the number of osteocytes or osteoblasts:

This was done with the help of the Leica Quin 500 analyzer computer system (Leica Microsytems, ltd , Heerbrugg, Switzerland) The cursor was used to outline the areas of newly formed bone trabeculae, which were then masked by a binary color that could be measured by the computer. The image analyzer is calibrated automatically to convert the measurement units (pixels) produced by the image analyzer program into actual micrometer units. Similarly the cursor was used to point at the designated cell (osteoblast or osteoclast), and then cells were automatically count by the computer system. Osteocytes are entrapped in lacuna within the bone trabeculae, whereas osteoblasts are located at the borders of newly formed bone. The area percent of newly-formed bone and the number of osteoblasts and osteocytes were estimated in 2 different fields in each patient using magnification (x200). Mean values were calculated for each group.

Radiographic Assessment

Immediate postoperative periapical radiographs were taken to evaluate the position of the implant in relation to the adjacent anatomical structures. Then direct digital panoramic radiographs for all patients to allow for radiometric and radio-densitometric evaluation of bone around the implant after 14 days, 3months and six months postoperatively.

Radio-densitometric evaluations were done using IDRISI Kilimanjaro software (IDRISI 14.01 Kilimanjaro software, Clark Labs, Clark University, Worcester MA.USA.) Digital image processing and bone density calibration were carried out by quantifying the image on 250 gray scales and dividing the bone around the implant into two zones. Zone 1 comprised the implant-bone interface representing the osseointegration zone. Zone 2 comprised the area around Zone 1 and represented the bone surrounding the implant.

Statistical Analysis

Numerical data were explored for normality by checking the data distribution and using Kolmogorov-Smirnov and Shapiro-Wilk tests. Data was calculated for zones one and two in all groups. Bone area percentage (%) data showed parametric distribution while Osteocytes and Osteoblasts counts were treated as non-parametric data. Paired t-test was used to study changes by time and compare between bone area percent of the two groups. Wilcoxon signed-rank test was used to compare between Osteocytes and Osteoblasts counts in the two groups. The significance level was set at $P \leq 0.05$. Statistical analysis was performed with IBM® SPSS® Statistics Version 20 for Windows (® SPSS, Inc., an IBM Corporation, NY, USA).

RESULTS

Clinical results

All patients attended the follow up period recalls in time till the end of the study (6 months post operatively). The post-operative healing period for all patients showed no evidence of post surgical infection, although mild edema was detected after the surgery and was resolved within 10 days post-operatively.

Radiographic Results

A-Comparison between the mean bone densities of zone [1] &zone [2] in both groups:

Results of the Student's t-test revealed no statistical significant difference between the mean bone densities of zone 1 and zone 2 in both laser and control group at all follow-up time intervals, where the P value in the control group was 0.989 , 0.1, and 0.969 at 14 days, 3 months and 6 months postoperatively , while the P value in the laser group was 1.00, 0.975 and 0.988 at 14 days, 3 months and 6 months postoperatively (Tables 1 & 2).

B- Changes by time in each group:

Results of the paired t- test revealed an obvious increase in bone densities with time, which was higher in the laser than in the control group. When the density values of the first radiograph taken after 14 days was compared to that of the first follow-up radiograph taken after 3 months, a mean percentage difference of 7.99 was recorded in the laser group which was higher than that of the control group at 3.76 mean difference. Also when comparing the bone density values between the first and second follow-up radiographs at 3 and 6 months postoperatively the mean percentage difference in the control group was 5.264 which was lower than that of the laser group demonstrating a 7.733 mean bone density. Similarly when the mean bone densities of the radiograph taken at 14 days was compared to the mean bone density of that taken at 6 months follow up period, the laser group showed a higher mean bone density difference than the control group being 16.359 for the laser group and 9.228 in the control group [Table 3].

Histological Results

The Computer count of osteoblasts and osteocytes in both groups demonstrated an increase in the number of the cells in the laser group in comparison to the control group. Also, the area percent of bone trabeculae has increased in the laser group in comparison to the control group. Descriptive statistics of bone area % osteocytes' and osteoblasts' count and values are presented in Table [4]. Control group showed statistically significantly lower mean bone area % , osteocytes' and osteoblasts' count than the laser group.

Table (1): Showing comparison between the mean values of zone 1 & zone 2 in the control group.

	Zone	Mean	Std. Deviation	P value
14 days postoperative	1	138.530	15.469	0.989
	2	138.404	15.362	
3 months postoperative	1	143.614	15.463	0.100
	2	143.612	15.156	
6 months postoperative	1	151.864	14.774	0.969
	2	150.195	14.411	

*: Significant at P ≤ 0.05

Table (2): Showing comparison between the mean values of zone 1 &zone 2 in the laser group.

	Zone	Mean	Std. Deviation	P value
14 days postoperative	1	144.375	13.863	0.100
	2	144.371	13.726	
3 months postoperative	1	155.850	12.793	0.975
	2	155.615	12.705	
6 months postoperative	1	167.548	10.016	0.999
	2	167.456	9.948	

*: Significant at P ≤ 0.05

Table 3. The percent changes with time in control and laser groups.

	Group	Mean	Std. Deviation	P value
14 days with 3 months	Control	3.760	0.920	0
	Laser	7.990	1.962	
3 months with 6 months	Control	5.264	1.251	0.01
	Laser	7.733	2.788	
14 days with 6 months	Control	9.228	2.022	0
	Laser	16.359	4.266	

*: Significant at P ≤ 0.05

Table 4. Bone area percentage, osteocytes' and osteoblasts' count and values in control and laser groups

	Group	Mean	SD	Median	Min	Max	95% CI		*P*-value
							Lower bound	Upper bound	
Bone area %	Control	37.9	4.2	39.6	31.2	42.1	33.5	42.3	0.004*
	Laser	57.4	10.1	55.0	44.5	71.8	46.8	68.0	
Osteocytes	Control	30.8	3.5	31.0	25.0	36.0	27.1	34.6	0.026*
	Laser	42.7	5.4	42.0	35.0	51.0	37.0	48.4	
Osteoblasts	Control	23.5	2.7	23.0	20.0	28.0	20.6	26.4	0.028*
	Laser	32.8	4.4	32.0	27.0	40.0	28.2	37.5	

*: Significant at P ≤ 0.05

Fig.1: IDRISI Kilimanjaro software assessing density of the bone surrounding the implant by quantifying the image on 250 gray scales and dividing the area surrounding the implant into two zones.

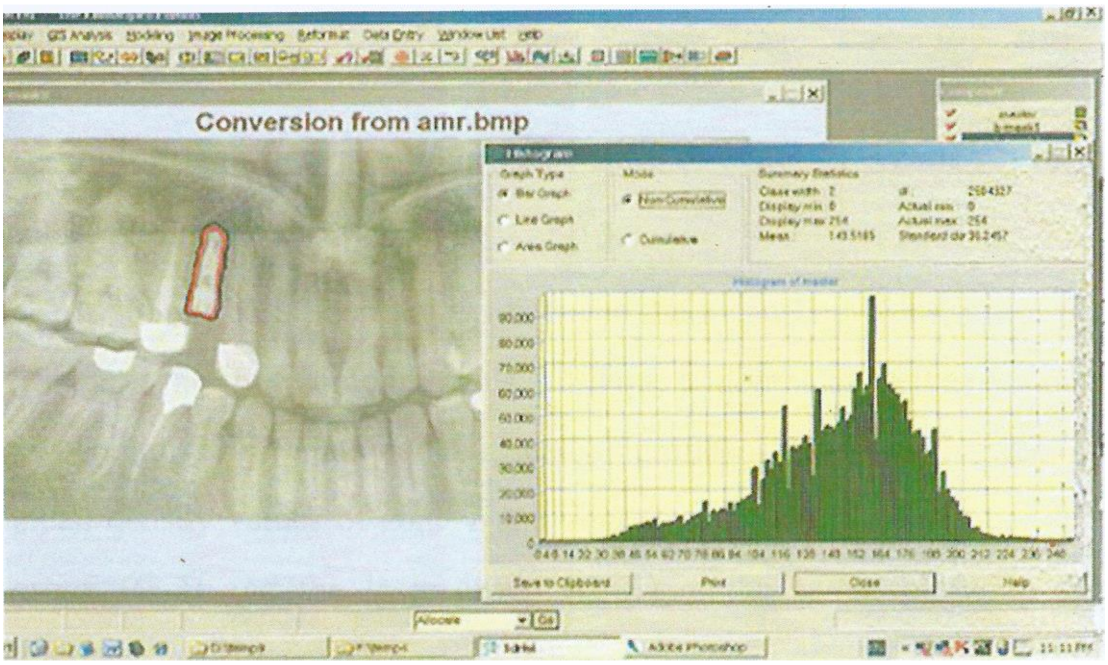


Fig. 2 : Comparison between mean bone densities of Z1 and Z2 in control and laser groups at different time intervals

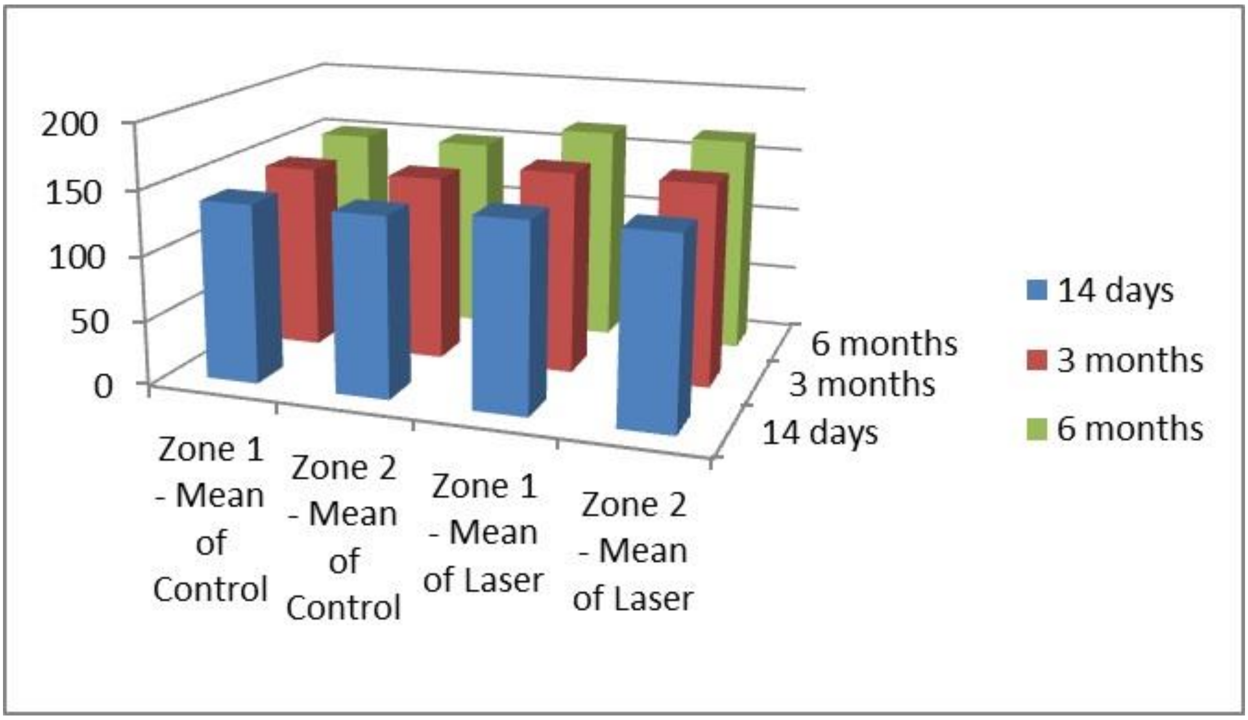


Fig.3: Comparison between percentage increase in bone density with time in laser and control groups at different time intervals

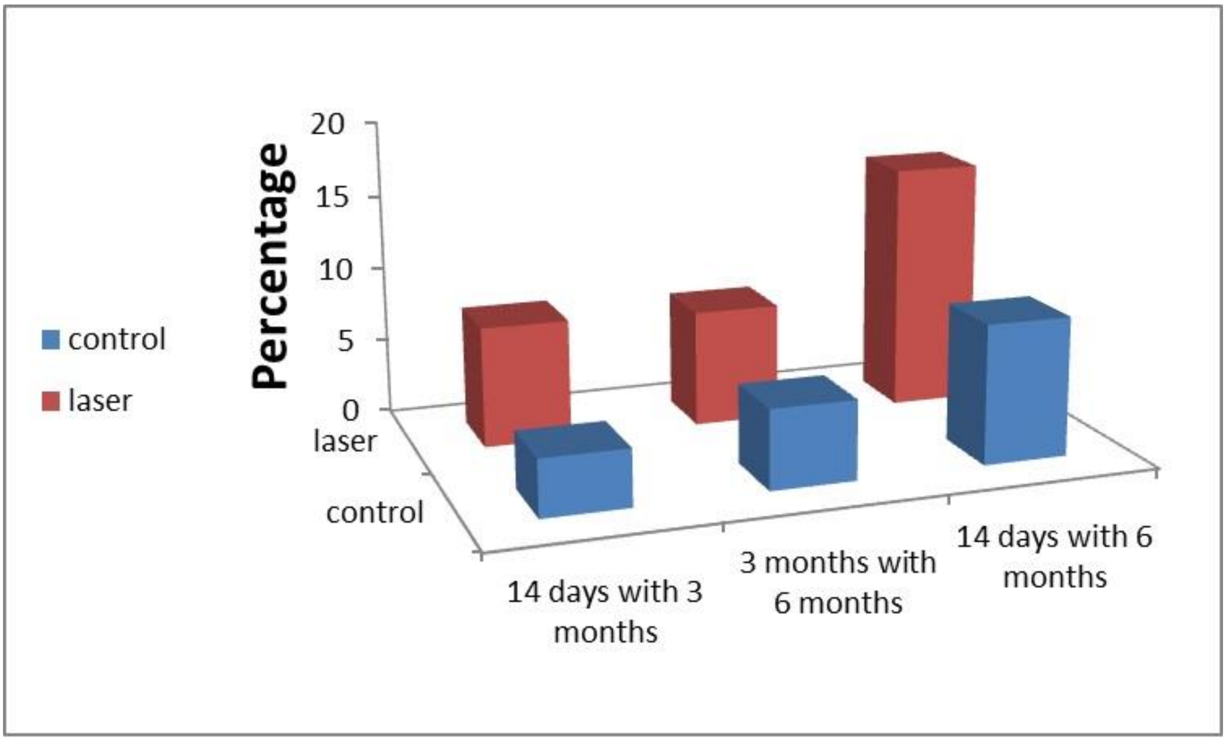


Fig.4: Photomicrograph showing bone trabeculae with entrapped osteocytes (big arrow) and osteoblasts (small arrow) rimming the surface of bone

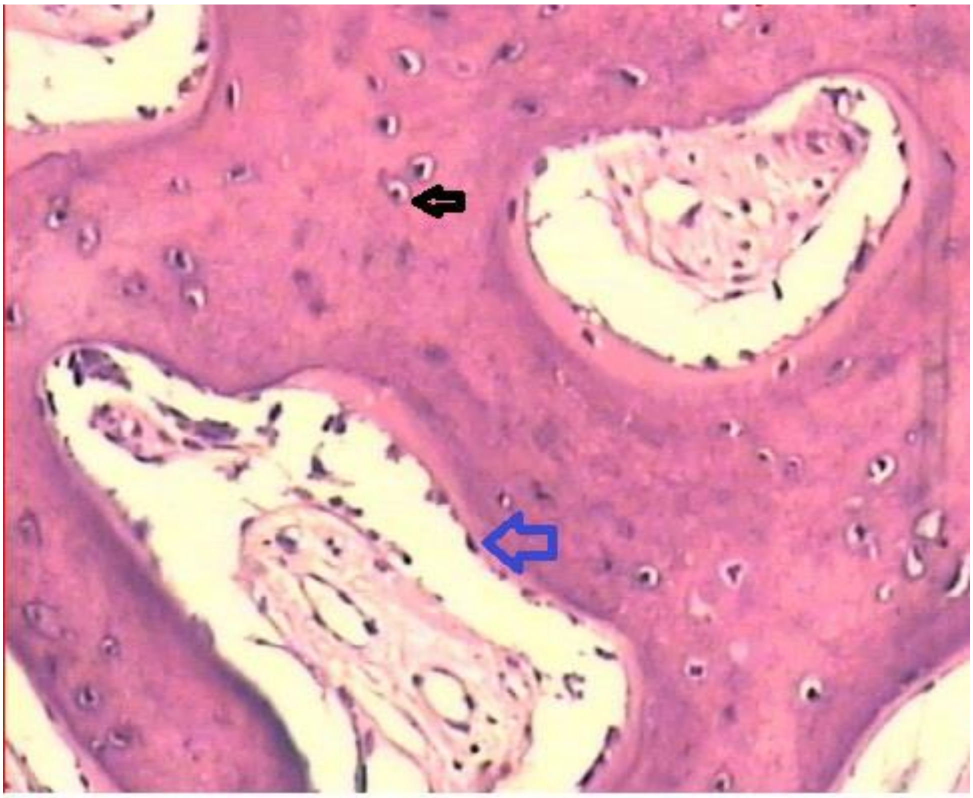


Fig.5: Photomicrograph showing selection and counting of the osteoblasts in the laser group by the computerized image analyzer .

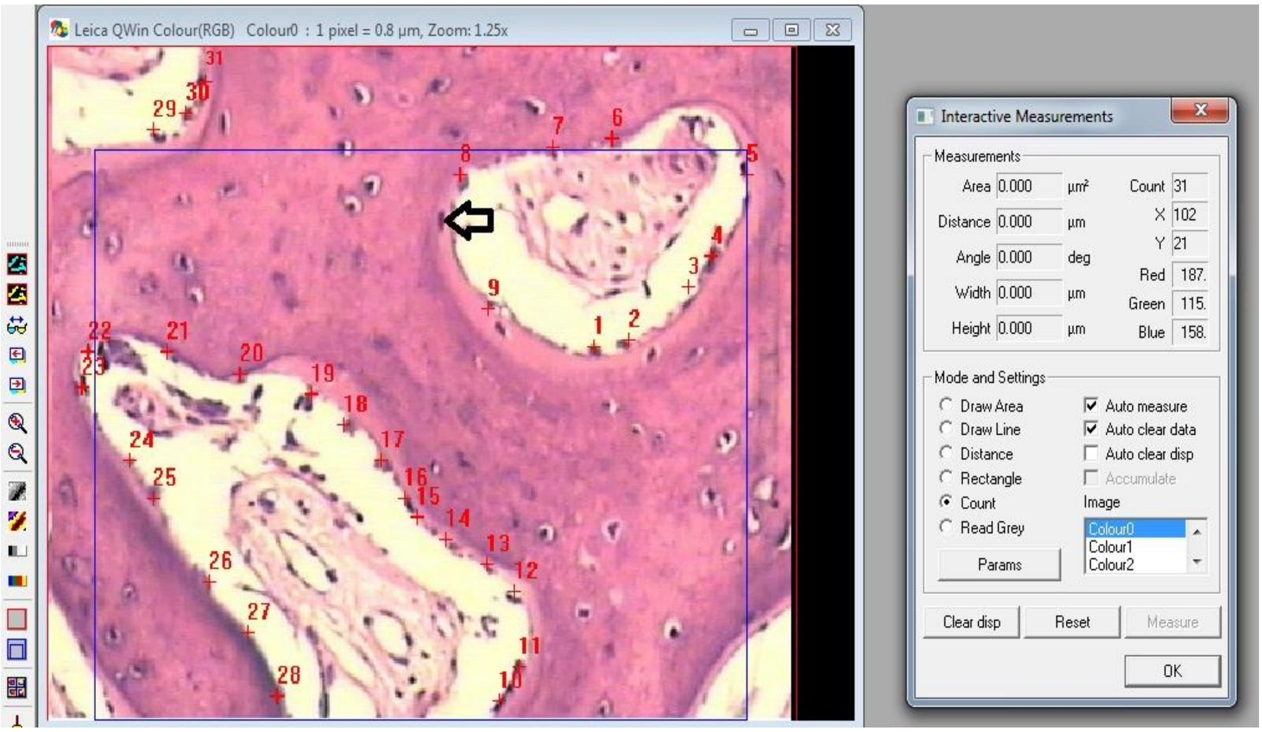


Fig.6: Photomicrograph showing selection and counting of the osteocytes in the control group by the computerized image analyzer

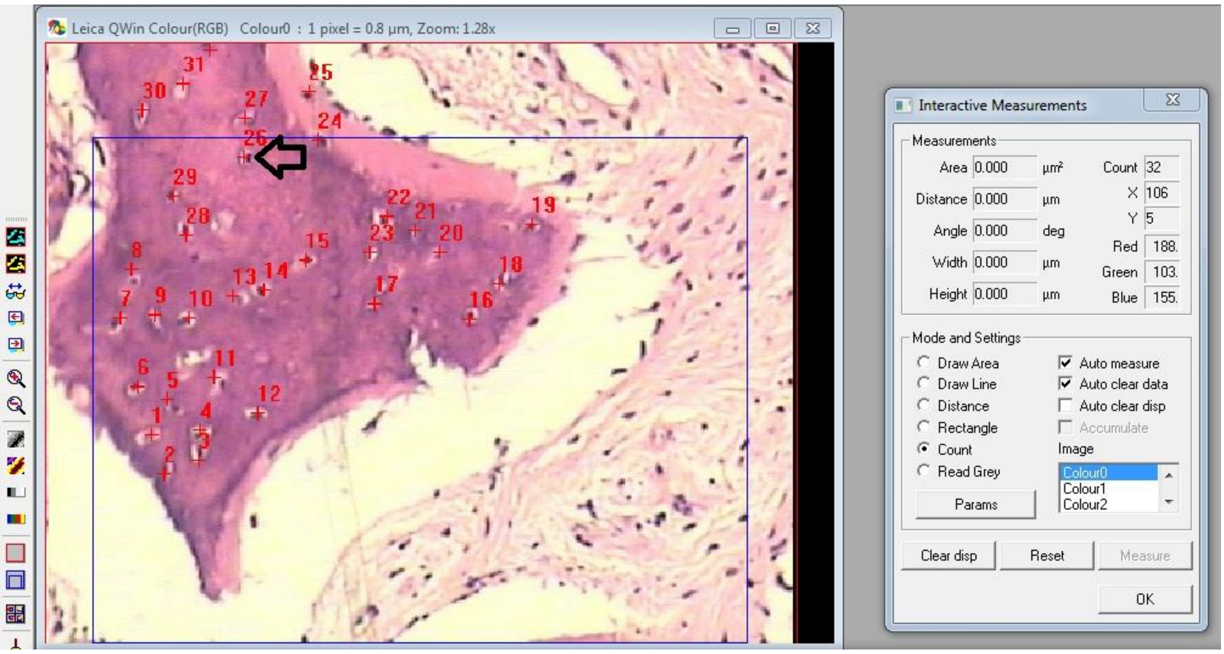
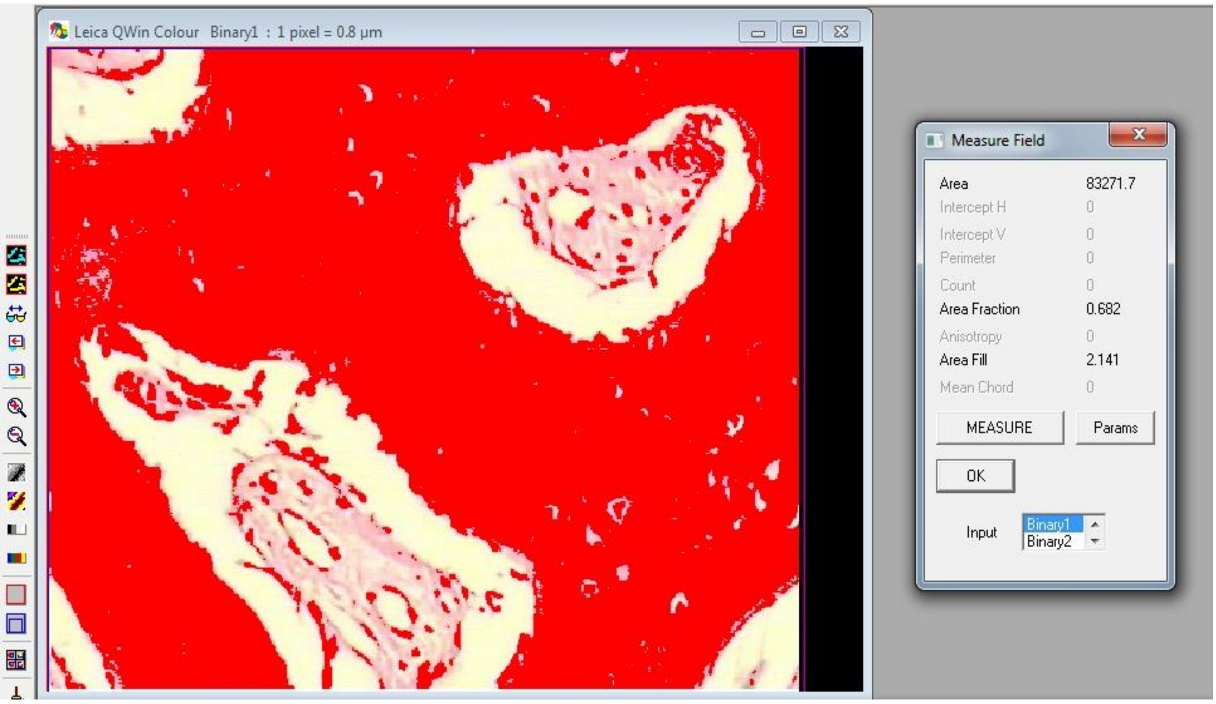


Fig 7: Photomicrographs showing the area of bone trabeculae marked and measured by the computerized image analyzer .



DISCUSSION

Dental implants have proved a valuable therapy for diabetic patients. However many consider diabetes to be a risky condition as implants can fail because of defects in osseointegration or premature loss of the implants [12]. Because LLLT has been known to have a biostimulatory effect enhancing the healing process and accelerating bone regeneration, this study attempted to investigate the effect of Gallium laser irradiation on the bone density in diabetic patients.

A gentle surgical protocol was applied during insertion of implants, to minimize crestal bone loss [13]. Dentium implant system was used in the present study because the surface of the implant is sand blasted and acid-etched, which increases the implant surface roughness thus increasing bone-implant interface and promoting osseointegration [14].

A 904 nm Gallium arsenide laser with adjusted power of 0.02W was used in the present study and exposure time of 30 sec. The effect of LLLT with a wavelength ranging between 670 to 1064 nm on bone tissue was investigated in many dental and medical fields. Laser irradiation in the infrared lesion (904 nm) has been recommended for bone rehabilitation therapy as it was demonstrated to have a low absorption coefficient and hence can penetrated deeper into the tissue , increasing the resistance and volume and enhancing bone regeneration. Moreover the strongest biomodulatory effects were observed at exposure time ranging from 30-120 seconds [15-18].

The dose of irradiation was 4.7 J/cm^2 that was in accordance with a previous studies that recommended doses ranging from 1.8-5.4 J/cm2. When the effect of low laser irradiation was studied on the repair of standardized bone defects in rats with inorganic bovine graft, radiation was given at four points around the defect where each point received a dose of 4 J/cm^2 (Ø-0.6 mm, 40 mW) . Histological results revealed enhanced bone repair in the irradiated group when compared to the non-radiated one as evidenced by enhanced bone and collagen formation around the graft at 15 days after surgery [19,20].

In the present study the bone density values along the zone of osseointegration (zone 1) as well as the surrounding bone (zone 2) were investigated to determine the extent of laser biomodulation. Image analysis was performed using the IDRISI Kilimanjaro software , as it provides a unique facility for image restoration, enhancement transformation and classification and was therefore used in monitoring the changes in bone density at two zones around implant images.

Although the mean bone density was higher in zone 1 than zone 2, no statistical significant difference was found between both zones at all follow-up time intervals. However when the mean bone density values of both zones were compared between both sides a statistical significant increase in bone formation was found in the laser side when compared to the control side. Moreover histological results revealed higher mean bone area percentage and increased osteocytes' and osteoblasts' count in the laser group when compared with the control group.

The findings of the present study are in agreement with previous results demonstrated LLLT to have a stimulatory effect on the proliferation and differentiation of osteoblasts and bone formation, thus enhancing bone regeneration and apposition as well as implant osseointegration [21,22]. The effect was more profound along the osseointegration zone (Zone 1) due to the fact that laser exhibits an enhanced effect at sites that show inflammation, regeneration and high cellular activity, where it was observed that the mitotic activity and viability of osteocytes were higher in the osseointegration or implant –bone interface as a result of the normal body defense mechanism that repaired the osteotomy site [22,23]. Other investigators demonstrated that LLLT improves vascular response by influencing the mitochondrial respiratory chain or membrane calcium channels . It was also shown to enhance collagen synthesis, angiogenesis and growth factor release which eventually promote wound healing and cell proliferation LLLT could stimulate undifferentiated mesenchymal stem cells to differentiate into osteoblasts that would rapidly change to osteocytes [24,9].

In a former study the authors stated that laser therapy enhances the osseointegration process (25). Mechanism explaining the enhanced effect of laser on bone formation was explained in several recent studies which demonstrated the stimulatory effect of LLLT on the expression of bone morphogentic protein-2 , bone sialoprotein and alkaline phosphatase activity thus stimulating cells with osteogenic potential [26,27].

Correlation between the changes in bone density by time in both groups revealed a highly statistically increase in the mean bone density values in laser side than the control side in all follow-up intervals denoting the positive effects of LLLT on wound healing and bone differentiation.

In a previous study, it was found that when Gallium-aluminum-arsenide diode laser was applied to the midpalatal suture during expansion over 7 days, the laser was more effective in enhancing bone regeneration in the early period of expansion (0-2 days) and in the later period (days 4 to 6) of expansion denoting that this effect is not only dependent on the total irradiation dosage but also on the timing and frequency of irradiation [28]. other studies demonstrated the effectiveness of LLLT at the start of the healing period of the tissues and with repeated application [29].

CONCLUSION

The low intensity laser irradiation promoted bone healing and enhanced osseointegration emphasizing the laser's biostimulatory effect.

CONFLICT OF INTEREST

The authors declare no conflict of interest . This research didn't receive any specific grant from funding agencies in the public , commercial or not-to-profit sectors.

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