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Evaluation of the effect of laser biostimulation on implant covered with PRF in controlled diabetic patients: A randomized clinical trial.

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Abstract

The success of implants' osseointegration in diabetic patients with compromised healing potential, is a major challenge. The study **aimed** at evaluation of laser biostimulation effect on osseointegration of implant covered by PRF in controlled diabetic patients with compromised healing. **Methods**: Patients received 22 implants covered with PRF inserted in posterior maxilla or mandible. Implants were divided randomly into 2 groups. Group1: control (no laser irradiation), group2: diode laser. Peri-implant new bone density and secondary stability were assessed using cone-beam computed tomography and Anycheck device respectively. Density was evaluated immediately post implant insertion and after 5 months, while stability was evaluated 5 months post implant insertion. Statistical analysis was executed significance level $P \leq 0.05$. **RESULTS**: Significantly improved density over time was recorded within each group from immediate to 5 months ($p \leq 0.05$). Comparing both groups revealed no statistical significance in bone density regarding per-implant buccal, lingual, distal, and mesial surfaces of bone after 5 months ($p > 0.05$). Stability measurements revealed no significance between both groups after 5 months. **CONCLUSION**: Either applying PRF solely or in conjunction with laser, could be used as effective tools in diabetic patients with compromised healing potential improving osseointegration by time. Laser biostimulation combined with PRF had a modest effect on PRF.

Keywords: laser biostimulation; PRF; dental implants; osseointegration; diabetic patient.

Introduction

Reconstruction of oral and maxillofacial skeleton defects and/or loss of teeth, represent a major challenge especially in conditions with impaired and compromised bone healing potential as in diabetic patients. Diabetes is a metabolic carbohydrate disease associated with hyperglycaemia and has been accounted for being a relative risk for implant rehabilitations as a result of morbidities which may jeopardize healing capacity, such as micro and macroangiopathy, infection susceptibility, increased frequency of periodontitis and delayed wound healing. (ELsyad et al., 2019)

Formerly, the defective healing mechanism and insufficient bone quality and quantity in diabetic patients necessitated the use of either partial or complete removable prosthesis as the feasible treatment modality for such patients. Even with such treatment modality, the patient is in continuous risk of more alveolar bone resorption in denture supporting areas. Moreover, compromised patient satisfaction is attained with removable prosthesis associated with frequent complications. As the numbers of diabetic patients tremendously increase worldwide, implant prosthodontics is considered a major challenging target for oral rehabilitation in such patients with variable success rates affected by chronology or duration of diabetes, impaired healing, age, blood supply, parafunctional habits, smoking, and alcohol consumption …etc. (Naujokat et al., 2016)

Accordingly, different supportive treatment modalities were innovated to improve soft and hard tissue healing in in diabetic patients concerned with oral rehabilitation, such as: bone grafts, ozone therapy, hyperbaric oxygen therapy, platelet-rich plasma (PRP), low-level laser treatment (LLLT), and platelet-rich fibrin (PRF)…. etc. (Taha et al.,2018, El-banna et al.,2018, Ghazal et al., 2023).

Different types of lasers, already established as promising treatment modalities, are used in oral and maxillofacial reconstruction to increase the osteogenic potential and improve bone healing. The use of soft laser or low power laser is mainly for bone biostimulation, increasing vascular angiogenesis and growth factors, and hence increases the success rate of implant osseointegration in compromised bone quality patients. (Allam et al., 2023, Taha et al.,2018).

PRP and PRF have been developed as biosources containing an abundant amount of growth factors favoring regeneration of bone (El-banna et al.,2018). PRF represents an autologous prepared platelet concentrate extracted from blood. It includes a densely formed fibrin matrix trapping leukocytes and platelets (El-banna et al.,2018, Khorshidi et al.,2016, Mowla et al., 2020). The improvement of cytokines stability and growth factors is attributable to the high content of fibrin by enhancing their longevity furthermore protecting them against proteolytic degradation . In addition, leukocytic content has a vital role in infection prevention and minimizing inflammation (El-banna et al.,2018)

Recently, researches spotting the synergistic effect related to low-level laser augmented with PRF on regenerating bone are scarce which led to a wide debate and controversy about the hypothesis of whether LLLT could biostimulate trapped platelets and leucocytes in PRF releasing more growth factors and subsequently more improved healing in terms of bone density and implant stability. The present study aim is to evaluate the influence of laser biostimulation on osseointegration of implant covered with PRF in controlled diabetic patients with compromised healing potential.

Patients and Methods

Study type:

randomized controlled clinical trial.

Study design:

Controlled type II diabetic patients with edentulous posterior areas were randomly selected from dental clinic of Medical and Scientific Centre of Excellence (MSCE), National Research Centre (NRC), Cairo, Egypt, according to inclusion and exclusion criteria to receive a total number of twenty-two dental implants. Implants were randomly distributed with 1:1 allocation ratio into two groups according to exposure to laser irradiation. Group 1 was not exposed to laser irradiation (control group), while group 2 was exposed to laser irradiation.

Peri-implant new bone density was evaluated immediately post implant insertion and after 5 months, while secondary implant stability was performed 5 months post implant insertion.

This study was prosecuted with the Code of Ethics of the World Medical Association, they were stated in the Declaration of Helsinki in 1975. Medical Research Ethical Committee of the National Research Centre, Cairo, Egypt permitted this study with approval number (03430423). All patients were familiar with the study's treatment phases and signed a consent form. The study was conducted from January 2023 to April 2024 and registered in ClinicalTrials.gov Identifier: NCT06444334.

Sample Size Calculation:

Based on the study of Mayer et al, the percentage volume of newly formed bone at 5months were 75.523 ± 8.510 in irradiated animals and 55.012 ± 19.840 in control group (Mayer et al., 2016). The required minimally accepted sample size in each group is 11 participants for a two-tailed study, with 1.34 effect size, 0.05 α error, 85% sample power and 1:1 allocation ratio. The sample size was calculated by G power 3 software (Faul et al., 2007). Total sample size enlarged to 13 per group to reward 20 % drop out.

Inclusion criteria: 1) nonsmoker patients, 2) age range 30 -60 years, 3) Glycosylated hemoglobin (Hb1C) ranges between 7-8, 4) no other systemic disease, 5) vital signs are normal (blood pressure, temperature, pulse rate, respiratory rate), 6) missing 1st or 2nd mandibular or maxillary premolars or molars, 7) lab investigations are within normal (CBC. liver function AST &ALT, kidney function urea &creatinine, Ca level, 25OH Vit D), 8) no need for alveolar bone grafting, and 9) no soft or hard tissue pathology.

Exclusion criteria: 1) smoker patient, 2) Glycosylated hemoglobin (Hb1C) more than 8 or less than 7, 3) age less than 30 or more than 60, 4) presence of other systemic diseases, 5) vital signs are not normal, 6) lab investigations are not normal (CBC, liver function, kidney function), 7) need for alveolar bone grafting and 8) present soft or hard tissue pathology.

Radiographic procedures:

Every patient had undergone radiographic analysis pre-operatively using cone beam computed tomography (CBCT) (Planmeca Oy Asentajankatu 6,00880 Helsinki, Finland). The intended size and location of the implants were determined and planned virtually by digital software (Planmeca Romexis Viewer 6.2.1.19). Bone density around the implants will be evaluated using CBCT software at immediately postoperative as baseline and 5 months postoperative. Both groups were radiographed by CBCT for evaluation and assessment of bone density around implants by professional blinded investigators. Planmeca Romaxies machine was used with the following specifications: Field of View (FOV) = 8.0x5.0 cm, resolution = 0.300, orientation = portrait, 90 kV, 80mA, and exposure time = 15.019 sec.

Surgical procedures

Implants (K1 line conical connection double thread, OXY, Italy) were inserted under profound local anesthesia using free hand open flap technique; where mucoperiosteal gingival envelop full thickness flap was performed by crestal incision & mucoperiosteal reflection exposing bone (Fig 1 a). The preplanned location was confirmed by the aid of CBCT, then sequential drilling was exerted using graduated drills with stoppers under copious amount of saline coolant with the aid of paralleling pins if multiple implants were inserted to be splinted in the same patient.

Implants were screwed with torque between 35-45N to ensure primary stability. Various implants' sizes were utilized ranging from 4 mm to 5.5mm in diameter and from 8mm to 11mm in length in accordance with the virtual pre-plan based on bone geometrical availability. The procedure was executed by a single well-experienced operator who was blinded to the groups.

PRF preparation protocol:

The preparation method of PRF was performed in accordance with the protocol developed by Choukroun (Choukroun et al., 2001). PRF was withdrawn and processed from the same operated-on patient's blood; 6ml IV blood withdrawn from the antecubital vein in to two sterile 3ml red vacutainer tubes without anticoagulant, followed by a 12 minute centrifuge with 3000 RPM producing a PRF clot, which is then incised & separated with 2mm basal layer of RBCs rich in growth factors (Fig 1 b,c). Following the cover screw placement, PRF was extended bucco-lingually and mesio-distally over the alveolar ridge (Fig1 d). Finally, approximation of the flap was achieved using non-resorbable 3/0 suture, removed after 7-10 days postoperative. Nonsteroidal anti-inflammatory drugs and antibiotics were administered for seven days. Delayed loading was initiated after 5 months of osseointegration process.

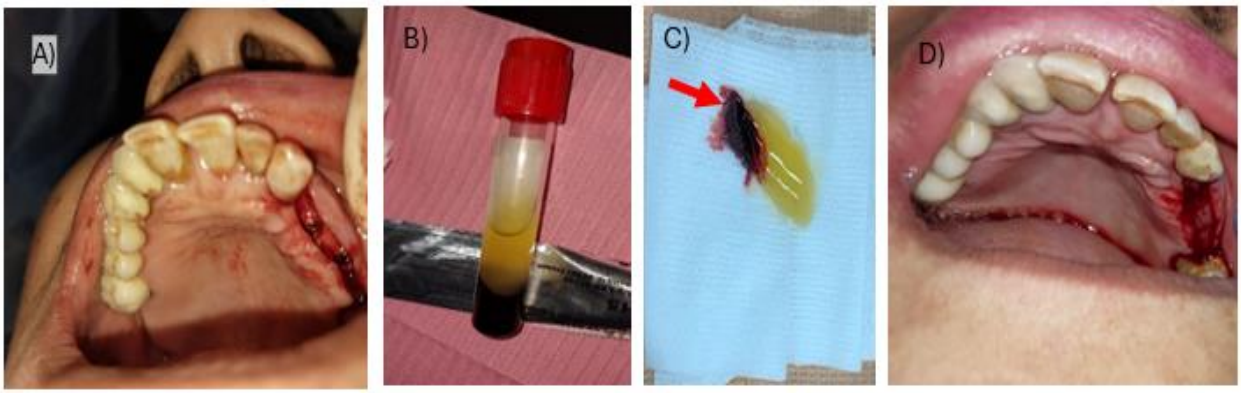

Figure1: a) mucoperiosteal crestal incision was performed in maxillary premolar and molar regions with implants in place, b) prepared PRF gel in the middle of the tube, c) extracted PRF with 2mm basal layer of RBC rich in growth factors (red arrow), and d) implants covered with PRF.

Laser irradiation protocol:

Group 2 was exposed to laser irradiation following implant insertion for 3 sessions: Immediately after implant insertion, 2 days after implant insertion and 1 week after insertion (Fig 2 a), using a red Diode(gallium-aluminum-arsenide) LLLT using calibrated diode laser device (Smart M, Lasotronix, Poland) at 635nm wavelength delivered by biomodulating handpiece with the following set parameters: 100mw power output, 8mm handpiece diameter, 0,5024 cm^2 spot area, 199.04 mw/cm^2 power density, continuous mode, and time 40 second per point and contact mode (Matys et al., 2019)(Fig 2 b). The laser probe was directed towards the implant site, gently touching the tissues mesially, distally, buccally, and lingually to assure the full exposure of the target surface to laser beam (Fig 2c).

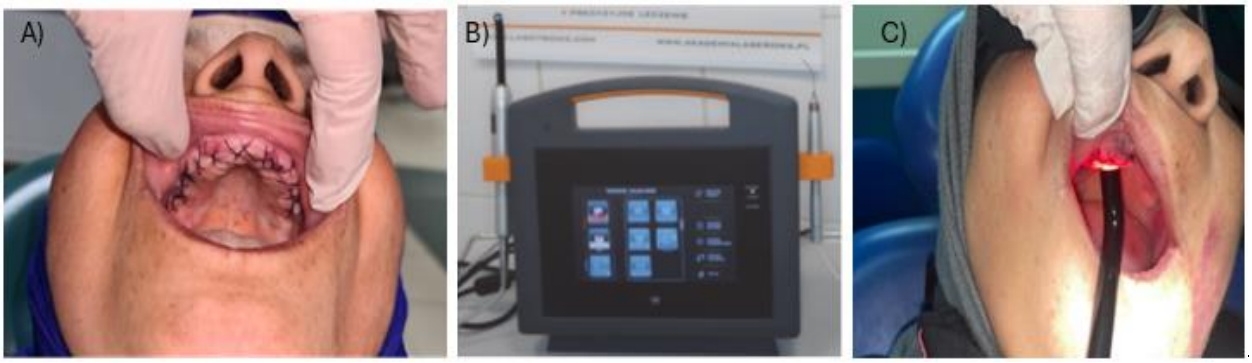

Figure2: a) maxillary premolar and molar implants with covered screws before laser irradiation, b) Diode laser device (Smart M, Lasotronix), and c) low level diode laser was active with safety protective eye goggles for both patient and operator.

Peri-implant bone density and implant stability measurements.

1- Bone density measurements

Digital software program was used for quantitative mean bone density measurements of all the captured radiographs produced by CBCT for both groups. Multiplanar resolution screen (MPR) was selected showing radiographic images of the mandible and maxilla in sagittal and coronal views to quantify bone radiodensity in mesiodistal and buccolingual surfaces respectively , a constant exact area was selected for each view with area =1 5.2mm^2, width = 2.00mm, and height = 7.60mm. Five readings of radiodensity in Hounsfield units (HU statistics) were collected by blind investigator for each peri-implant surface (mesial, distal, buccal, and lingual) with slice thickness of 0.5mm. Finally, the mean density values were calculated and tabulated concerning sagittal and coronal views in each implant (Fig 3).

2-**Implant stability measurements**

-AnyCheck device (AnyCheck, Neobiotech Co., Ltd. E-space #1001, 36, Digital-ro 27-gil, Guro-gu, Seoul, Korea, 08381) was utilized 5 months (2nd stability) post implant insertion. AnyCheck is an implant stability meter that measures the stiffness of the alveolar bone-implant interface through a tapping-motion. The degree of osseointegration is calculated in terms of **IST** (Initial Stability Test) value between 30 to 85. AnyCheck was turned on, then, the tip of the tapping rod maintained contact angle between 0 to 30 degrees with healing abutment. The START button was gently pressed while holding the device stable, the measured value displayed on the LCD screen was recorded (Fig.4). The smaller the measured value, the weaker the degree of osseointegration. Mesiodistal and buccolingual sides of the implant were measured, five IST readings for each side were repetitively recorded by blind examiner and the mean reading was calculated and tabulated.

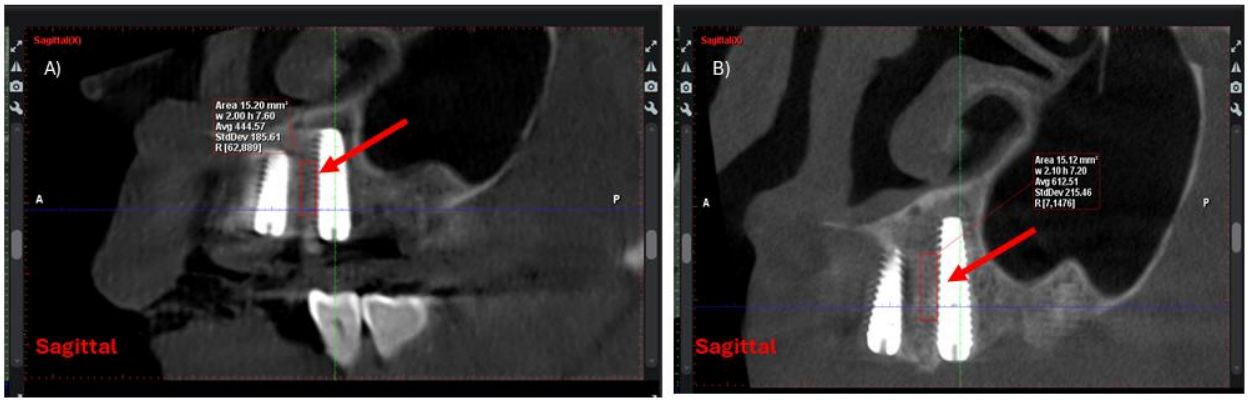

Figure 3: Preview screen MPR (multiplanar resolution) screen was selected showing radiographic images of maxillary premolar in sagittal view to quantify peri-implant bone radiodensity in mesiodistal surfaces respectively, a constant exact area (red arrow) was selected for each view (sagittal) in immediate(a) and 5 months(b) post- implant insertion in the same patient(group2). Note the increase in density in Hounsfield units.

*Figure 4: **AnyCheck** device was directed to the implant side to measure secondary stability, the measured value displayed on the LCD screen was recorded (red arrow)*

Statistical analysis

Data were collected, tabulated, statistically analyzed using an IBM personal computer with Statistical Package of Social Science (SPSS) version 20 (IBM Corporations, 2011), Armonk, NY and Epi Info 2000 programs, at

P<0 .05 significance level. Data normalities were assessed by Kolmogorov and Shapiro-Wilk tests (P< .05). All data was normally distributed and presented as mean ($\overline{X}$), standard deviation (SD), range, median and interquartile range. Student's t- test was used to compare between two groups, while Paired T test was used to compare between successive intervals within each group.

Results

Peri-implant bone density measurements

Results of peri-implant new bone density measurements (mean ±SD) in each group, revealed that there was no statistical significance in density of bone among group 1 and 2 regarding peri-implant buccal, lingual, distal, mesial surfaces of bone at time of immediate implant insertion (baseline)(p>0.05) (table 1).

In addition, table 2 showed that despite increased peri-implant new bone density with time in group2 (5 months), there was no statistical significance in density of bone between group1 and group2 after 5 months post implant insertion (p>0.05) regarding buccal, lingual, distal, mesial surfaces of bone while the result of the present study revealed statistically significant increase of peri-implant new bone density within each group (1 and 2) over time; from implant insertion (baseline) to five months post-operative (fig3).

Furthermore, table 3 showed high statistically significant increased peri-implant new bone density in buccal surfaces of bone at group1 with time (control baseline vs control 5 months) (p≤0.001) and statistically significant increased new bone density in lingual, distal, mesial surfaces of bone (p≤0.05) (table 3)

Similarly, table 4 revealed high statistically significant increased peri-implant new bone density in buccal surfaces of bone at group2 with time (Laser baseline vs Laser 5 months) (p≤0.001) and statistically significant increased new bone density in lingual, distal, mesial surfaces of bone (p≤0.05) (table 4)

Optimal implant osseointegration and success was found in all implants covered with PRF with-or without laser biostimulation in the study period.

implant stability measurements.

Considering secondary stability measurements (mean ±SD) in mesiodistal and buccolingual sides of the implant; table (5) stated that there was no statistical significance among group1 (84±4.3, 84.2±4.4) and group2(83.7±1.5, 83.72±1.2) regarding mesiodistal (**P =0.900)** &buccolingual (**P =0.837)** surfaces measurements respectively after 5 months post implant insertion**, (P>0.05)**

Optimal soft and hard tissue uneventful healing was observed around implants without adverse reactions or any compromised soft or hard tissue healing (dehiscence and/or fenestration) in such type of patients (diabetic with poor vascularization.). Moreover, no implants were lost or revealed signs of failure, infection, or inflammatory reactions during the study period.

Based on **AnyCheck** device measurements, all the group1 and 2 implants survived in the study period with excellent success and survival.

Table 1. Comparison between group 1and 2 including measurements of peri-implant new bone density immediately post implant insertion (baseline).

Items	Group1(control) (No=11)	Group 2(Laser) (No=11)	Test of sig. & p-value	Significance
Distal				
- Mean ± SD - Min- Max - Median(IQ)	599.4±378.2 166.8-1424 485.2(370.1-826.3)	616.3±338.9 203.8-1235.6 456.8(391.5-728.3)	**t test=0.111** **P =0.913(>0.05)**	**Non sig.**
Mesial				
- Mean ± SD - Min- Max - Median(IQ)	569.7±373.6 66-1108 472.3(277.1-932.1)	596.2±393 43.3-1222.9 612.8(262.2-882.9)	**t test=0.166** **P =0.870(>0.05)**	**Non sig.**
Buccal				
- Mean ± SD - Min- Max - Median(IQ)	694.4±268.9 320-1045 687.6(461.1-932.02)	648.4±275.3 210.3-1072 704.6(425.4-844.3)	**t test=0.403** **P =0.691(>0.05)**	**Non sig.**
Lingual				
- Mean ± SD - Min- Max - Median(IQ)	938.7±328.4 355-1538 963.3(758.1-1151.8)	850.1±235.4 347.5-1182.5 848.8(751.9-977.9)	**t test=0.738** **P =0.469(>0.05)**	**Non sig.**

Unit of measurement is HU=Hounsfield Unit.

Table 2. Comparison between group 1 and 2 including measurements of peri-implant bone density at 5 months post-implant insertion.

Items	Group1(control) (No=11)	Group 2(Laser) (No=11)	Test of sig. & p-value	Significance
Distal				
- Mean ± SD - Min- Max - Median(IQ)	800.7±319.9 370.6-1295.2 827.5(472.9-1066.9)	940.8±386.3 410.5-1618 894.2(642.7-1184.5)	**t test=0.947** **P =0.354(>0.05)**	**Non sig.**
Mesial				
- Mean ± SD - Min- Max - Median(IQ)	809.9±386.1 153-1339.8 701.8(534.3-1167.4)	876.7±384.2 364.7-1367 894.7(499.6-1248.5)	**t test=0.415** **P =0.682(>0.05)**	**Non sig.**
Buccal				
- Mean ± SD - Min- Max - Median(IQ)	962.8±320.1 423.4-1474.1 1003.4(735.2-1162.2)	908.7±200.9 630-1251.2 916.4(750.5-1027.7)	**t test=0.480** **P =0.636(>0.05)**	**Non sig.**
Lingual				
- Mean ± SD - Min- Max - Median (IQ)	1083.8±425.6 628-1456.4 1089.8(1006.2-1254.1)	1163±217.02 884.8-1618.2 1078(1006.3-1291.9)	**t test=0.817** **P =0.423(>0.05)**	**Non sig.**

Unit of measurement is HU=Hounsfield Unit.

Table 3. Comparison of peri-implant bone density in group 1 over time (from immediate baseline to 5months)

Items	Group1 immediate	Group1 at 5 months	Test of sig. & p-value	Significance
Distal side				
- Mean ± SD - Min- Max - Median(IQ)	599.4±378.2 166.8-1424 485.2(370.1-826.3)	800.7±319.9 370.6-1295.2 827.5(472.9-1066.9)	**Paired t test=2.31** **P =0.042*(≤0.05)**	**Sig.**
Mesial side				
- Mean ± SD - Min- Max - Median(IQ)	569.7±373.6 66-1108 472.3(277.1-932.1)	809.9±386.1 153-1339.8 701.8(534.3-1167.4)	**Paired t test=3.15** **P =0.009*(≤0.05)**	**Sig.**
Buccal side				
- Mean ± SD - Min- Max - Median(IQ)	694.4±268.9 320-1045 687.6(461.1-932.02)	962.8±320.1 423.4-1474.1 1003.4(735.2-1162.2)	**Paired t test=4.35** **P =0.001**(≤0.001)**	**Highly sig.**
Lingual side				
- Mean ± SD - Min- Max - Median(IQ)	938.7±328.4 355-1538 963.3(758.1-1151.8)	1083.8±425.6 628-1456.4 1089.8(1006.2-1254.1)	**Paired t test=2.38** **P =0.036*(≤0.05)**	**Sig.**

*Significant, ** highly significant, Unit of measurement is HU=Hounsfield Unit).

Table 4. Comparison of peri-implant new bone density in group 2 over time (laser baseline vs laser 5 months)

Items	Group2 (Laser) immediate	Group2 at 5 months	Test of sig. & p-value	Significance
Distal side				
- Mean ± SD - Min- Max - Median(IQ)	616.3±338.9 203.8-1235.6 456.8(391.5-728.3)	940.8±386.3 410.5-1618 894.2(642.7-1184.5)	**Paired t test=3.58** **P =0.005*(≤0.05)**	**Sig.**
Mesial side				
- Mean ± SD - Min- Max - Median(IQ)	596.2±393 43.3-1222.9 612.8(262.2-882.9)	876.7±384.2 364.7-1367 894.7(499.6-1248.5)	**Paired t test=3.99** **P =0.003*(≤0.05)**	**Sig.**
Buccal side				
- Mean ± SD - Min- Max - Median(IQ)	648.4±275.3 210.3-1072 704.6(425.4-844.3)	908.7±200.9 630-1251.2 916.4(750.5-1027.7)	**Paired t test=6.72** **P =0.00**(≤0.001)**	**Highly sig.**
Lingual side				
- Mean ± SD - Min- Max - Median(IQ)	850.1±235.4 347.5-1182.5 848.8(751.9-977.9)	1163±217.02 884.8-1618.2 1078(1006.3-1291.9)	**Paired t test=4.09** **P =0.002*(≤0.05)**	**Sig.**

*Significant, ** highly significant, Unit of measurement is HU=Hunsfield Unit).

Table 5. Measurement of secondary stability of implant at 5 months using AnyCheck Device

Items	Group1(control) (No=11)	Group2(laser) (No=11)	Test of sig. & p-value	Significance
Mesiodistal				
- Mean ± SD - Min- Max - Median(IQ)	84±4.3 77-91 85(81-86)	83.7±1.5 82-85 84(83-84.5)	**t test=0.129** **P =0.900(>0.05)**	**Non sig.**
buccolingual				
- Mean ± SD - Min- Max - Median(IQ)	84.2±4.4 78-92 85(82-86)	83.72±1.2 83-85 83(83-84)	**t test=0.211** **P =0.837(>0.05)**	**Non sig.**

Discussion

Diabetes is referred to as a metabolic chronic disease defined by blood glucose elevated levels which causes overtime profound damage in different organs of the body; including eyes, kidneys, nerves, heart, and blood vessels. It is associated with compromised healing process in soft and hard tissues (Naujokat et al., 2016) that require effective supporting modalities during oral rehabilitation and reconstruction.

Bio-stimulation is a promising concept in dentistry evidenced in acceleration of biodynamics of regeneration and healing (Taha et al.,2018). As the present study explored the effectiveness of the combination of bio-stimulants, referring to PRF and diode laser in comparison to sole PRF application, scarce studies were found to address this new concept of synergistic combination of two biostimulatory treatments.

PRF is an interesting-non-invasive modality, discovered by choukroun et al. 2001 as a rich source of growth factors like platelets derived growth factors (PDGF), transforming growth factor beta (TGFB), insulin like growth factors (IGF), fibroblast growth factors (FGF), leukocytes, adhesion proteins like Fibronectin, and Vitronectin etc. (Rafael et al., 2021). It stimulates cellular differentiation and proliferation of precursor cells into osteoblasts, fibroblasts, and endothelial cells for both hard and soft tissue healing (Rafael et al., 2021). These outcomes explained the findings in the present research and explored the supreme increase of new bone density and implant stability over time in the group1 applying PRF alone. On the other hand, laser therapy is already established an enthusiastic modality tool to ameliorate bone healing through collagenesis, neovascularization, optimized osseointegration around implant. The current study involved a combination of two biostimulatory techniques (laser and PRF) for improvement of osseointegration in such challenging diabetic patient with compromised healing process.

Selected laser is diode red laser with wavelength 635nm, a low-cost semi-conductor, available for standard dental practice use, selection was based upon its deep tissue penetration in comparison with different sorts of lasers, offering a highly effective tool for the practitioner as reported by Habash & Jayash (2021). This selected wavelength is reported to be associated with increased depth of hard and soft tissue penetration in agreement with previous study (Matys et al., 2019). Three sessions laser application protocol in the current study was adopted to allow biostimulation of bone in the early phases of healing (inflammatory, proliferative cellular). As well as biostimulation of PRF in the early period of release of growth factors by leukocytes and platelets in the PRF matrix which is about 7 days (El-banna et al., 2017).

Furthermore, this study was designed to separate patients receiving just PRF from those receiving PRF in conjunction with laser to avoid laser favourable systemic effect which could exist as reported by several studies leading to false results to implants covered with PRF solely if done in the same patient but in the contralateral site (ELsyad et al., 2019, Karaca et al.,2018).

For proper evaluation of implant successful osseointegration, peri-implant bone density and implant stability were measured using CBCT and radio frequency analysis (Anycheck device) respectively as they are considered key factors in implant success. Anycheck device was used in the current study as an effective tool to measure implant stability with the following advantages: Excellent measurement of lateral mobility, acceptable for the patient, no extra cost of variable smartpegs for the implants, no need to remove suprastuctures for measurement, doesn't violate healing process by limiting the number of taps if stability is still low.

In the current study, significant increase in bone density was observed within each group from immediate (baseline) to five months post-implant insertion, this indicated the effective role and osteogenic potential of both treatment modalities PRF & LLLT in stimulation of biodynamics of bone healing (osteoblastic differentiation, proliferation, neovascularization) with subsequent increase of peri-implant bone density (bone maturation and remodelling) and implant stability. While the insignificant difference between laser and control group at five months follow up regarding density and the implant stability, unveiled that the combined effect related to laser and PRF has the same clinical significance as compared to using PRF alone i.e. no synergistic effect of LLLT on PRF. This might be due to insufficient or less effective laser dose and parameters. In addition, the early effect of lasers was undetected in the later stages of bone healing regarding rate of maturation and remodelling. In defence, the mild effect of LLLT could be ascribed to not having a single unified proved effective protocol document regarding using LLLT (Arakeeb el al., 2019), despite well-established evidence favouring its role in increased implant secondary stability &new bone deposition (Sleem et al .,2019, Soares et al.,2013, Batista et al.,2015).

Insignificant difference in 5 months secondary stability of group1 vs group2 was in corroboration with peri-implant bone density findings. This finding is considered to be in line with EL sayed et al and Mandic et al stating a nonsignificant influence of LLLI on secondary stability of implants (ELsyad et al., 2019, Mandić et al., 2015). Both treatment modalities, PRF and laser accelerated the process of healing regarding cellular differentiation, proliferation, granulation tissue formation, organization, revascularization, maturation, & remodelling around the implants and bactericidal action of PRF due to existing leukocyte as well as the inhibitory effect of laser on the bacterial counts around the implants as suggested by Kusek (2011). This explained the excellent soft and hard tissue uneventful healing without any adverse reactions in these moderately controlled diabetics.

All the test and control implants survived with good success rate throughout the study period as evidenced by Anycheck device that was used to attain secondary stability measurements for its sensitivity in detecting either

failure regarding implants or their excellent stability during the integration phase. Furthermore, early implant success was assessed utilizing the next criteria reported by Buser et al; 1) no recurring peri-implant suppurative infection, 2) absence of pain among other persistent signs, dysesthesia, and/or foreign body sensation, 3) absence of any sort of detectable mobility of implants, and 4) no continuous peri-implant radiolucency (Buser el .,1990). In addition, the outcomes could be imputed to increased density of bone in the maxillary and mandibular bone regions by the effect of PRF and laser modalities over time.

The outcomes of the current study are in line with a research performed by Sleem et al who executed a randomized clinical trial design, on a group of nine patients with missing mandibular bilateral posterior teeth. The whole group received single implant on each side subjected to PRF application (Sleem et al.,2019). Laser treatment was applied to a single side where laser parameters included: 830 nm wavelength continuous emission of diode low-level laser, 0.28 cm^2 spot size, 100 mW power, and 92.1 J/cm^2 density of energy. Authors of that research concluded that no significant statistical difference was found regarding stability of implants, bone density, and post-operative pain values between sites receiving PRF combined with Diode laser in comparison with PRF alone.

Additionally, focusing on the stability of implants and density of bone, this research outcomes illustrated statistical significance difference in bone density within every group initially at insertion day till the termination of the follow-up period, these findings were in accordance with those published by Jang et al. (Jang et al., 2010). The research tested the influence of PRF in filling peri-implant defects of bone in rabbits. Success in repair of peri-implant defects was achieved by Choukroun PRF application, supporting the principle of favorable PRF influence on osteogenesis.

Moreover, the present study revealed no statistical significance among comparison of both groups (1 control, 2 laser) regarding newly formed bone density around implant 5 months post implant insertion, this agrees with the study made by Shanei et al in rabbits' calvarial defects; who reported

insignificant difference between laser + PRF group compared to PRF alone group regarding percentage of new bone and number of osteoblasts (Shanei et al., 2022). Furthermore, numerous researches concluded that the sole action of LLLT was effective on osteogenesis, while the synergistic impact of LLLT combination did not significantly enhance osteogenesis. Another research based on the synergistic influence of LLLT combined with mesenchymal stem cells on osteogenesis in rabbits associated with calvarial defects clarified that despite LLLT improved osteogenesis significantly, there was no synergistic significantly recorded effect of utilizing mesenchymal stem cells augmented with low level laser (Fekrazad et al., 2015).

Findings of the current research are in contradiction with that conducted by ELsyad et al [1] who evaluated the effect of LLLI on stability of implants and bone crest involving mandibular overdentures retained by small-diameter dental implants inserted in moderately controlled diabetic patients. Authors reported significant

difference in stability of laser group compared to control at 6 months only. This difference and disagreement with results of the current study in implant stability and subsequently density, is mainly due to the comparison with control not measuring the synergistic effect of combination of PRF + laser supporting this study outcome in the favourable influence of LLLI on osteogenesis with time.

Conclusions.

Both PRF alone and laser combined with PRF improved osseointegration around implants and could be used as effective supportive tools in in diabetic patients with compromised healing potential. According to the limitations of these studies and the obtained results of PRF alone that is comparable to PRF combined with laser, it is concluded that the synergistic effect of laser biostimulation specifically on PRF is weak and needs further studies considering different doses, wave lengths and time of Diode laser application. There was no difference in treatment of PRF alone or combined with laser modality, both techniques resulted in good survival and success rate of all implants throughout the study period. PRF has the advantages of being a completely autogenous material, cost effective, easy manipulation and application. The sole application of PRF has improved implant osseointegration, stability and bone density around the implants over the selected study period. This proved that PRF is an effective regenerative therapy in diabetic patient.

Recommendations

Further studies with different laser parameters, advanced PRF preparations, longer study periods and variable groups in diabetic patients, were recommended.

Measure the effect of laser on rapid osseointegration and attaining implant stability in earlier postoperative periods.

Conflict of Interest: The authors declare that they have no conflict of interest.

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Figure Captions.

Figure1: a) mucoperiosteal crestal incision was performed in maxillary premolar and molar regions with implants in place, b) prepared PRF gel in the middle of the tube, c) extracted PRF with 2mm basal layer of RBC rich in growth factors (red arrow), and d) implants covered with PRF.

Figure2: a) maxillary premolar and molar implants with covered screws before laser irradiation, b) Diode laser device (Smart M, Lasotronix), and c) low level diode laser was active with safety protective eye goggles for both patient and operator.

Figure 3: Preview screen MPR (multiplanar resolution) screen was selected showing radiographic images of maxillary premolar in sagittal view to quantify peri-implant bone radiodensity in mesiodistal surfaces respectively, a constant exact area (red arrow) was selected for each view (sagittal) in immediate(a) and 5 months(b) post- implant insertion in the same patient(group2). Note the increase in density in Hounsfield units.

Figure 4: **AnyCheck** device was directed to the implant side to measure secondary stability, the measured value displayed on the LCD screen was recorded (red arrow)