RESEARCH PAPER

Assessment of Osteoblastic Activity Around Dental Implants Following Low-Intensity Pulsed Ultrasound (LIPUS) Therapy in Patients with Type 2 Diabetes Mellitus: A Split-Mouth Randomized Controlled Trial

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Introduction:

Partial or complete edentulism resulting from tooth loss leads to compromised masticatory function, altered speech, impaired aesthetics, and a decline in overall oral health–related quality of life. In addition, loss of teeth is often followed by progressive resorption of the alveolar bone, further complicating prosthetic rehabilitation. Over the past few decades, dental implants have emerged as a predictable and widely accepted treatment modality for the replacement of missing teeth. Compared with conventional fixed partial dentures and removable prostheses, dental implants offer several advantages, including preservation of adjacent teeth, improved retention and stability, maintenance of alveolar bone, and enhanced patient comfort and satisfaction. Long-term clinical studies have consistently demonstrated high survival and success rates of dental implants, establishing them as a reliable treatment option in modern prosthodontics.

The biological foundation of implant success lies in the process of osseointegration. Osseointegration is defined as a direct structural and functional connection between living bone and the surface of a load-bearing implant, without the interposition of fibrous connective tissue. Since its description by Brånemark, osseointegration has remained the cornerstone of implant dentistry. Successful osseointegration depends on a combination of factors, including atraumatic surgical technique, implant design and surface characteristics, loading protocols, and host-related biological factors. Any disturbance in these parameters may adversely affect bone healing at the bone–implant interface and ultimately compromise implant stability.

Although dental implants demonstrate high success rates, certain systemic conditions are known to influence the biological process of osseointegration. Diabetes mellitus has long been considered a relative contraindication for dental implant therapy. It is a chronic metabolic disorder characterized by persistent hyperglycemia resulting from impaired insulin secretion, insulin resistance, or both. Diabetes mellitus is associated with delayed wound healing, impaired angiogenesis, altered immune response, and disturbances in bone metabolism. These systemic alterations may negatively affect peri-implant tissue healing and osseointegration.

The detrimental effects of diabetes mellitus on bone healing are multifactorial. Chronic hyperglycemia leads to microvascular disease, resulting in reduced blood supply and oxygenation of healing tissues. In addition, diabetes is associated with increased levels of inflammatory mediators such as interleukin-1β, interleukin-6, and tumor necrosis factor-α, which interfere with normal bone remodeling by inhibiting osteoblastic differentiation and enhancing osteoclastic activity. The formation of advanced glycation end products further impairs collagen synthesis and bone matrix formation. Collectively, these factors contribute to delayed bone healing and compromised osseointegration around dental implants in diabetic patients.

Clinical studies evaluating implant outcomes in patients with diabetes mellitus have reported variable success rates, with a higher incidence of early implant failures compared to non-diabetic individuals. These early failures are primarily attributed to impaired osseointegration during the initial healing phase rather than late mechanical complications. With advances in implant surface technology, surgical protocols, and improved glycemic control strategies, dental implant therapy has become increasingly common in patients with well-controlled type 2 diabetes mellitus. Nevertheless, the need to enhance peri-implant bone healing and reduce early failure risk in this patient population remains a significant clinical concern.

Over the years, several approaches have been investigated to promote osseointegration in patients with type 2 diabetes mellitus, including autologous platelet concentrates, recombinant growth factors, cell-based regenerative therapies, surface modifications, biomaterial scaffolds, and gene-based techniques. While many of these methods have shown promising osteogenic potential, their routine clinical application is often limited by factors such as biological variability, high cost, technical sensitivity, regulatory constraints, and inconsistent outcomes. As a result, there is increasing interest in adjunctive therapies that are safe, non-invasive, cost-effective, and capable of enhancing bone healing without adding procedural complexity.

Low-intensity pulsed ultrasound (LIPUS) is a non-invasive biophysical modality that has been extensively used in orthopedic practice to accelerate fracture healing. LIPUS delivers low-energy acoustic waves that generate mechanical stimulation at the cellular level, leading to activation of mechanotransduction pathways involved in bone regeneration. Experimental studies have demonstrated that LIPUS enhances osteoblastic proliferation and differentiation, increases angiogenesis, and promotes extracellular matrix production and mineralization. These biological effects suggest that LIPUS may have a beneficial role in enhancing peri-implant bone healing.

Several animal studies have demonstrated that LIPUS improves peri-implant bone formation by increasing bone volume, bone–implant contact, and mechanical stability of dental implants. Importantly, studies conducted in compromised systemic conditions, including diabetes mellitus, have shown that LIPUS can partially reverse impaired bone healing and enhance osseointegration. Despite these encouraging findings, evidence from human clinical studies remains limited.

Evaluation of peri-implant bone healing traditionally relies on histological analysis, which is considered the gold standard but is invasive and unsuitable for human subjects. Conventional radiographic methods provide limited information regarding bone density and fail to reflect dynamic bone metabolism. Bone scintigraphy using Technetium-99m-labeled methylene diphosphonate (Tc-99m-MDP) offers a non-invasive functional assessment of bone activity. Tc-99m-MDP preferentially accumulates in areas of increased osteoblastic activity and vascularity, thereby enabling evaluation of dynamic changes in bone metabolism around dental implants.

Despite promising preclinical evidence, there is a paucity of well-designed human clinical trials evaluating the effect of LIPUS on peri-implant bone healing in patients with type 2 diabetes mellitus. As a result, following study for proposed. The aim of this split-mouth randomized controlled trial was to assess osteoblastic activity around dental implants following adjunctive low-intensity pulsed ultrasound (LIPUS) therapy in patients with type 2 diabetes mellitus.

Materials and Methods:

Ethical approval-

Formal Ethical approval was obtained from the Institutional Ethics Committee of Sri Ramachandra Institute of Higher Education and Research. (REFERENCE NO: CSP/24/NOV/153/408)

Study design-

The study population included 10 well-controlled diabetic patients with bilaterally missing mandibular posterior teeth (glycosylated hemoglobin levels < 8.0). Men and women within the age group of 21 to 60 years were included in this study. A Splitmouth, randomized control trial was planned.

Inclusion Criteria-

1. Partially edentulous participants with bilaterally missing mandibular posterior teeth willing for implant-supported prosthesis.
2. Patients aged between 21 to 60 years.
3. Patients with Controlled Type 2 Diabetes Mellitus (HbA1c level 5.7 to 8%).

Exclusion Criteria-

1. Smokers.
2. Patients under bisphosphonate therapy.
3. Patients with psychological problems.
4. Pregnant or lactating women.

METHODOLOGY -

Phase 1: Pre-operative Investigation

- Extra-oral pre-operative photographs.
- Intra-oral photographs.
- Blood investigations that were evaluated :
    - Complete blood count
    - Blood glucose: Fasting and Post-prandial
    - Bleeding time
    - Clotting time
    - HbAIC
- Radiograph :

  A pre-operative Cone Beam Computed Tomography (CBCT) scan was made to evaluate:

  1. Quality and quantity of mandibular bone in relation to the edentulous region.
  2. Exclude the presence of any impacted tooth or retained root fragment.
  3. Rule out any pathology present in relation to the remaining dentition.

After completion of the pre-prosthetic and pre-surgical evaluation, the patients were informed about the entire study protocol, and a written informed consent form was obtained.

Phase 2: Surgical Phase

Implant Placement:

A full-thickness muco-periosteal flap was elevated on the control and test site after administration of local anesthesia. The osteotomy preparation for

bilateral posterior edentulous site was done with sequential drills of 2.0 mm, 2.7mm, 3.0mm, and 3.2mm to the full length of the selected implant length, i.e., 10 mm.

Implants of diameter 4.0*10 mm were torque-wrenched into the osteotomy sites, after which healing collars were placed. The flap was then approximated using simple interrupted sutures. Patients were advised to report after 1 week for suture removal.

LIPUS therapy:

One week later, suture removal was performed, and LIPUS therapy was administered for 20 minutes (first session of LIPUS therapy). The patient was recalled after three days for the second session of LIPUS therapy. The third and fourth sessions were completed during the subsequent week.

Phase 3: Evaluation Phase

The patients were subjected to bone scintigraphy test before prosthetic rehabilitation to evaluate the bone changes around the implants.

Bone scintigraphy scan:

The patients were recalled at the 30th day, 60th day, and 90th day after implant placement for bone scintigraphy in order to evaluate the osteoblastic activity around the implant site. Multiphase bone scintigraphy consists of dynamic images, blood pool images, and delayed skeletal images of the whole body or localized areas. All patients were subjected to an anterior view bone scintigraphy scan of the head and neck region.

Protocol-

- The patients were given 10mCi of Tc-99m-MDP intravenously in a supine position.
- Immediate blood pool images consisting of one or more static images of the head and neck region were taken 10 minutes following tracer injection.
- Patients were then well hydrated and instructed to drink two or more glasses of water between the time of injection and the time of delayed imaging.

In the images obtained, a region of interest (ROI) is drawn around the implant, adjacent bone, and the mandibular ramus to evaluate the osteoblastic activity at different time intervals.

Alkaline phosphatase(ALP) biomarker:

The patients were recalled at the 30th day, and 90th day after implant placement for PICF sample collection in order to evaluate the osteoblastic activity around the implant site.

Sample Collection and ELISA assay -

Open ended capillary tubes of length 75mm were used to collect Peri-implant Crevicular Fluid (PICF) for ten minutes. PICF samples were placed in sterile Eppendorf tubes containing 300 µL of phosphate buffered saline (PBS). Samples were then kept at room temperature for 30 minutes before removing the capillary tubes and subsequently transported to lab and stored at -70◦ C until analysis. The samples collected on 30th day and 90th day were subjected to commercially available ELISA to assess Alkaline phosphatase (ALP), a biomarker for osteoblastic activity. The baseline value of ALP to be detected as a biomarker is 0.156-10 IU.

STATISTICAL TOOLS:

The collected data were analyzed using SPSS software version 21. Descriptive statistics was analyzed using mean & standard deviation (S.D). Shapirov Wilks and Kolmogrov Smirnov test was used to analyze the normality of the data. Independent t test was used to compare between the groups. In the above statistical tool, p value of less than 0.05 was considered significant.

RESULTS:

The present study aims to evaluate the efficiency of LIPUS therapy on enhancing the osteoblastic activity around dental implants in patients with type 2 diabetes mellitus.

Bone scintigraphy was performed to evaluate osteoblastic activity at the following intervals 30th, 60th and 90th day after implant placement. Osteoblastic activity was evaluated by drawing a ROI around the implant site. The ROI for all patients, on both implant sites was maintained at 110 pixels. The counts/pixel was then calculated for each site and was expressed in percentage.

Peri-implant crevicular fluid (PICF) was collected at the 30th and 90th day following implant placement to assess osteoblastic activity. Osteoblastic activity was evaluated by estimating alkaline phosphatase (ALP) levels using a commercially available ELISA kit.

The data obtained were then tabulated.

Table 1: Osteoblastic Activity Values at 30, 60, 90 Days in Experimental Group

Sr.No	Experimental		
	30	60	90
1	55.84	55.85	53.39
2	52.11	54.62	53.73
3	45.96	54.66	53.21
4	49.82	52.91	52.66
5	46.23	52.96	52.15
6	52.45	55.16	55.62

7	50.58	53.12	52.31
8	48.27	53.17	53.09
9	49.38	54.12	53.71
10	52.12	53.39	53.11
Mean	50.276	53.996	53.298
SD	3.028998	1.038559	0.974335

Table 2: Osteoblastic Activity Values at 30, 60, 90 Days in Experimental Group

Sr.No	Control		
	30	60	90
1	46.2	47.83	46.02
2	45.97	48.16	46.63
3	46.14	48.02	46.98
4	50.11	51.08	49.56
5	46.61	47.8	47.98
6	46.9	47.96	48.02
7	46.86	47.83	48.67
8	46.84	48.14	48.56
9	45.51	48.23	48.9
10	50.07	51.16	49.91
Mean	47.121	48.621	48.123
SD	1.626806	1.325539	1.262335

Table 3: Serum Alkaline Phosphatase Values in Experimental and Control Groups at 30 Days and 90 Days

Sr. No	Experimental		Control	
	30	90	30	90
1	12.48	22.14	10.77	12.16
2	13.53	21.44	9.88	12.28
3	14.04	22.6	9.05	11.64
4	14.81	20.71	10.76	12.56
5	13.62	22.69	9.24	13.35
6	12.17	22.05	10.83	11.01
7	12.4	20.26	9.67	11.94
8	12.8	18.42	10.91	12.34
9	13.83	20.26	9.76	12.27
10	14.47	22.73	10.85	11.55
Mean	13.415	21.33	10.172	12.11
SD	0.91347	1.404414	0.728405	0.634508

Table 4: Mean Distribution and Within-Group Comparison of the Osteoblastic Activity at 30 Days, 60 Days, and 90 Days in Experimental and Control Groups.

		N	Minimum	Maximum	Mean	Std. Deviation	F	pValue
Experimental	30Days	10	45.96	55.84	50.2760	3.02900	10.470	.000*

	60 Days	10	52.91	55.85	53.99 60	1.0385 6		
	90 Days	10	52.15	55.62	53.29 80	.97434		
Control	30 Days	10	45.51	50.11	47.12 10	1.6268 1	2.920	.071
	60 Days	10	47.80	51.16	48.62 10	1.3255 4		
	90 Days	10	46.02	49.91	48.12 30	1.2623 4		

*- ($p < 0.05$) using one way Analysis of Variance

The osteoblastic activity for the experimental group showed a statistically significant difference over time. At 30 days, the mean osteoblastic activity was 50.28 ± 3.03, with a significant rise to 53.99 ± 1.04 at 60 days and remaining more or less constant at 53.30 ± 0.97 at 90 days. One-way ANOVA showed that the within-group difference across the three-time intervals was highly significant: $F = 10.470$, $p < 0.001$. However, the control group had no statistically significant change in osteoblastic activity. The mean value was 47.12 ± 1.63 units at 30 days, 48.62 ± 1.33 units at 60 days, and 48.12 ± 1.26 units at 90 days. One-way ANOVA indicated no statistically significant difference within the group ($F = 2.920$; $p = 0.071$).

Table 5: Pairwise Comparison of the Osteoblastic Activity at 30 Days, 60 Days, and 90 Days in Experimental and Control Groups.

Multiple Comparisons							
Tukey HSD							
Dependent Variable	(I) Time Interval	(J) Time Interval	Mean Difference (I-J)	Std. Error	pValue	95% Confidence Interval	
						Lower Bound	Upper Bound
Experimental	30 Days	60 Days	-3.72000	.86421	.001*	-5.86273	-1.57773
		90 Days	-3.02200	.86421	.005*	-5.16447	-.87953
	60 Days	90 Days	.69800	.86421	.702	-1.44447	2.84007
Control	30 Days	60 Days	-1.50000	.63230	.063	-3.06777	.06777
		90 Days	-1.00200	.63230	.269	-2.56997	.56557

	60 Days	90 Days	.49800	.63230	.714	-1.0697	2.0657

*- (p <0.05) using Tukey's post hoc comparison

In the post-hoc Tukey analysis for the experimental group, the increase from 30 to 60 days (mean difference = 3.72, p = 0.001) and from 30 to 90 days (mean difference = 3.02, p = 0.005) was statistically significant, but from 60 to 90 days, it did not attain statistical significance (p = 0.702).

The results of the Tukey test also indicated the lack of statistical significance in the difference between 30 and 60 days, 30 and 90 days, and 60 and 90 days (p < 0.05), showing the least variation in osteoblastic activity in the control group.

Table 6: Between-group comparison of Osteoblastic Activity at 30 Days, 60 Days, and 90 Days in Experimental and Control Groups.

Groups		N	Mean	Std. Deviation	Std. Error Mean	Mean Difference	t	pValue
30 Days	Experimental	10	50.2760	3.02900	.95785	3.15500	2.902	.010*
	Control	10	47.1210	1.62681	.51444			
60 Days	Experimental	10	53.9960	1.03856	.32842	5.37500	10.094	.000*
	Control	10	48.6210	1.32554	.41917			
90 Days	Experimental	10	53.2980	.97434	.30811	5.17500	10.262	.000*
	Control	10	48.1230	1.26234	.39919			

*- p<0.05 students' independent t-test

Comparative analysis between the groups indicated a greater osteoblastic activity in the experimental group at each of the points in time examined. At the 30-day mark, the osteoblastic activity was recorded to be higher in the experimental (50.28 ± 3.03) compared to the control (47.12 ± 1.63) with a mean difference of 3.16; t = 2.902, p = 0.010. This difference expanded to be greater in magnitude at the 60-day mark, with a recorded value of 53.99 ± 1.04 in the experimental group against a value of 48.62 ± 1.33 in the control group, with a mean difference of 5.38; t = 10.094, p < 0.001. At the 90-day mark, a similarly greater difference was seen, with a value of 53.30 ± 0.97 in the experimental group against a value of 48.12 ± 1.26 in the control group, and a mean difference of 5.18; t = 10.262, p < 0.001.

Table 7: Between-group comparison of Mean Change in Osteoblastic Activity from 30 Days – 60 Days, 30 Days – 90 Days, and 60 Days to 90 Days.

Groups		N	Mean	Std. Deviation	Std. Error Mean	Mean Difference	t	pValue
Mean Difference in Osteoblastic Activity (30 Days - 60 Days)	Experimental	10	3.7200	2.58915	.81876	2.22000	2.643	.017*
	Control	10	1.5000	.59492	.18813			
Mean Difference in osteoblastic activity (30 Days - 90	Experimental	10	3.0220	2.76847	.87547	2.02000	2.125	.048*

Days)								
	Control	10	1.00020	1.16997	.36998			
Mean Difference in Osteoblastic Activity (60 Days - 90 Days)	Experimental	10	-.6980	.81058	.25633	-.20000	-.484	.634
	Control	10	-.4980	1.02515	.32418			

*- p<0.05 students' independent t-test

Analysis of the mean change in osteoblastic activity demonstrated that the experimental group had significantly higher increases compared to the control group. For the period between 30 and 60 days, the mean increase for the experimental group was 3.72 ± 2.59, while the increase for the control group was 1.50 ± 0.59; this difference was significant at t = 2.643 and p = 0.017. This shows that between 30 and 90 days, the mean increase was 3.02 ± 2.77 for the experimental group and 1.00 ± 1.17 for the control group, with a statistically significant between-group difference at t = 2.125 and p = 0.048. However, from 60 to 90 days, there was hardly any change within the groups (experimental: –0.70 ± 0.81; control: –0.50 ± 1.03), and no statistically significant difference can be reported at p = 0.634.

Discussion:

The present split-mouth randomized controlled clinical trial was conducted to evaluate the effect of adjunctive low-intensity pulsed ultrasound (LIPUS) therapy on osteoblastic activity around dental implants in patients with type 2 diabetes mellitus. Diabetes mellitus is a well-recognized systemic condition that adversely affects bone metabolism and wound healing, thereby posing challenges to successful osseointegration of dental implants. The rationale for the present study was based on the need to explore non-invasive and clinically feasible adjunctive therapies that can enhance peri-implant bone healing in this medically compromised patient population.

A split-mouth study design was adopted to minimize inter-subject variability and to ensure better internal validity of the results. By placing implants in bilaterally comparable sites within the same patient, confounding factors such as glycemic status, bone quality, systemic health, and healing potential were effectively controlled. This allowed a more accurate assessment of the influence of LIPUS therapy on osteoblastic activity.

Bone scintigraphy using Tc-99m methylene diphosphonate was employed to assess osteoblastic activity around the implant sites at 30, 60, and 90 days following implant placement. Bone scintigraphy provides a functional evaluation of bone metabolism by detecting areas of increased osteoblastic activity and vascularity, making it particularly suitable for assessing early bone healing changes that may not be apparent on conventional radiographs.

In the experimental group, a progressive increase in osteoblastic activity was observed from 30 days to 60 days, followed by a relative stabilization from 60 days to 90 days. Statistical analysis using one-way ANOVA revealed a highly significant difference in osteoblastic activity across the three time intervals ($p < 0.001$). This finding indicates that LIPUS therapy significantly enhances peri-implant bone activity during the early and intermediate phases of healing.

In contrast, the control group demonstrated only minimal changes in osteoblastic activity over time, and these changes were not statistically significant. This observation supports existing evidence that diabetes mellitus is associated with impaired bone healing, likely due to reduced osteoblastic differentiation, compromised angiogenesis, and prolonged inflammatory response.

Post-hoc Tukey analysis revealed that, in the experimental group, the increase in osteoblastic activity from 30 to 60 days and from 30 to 90 days was statistically significant, whereas the change from 60 to 90 days was not significant. This pattern suggests that the primary effect of LIPUS therapy occurs during the early stages of osseointegration, when active bone formation predominates. Once the healing process transitions into the remodeling phase, the rate of increase in osteoblastic activity tends to plateau. The absence of statistically significant changes between time intervals in the control group further emphasizes the limited regenerative response in diabetic patients when no adjunctive stimulation is employed.

Intergroup comparison demonstrated significantly higher osteoblastic activity in the experimental

group compared to the control group at all evaluated time points. At 30 days, the experimental group already exhibited a statistically significant increase in activity, indicating an early stimulatory effect of LIPUS therapy. This difference became more pronounced at 60 and 90 days, highlighting the sustained influence of LIPUS on peri-implant bone metabolism. The greater magnitude of difference observed at 60 and 90 days suggests that LIPUS not only accelerates early bone formation but also results in a more favorable biological environment for ongoing bone remodeling in diabetic patients.

The analysis of mean changes in osteoblastic activity between different time intervals further corroborated the positive effect of LIPUS therapy. The experimental group showed significantly greater increases in osteoblastic activity from 30 to 60 days and from 30 to 90 days compared to the control group. However, the change from 60 to 90 days was minimal and not statistically significant in both groups, reflecting the natural progression from bone formation to maturation and remodeling. These findings reinforce the concept that adjunctive LIPUS therapy is most beneficial during the early healing period, which is critical for achieving stable osseointegration.

Alkaline phosphatase (ALP) levels in peri-implant crevicular fluid were assessed as a biochemical marker of osteoblastic activity at 30 and 90 days. The experimental group demonstrated higher ALP levels at both time points compared to the control group. Additionally, a marked increase in ALP levels was observed from 30 to 90 days in the experimental group. ALP plays a crucial role in bone mineralization and is a reliable indicator of osteoblastic differentiation. The elevated ALP levels observed in the LIPUS-treated sites are in agreement with the scintigraphic findings and confirm enhanced osteogenic activity at the cellular level. In contrast, the comparatively lower ALP levels in the control group reflect compromised bone metabolism associated with diabetes mellitus.

The enhanced osteoblastic activity observed in the experimental group may be attributed to the mechanotransductive effects of LIPUS. Low-intensity ultrasound waves generate mechanical microstimulations that activate intracellular signaling pathways involved in osteogenesis. These effects include increased osteoblastic proliferation, upregulation of bone-specific markers, enhanced angiogenesis, and improved extracellular matrix mineralization. In diabetic patients, where osteoblastic function is suppressed due to chronic hyperglycemia and inflammatory imbalance, LIPUS may help restore a more favorable bone healing environment by stimulating cellular activity and improving local vascularity.

The findings of the present study suggest that adjunctive LIPUS therapy can significantly enhance peri-implant osteoblastic activity in patients with type 2 diabetes mellitus. By improving early bone healing, LIPUS may reduce the risk of delayed osseointegration and early implant failure in this high-risk group. Given its non-invasive nature and ease of application, LIPUS may be considered a valuable adjunct in implant therapy for diabetic patients. The study was limited by a relatively small sample size. Future studies with larger sample sizes are recommended to further validate the role of LIPUS in implant therapy for diabetic patients.

Conclusion:

Within the limitations of this split-mouth randomized controlled trial, adjunctive low-intensity pulsed ultrasound (LIPUS) therapy significantly enhanced osteoblastic activity around dental implants in patients with type 2 diabetes mellitus. Bone scintigraphy demonstrated a significant increase in osteoblastic activity in the experimental group, particularly during the early healing phase, whereas the control group showed no significant change. Higher alkaline phosphatase levels in the LIPUS-treated sites further supported enhanced osteoblastic activity at the cellular level. These findings suggest that LIPUS may serve as a safe, non-invasive adjunct to improve peri-implant bone healing and osseointegration in diabetic patients.