

ORIGINAL ARTICLE

# Periodontal Laser Therapy Modulates Rankl-Associated Bone Resorption in Diabetic Patients

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## ABSTRACT

**OBJECTIVE:** To evaluate the impact of periodontal laser therapy on RANKL levels, pro-inflammatory cytokines, and oxidative stress markers in diabetic patients with chronic periodontitis.

**METHODOLOGY:** We conducted a comparative study of diabetic patients with chronic periodontitis from October 2023 to April 2024 at the Institute of Molecular Biology and Biotechnology (IMBB), University of Lahore, Lahore, Pakistan. We performed complete blood counts for red and white blood cells using an automated analyzer. We assessed oxidative stress by measuring malondialdehyde (MDA) using the thiobarbituric acid reactive substances (TBARS) assay. Receptor activator of nuclear factor kappa-b ligand (RANKL), tumor necrosis factor-alpha (TNF-alpha), and interleukin-6 (IL-6) levels were quantified using sandwich ELISA. We used one-way analysis of variance and the Tukey test to analyze the data in GraphPad Prism.

**RESULTS:** Diabetic periodontitis patients showed significantly ( $p < 0.001$ ) high levels of IL-6 ( $1.85 \pm 0.05$  ng/mL), TNF- $\alpha$  ( $1.83 \pm 0.08$  ng/mL), RANKL ( $2.15 \pm 0.20$  ng/mL), and MDA ( $1.90 \pm 0.22$  ng/mL) before treatment compared to control, while laser-treated significantly ( $p < 0.001$ ) reduced IL-6 ( $1.08 \pm 0.07$  ng/mL), TNF- $\alpha$  ( $1.08 \pm 0.18$  ng/mL), RANKL ( $1.05 \pm 0.10$  ng/mL) and MDA ( $1.20 \pm 0.10$  ng/mL) levels compared to periodontitis group. SOD levels dropped in the periodontitis group ( $0.68 \pm 0.10$  ng/mL) but increased after laser treatment ( $1.02 \pm 0.05$  ng/mL).

**CONCLUSION:** Periodontal laser therapy markedly reduced inflammatory burden and bone resorption potential, underscoring its effectiveness as an adjunctive treatment for periodontitis in diabetic patients.

**KEYWORDS:** Periodontitis, Laser Therapy, RANKL, SOD, TNF- $\alpha$ , IL6, MDA

## INTRODUCTION

Periodontitis is a chronic inflammatory disease that affects the supporting structures of the teeth, such as the gingiva, periodontal ligament, cementum, and alveolar bone, leading to tooth mobility and tooth loss if left untreated<sup>1</sup>. It begins with the accumulation of bacterial dental plaque, which gradually mineralizes into calculus when oral hygiene is inadequate<sup>2</sup>. The presence of calculus promotes the formation of periodontal pockets, creating a favorable environment for pathogenic bacteria that release toxins and stimulate a persistent host inflammatory response that results in progressive destruction of periodontal tissues<sup>2,3</sup>.

A bidirectional relationship exists between periodontitis and diabetes mellitus<sup>4</sup>. Chronic periodontal inflammation contributes to systemic inflammation, impairing glycemic control and increasing the risk of diabetes progression<sup>4</sup>. Conversely, persistent hyperglycemia promotes the formation of advanced glycation end-products (AGEs), which interact with their receptors (RAGEs) in periodontal tissues. Activation of the AGE–RAGE signaling pathway stimulates monocytes, macrophages, and endothelial cells to produce pro-inflammatory cytokines, including interleukin (IL)-1 $\beta$ , IL-6, and tumor necrosis factor-alpha (TNF- $\alpha$ ). This inflammatory cascade disrupts the receptor activator of nuclear factor kappa-B ligand/osteoprotegerin (RANKL/OPG) balance, thereby accelerating alveolar bone destruction and periodontal tissue breakdown. Studies have consistently reported elevated RAGE expression in diabetic patients with periodontitis compared with non-diabetic individuals with periodontal disease, highlighting the synergistic effects of diabetes on periodontal inflammation<sup>4</sup>.

Alveolar bone resorption is one of the most significant pathological consequences of periodontitis and is primarily regulated by the RANK/RANKL/OPG signaling pathway<sup>5</sup>. RANKL, a member of the TNF superfamily produced by osteoblasts, fibroblasts, stromal cells, and activated immune cells, binds to its receptor RANK on osteoclast precursors, promoting osteoclast differentiation and activation. OPG acts as a decoy receptor that inhibits this interaction, thereby suppressing bone resorption. In periodontitis, increased RANKL expression together with reduced OPG levels shifts the balance toward excessive osteoclastic activity, resulting in progressive alveolar bone loss. This imbalance is further amplified in diabetic patients due to chronic inflammation and oxidative stress<sup>4,6</sup>.

Periodontal laser therapy has emerged as a minimally invasive adjunct to conventional non-surgical periodontal treatment<sup>7</sup>. Laser-assisted therapy facilitates selective removal of inflamed periodontal tissues, reduces bacterial load, promotes root surface decontamination, and enhances wound healing while minimizing postoperative discomfort<sup>7</sup>. In addition to its antimicrobial effects, low-level laser therapy exhibits photobiomodulatory properties that modulate inflammatory responses, reduce oxidative stress, and promote tissue regeneration<sup>8</sup>. Although several studies have demonstrated short-term clinical improvements following laser therapy, its effects on molecular pathways involved in periodontal destruction, particularly the RANKL-mediated bone remodeling pathway, remain incompletely understood<sup>6,8</sup>.

Despite the established association among diabetes, periodontal inflammation, oxidative stress, and RANKL-mediated bone resorption, evidence on the molecular effects of periodontal laser therapy in diabetic patients with chronic periodontitis remains limited<sup>9</sup>. Specifically, the impact of laser therapy on RANKL expression, inflammatory cytokines, and oxidative stress biomarkers remains unclear. Therefore, the present study aimed to evaluate the effects of periodontal laser therapy on RANKL levels, pro-inflammatory cytokines, and oxidative stress markers in diabetic

patients with chronic periodontitis, providing insights into its potential role in reducing inflammation and preventing periodontal bone loss.

## METHODOLOGY

### *Study settings*

We conducted a quasi-experimental, pre-post interventional study among diabetic patients with chronic periodontitis from October 2023 to April 2024 at the Institute of Molecular Biology and Biotechnology (IMBB), University of Lahore, Lahore, Pakistan. We obtained informed consent from all participants in accordance with the Declaration of Helsinki. A total of 60 unique individuals were enrolled, consisting of an experimental group of 50 diabetic patients with chronic periodontitis (25 before treatment and 25 post-treatment) and a control group of 10 systemically and periodontally healthy subjects. **Table I** lists the treatment groups used in this study.

**Table I: Treatment groups and acronyms**

| No. | Treatments group        | Acronyms |
|-----|-------------------------|----------|
| 1   | Healthy Control         | H.C      |
| 2   | Untreated periodontitis | P        |
| 3   | Laser-treated           | L        |

### *Sample size*

To evaluate sample size adequacy, we performed a retrospective sensitivity power analysis for a one-way analysis of variance (ANOVA) in G\*Power (version 3.1.9.7). Based on the final sample distribution across the three distinct groups ( $n_1 = 10$ ,  $n_2 = 25$ ,  $n_3 = 25$ ; Total  $N = 60$ ) and a significance level of  $\alpha = 0.05$ , the study was evaluated for its capacity to detect a large clinical effect size (Cohen's  $f = 0.50$ ), which is typically observed in severe chronic tissue inflammation and biomarker disparity. Results indicate that the final sample of  $N = 60$  provides 92.34% statistical power to detect an effect of this magnitude reliably.

### *Inclusion and exclusion criteria*

The study includes patients above the age of 25 years who have both periodontitis and diabetes, provided they are managed with oral hypoglycemic drugs or insulin, while participants who have used steroids in the last three months, received periodontal therapy within the past six months, are pregnant or lactating women, or are tobacco/alcohol users were excluded from this study. A healthy control group without periodontitis or diabetes, aged 25 years or older, and able to give informed consent before inclusion were included.

### *Diagnostic criteria*

We diagnosed, staged, and graded periodontitis using the 2017 AAP/EFP system. Diagnosis required periodontal pocket depth (PPD)  $\geq 4$  mm at  $\geq 2$  non-adjacent teeth with bleeding on probing (BOP), or widespread gingival inflammation with pathological pocketing. We classified severity as Stage I (Initial), Stage II (Moderate), Stage III (Severe), or Stage IV (Advanced)

based on maximum pocket depth and inflammation. Extent was categorized as localized (<30% of teeth) or generalized ( $\geq 30\%$  of teeth). Diabetes mellitus was diagnosed and monitored following the american diabetes association (ADA) guidelines. Participants were classified using standard diagnostic thresholds, including a glycated hemoglobin (HbA1c) level  $\geq 6.5\%$  (48 mmol/mol), a fasting plasma glucose (FPG) level  $\geq 126$  mg/dL ( $\geq 7.0$  mmol/L) after an 8-hour fast, or a random plasma glucose level  $\geq 200$  mg/dL ( $\geq 11.1$  mmol/L) accompanied by classic clinical symptoms of hyperglycemia.

### ***Periodontal laser therapy***

Adjunctive laser therapy was performed immediately following scaling and root planing (SRP) using a soft tissue diode laser system (Biolase Technology Inc., CA, USA). The operational parameters were standardized as follows: a wavelength of 940 nm, a power output of 1.5 W in pulsed emission mode (duty cycle: 50%, pulse duration: 10 ms), and a fiber tip diameter of 300  $\mu\text{m}$ . The fiber tip was kept uninitiated and inserted parallel to the long axis of the tooth into the periodontal pocket, then moved in a continuous circumferential, slow sweeping motion from distal to mesial aspects to prevent thermal injury. Exposure was limited to 15 seconds per pocket surface, for a maximum of 60 seconds per tooth. Laser application was repeated at a frequency of two sessions, spaced 4 weeks apart during the active phase of treatment. We measured bleeding, pocket depth, and gingival tone 3 months post-treatment.

### ***Blood collection and processing***

Fasting venous blood (10 mL) was drawn from each subject by venipuncture into two tubes: (1) 3 mL into an EDTA tube for complete blood count (CBC) and (2) 7 mL into a plain tube for serum separation. Plain tubes were allowed to clot at room temperature for 30 minutes and then centrifuged at  $1,500 \times g$  for 10 minutes at  $4^\circ\text{C}$ . Serum was aliquoted into 0.5–1.0 mL fractions and stored at  $-80^\circ\text{C}$  until analysis. All samples underwent no more than one freeze-thaw cycle before assay.

### ***Hematology: RBC and WBC measurement***

Complete blood counts, including red blood cell (RBC) and white blood cell (WBC) counts, were performed on fresh EDTA whole blood using an automated hematology analyzer (model: Sysmex XN-1000, manufacturer: Sysmex Corporation, Kobe, Japan). We performed quality control daily using the manufacturer's control materials. RBC and WBC values are reported as  $\times 10^{12}/\text{L}$  and  $\times 10^9/\text{L}$ , respectively. All assays were run in the clinical laboratory according to standard operating procedures.

### ***MDA and SOD evaluation***

To evaluate oxidative stress, malondialdehyde (MDA) was measured using a thiobarbituric acid reactive substances (TBARS) colorimetric assay, whereby the serum samples were incubated with TBA reagent at high temperature to form a malondialdehyde to Thio barbituric acid complex, which is detected at 532 nm. Antioxidant activity was determined by assessing the ability of superoxide dismutase (SOD) using a colourimetric test, measuring the ability to suppress superoxide-induced reduction of a tetrazolium salt by absorbance at 450 nm, expressed in units per millilitre (U/ml) of activity. Each assay was performed in duplicate using freshly thawed serum aliquots, and internal controls were included on every plate to ensure accuracy of analysis and reproducibility.

### ***ELISA of IL-6, RANKL, and TNF- $\alpha$***

We analyzed all participants' serum samples in terms of inflammatory, oxidative stress, and antioxidant biomarkers. We determined concentrations of human RANKL (Cat. No. E0620Hu), TNF- $\alpha$  (Cat. No. E0082Hu), and IL-6 (Cat. No. E0090Hu) using commercial 96-well sandwich ELISA kits (Bioassay Technology Laboratory, China) according to the manufacturer's instructions. All reagents, standards, and serum samples were incubated at room temperature before analysis, and standard curves were prepared using serial dilutions of recombinant cytokines for proper quantification. Serum samples were thawed on ice, centrifuged to remove particulates, and loaded into antibody-coated duplicate wells. Plates were incubated with capture and detection antibodies, washed, then streptavidin-HRP conjugate was added; TMB substrate was used to develop colour, and absorbance at 450 nm was measured. A four-parameter logistic (4-PL) standard curve was used to compute concentrations, and a variation of more than 15% in suspected duplicate samples was subject to re-assay.

### ***Statistical Analysis***

Statistical analysis was done in GraphPad Prism version 8.0 (GraphPad Software, USA). We assessed data normality using the Shapiro–Wilk test before inferential analyses. Quantitative variables are presented as mean  $\pm$  standard deviation (SD). We initially compared the healthy control, pre-treatment diabetic periodontitis, and post-treatment diabetic periodontitis groups using one-way analysis of variance (ANOVA), followed by Tukey's multiple comparison test where appropriate. For within-subject pre- and post-treatment comparisons in the same diabetic patients, we used a paired Student's t-test for normally distributed data. Statistical significance was set at  $p < 0.05$ , and exact p-values are reported wherever possible. A 95% confidence interval (CI) was used, and effect sizes were calculated using Cohen's d for paired comparisons and Cohen's f for ANOVA.

## RESULTS

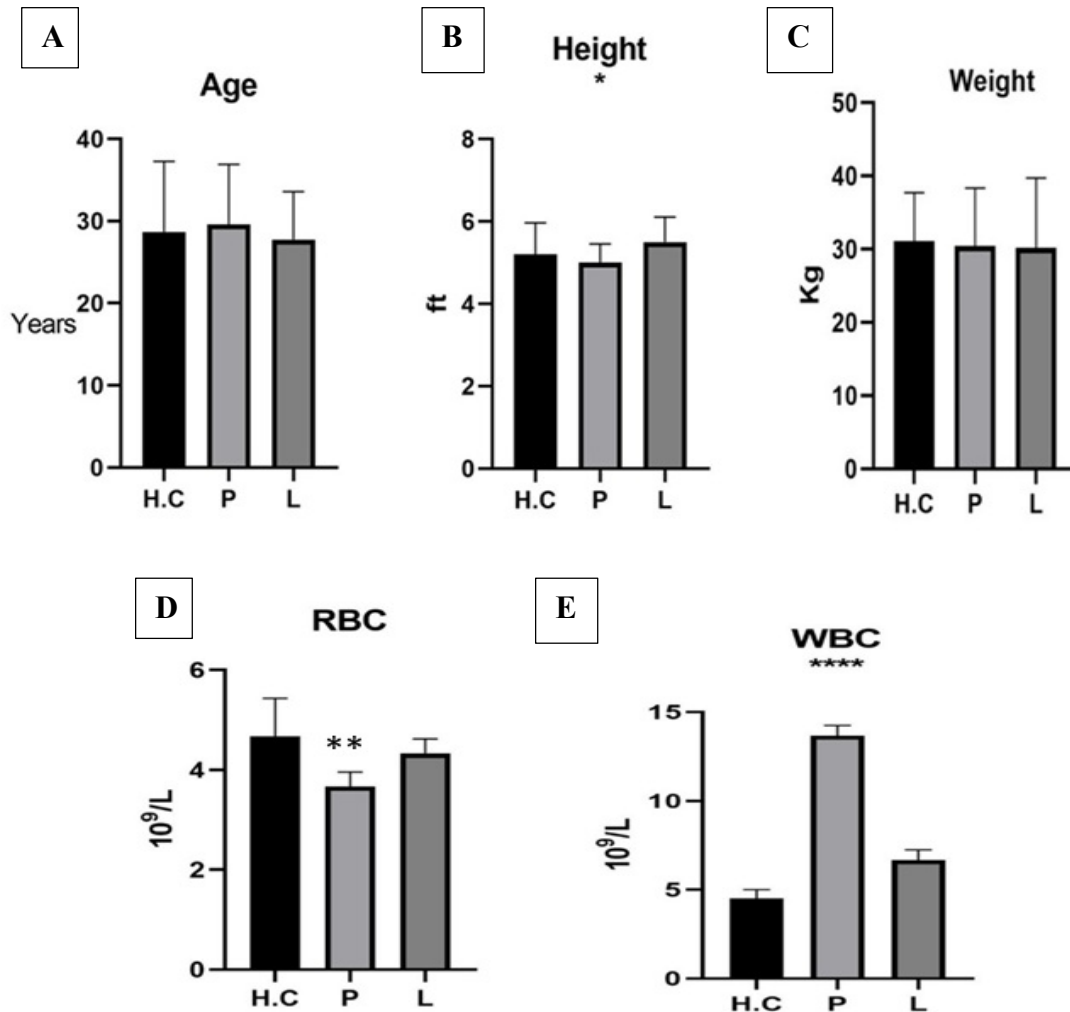
The present study included 60 participants: a healthy control group (H.C., n=10), an untreated periodontitis group (P, n=25), and a laser-treated periodontitis cohort (L, n=25). Baseline clinical characteristics remained uniform across all study groups with no statistically significant differences (**Table II**). The mean age was  $28.5 \pm 4.2$  years for healthy controls,  $29.2 \pm 5.1$  years for the untreated periodontitis group, and  $27.5 \pm 4.6$  years for the laser-treated group ( $p > 0.05$ ) (**Figure 1A**). Similarly, anthropometric variations in height ( $5.2 \pm 0.3$  ft vs  $5.0 \pm 0.4$  ft vs  $5.4 \pm 0.3$  ft) (**Figure 1B**) and weight ( $30.8 \pm 3.5$  kg vs  $30.0 \pm 4.1$  kg vs  $29.8 \pm 3.9$  kg) (**Figure 1C**).

Short-term post-therapy evaluation demonstrated significant resolution of periodontal destruction. Laser therapy resulted in a 77.78% reduction in bleeding on probing ( $15.2 \pm 4.3\%$  vs  $68.4 \pm 12.5\%$ ,  $p < 0.001$ ) and a 57.14% decrease in mean Probing Pocket Depth ( $2.4 \pm 0.4$  mm vs  $5.6 \pm 0.8$  mm,  $p < 0.001$ ). Furthermore, tissue tone improved markedly, as reflected by a 75.00% reduction in the Gingival Index ( $0.6 \pm 0.2$  vs  $2.4 \pm 0.4$ ,  $p < 0.001$ ). These findings indicate accelerated wound healing and a rapid return to periodontal stability (**Table II**).

Untreated periodontitis caused significant systemic physiological and metabolic changes in diabetic patients. The periodontitis cohort ( $n = 25$ ) exhibited significantly depressed RBC ( $3.7 \pm 0.5 \times 10^9/L$ ) compared to the healthy control group ( $4.7 \pm 0.4 \times 10^9/L$ ,  $p < 0.01$ ) (**Figure 1D**). Concurrently, WBC counts spiked more than threefold in the periodontitis (P) group, reaching  $13.6 \pm 2.4 \times 10^9/L$  compared with the healthy control (H.C) group ( $4.4 \pm 0.9 \times 10^9/L$ ;  $p < 0.001$ ) (**Figure 1E**). Untreated patients also had severe, uncontrolled fasting blood glucose levels. **Figure 1** presents the demographic profile and haematological parameters of the study population, diabetic periodontitis patients, and healthy controls.

The figure shows age (A), height (B), weight (C), RBC (D), and WBC (E) across all groups. We used one-way analysis of variance (ANOVA), followed by Tukey's multiple comparison test, to analyze the data in GraphPad Prism. Asterisks (\*) denote statistically significant differences with healthy controls.

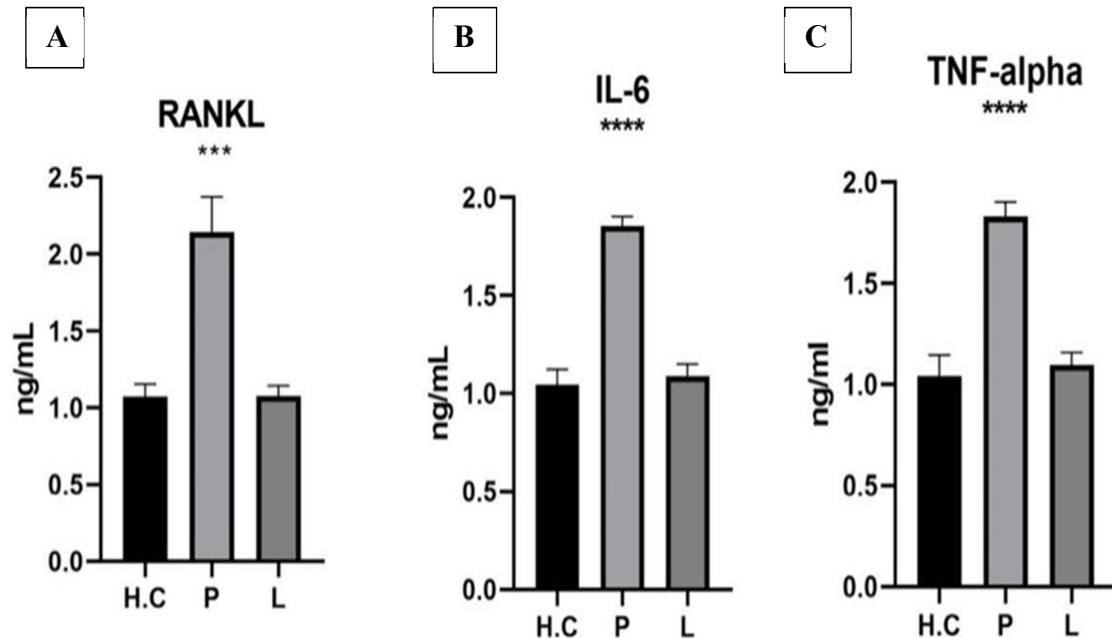
peaking at  $165.2 \pm 18.8$  mg/dL ( $p < 0.001$ ). Following the application of adjunctive periodontal laser therapy, these parameters stabilized significantly. In the laser-treated cohort, WBC counts normalized completely to  $6.7 \pm 1.2 \times 10^9/L$ , and RBC counts recovered toward normal clinical parameters ( $4.3 \pm 0.4 \times 10^{12}/L$ ). Furthermore, glycemic stress was significantly down-regulated, with fasting blood glucose falling to  $110.1 \pm 12.5$  mg/dL ( $p < 0.001$ ) (**Table II**).



**Table II** summarises the biochemical findings. Compared with healthy controls, patients with untreated periodontitis (P group) exhibited significantly higher levels of osteoclastogenic and inflammatory biomarkers. RANKL levels significantly ( $p < 0.001$ ) increased in the P group ( $2.15 \pm 0.20$  ng/mL) compared to healthy controls ( $1.05 \pm 0.10$  ng/mL) (**Figure 2A**). Likewise, IL-6 and TNF- $\alpha$  concentrations increased significantly ( $p < 0.001$ ) to  $1.85 \pm 0.05$  ng/mL and  $1.83 \pm 0.08$  ng/mL, respectively, compared with healthy controls (**Figure 2**).

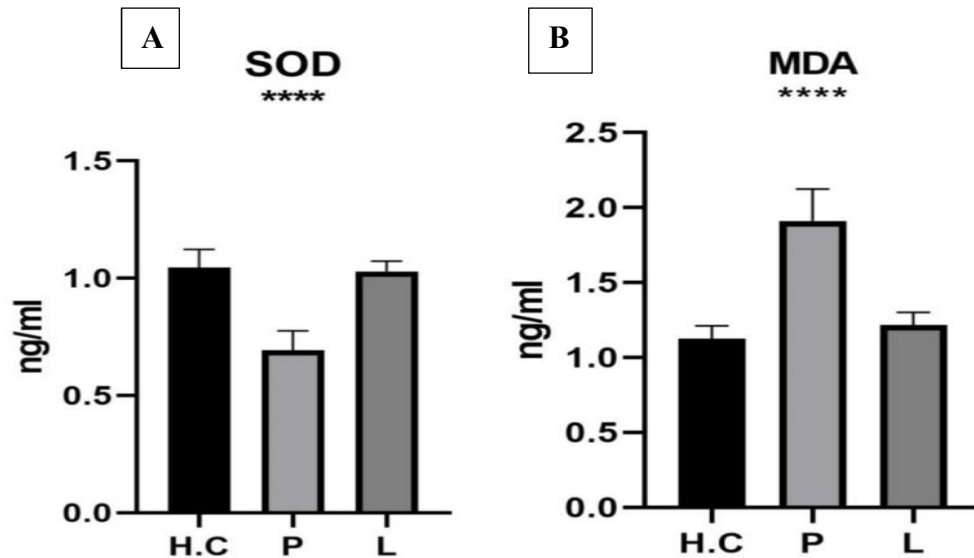
Compared with the untreated periodontitis group, adjunctive periodontal laser therapy (L group) significantly reduced all inflammatory biomarkers. RANKL significantly ( $p < 0.001$ ) decreased by 51.16%, from  $2.15 \pm 0.20$  ng/mL to  $1.05 \pm 0.10$  ng/mL. Similarly, IL-6 significantly ( $p < 0.001$ ) declined by 41.62%, from  $1.85 \pm 0.05$  ng/mL to  $1.08 \pm 0.07$  ng/mL (**Figure 2B**), while TNF- $\alpha$  significantly ( $p < 0.001$ ) decreased by 40.98%, from  $1.83 \pm 0.08$  ng/mL to  $1.08 \pm 0.18$  ng/mL (**Figure 2C**). Furthermore, no significant differences were observed between the laser-treated and healthy control groups for RANKL, IL-6, or TNF- $\alpha$ , which indicates that laser therapy effectively restored these biomarkers to near-normal levels (**Figure 2**). Untreated periodontitis was associated with marked oxidative stress, characterized by significantly reduced SOD activity and increased MDA concentrations compared with healthy controls. In the P group,

SOD levels significantly ( $p < 0.001$ ) decreased from  $1.05 \pm 0.07$  ng/mL in healthy controls to  $0.68 \pm 0.10$  ng/mL, whereas MDA significantly ( $p < 0.001$ ) increased from  $1.12 \pm 0.09$  ng/mL to  $1.90 \pm 0.22$  ng/mL (**Figure 3**).



**Figure 2: The levels of RANKL (A), IL-6 (B), and TNF-alpha (C) among all treatments:** One-way analysis of variance (ANOVA), followed by Tukey's multiple comparison test, was used to test the data in GraphPad Prism. Asterisks (\*) denote statistically significant differences compared with the healthy control.

Compared with the P group, adjunctive laser therapy (L group) significantly improved oxidative stress status. SOD activity significantly ( $p < 0.001$ ) increased by 50.00%, rising from  $0.68 \pm 0.10$  ng/mL in the P group to  $1.02 \pm 0.05$  ng/mL in the L group (**Figure 3A**). Conversely, MDA concentrations significantly ( $p < 0.001$ ) decreased by 36.84%, from  $1.90 \pm 0.22$  ng/mL to  $1.20 \pm 0.10$  ng/mL (**Figure 3B**). No significant ( $p > 0.05$ ) differences were detected between the laser-treated and healthy control groups for either SOD or MDA, which suggests that adjunctive laser therapy effectively restored antioxidant capacity and reduced oxidative damage.



**Figure 3: SOD (A) and MDA (B) levels among all treatments:** One-way analysis of variance (ANOVA), followed by Tukey's multiple comparison test, was used to test data in GraphPad Prism. Asterisks (\*) denote statistically significant differences with healthy controls.

**Table II: The demographic profile and all parameters of the study population, diabetic periodontitis patients, and the healthy controls are presented**

| Parameter                                   | Healthy Control (H.C) (n=10)   | Periodontitis (P) (n=25)         | Laser-treated (L) (n=25)        | Percentage change: L vs. P | p-value (P vs H.C) | p-value (L vs H.C) | p-value (L vs P) |
|---------------------------------------------|--------------------------------|----------------------------------|---------------------------------|----------------------------|--------------------|--------------------|------------------|
| <b>Age (Years)</b><br>95% CI                | 28.5 ± 8.5<br>[23.23 to 33.77] | 29.5 ± 7.5<br>[26.56 to 32.44]   | 27.5 ± 6.0<br>[25.15 to 29.85]  | -6.78%                     | > 0.05             | > 0.05             | > 0.05           |
| <b>Height (ft)</b><br>95% CI                | 5.2 ± 0.8<br>[4.70 to 5.70]    | 5.0 ± 0.5<br>[4.80 to 5.20]      | 5.5 ± 0.6<br>[5.26 to 5.74]     | +10.00%                    | > 0.05             | < 0.05             | < 0.05           |
| <b>Weight (Kg)</b><br>95% CI                | 31.0 ± 7.0<br>[26.66 to 35.34] | 30.0 ± 8.0<br>[26.86 to 33.14]   | 30.0 ± 10.0<br>[26.08 to 33.92] | 0.00%                      | > 0.05             | > 0.05             | > 0.05           |
| <b>Bleeding on probing (BOP)</b><br>95% CI  | 8.5 ± 2.1%<br>[7.20 to 9.80]   | 68.4 ± 12.5%<br>[63.50 to 73.30] | 15.2 ± 4.3%<br>[13.51 to 16.89] | -77.78%                    | < 0.001            | > 0.05             | < 0.001          |
| <b>Probing pocket depth (PPD)</b><br>95% CI | 1.8 ± 0.3 mm<br>[1.61 to 1.99] | 5.6 ± 0.8 mm<br>[5.29 to 5.91]   | 2.4 ± 0.4 mm<br>[2.24 to 2.56]  | -57.14%                    | < 0.001            | < 0.01             | < 0.001          |
| <b>Gingival index (GI)</b><br>95% CI        | 0.2 ± 0.1<br>[0.14 to 0.26]    | 2.4 ± 0.4<br>[2.24 to 2.56]      | 0.6 ± 0.2<br>[0.52 to 0.68]     | -75.00%                    | < 0.001            | > 0.05             | < 0.001          |
| <b>RBC (10<sup>9</sup>/L)</b><br>95% CI     | 4.7 ± 0.4<br>[4.45 to 4.95]    | 3.7 ± 0.5<br>[3.50 to 3.90]      | 4.3 ± 0.4<br>[4.14 to 4.46]     | +16.22%                    | < 0.01             | > 0.05             | < 0.01           |
| <b>WBC (10<sup>9</sup>/L)</b>               | 4.4 ± 0.9                      | 13.6 ± 2.4                       | 6.7 ± 1.2                       | -50.74%                    | < 0.001            | > 0.05             | < 0.001          |

|                                             |                               |                                  |                                  |         |         |        |         |
|---------------------------------------------|-------------------------------|----------------------------------|----------------------------------|---------|---------|--------|---------|
| 95% CI                                      | [3.84 to 4.96]                | [12.66 to 14.54]                 | [6.23 to 7.17]                   |         |         |        |         |
| <b>Fasting glucose</b><br>(mg/dL)<br>95% CI | 88.5 ± 6.4<br>[84.5 to 92.5]  | 165.2 ± 18.8<br>[157.8 to 172.6] | 110.1 ± 12.5<br>[105.2 to 115.0] | -33.35% | < 0.001 | < 0.01 | < 0.001 |
| <b>RANKL</b> (ng/mL)<br>95% CI              | 1.05 ± 0.10<br>[0.99 to 1.11] | 2.15 ± 0.20<br>[2.07 to 2.23]    | 1.05 ± 0.10<br>[1.01 to 1.09]    | -51.16% | < 0.001 | > 0.05 | < 0.001 |
| <b>IL-6</b> (ng/mL)<br>95% CI               | 1.04 ± 0.08<br>[0.99 to 1.09] | 1.85 ± 0.05<br>[1.83 to 1.87]    | 1.08 ± 0.07<br>[1.05 to 1.11]    | -41.62% | < 0.001 | > 0.05 | < 0.001 |
| <b>TNF-alpha</b> (ng/mL)<br>95% CI          | 1.04 ± 0.10<br>[0.98 to 1.10] | 1.83 ± 0.08<br>[1.80 to 1.86]    | 1.08 ± 0.07<br>[1.05 to 1.11]    | -40.98% | < 0.001 | > 0.05 | < 0.001 |
| <b>SOD</b> (ng/mL)<br>95% CI                | 1.05 ± 0.07<br>[1.01 to 1.09] | 0.68 ± 0.10<br>[0.64 to 0.72]    | 1.02 ± 0.05<br>[1.00 to 1.04]    | +50.00% | < 0.001 | > 0.05 | < 0.001 |
| <b>MDA</b> (ng/mL)<br>95% CI                | 1.12 ± 0.09<br>[1.06 to 1.18] | 1.90 ± 0.22<br>[1.81 to 1.99]    | 1.20 ± 0.10<br>[1.16 to 1.24]    | -36.84% | < 0.001 | > 0.05 | < 0.001 |

## DISCUSSION

The present study demonstrated that adjunctive periodontal laser therapy produced significant clinical and biochemical improvements in diabetic patients with chronic periodontitis. Clinically, laser-treated patients showed marked reductions in gingival inflammation, bleeding on probing, and periodontal pocket depth compared with untreated periodontitis patients, indicating improved periodontal healing and stabilization. These findings are consistent with Aggarwal R et al.<sup>10</sup> who reported that adjunctive laser therapy achieved greater reductions in probing depth, bleeding on probing, and periodontal pathogens than conventional scaling and root planing alone. The favorable clinical outcomes observed in the present study likely reflect the combined antimicrobial, anti-inflammatory, and biostimulatory effects of laser irradiation, which accelerate soft-tissue repair and enhance periodontal wound healing.

A major finding of this study was the significant modulation of inflammatory mediators following laser therapy. Diabetic patients with periodontitis showed markedly elevated levels of IL-6, TNF- $\alpha$ , and RANKL before treatment compared with healthy controls, which reflects the chronic inflammatory state and enhanced osteoclastogenic activity associated with diabetes-associated periodontal disease. These observations are consistent with the findings of Cutando A 2015<sup>11</sup>, who reported significantly increased circulating IL-6 and TNF- $\alpha$  levels in patients with both diabetes and periodontitis. Similarly, Mrasori S et al.<sup>12</sup> found that low-level laser therapy significantly reduced serum IL-6 concentrations in patients with periodontitis and type 2 diabetes mellitus. Following laser treatment, the significant reductions in IL-6 and TNF- $\alpha$  observed in the present study suggest effective modulation of the host inflammatory response through photobiomodulation. These findings support previous evidence that laser irradiation suppresses pro-inflammatory cytokine production while promoting tissue repair and periodontal homeostasis<sup>13</sup>.

The reduction in RANKL observed after laser therapy is particularly important because activation of the RANKL/RANK/OPG signaling pathway is a central mechanism underlying osteoclast differentiation and alveolar bone destruction in periodontitis<sup>14,15</sup>. López Roldán A 2020<sup>16</sup> similarly reported decreased RANKL expression following periodontal therapy. Since diabetes enhances osteoclastogenesis through persistent hyperglycemia, advanced glycation end-products (AGEs), and chronic inflammatory signaling, suppression of RANKL may help reduce

osteoclastic activity and limit progressive alveolar bone loss in diabetic patients<sup>17,18</sup>. Therefore, the observed reduction in RANKL suggests that laser therapy may contribute not only to inflammation control but also to preservation of periodontal supporting tissues.

Oxidative stress biomarkers further supported the therapeutic effects of laser treatment. Before treatment, diabetic periodontitis patients showed significantly elevated MDA concentrations and reduced SOD activity, indicating increased lipid peroxidation and impaired antioxidant defence. These findings are consistent with those of Surdu A 2024<sup>19</sup>, who reported elevated MDA levels accompanied by reduced antioxidant enzyme activities in diabetic patients with periodontitis. Following periodontal laser therapy, MDA concentrations decreased significantly, whereas SOD activity increased substantially in our study as compared to healthy controls. The absence of significant differences between laser-treated patients and healthy controls suggests that adjunctive laser therapy effectively restored redox homeostasis by reducing oxidative damage while enhancing endogenous antioxidant capacity. Ribeiro IM et al.<sup>20</sup> reported similar reductions in oxidative stress following photobiomodulation, demonstrating decreased MDA levels after laser-assisted periodontal treatment. Previous studies have also shown improvements in antioxidant enzyme activity after laser irradiation, which supports the concept that laser therapy attenuates oxidative injury by reducing inflammatory burden and improving cellular metabolism<sup>21,22</sup>.

The beneficial biochemical changes observed in the present study are biologically plausible considering the known mechanisms of periodontal laser therapy. Different laser systems, including Er: YAG, diode, and Nd: YAG lasers, have been shown to facilitate root surface decontamination, bacterial reduction, soft-tissue curettage, and biostimulation of periodontal tissues<sup>23,24</sup>. Photobiomodulation enhances mitochondrial ATP production, collagen synthesis, angiogenesis, and microcirculation, thereby accelerating tissue regeneration and wound healing<sup>25</sup>. In diabetic patients, where persistent hyperglycemia impairs immune function, collagen turnover, and tissue repair<sup>26</sup>, these biological effects may be particularly advantageous in improving periodontal healing while simultaneously reducing inflammation and oxidative stress<sup>27,28</sup>.

Overall, the present findings provide evidence that adjunctive periodontal laser therapy beneficially influences multiple pathogenic pathways involved in diabetic periodontitis. Beyond improving conventional clinical periodontal parameters, laser treatment significantly reduced systemic inflammatory cytokines, suppressed RANKL-mediated osteoclastogenic signaling, restored antioxidant defenses, and reduced oxidative stress. Collectively, these effects suggest that laser therapy represents a promising minimally invasive adjunct to conventional periodontal treatment in diabetic patients by targeting inflammation, oxidative stress, and bone resorption simultaneously. Nevertheless, the present study was limited by its relatively short follow-up period and modest sample size. Future multicenter randomized controlled trials with longer observation periods and additional molecular biomarkers are warranted to confirm the long-term clinical benefits and biological mechanisms of periodontal laser therapy in diabetic populations.

## CONCLUSION

In conclusion, this study underscores that periodontal laser therapy effectively reduces key markers of inflammation, oxidative stress, and bone resorption in diabetic patients. Laser treatment serves as a potent adjunctive modality by significantly lowering levels of IL-6, TNF- $\alpha$ , RANKL, and MDA, targeting the biochemical pathways driving periodontal destruction. Although SOD levels improved only modestly, the overall reduction in inflammatory burden and osteoclastic activity underscores the therapy's clinical utility. As a minimally invasive intervention, laser therapy not only enhances soft-tissue healing but also offers a viable strategy for preventing disease progression and maintaining periodontal health in this high-risk population.

## LIMITATION

Although the results are encouraging, several limitations restrict their generalizability and the long-term assessment of outcomes. First, the study had a small sample size, a single-centre design, and of randomization. Second, the short follow-up period and lack of long-term monitoring make it impossible to determine whether biochemical improvements or potential bone regeneration are maintained over time. Third, the omission of radiographic tests prevented collection of detailed data on changes in alveolar bone density. Finally, the study did not incorporate a diabetic-only control group or a non-diabetic periodontitis control group, which limits the ability to isolate the independent impacts of diabetes versus periodontal disease on the evaluated biomarkers.

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**Data Sharing Statement:** The corresponding author can provide the data proving the findings of this study on request. Privacy or ethical restrictions bound us from sharing the data publicly.

## AUTHOR CONTRIBUTIONS

Qureshi Z: Conceived and designed the study, data collection and interpretation, writing – original draft, and finalized the manuscript.

Maqbool T: Supervised the study, helped in coordinating and analyzing testing, contributed to the literature review and methodology section.

Azhar M: Performed formal analysis and drafted the results section of the manuscript.

Umair S: Assisted in patient recruitment, data entry, and reviewed the manuscript for accuracy.

Ubaid A: Helped in conducting the experiment and manuscript writing – review & editing.

Ejaz S: Critical review of the manuscript and help in reference correction.

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