

## ORIGINAL PAPER

# Effects of Low Level Laser Therapy (LLLT) on Serum Values of Interleukin 6 (IL-6) in Patients with Periodontitis and Type 2 Diabetes Mellitus (T2DM)

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## ABSTRACT

**Background:** In patients with T2DM, the therapeutic effects of conservative treatment are quite limited, and there is a need for additional therapeutic procedures to achieve the desired satisfactory and solid effect. Low-level laser therapy (LLLT) has an anti-inflammatory effect, and is used to heal lesions. This mechanism is realized through inhibition of lipopolysaccharides (LPS), so it can be used in the treatment of periodontal disease in patients with diabetes. **Objective:** The aim of this study is to assess the effect of level laser therapy (LLLT) on serum IL-6 values in patients with periodontitis and T2DM. **Methods:** Patients at age between 35-60 years old, with chronic periodontitis (CH) where the clinical loss of attachment (CAL) was  $\geq 4$  mm therefore covering at least 50 % of affected teeth. In this study we included 80 patients, divided into two groups: 40 patients with type 2 diabetes mellitus (2TDM) treated with conservative periodontal treatment supplemented with laser therapy (LLLT), group A, and 40 patients with 2TDM, conservatively treated without LLLT. therapy i.e. group B. The laser light was applied to the gingiva in separate quadrants in 5 sessions for the next five days in a row. Blood samples were taken from all subjects at the first treatment, then in 6 weeks and 3 months after treatment, and interleukin 6 (IL-6) levels were measured. The blood samples in the test tubes remained for about 30 minutes and were then distributed in a biochemical laboratory, where they were centrifuged at 6,000 rpm for 10 minutes. The serum was separated from the test tube and transferred to the eppendorph. All serum samples were stored at  $-80^{\circ}\text{C}$  until complete analysis and determination of IL-6, according to the standardized methodology. **Results:** In group A, on the first examination serum IL-6 levels varies in the interval  $11.54 \pm 1.11$  pg / mL, after 6 weeks of therapy the values range between  $11.26 \pm 0.77$  pg / mL, and after 3 months of therapy levels oscillate at intervals of  $11.02 \pm 0.67$  pg / mL. In group B the findings are similar. At the first examination, the serum IL-6 values were  $11.56 \pm 0.81$  pg / mL, after 6 weeks of therapy ranged from  $11.59 \pm 0.71$  pg / mL, and after 3 months of therapy levels were recorded at intervals.  $11.41 \pm 0.78$  pg /mL. The serum IL-6 value after 6 weeks of therapy in patients in group B for  $Z = -2.04$  and  $p < 0.05$  ( $p = 0.04$ ) was significantly higher than in patients in group A, while after 3 months of therapy in patients in group B for  $Z = -2.42$  and  $p < 0.05$  ( $p = 0.02$ ) is significantly higher than the value in patients in group A. **Conclusion:** LLLT resulted in significantly reduced serum IL-6 values in patients with periodontitis and T2DM after 6 weeks and 3 months of therapy in which conservative treatment was supplemented with LLLT.

**Keywords:** low power laser radiation (LLLT), interleukin 6 (IL-6), serum, periodontitis, type 2 diabetes mellitus (T2DM).

## 1. BACKGROUND

According to the data from the Center for control and prevention of diabetes, which treats people with diabetes in the United States, diabetes affects about 25.6 million people, or 11.3% of the country's population (1). The disease gradually

and quietly but systematically attacks the organs and tissues, causing micro and macro circulatory disorders, which affect the health status of the individuals. These changes also occur locally, soft and hard structures in the oral cavity (2-3). It has been clinically proven that type

2 diabetes mellitus (T2DM) is a risk factor for periodontitis, sometimes defined as a moderate association (4), sometimes as an increased risk of initiation and progression of periodontal disease (5) or very often the case is presence of both diseases in the same patient (6-7). In conditions of chronic disease and systemic disorder, the periodontium becomes a barrier which is easy to overcome, which allows the penetration of certain harmful pathogens first into the gingival tissue and then into the remaining periodontal structures. This condition results in a host response, activation of enzymes, and release of pro-inflammatory cytokines, including IL-6. IL-6 is responsible for regulating the immune and inflammatory tissue response and participates in the acute phase of the inflammatory response where it acts together with TNF- $\alpha$  (8). Through regulatory mechanisms, the production of TNF- $\alpha$  (9) is impaired, hence its effect on diabetes is indisputable. It has been shown to have a potential synergistic effect on fibroblasts, and is a stimulator of alveolar bone destruction by stimulating osteoclasts (10). In patients with T2DM, the therapeutic effects of conservative treatment are quite limited, and there is a need for additional therapeutic procedures to achieve the desired satisfactory and solid effect. Low-level laser therapy (LLLT) has very wide use in medicine, and is used to heal lesions. This mechanism is realized through inhibition of lipopolysaccharides (LPS), so it can be used in the treatment of periodontal disease in patients with diabetes (11).

The role and effect of lasers on fibroblasts and osteoblasts have been investigated and proven in hyperglycemic conditions (12-13). At the systemic level, the impact of LLLT on the secretion of pro-inflammatory mediators TNF- $\alpha$  and IL-6 from endothelial cell cultures has been investigated. According to some research, the link between these components is due to the structural placement of endothelial cells on the walls of blood vessels and the initial contact with blood rich in glucose (14).

LLLT as an addition to conservative treatment minimized the effects of 5-FU on the periodontium (15-16), the diode laser provided significant improvements in clinical parameters, confirming that lasers have a positive effect along with non-surgical periodontal therapy (17). The researchers suggest that LLLT reduces gingivitis and contributes to better therapeutic results when LLLT is used in conjunction with basic periodontal therapy, opposing to the classical conservative treatment only (18).

## 2. OBJECTIVE

The aim of this study was to evaluate the effect of LLLT on serum IL-6 values in patients with periodontitis and T2DM.

## 3. MATERIAL AND METHODS

### Study design

Patients in this study were selected by the Department of Periodontology and Oral Diseases at the University of Kosovo, University Dental Clinical Center in Pristina, aged 35-60, with chronic periodontitis (CH) where the clinical attachment loss (CAL) was  $\geq 4$  mm covering at least 50% of affected teeth. The research was approved

by the Ethics Commission of the Faculty of Dentistry in Skopje(01/434/17).

The selected patients in this study were informed about the motive and course of the study. Only volunteers who agreed to be part of this research took part in the study, and written consent was submitted, signed by hand. The study included survey of 80 patients, who were divided into two groups:

- patients with type 2 Diabetes mellitus where conservative periodontal treatment was supplemented with laser therapy in 40 patients (group A);
- patients with type 2 Diabetes mellitus where conservative periodontal treatment has been performed without applied laser therapy, which also counted 40 patients (group B).

All patients in both groups regulated hyper-glycaemia with oral antidiabetic drugs (Glucophage XR tablets of 750 mgr. 2x daily, manufacturer Merck Sante, France).

Certain criteria were used in selection of the patients, respectively proposed by the World Health Organization as criteria for inclusion and exclusion in the study.

Criteria for exclusion from the study are: a) use of antibiotics in the previous 4 months; b) pregnancy; c) patients - smokers; d) malignant diseases; e) use of immunosuppressive drugs; f) medications that may affect periodontal status; and g) Fentoin, cyclosporine, calcium channel blockers, etc.

Criteria for inclusion in the study are: a) diagnosed with diabetes mellitus type 2; b) regulation of diabetes with oral antidiabetics; and c) diagnosed periodontitis with depth of periodontal pockets  $\geq 4$  mm in at least 50% of affected teeth.

After determined diagnosis in all patients who were part of the study, conservative treatment (removal of hard and soft deposits) of periodontal pockets was performed. After the initial measurements and after the determining of the clinical parameters, non-surgical treatment of the periodontal pockets was performed in all participants in the study. Periodontal pockets were irrigated with 1% chlorhexidine solution (three times for 5 minutes). Scaling and root planning was carried out in 5 sessions, in separate quadrants each session. The supra-gingival tartar was removed by ultrasound, and the treatment was performed with Grace's curette, model Hu-Friedy, Chicago, IL, USA by the same therapist.

All patients were instructed to maintain daily oral hygiene: tooth brushing, use of dental floss, Listerine solution use. In the first group of respondents, conservative treatment was supplemented with laser therapy. For this purpose, low level laser therapy (LLLT), laser light (660 nm, 10 mW, 8 min/day, in contact with the gingiva) was applied; model: (Hager & Werken LASER HF " confort " Vo23-17, Duisburg, Germany) for the next five days in a row. Blood was taken from all patients during the first treatment, and after in the 6th week an 3rd month of treatment.

### Collecting serum

Samples of venous blood from the cubital vein were taken from each patient. Collected blood samples were transferred into a test tube with an anticoagulant

(pre-fabricated). The blood samples remained in the test tubes for about 30 minutes and were then distributed in a biochemical laboratory, where they were centrifuged at 6,000 rpm for 10 minutes. The serum was separated from the test tube and transferred to the eppendorph tube. All serum samples were stored at -80 °C until complete analysis and measurement of IL-6 levels.

**Determining IL-6 levels in serum**

In the test procedure, the reagents are prepared firstly. Namely, first, before use, all reagents should be brought to room temperature (10-25 oC). The standard reagent is prepared 15 minutes before starting with work. The concentration of the solution is 1000 pg/mL. Then 7 tubes are prepared containing 1.0 µL dilution for standard and are used to make a double dilution series according to the picture shown below. Each tube is vigorously mixed before the next transfer. 7 tubes with dissolved standard are obtained with the following concentrations: 1000 pg/mL, 500 pg/mL, 250 pg/mL, 125 pg/mL, 62,5 pg/mL, 31,25 pg/mL, 250 pg/mL, 15,625 pg/mL and 0 pg/mL.

Rinsing buffer - diluted with 30 mL of concentrated rinse buffer in 750 mL rinse buffer with deionized or distilled water.

**Biotinylated Detection Ab** - The exact amount needed (100µL / well) is calculated before the experiment begins. Before use, centrifuge the tube with the solution, and dilute with the concentrated Biotinylated Detection Ab to the working concentration using the Biotinylated Detection Ab Diluent (1: 100).

**Concentrated HRP Conjugate** - Before the start of the experiment, the exact amount needed (100µL / well) is calculated. Before use, the tube is centrifuged with the solution, and diluted with the concentrated HRP Conjugate to working concentration needed using the Concentrated HRP Conjugate Diluent (1: 100).

**Test protocol**

We added 100µL standard or samples to the appropriate well. Reference Standard and Sample diluent is added to standard wells. The solutions are added at the bottom of the microplate. Gently mix and cover with protective foil. Then incubate for 90 minutes at 37°. The liquid is then removed from each well. Immediately afterwards, 100 µL of the Biotinylated Detection Ab working solution is added to each well. Cover the microplate with protective foil, gently touch the tile to ensure thorough mixing. Incubate for 1 hour at 37° C.

Each well is aspirated and rinsed, repeating the process three times. Each well is rinsed with a rinsing buffer (approximately 350µL). After the last rinse, remove the remaining rinse buffer by aspiration or decantation. Then 100 ml of HRP Conjugate working solution is added to each well. Cover with foil and incubate for 30 minutes at 37 ° C. Each well is aspirated and rinsed, repeating the process five times. Each well is flushed with a wash buffer (approximately 350µL).

After the last rinse, remove the remaining rinsing buffer by aspiration or decantation. Add 90 µL of Substrate Solution to each well, cover the plate with foil and incubate in the dark for about 15-25 minutes at 37 ° C. Add 50 µL of Stop Solution to each well. The color immediately

turns yellow. The optical density (OD value) of each well is determined simultaneously, using a 450 nm microplate tile reader, and at the end we calculate the results.

**Statistical processing and data analysis**

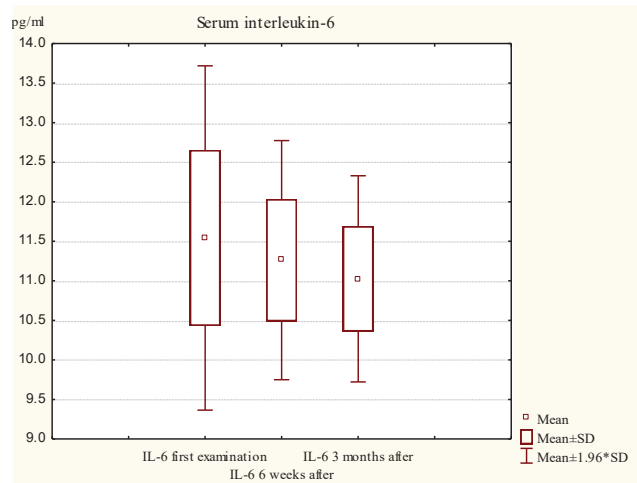
The statistical processing is performed in the statistical program Statistica 7.1 for Windows. The data is displayed as a table and graphically.

In the analysis of the data we used: a) The differences in the analyzed parameters in the first examination, after 6 weeks of therapy and after 3 months of therapy were tested using Friedman ANOVA Chi Sqr. / p; b) Differences in relations: first examination and after 6 weeks of therapy; first examination, after 3 months of therapy; after 6 weeks of therapy and after 3 months of therapy, they were tested using T-test for Dependent Samples (t / p), Wilcoxon Matched Pairs Test (Z / p) depending on the data distribution; c) The differences in the analyzed parameters between group A and group B were tested with T-test, independent, by groups (t / p) and Mann-Whitney U Test (Z / p), depending on the data distribution.

**4. RESULTS**

Figure 1 shows the descriptive statistics of serum IL-6 in group A. At the first examination IL-6 in serum varies in the interval 11.54 ± 1.11 pg / ml, after 6 weeks of therapy the values range between 11.26 ± 0.77 pg / mL and after 3 months of therapy they oscillate in the interval 11, 02 ± 0.67 pg / mL.

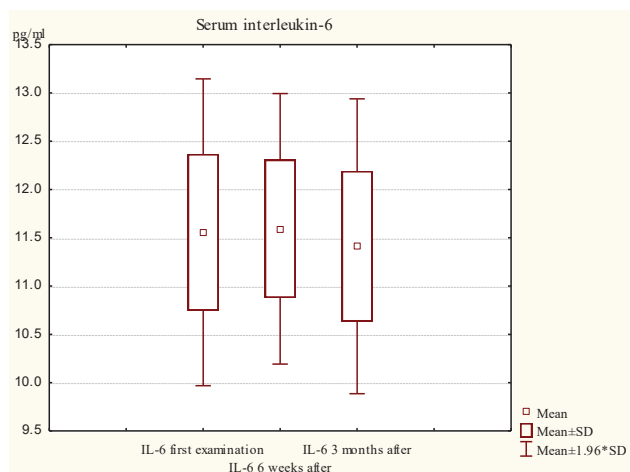
Among the IL-6 serum values in group A (first examination, six weeks, and three months) for Friedman ANOVA



**Graph 1. View the values of IL-6 in serum at different time intervals in group A**

IL-6 in serum	Average Rank	Sum of Ranks	Mean	Std.Dv.
<b>Group A</b>				
First examination	2.33	93.00	11.54	1.11
After 6 weeks	2.13	85.00	11.26	0.77
After 3 months	1.55	62.00	11.02	0.67
<b>Group B</b>				
First examination	1.96	78.50	11.56	0.81
After 6 weeks	2.36	94.50	11.59	0.71
After 3 months	1.68	67.00	11.41	0.78

**Table 1. Differences between serum IL-6 values in groups A and B at different time intervals after therapy**



Graph 2. View the values of IL-6 in serum at different time intervals in group B

IL-6 in serum	N	T	Z/ t	p-level
<b>group A</b>				
First examination and after 6 weeks	40	/	t=1.80	0.08
After 6 weeks and 3 months	40	141.50	3.61	0.0003
First examination and after 3 months	40	151.00	3.18	0.001
<b>group B</b>				
First examination and after 6 weeks	40	316.50	0.53	0.60
After 6 weeks and 3 months	40	90.00	4.30	0.000
First examination and after 3 months	40	239.50	0.99	0.32

Table 3. Differences in serum IL-6 values after 6 weeks and 3 months of treatment between groups A and B

Chi Sqr. (N = 40, df = 2) = 13.11 and p <0.01 (p = 0.001) there is a significant difference. In group B, IL-6 in serum (first examination and six weeks and three months after therapy) for Friedman ANOVA Chi Sqr. (N = 40, df = 2) = 10.11 and p <0.01 (p = 0.006) there is a significant difference (Table 1).

Figure 2 shows the descriptive statistics of serum IL-6 in group B. At the first examination, IL-6 serum values ranged from 11.56 ± 0.81 pg / mL, after 6 weeks of therapy ranged from 11.59 ± 0.71 pg / mL, and after 3 months of therapy were recorded at intervals. 11.41 ± 0.78 pg/mL.

Table 2. Intergroup differences between serum IL-6 values in groups A and B at different time intervals after therapy

In group A, the serum IL-6 value after 6 weeks of therapy for t = 1.80 and p > 0.05 (p = 0.08) was significantly lower than the value at first examination, after 3 months for Z = 3,61 and p <0.001 (p = 0.000) is significantly lower than the value after 6 weeks of therapy, and after 3 months of therapy is significantly lower than the value at the first examination, ie. Z = 3.18 and p <0.01 (p = 0.001).

In group B, after 6 weeks of therapy for Z = 0.53 and p > 0.05 (p = 0.60) the values for IL-6 were insignificantly higher than the values at first examination. Comparison made between 6 weeks and 3 months of IL-6, after 3 months of therapy for Z = 4.30 and p <0.001 (p = 0.000) is significantly lower than the value after 6 weeks, while

after 3 months of therapy for Z = 0.99 and p > 0.05 (p = 0.32) values of IL-6 are slightly lower than those at first examination (Table 2).

The serum IL-6 value after 6 weeks of therapy in patients in group B for Z = -2.04 and p <0.05 (p = 0.04) was significantly higher than in patients in group A, while again after 3 months of therapy in patients in group B for Z = -2.42 and p <0.05 (p = 0.02) is significantly higher than in patients with group A (Table 3).

## 5. DISCUSSION

Between chronic periodontitis and diabetes, a two-way relationship has been proven. On the one hand, destruction of the supporting apparatus of the teeth is more advanced in patients with T2DM (19, 20), while on the other hand CH may worsen glycemic control in patients with T2DM (21). This two way street is thought to be due to the presence of pro-inflammatory mediators, such as TNF-α and IL-6. It is believed that their presence is a consequence of constant microbiological stimulation or as a response of the host. In circulation, pro-inflammatory mediators come in contact with the insulin receptors, disrupting insulin function and signalization (20).

In addition to IL-10, the study of the effect of IL-6 is quite complicated and completely unclear (22, 23). The role of IL-6 is crucial because it is involved in osteoclastic activity and has a strong effect on Th-17 cells (24). In addition to this exceptional activity, it simultaneously stimulates the production of IL-1 α, which contributes to the stimulation of the anti-inflammatory process (25). There is varying information about the association between these diseases. Khosravi (26) says there is insufficient evidence to support a link between elevated IL-6 levels and alveolar destruction in periodontal disease in individuals with hyperglycaemia. While Javed et al. (27) reported that cytokines in GCF in patients with and without T2D are regulated by the intensity of periodontal infection, while the role of T2DM is quite secondary.

In group A, ie. in patients with T2DM where conservative periodontal treatment with LLLT application has been performed, the value of IL-6, TNF-α in serum after 6 weeks (11.26 ± 0.77 pg / mL, and after 3 months of therapy is significantly lower than the values of the first examination. At the first examination IL-6 in serum varies in the interval 11.54 ± 1.11 pg / mL, after 6 weeks of therapy the values range between and after 3 months of therapy they oscillate in the interval 11.02 ± 0.67 pg / mL.

Quantitative analyzes have shown that serum IL-6 values after 3 months of treatment are significantly lower than the value after 6 weeks of therapy in patients with 2TDM whose conservative therapy was supplemented with LLLT.

In group B, ie. in patients with T2DM where conservative periodontal treatment was performed without LLLT application, IL-6 values after 6 weeks (11.59 ± 0.71 pg / mL) were insignificantly higher, while after 3 months (11.41 ± 0.78 pg / mL.), they are insignificantly lower than the value at first examination (11.56 ± 0.81 pg / mL). Serum IL-6 values are significantly lower after 3 months of therapy than 6 weeks after therapy.

The results indicate that serum values of IL-6 were corrected 6 and 3 months after treatment in both groups, with a significant difference in group A  $p < 0.01$  ( $p = 0.001$ ) and in group B  $p < 0.01$  ( $p = 0.006$ ). Statistical analysis showed that in the second group, IL-6 values were significantly higher than those in group A patients after 6 weeks and 3 months of therapy. Regarding the quantification of values of IL-6 in serum after 6 weeks of therapy in patients in group B for  $Z = -2.04$  and  $p < 0.05$  ( $p = 0.04$ ) is significantly higher than the value in patients with group A. After 3 months of treatment, differences between the two groups showed that the values after treatment between groups A and B for IL-6 in serum in group B were higher, ie. for  $Z = -2.42$  and  $p < 0.05$  ( $p = 0.02$ ) in relation to the value in patients of group A. Intergroup differences at all time intervals showed better results in group A, confirming the effectiveness of LLLT in the treatment of periodontal disease in patients with T2DM.

The use of lasers in the treatment of periodontitis dates back not long ago, but in the beginning the recommendations for the use of LLLT in the treatment of many diseases including T2DM and periodontitis for many years were based only on vague assumptions, conclusions, reports or pilot clinical trials (28). However, experience showed that LLLT was applied to wound healing (29), against inflammation (30, 31), pain relief (32-33), swelling reduction (34-35), according to specific guidelines.

In recent years, the interest in the use of laser therapy has increased gradually, although there is still heterogeneity in research data and findings, the justification for their application has been unequivocally confirmed (36). Our findings agree with the findings of Boschi (14, 37, 38). Identical to the values obtained after applying LLLT (In GaAlP, 660 nm) in the study, the values of IL-6 and TNF- $\alpha$  37 were significantly reduced.

Complexed progression of periodontal disease, with advanced inflammatory and destructive processes, as well as inadequate therapeutic effect are the basic features in patients with periodontitis in which T2DM (39-40) is diagnosed. These results are due to the presence of perio-pathogens that secrete endotoxin, which can increase the amount of many pro-inflammatory markers, including IL-6 (41-42).

In this study, in the group of patients treated conservatively with LLLT - therapy applied as adjuvant, we received 6 weeks and 3 months of reduced IL-6 values in serum. We believe that the results are due to the numerous positive properties of LLLT that are reflected on the examined pro-inflammatory mediator.

## 6. CONCLUSION

The low power laser radiation LLLT resulted in significantly reduced serum IL-6 values in patients with periodontitis and T2DM after 6 weeks and 3 months of therapy.

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• **Conflict of interest:** None declared.

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