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Serum Level of Il-2 in Patients with Type 2 Diabetes Mellitus and Periodontopathy

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Abstract

The purpose of this study was to investigate the influence of low-level laser therapy (LLLT) in patients with type 2 diabetes mellitus (T2DM) and chronic periodontopathy (ChP) on serum levels of IL-2.

***Methods and Results*:** A total of 80 patients aged 35-60 years were followed; all of them had T2DM diagnosed (HbA1C≤7.5%) with ChP, where clinical attachment loss (CAL) was ≥ 4mm on at least 50% of affected teeth. All participants are divided into two groups. Group A included 40 patients who underwent conservative (non-surgical) periodontal treatment supplemented with LLLT. Group B included 40 patients who underwent only conservative therapy. Patients used oral antidiabetic medications to control glycemia: Metformin (Alkaloid, Skopje S. Macedonia) 500 mg two times a day. LLLT (Laser HF® comfort, Hager. Werken, Duisburg, Germany) was applied (660 nm, 10 mW, 8 min/day) with contact to the gingiva for five consecutive days. Serum IL-2 was determined by ELISA in 3 time intervals: at the first examination, 6 weeks, and 3 months after treatment in both groups. In Group A and Group B, at the first examination, 6 weeks after therapy, and 3 months after treatment, the serum IL-2 was 17.20±0.54 pg/ml and 17.22±0.66 pg/ml, 17.12±0.63 pg/ml and 17.17±0.63 pg/ml, and 17.03±0.64 pg/ml and 16.98±0.65 pg/ml, respectively.

In Group A, there was a significant difference between the serum IL-2 values in specified time points (first examination, 6 weeks, and 3 months after the therapy) (Friedman's ANOVA: χ^2 (n=40, df=2) = 17.22 and *P*=0.0002). In Group B, between the serum IL-2 levels, there also was a significant difference in specified time points (Friedman's ANOVA: χ^2 (n=40, df=2) = 42.33 and *P*=0.0000). The intergroup analysis, according to the temporal dynamics of the measurements, showed an evident difference between the two groups, but the serum IL-2 values in the two groups treated with and without LLLT were close, and no statistical significance was recorded between them.

Conclusion: No significant differences were recorded in the serum IL-2 levels in T2DM patients with ChP non-surgically treated with and without the application of LLLT.(**International Journal of Biomedicine. 2022;12(4):611-616.**).

Keywords: chronic periodontopathy • type 2 diabetes mellitus • IL-2 • low-level laser therapy

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Abbreviations

ChP, chronic periodontopathy; **CAL**, clinical attachment loss; **GCF**, gingival crevicular fluid; **LLLT**, low-level laser therapy; **T2DM**, type 2 diabetes mellitus.

Introduction

Periodontal disease is a common disease in the population. It starts as gingivitis that can progress and affect the remaining structures of the periodontium, causing destructive processes, luxation, and tooth loss. The main cause of destructive inflammatory processes in the periodontium is dental plaque. The connection between plaque and the periodontium has been proven many times, and the immune response of the host has a central role.[1] However, it is considered that certain systemic diseases that have an inflammatory component can be a risk factor for periodontal disease because, through their mechanisms, they manage to change the oral microbiological environment, creating conditions for higher susceptibility to periodontopathy.[2]

During inflammation, inflammatory cytokines IL-1, IL-2, IL-8, TNF-α, and many other factors are produced, which cause an even higher inflammatory destructive reaction of the periodontium.[3]

It is considered that hyperglycemia can affect some biological pathways that stimulate the release and activation of inflammatory cytokines,[4] so poorly controlled type 2 diabetes mellitus (T2DM) negatively affects the periodontal status.[5-7] The disturbed balance between pro-inflammatory and anti-inflammatory cytokines is the cause of serious disorders in the periodontium. IL-1, IL-8, and TNF-α play a significant role in that process.[8] IL-6 can stimulate stromal cells to produce RANKL;[8,9] in contrast, IL-2 has a key role during immune homeostasis through its mediating role on T cells.[10] There is much data in the literature that confirms the connection between T2DM and periodontopathy.[11,12]

It is still a question for consideration whether diabetes affects the periodontal status or the diseased periodontium through the salivary factors and released pro-inflammatory cytokines into the gingival crevicular fluid (GCF) and worsens this systemic disease and tends toward possible complications. On the one hand, the impact of chronic periodontopathy (ChP) on the levels of glucoregulatory biomarkers in GCF[13] has been proven, and on the other hand, it is known that T2DM affects the composition of saliva and other tissue fluids that manifest in the mouth with different clinical symptomatology from the oral and periodontal aspect.[14]

In conditions of poor systemic health where T2DM and chronic periodontopathy are diagnosed, the main problem for every therapist is the choice of successful periodontal treatment. Often conventionally applied therapy is insufficient to achieve a clinical effect that would certainly regulate cytokine levels at a systemic or local level. In recent years, low-level laser therapy (LLLT) has been applied, which has been proven as an effective procedure in reducing inflammation and edema, and correcting clinical periodontal parameters.[15-17]

Histological findings of the gingival tissue treated with LLLT showed expressed healing, evident by the absence of inflammatory cells. Tissue edema could not be registered, and the number of blood vessels was reduced. Expressed collagenization and homogenization were present in the lamina propria of the gingiva.[18] Also, the correction of certain inflammatory mediators in saliva and GCF was confirmed after the application of the LLLT therapy.[19,20]

The purpose of this study was to investigate the influence of LLLT in patients with T2DM and ChP on serum levels of IL-2 as one of the inflammatory biomarkers important in the pathogenesis of periodontal disease.

Materials and Methods

We selected participants in this study by choosing suitable patients from the Department of Periodontology and Oral Diseases at the University Dental Clinical Center of Kosovo in Pristina. A total of 80 patients aged 35-60 years were followed; all of them had T2DM diagnosed (HbA1C≤7.5%) with ChP, where CAL was ≥ 4mm on at least 50% of affected teeth.

All participants are divided into two groups, A and B. Group A included 40 patients who underwent conservative (non-surgical) periodontal treatment supplemented with laser therapy. Group B included 40 patients who underwent only conservative therapy. Patients used oral antidiabetic medications to control glycemia: Metformin (Alkaloid, Skopje S. Macedonia) 500 mg two times a day. After the initial measurements and the observation of the clinical parameters, non-surgical treatment of the periodontal pockets was carried out in all study participants. Pockets were irrigated with 1% chlorhexidine gel (three times for 10 minutes). Then, LLLT was applied to the gingival part of the affected side. LLLT (Laser HF® comfort, Hager. Werken, Duisburg, Germany) was applied (660 nm, 10 mW, 8 min/day) with contact to the gingiva for five consecutive days.

To determine serum IL-2, the blood was collected after 12 hours of fasting and centrifuged for 20 minutes at 6000 rpm at 2-8°C. Serum was used for all further analyses. Serum IL-2 was determined by ELISA, based on the Biotin double antibody sandwich technology. A biotinylated detection antibody specific for Human IL-2 and Avidin-Horseradish Peroxidase (HRP) conjugate were added successively to each microplate well and incubated. The test protocol included a series of procedures that culminated in determining the optical density (OD) of each well simultaneously, using a microplate reader set at 450 nm. The OD value was proportional to the concentration of Human IL-2. Finally, a concentration of Human IL-2 in the samples was calculated by comparing the OD of the samples to the standard curve. IL-2 values in serum were determined in 3 time intervals: at the first examination, 6 weeks, and 3 months after treatment in both groups.

Statistical analysis was performed using statistical software package SPSS version 26.0 (SPSS Inc, Armonk, NY: IBM Corp). For descriptive analysis, results are presented as mean±standard deviation (SD). Student's paired t-test was applied to compare two groups for data with normal distribution. The Friedman ANOVA was used to compare three or more matched groups. A probability value of $P<0.05$ was considered statistically significant.

Ethical approval for this study was obtained from the Ethical Committee at UBT - Higher Education Institution

(Faculty of Dentistry), Pristina, Republic Kosovo. Written informed consent was obtained from all patients before inclusion in the study.

Results

Table 1 shows descriptive statistics of serum IL-2 for two study groups. In Group A and Group B, at the first examination, 6 weeks after therapy, and 3 months after treatment, the serum IL-2 was 17.20±0.54 pg/ml and 17.22±0.66 pg/ml, 17.12±0.63 pg/ml and 17.17±0.63 pg/ml, and 17.03±0.64 pg/ml and 16.98±0.65 pg/ml, respectively.

Table 1.

Serum values (pg/ml) of IL-2 in patients of two groups.

IL-2 in serum	n	Mean	Confidence -95.00%	Confidence +95.00%	Minimum	Maximum	SD
Group A							
First examination	40	17.20	17.03	17.37	16.15	18.45	0.54
6 weeks after the therapy	40	17.12	16.92	17.32	15.85	18.55	0.63
3 months after the therapy	40	17.03	16.83	17.24	15.55	18.45	0.64
Group B							
First examination	40	17.22	17.01	17.43	16.05	18.95	0.66
6 weeks after the therapy	40	17.17	16.97	17.38	16.15	18.75	0.63
3 months after the therapy	40	16.98	16.78	17.19	16.00	18.55	0.65

In Group A, there was a significant difference between the serum IL-2 values in specified time points (first examination, 6 weeks, and 3 months after the therapy) (Friedman's ANOVA: χ^2 (n=40, df=2) = 17.22 and *P*=0.0002). In Group B, between the serum IL-2 levels, there also was a significant difference in specified time points (Friedman's ANOVA: χ^2 (n=40, df=2) = 42.33 and *P*=0.0000) (Table 2).

The serum IL-2 value 6 weeks after therapy was significantly lower than at the first examination in Group A (t=2.36 and *P*=0.02), while in Group B, the difference between IL-2 levels was not significant (t=1.12 and *P*=0.27) (Table 3).

The serum IL-2 values 3 months after therapy were significantly lower than at the first examination in both groups (Group 1: t=2.50 and *P*=0.02; Group B: t=5.67 and *P*=0.00) (Table 4).

In Group A, the serum IL-2 value 3 months after therapy was slightly lower than 6 weeks after therapy (t=1.69 and *P*=0.10), while in Group B, the IL-2 level 3 months after therapy was significantly lower than 6 weeks after therapy (t=17.17 and *P*=0.000) (Table 5).

Table 2.

Differences between IL-2 values in serum in two groups at different time intervals.

IL-2 in serum	Average Rank	Sum of Ranks	Mean	SD
Group A				
First examination	2.46	98.50	17.20	0.54
6 weeks after therapy	2.00	80.00	17.12	0.63
3 months after therapy	1.54	61.50	17.03	0.64
Group B				
First examinatin	2.38	95.00	17.22	0.66
6 weeks after therapy	2.44	97.50	17.17	0.63
3 months after therapy	1.19	47.50	16.98	9.65

Table 3.

Differences in serum IL-2 values at the first examination and 6 weeks after the treatment in both groups

IL-2 in serum	Mean	SD	n	Diff.	SD Diff.	t	df	P
Group A								
First examination	17.20	0.54						
6 weeks after therapy	17.12	0.63	40	0.08	0.22	2.36	39	0.02
Group B								
First examination	17.22	0.66						
6 weeks after therapy	17.17	0.63	40	0.04	0.25	1.12	39	0.27

Table 4.

Differences in serum IL-2 values at the first examination and 3 months after the treatment in both groups

IL-2 in serum	Mean	SD	n	Diff.	SD Diff.	t	df	P
Group A								
First examination	17.20	0.54						
3 months after therapy	17.03	0.64	40	0.17	0.42	2.50	39	0.02
Group B								
First examination	17.22	0.66						
3 months after therapy	16.98	0.65	40	0.23	0.26	5.67	39	0.00

The intergroup analysis, according to the temporal dynamics of the measurements, showed an evident difference

between the two groups, but the serum IL-2 values in the two groups treated with and without LLLT were close, and no statistical significance was recorded between them.

Table 5.

Differences in serum IL-2 values 6 weeks and 3 months after the treatment in both groups

IL-2 in serum	Mean	SD	n	Diff.	SD Diff.	t	df	P
Group A								
6 weeks after therapy	17.12	0.63						
3 months after therapy	17.03	0.64	40	0.08	0.31	1.69	39	0.10
Group B								
6 weeks after therapy	17.17	0.63						
3 months after therapy	16.98	0.65	40	0.19	0.07	17.17	39	0.000

Discussion

The high-quality standard in the therapy of periodontal disease is the non-surgical treatment of the periodontium, which includes curettage of the soft wall of the periodontal pocket, removal of necrotic cementum, subgingival concretions from the root of the tooth and elimination of the complete contents (exudate, dental plaque, detritus, and microorganisms).[21] With mechanical instrumentation, there is a qualitative and quantitative change in the oral microbial flora,[22,23] which in turn results in a reduction of bacterial toxins and enzymes that change the level of local and systemic inflammatory mediators.[24] In conditions of good systemic health without comorbidities, the irritating local factors can be the cause of inflammation and destruction of the periodontium due to releasing the pro-inflammatory cytokines. Systemic diseases are an additional cause of deterioration of clinical and cytokine findings. Namely, the disturbed balance between pro- and anti-inflammatory cytokines and the immune response can be the cause of periodontal damage.[25] This study showed that serum IL-2 values in 2TDM patients treated with and without LLLT at all investigated time intervals (at 6 weeks and 3 months) after non-surgical periodontal treatment were not statistically significant. Namely, hyperglycemia, among others, affects the glycation pathway, thereby potentiating the release of inflammatory mediators at the systemic level,[26] also reflecting on the oral and periodontal tissues.

Our findings agree with the data obtained from Koçak's study.[27] But the non-surgical treatment was expected to correct the IL-2 levels, which it did after 6 weeks and 3 months in both groups. In Group A, there was a significant difference between the serum IL-2 values (first examination, 6 weeks, and 3 months after the therapy) (Friedman's ANOVA: χ^2 (n=40, df=2)=17.22 and *P*=0.0002). In Group B, between the serum IL-2 levels, there also was a significant difference in specified time points (Friedman's ANOVA: χ^2 (n=40, df=2)=42.33 and *P*=0.0000).

The authors who determined the serum biomarkers agree with these findings and confirmed that conventionally applied therapy is associated with systemic and local reduction of inflammatory markers.[28,29] In Group A, the serum IL-2 value 3 months after therapy was slightly lower than 6 weeks after therapy (t=1.69 and *P*=0.10), while in Group B, the IL-2 level 3 months after therapy was significantly lower than 6 weeks after therapy (t=17.17 and *P*=0.000).

Our results point to the fact that LLLT has no effect on the serum levels of IL-2, which has an important immune-regulatory role, promotes the growth and development of peripheral immune cells during the initiation of the (defensive) immune response, and keeps them alive as effector cells.

Researchers suggest that LLLT reduces gingival inflammation when applied as an adjunct to non-surgical treatment, calms gingival inflammation, and corrects the depth of periodontal pockets due to the anti-inflammatory, analgesic, and bio-stimulating effects.[30,31] Stadler et al.[32] registered a correction of IL-1β, IL-17, and IL-4 in GCF. The values of IL-1β and TNF-α have significantly reduced independently of blood glycemia and diabetic status in patients treated only conservatively.[33] Non-surgical periodontal treatment resulted in reduced salivary values of IL-1β and TNF-α.[34]

Based on the obtained findings and the data from several studies that showed a significant reduction of individual markers in GCF and saliva, we approach the fact that periodontal treatment effectively corrects biomarkers in local oral fluids (GCF and saliva) but not at the systemic level (serum). These changes in the oral medium are manifested through clinical improvements of the periodontium that can be partially explained by the normalization of homeostasis in tissue metabolism and inhibition of mast cell degranulation, where immune changes are in focus.

In conclusion, in our study, no significant differences were recorded in the serum IL-2 levels in T2DM patients with ChP non-surgically treated with and without the application of LLLT.

Competing Interests

The authors declare that they have no competing interests.

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