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ORIGINAL ARTICLE

Low-Level Lasers as an Adjunct in Periodontal Therapy in Patients with Diabetes Mellitus

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Abstract

Background: Diabetes mellitus (DM) increases the risk of periodontitis, and severe periodontitis often coexists with severe DM. The proposed dual pathway of tissue destruction suggests that control of chronic periodontal infection and gingival inflammation is essential for achieving long-term control of DM. The purpose this study is to evaluate the effects of low-level laser therapy (LLLT) by exfoliative cytology in patients with DM and gingival inflammation.
Subjects and Methods: Three hundred patients were divided in three equal groups: Group 1 consisted of patients with periodontitis and type 1 DM, Group 2 of patients with periodontitis and type 2 DM, and Group 3 of patients with periodontitis (control group). After oral examination, smears were taken from gingival tissue, and afterward all of the patients received oral hygiene instructions, removal of dental plaque, and full-mouth scaling and root planing. A split-mouth design was applied; on the right side of jaws GaAlAs LLLT (670 nm, 5 mW, 14 min/day) (model Mils 94; Optica Laser, Sofia, Bulgaria) was applied for five consecutive days. After the therapy was completed, smears from both sides of jaws were taken. The morphometric analysis was done using the National Institutes of Health Image software program and a model NU2 microscope (Carl Zeiss, Jena, Germany).
Results: Investigated parameters were significantly lower after therapy compared with values before therapy. After therapy on the side subjected to LLLT, there was no significantly difference between patients with DM and the control group.
Conclusions: It can be concluded that LLLT as an adjunct in periodontal therapy reduces gingival inflammation in patients with DM and periodontitis.

Introduction

Diabetes mellitus (DM) is a chronic metabolic disorder caused by inherited or acquired deficiency in production of insulin by the pancreas or by the ineffectiveness of the insulin produced. The high level of glucose present in the blood has profound effects on various systems of the human body.[1] Periodontitis is a chronic infectious disease of the supporting tissues around the teeth leading to its progressive destruction. DM increases the risk of periodontitis, and severe periodontitis with pronounced gingival inflammation often coexists with severe DM.[2,3] It is suggested that hyperglycemia could indirectly exacerbate inflammatory tissue destruction through the body's scavenger system against advanced glycation end-products and that hyperglycemia might directly impair the biological functions of periodontal connective tissues through cell–matrix interactions.[4] Less clear is the impact of periodontal disease on glycemic control of DM and the mechanisms through which this occurs. It is suggested that an infection-mediated regulation cycle of cytokine synthesis and secretion by chronic stimulus from lipopolysaccharide and products of periodontopathogenic organisms may amplify the magnitude of the advanced glycation end-product–mediated cytokine response operative in DM. The proposed dual pathway of tissue destruction suggests that control of chronic periodontal infection and gingival inflammation is essential for achieving long-term control of DM.[2–6]

Low-level laser therapy (LLLT) was introduced as a therapeutic modality as early as 1968.[7] LLLT includes wavelengths between 500 and 1,100 nm and typically involves a dose of 1–4 J/cm^2 using lasers with output powers of 10–90 mW.[7] The infrared portions of the spectrum (e.g., GaAlAs, 800–900 nm) have been shown to have highly absorbent and unique therapeutic effects in living tissues and seem to provide the best results.[7] LLLT has shown to be effective in the treatment of impaired microcirculation, wound healing,[8,9] pain relief,[10–12] fracture healing, and reduction of inflammation and swelling.[13,14] It is widely accepted today that the

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inflammation is a basic response of periodontal tissue to damage and serves as a fast first line of defense. Investigators have suggested that LLLT reduces gingival inflammation and that better therapeutic results were achieved when LLLT was applied during basic periodontal therapy compared with applying basic periodontal therapy alone.[15] Until now, there has been little knowledge pertaining to a study of LLLT in periodontal disease in patients with DM.

Most of the pathologic conditions of the oral mucosa can be easily identified, but some of them need detailed examination. In specific conditions such as DM, routinely applied techniques like biopsy sometimes cannot be applied because of glycemic variations.[16–18] Accordingly, exfoliative cytology, which is a straightforward noninvasive diagnostic method that can be applied during inflammation, can be considered as a more practical technique to evaluate the oral mucosa in DM.[19,20] It collects superficial desquamated cells, which can easily be analyzed microscopically. Desquamation of gingival epithelium depends on mitotic activity of basal cells, their enzyme processes, and mechanical irritations of gingival tissue.[21] In recent years exfoliative biopsy has been introduced with good results in specification and diagnosis of the lesions.[22–26] A few studies have used exfoliative cytology to evaluate changes in the oral mucosa in DM and have shown that this disease can produce alterations in oral epithelial cells that are detectable by cytomorphometric analysis.[26] Gingival inflammation in periodontal disease is common in patients with DM. Depending on the degree of differentiation, epithelium cells show variations in size and shape. Also, enlargement of cell nuclear area and decrease of keratinization are noticed.[20] Morphometric analysis of inflamed gingival tissue can suggest changes in the periodontium and whole body that are manifested in the superficial gingival epithelium.[26]

The aim of the present study was to evaluate the effects of LLLT as an adjunct in periodontal therapy in patients with DM.

Subjects and Methods

This clinical study was carried out as a joint collaboration between the Department of Endocrinology and the Department of Periodontology and Oral Medicine, Faculty of Medicine, Niš University, Niš, Serbia. The study was registered and approved by the Niš University Faculty of Medicine Institutional Ethical Committee (protocol number 01-2800-7).

Three hundred twenty patients with periodontitis and DM were selected randomly from the pool of patients followed up at the Department of Endocrinology, Niš University Medical Center. Also, 156 patients with periodontitis who regularly came to the Dental Clinic of Niš University participated in the study. Patients (120 patients with DM and periodontitis and 56 patients with periodontitis) who underwent antibiotic and corticosteroid therapy in the last 3 months or had acute systemic illness, hemorrhagic disorders, or autoimmune diseases, as well as pregnant patients and patients who had periodontal treatment in the last 3 months, were excluded from the study. The following information was collected from medical records: type of DM and duration (years since diagnosis), glycosylated hemoglobin (HbA1c), and patient's age and sex.

Three hundred patients who participated in the study signed an informed consent form and were divided in three groups. Group 1 consisted of patients with periodontitis and type 1 DM: 54 (54%) women and 46 (46%) men, with a mean age of 35.54 ± 3.65 years. Group 2 consisted of patients with periodontitis and type 2 DM: 48 (48%) women and 52 (52%) men, with a mean age of 62.57 ± 8.57 years. Group 3 consisted of systemically healthy patients with periodontitis: 50 (50%) women and 50 (50%) men, with a mean age of 45.68 ± 8.91 years.

Oral examination and identification of periodontitis were done by a single examiner for all fully erupted permanent teeth. The presence of gingivitis was determined using the Gingival Index (GI) averaged per patient.[27] Before samples were taken, patients washed their mouths with normal saline for about 5 min, and then a surface smear was taken with the tip of a lancet from gingival tissue surrounding fully erupted permanent premolar teeth. The smears were transferred to a clean dry glass slide, immediately spray-fixed with 95% ethyl alcohol, and prepared with the Papanicolaou staining process for cytomorphometric analysis. Afterward all of the patients received oral hygiene instructions, removal of dental plaque (using machine toothbrushes and abrasive dental paste), and full-mouth scaling and root planing. Then a split-mouth design was applied; on the gingival tissue of the premolar region of the right side of the jaw GaAlAs LLLT (670 nm, 5 mW, 14 min/day, with contact to gingiva) (model Mils 94; Optica Laser, Sofia, Bulgaria) was applied for five consecutive days. Not one of the patients was lost during the follow-up and after all of them completed the therapy. After the therapy was finished once again the presence of gingivitis was determined using the GI procedure,[27] and smears from both sides of treated jaws were taken. Morphometric analysis was done on all of the samples using the National Institutes of Health Image software program and an NU2 microscope (Carl Zeiss, Jena, Germany) objective ×63 (numerical aperture 0.8). The person examining the cytology slides was not aware about the clinical history of the participants.

The statistical analysis was performed using the SPSS software program (SPSS, Inc., Chicago, IL). Parameters are shown as mean and SD values. Student's *t* test, Leven's method, Tukey's Honestly Significantly Difference test, and Dunnett's T3 test were used for analysis of statistically important differences between mean values of two groups. Results are shown tabularly using Microsoft® (Redmond, WA) Office Excel and SPSS version 15.0 software.

Results

In Group 1 the mean HbA1c was 9.87 ± 0.32%, and DM duration was 19.01 ± 1.22 years; in Group 2 the mean HbA1c was 8.70 ± 0.45%, and DM duration was 14.68 ± 3.43 years (Table 1). It was noted that gingival inflammation was reduced after the therapy was completed. More pronounced GI reduction was noticed on the lasered side of jaws (Table 2).

Multivariable analyses showed that there were no differences in the cellular parameters investigated (nuclear area, nuclear circularity, perimeter, Ferret's diameter, and integral optical density) between the two groups of patients with DM before therapy. Parameters were higher than those in the control group. After therapy in all investigated groups, cellular parameters were lower compared with values before therapy. There was no difference in the cellular parameters investigated in patients with DM with the same therapy

Table 1. Description of Subjects' Baseline Characteristics

Group	Age (years)	Sex		DM duration (years)	HbA1c
		Male	Female		
1 (DM type 1)	25.54 ± 3.65	46 (46%)	54 (54%)	19.01 ± 1.22	9.87 ± 0.32%
2 (DM type 2)	62.57 ± 8.57	54 (54%)	46 (46%)	14.68 ± 3.43	8.70 ± 0.45%
3 (control)	45.68 ± 8.91	48 (48%)	52 (52%)	—	—

Data are mean ± SD values unless indicated otherwise.
DM, diabetes mellitus; HbA1c, glycosylated hemoglobin.

protocol. In all groups, cellular parameters on the lasered jaw side were lower compared with the non-lasered side. There was no difference in nuclear area in patients with DM and the control group on the jaw side subjected to LLLT. On the side subjected only to basic periodontal therapy the nuclear area had a higher value in patients with DM compared with the control group (Table 3).

Discussion

DM is a risk factor for periodontitis, but also severe periodontitis increases the severity of DM and complicates metabolic control. Early diagnosis of gingivitis that occurs during periodontitis initiation and progression and its treatment are of the most importance to avoid progression of the disease and complications.[28] The relationship between these periodontitis events and DM appears bidirectional. The presence of one condition tends to promote the other, and the meticulous management of either may assist treatment of the other. Investigators have suggested that control of gingival inflammation during chronic periodontal infection is essential for achieving long-term control of DM and that all patients with DM should be encouraged to regularly visit a periodontist in order to maintain a high level of oral and periodontal health.[2,3]

A literature review revealed several studies that have found an improvement in periodontal wound healing with the use of LLLT, both in vitro and in vivo.[29–33] After scaling and root planing, adjuvant LLLT was shown to significantly reduce gingivitis, probing depth, and gingival crevicular fluid volume.[29] LLLT has been observed to produce an anti-inflammatory effect, a biostimulatory effect, and an analgesic effect. The anti-inflammatory effect and edema reduction can partially be explained by the normalized homeostasis in tissue metabolism and inhibition of mast cell degranulation.[28] Studies of the use of the low-level laser vary with regard to the types of laser and parameters of laser radiation,[34] but the clinical benefits observed when lasers are used are beyond doubt.[28] Houreld and Abrahamse[7] showed that wounded cells in patients with DM respond in a dose- and a wavelength-dependent manner to laser light, and the best results can be achieved on irradiation with a dose of $5\,J/cm^2$ at a wavelength of 632.8 nm. Similar doses were used in this study (4.2 J) for five consecutive days.

Table 2. Gingival Index Values Before and After Therapy

Timing, group	Gingival index (mean ± SD)
Before therapy	
1 (DM type 1)	1.86 ± 0.35
2 (DM type 2)	1.78 ± 0.44
3 (control)	1.20 ± 0.45[ab]
After therapy	
Lasered side	
1 (DM type 1)	0.16 ± 0.37*
2 (DM type 2)	0.16 ± 0.36*
3 (control)	0.14 ± 0.33*
Non-lasered side	
1 (DM type 1)	0.32 ± 0.44*†
2 (DM type 2)	0.31 ± 0.42*†
3 (control)	0.24 ± 0.40[cd]*†

Significant ($P < 0.01$) differences are indicated: before therapy, [a]Group 2 versus Group 3, [b]Group 1 versus Group 3; after therapy, [c]Group 2 versus Group 3, [d]Group 1 versus Group 3; *after therapy versus before therapy; †after therapy lasered versus non-lasered side.
DM, diabetes mellitus.

Gingival inflammation is common in periodontitis, especially in diabetes patients. Regardless of the degree of differentiation, epithelial cells demonstrate deviations in size and shape. Cytomorphological assays enable the association between structure and function of certain types of tissue and a better understanding of the reactions of the affected organism as manifested in the superficial gingival epithelium. It is known that the nuclei of the squamous stratified epithelium enlarge during gingival inflammatory reactions in periodontitis.[28] Inflammation is one of the factors that can increase nuclear area and lead to a poorly preserved cytoplasm.[19] The results from this study demonstrate the difference in nuclear size between inflamed and healthy gingival tissue. Similar results were noted by Igić et al.,[28] who conducted a pioneering attempt to demonstrate the events in the gingival cells during inflammation, as well as the changes occurring after LLLT treatment in children. They confirmed that gingivitis can be successfully treated with LLLT as a supplement to basic treatment. Cytomorphometric analysis confirmed that the nuclei of the squamous stratified gingival epithelium were reduced in size after basic treatment, although not to the size of the nuclei of healthy gingiva. However, after LLLT in addition to basic treatment of gingivitis, the nuclear size of the cells of the squamous stratified gingival epithelium corresponded to the size characteristic of healthy gingiva.

In this study comparing the cell nuclear size (nuclear area) in each group before and after therapy, a significant decrease of nuclear area after the therapy was noted, suggesting that basic periodontal treatment alone and periodontal treatment together with LLLT reduce gingival inflammation. On the side of jaws subjected to LLLT and basic periodontal therapy compared with the side subjected only to basic periodontal therapy, the nuclear area was smaller, suggesting that

Table 3. Values of Cellular Parameters Investigated Before and After Therapy

Timing, group	Mean ± SD for investigated parameter				
	Nuclear area (μm)	*Perimeter*	*Nuclear circularity*	*Ferret's diameter (μm)*	*Integral optical density (au)*
Before therapy					
1 (DM type 1)	107.28 ± 15.42	39.11 ± 2.77	0.87 ± 0.03	13.56 ± 1.08	28.51 ± 5.82
2 (DM type 2)	103.50 ± 14.52	38.42 ± 2.60	0.87 ± 0.03	13.57 ± 0.96	24.62 ± 4.71
3 (control)	77.29 ± 7.87[ab]	33.40 ± 1.80[ab]	0.86 ± 0.03[ab]	11.98 ± 0.87[ab]	18.20 ± 3.55[ab]
After therapy					
Lasered side					
1 (DM type 1)	40.69 ± 4.91*	24.87 ± 1.93*	0.82 ± 0.05*	9.01 ± 1.05*	14.23 ± 3.03*
2 (DM type 2)	39.91 ± 3.39*	24.63 ± 1.32*	0.82 ± 0.07*	8.82 ± 0.74*	14.21 ± 2.48*
3 (control)	39.40 ± 3.71*	24.30 ± 1.56*	0.84 ± 0.06*	8.72 ± 0.84*	10.62 ± 1.95*
Non-lasered side					
1 (DM type 1)	52.03 ± 4.97*[†]	27.79 ± 1.02*[†]	0.84 ± 0.03*[†]	10.25 ± 0.67*[†]	16.41 ± 3.16*[†]
2 (DM type 2)	54.30 ± 5.07*[†]	28.03 ± 1.43*[†]	0.86 ± 0.03*[†]	9.90 ± 0.87*[†]	17.52 ± 4.93*[†]
3 (control)	51.09 ± 5.99*[†cd]	27.64 ± 1.70*[†cd]	0.83 ± 0.06*[†cd]	10.09 ± 0.82*[†cd]	16.74 ± 2.84*[†cd]

Significant ($P < 0.01$) differences are indicated: before therapy, [a]Group 2 versus Group 3, [b]Group 1 versus Group 3; after therapy, [c]Group 2 versus Group 3, [d]Group 1 versus Group 3; *after therapy versus before therapy; [†]after therapy lasered versus non-lasered side.
au, absorbance units; DM, diabetes mellitus.

inflamed gingival cells in patients with DM respond to laser light, and better therapy results can be achieved combining basic periodontal therapy with LLLT. This finding is similar to literature data indicating that adjuvant LLLT therapy reduces gingival inflammation during periodontal treatment and gives better results than basic periodontal treatment alone.[15,35–41] On the side of jaws subjected to combined therapy there was no difference in nuclear area between groups, suggesting that when LLLT and basic periodontal therapy are combined, patients with DM can achieve gingival health similar to that in persons without diabetes.

The general opinion is that patients with DM exhibit poorer periodontal health and poorer therapeutic response than patients without DM.[42] It is suggested that control of gingivitis during periodontal infection is important for achieving long-term control of DM.[2–5] Therefore, a periodontist should be consulted in the treatment of periodontal patients with DM in aim to achieve better and longer-lasting therapeutic results.[43]

Conclusions

Cytomorphometric analysis confirmed that the nuclei of the squamous stratified gingival epithelium of patients with DM were reduced in size after LLLT in addition to basic treatment of gingival inflammation during periodontitis and corresponded to the size of ones in patients without DM.

It can be concluded that LLLT as an adjunct in periodontal therapy reduces gingival inflammation in patients with DM and periodontitis.

Close collaboration among the patient, primary healthcare professionals, and oral health professionals and further investigation of new therapeutic modalities such as LLLT could be a way of improving the general and periodontal health in patients with DM.

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Author Disclosure Statement

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