Lasers Med Sci (2013) 28:19–24
DOI 10.1007/s10103-012-1058-7

ORIGINAL ARTICLE

A histological evaluation of a low-level laser therapy as an adjunct to periodontal therapy in patients with diabetes mellitus

Radmila Obradović · Ljiljana Kesić · Dragan Mihailović · Slobodan Antić · Goran Jovanović · Aleksandar Petrović · Snežana Peševska

Received: 27 September 2011 / Accepted: 18 January 2012 / Published online: 5 February 2012

Abstract Diabetes mellitus (DM) and chronic periodontitis are common chronic diseases in adults in the world population. DM has a strong influence on the oral cavity and represents a risk factor for gingivitis and periodontitis. Low-level laser therapy (LLLT) has proven effective in the reduction of inflammation and swelling. The aim of the present study was to evaluate the efficacy of LLLT in diabetic periodontitis through histological analysis. A total of 300 diabetics with chronic periodontal disease and teeth indicated for extraction were assigned into six equal groups. In the groups 1 and 4, indicated teeth were extracted before treatment, and in the rest of the groups upon completion of the entire treatment. All patients received oral hygiene instructions and full-mouth conservative periodontal treatment. In groups 3 and 6, LLLT was applied (670 nm, 5 mW, 2 J/cm^2, 16 min, 5 days). Histologic findings of gingival tissue treated with LLLT showed expressed healing, as is evident by the absence of inflammatory cells. Tissue edema could not be seen, and the number of blood vessels was reduced. In the gingival lamina, propria pronounced collagenization and homogenization were present. It can be concluded that LLLT has shown efficacy in the treatment of periodontitis in diabetics. Because of more pronounced alterations of periodontium in diabetics, the use of LLLT is of particular importance.

Keywords Diabetes mellitus · Periodontitis · Low-level laser therapy · Histology

R. Obradović (✉) · L. Kesić
Department of Periodontology and Oral Medicine,
Faculty of Medicine, University of Niš,
45/19 Nikola Tesla Blvd,
18000 Niš, Serbia
e-mail: dr.rada@yahoo.com

D. Mihailović
Institute of Pathology, Faculty of Medicine, University of Niš,
Niš, Serbia

S. Antić
Clinic for Endocrinology, Faculty of Medicine, University of Nis,
Niš, Serbia

G. Jovanović
Department of Oral Surgery, Faculty of Medicine,
University of Nis,
Niš, Serbia

A. Petrović
Institute of Histology, Faculty of Medicine, University of Niš,
Niš, Serbia

S. Peševska
Department of Periodontology, Faculty of Dentistry,
University "St. Cyril and Methodius",
Skopje, Macedonia

Introduction

Diabetes mellitus (DM) and chronic periodontitis are common chronic diseases in adults in the world population. DM is a complex disease with both metabolic and vascular components characterized by hyperglycemia due to defects in insulin secretion, insulin action, or both. The influence of DM on the oral cavity has been well researched, and studies have reported DM as a risk factor for gingivitis and periodontitis [1, 2]. Several epidemiological studies have presented a positive correlation between degree of glycemic control and periodontitis prevalence and progression [3–5].

The mechanisms by which hyperglycemia can induce periodontal destruction are not yet fully understood, but is

thought to have an association with altered immune function, advanced glycation end products (AGEs), and changes in collagen [6–8]. The diabetic state impairs the gingival fibroblast synthesis of collagen and glycosaminoglycan, enhances crevicular fluid collagenolytic activity, results in the loss of periodontal fibers and loss of the alveolar supporting bone [9, 10]. This predisposes to chronic inflammation, progressive tissue breakdown, and diminished tissue repair capacity [11]. Taken collectively, these mechanisms cause periodontal tissue breakdown, loosening, and finally exfoliation of the teeth [9–11]. Patients with DM exhibit poorer periodontal health and poorer therapeutic response than systemically healthy patients [5]. Close collaboration between the patient, the primary health care and oral health professionals, and application of new therapeutic modalities could be a way of improving general and oral health in patients with DM.

Low-level laser therapy (LLLT) was introduced as a therapeutic modality as early as 1968 [12]. 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. The infrared portions of the spectrum have been shown to have highly absorbent and unique therapeutic effects in living tissues and seem to provide the best results [12]. LLLT has shown to be effective in the treatment of impaired microcirculation, wound healing, pain relief, fracture healing, and reduction of inflammation and swelling [13–19]. It is widely accepted today that the inflammation is a basic response of periodontal tissue to damage. LLLT reduces gingival inflammation and better therapeutic results are achieved when LLLT was applied during conservative periodontal therapy compared to applying conservative periodontal therapy alone [20]. Until now, there has been little histological evidence pertaining to a study of LLLT patients with DM.

The aim of the study

With an increased number of patients presenting with DM, a more proper understanding on its pathophysiology is a key for an appropriate treatment approach. Therefore, the aim of the present study was to evaluate the efficacy of low-level laser therapy and conservative periodontal therapy in patients with DM through histology.

Subjects and methods

Study design and subjects

This clinical study was carried out as a joint collaboration between the Department of Endocrinology and Department of Periodontology and Oral medicine, Niš University, Faculty of Medicine. The Ethics Committee of the Faculty of Medicine Niš approved the study protocol (evidential number 01-2800-7). Patients with periodontitis and DM were selected randomly from the pool of followed patients at the Department of Endocrinology, Niš University Medical Center.

Patients who underwent antibiotic and corticosteroid therapy within the last 3 months or who had acute systemic illness, hemorrhagic disorders, autoimmune diseases as well as pregnant patients, or underwent periodontal treatment less than 6 months prior to the study were not included in the study. After taking the history and clinical examination, 300 diabetic patients who had chronic periodontal disease and teeth indicated for extraction (because of advanced periodontitis and poor treatment prognosis) signed an informed consent form. There were 150 subjects with periodontitis and DM type 1 that were assigned into three groups (groups 1, 2, and 3), and 150 subjects with periodontitis and DM type 2 assigned into three groups (groups 4, 5, and 6).

Oral examination protocol

Oral examination was done by a single examiner for all fully erupted permanent teeth (third molars excluded). In groups 1 and 4 the teeth were extracted before treatment, and in the rest of the groups upon completion of the entire treatment. All patients in the study received oral hygiene instructions and full-mouth conservative periodontal treatment (removal of dental plaque followed by scaling and root planing). Afterwards, in groups 3 and 6, GaAlAs LLLT (Mils 94, 670 nm, 5 mW, 16 min, 2 J/cm^2) was applied to gingival tissue surrounding investigated tooth, for five consecutive days. After completing the therapy in groups 2, 3, 5 and 6, teeth were extracted and gingival material from the socket was taken. After standard processing, material H&E staining was applied.

Diabetes-related variables

The following information was collected from medical records: type of DM and duration (years since diagnosis), glycosylated hemoglobin (HbA_{1C}), patient age, and sex.

Results

Subjects with periodontitis and DM type 1 were 54% women and 46% men with the mean age of 25.54±3.65 years, HbA_{1C} value 9.87±0.32% and the mean DM duration was 19.01±1.22 years. Subjects with periodontitis and DM type 2 were 48% women and 52% men, with the mean age of

62.57±8.57 years, with HbA_{1C} value 8.70±0.45% and the mean DM duration was 14.68±3.43 years.

Histological observation before therapy (groups 1 and 4)

In the lamina propria between pseudocystic formations and fibrosclerotic areas, an inflammatory cell infiltrate was observed. The inflammation was intense and the inflammatory infiltrate was composed mainly of lymphocytes, fibroblasts, and a few plasma cells. Fibrosclerotic areas had a gnarly appearance, and were characterized by collagen bundle hyalinization. Thickening of the basement membrane of blood vessels was also noticed. Small, locally confluent and large, pseudocystic formations were seen at the place of the collagenolysis. Its content seemed to be a homogeneous, structureless material similar to serous liquid filled with fresh and old erythrocytes. Nerve bundles and collagen structures surrounded these areas. In the dilated capillaries, marginalization of agglomerated erythrocytes and rare Russell's cells were also seen. There was no histological difference between groups 1 and 4. (Figs. 1 and 2)

Histological observation after conservative periodontal therapy (groups 2 and 5)

A reduction of inflammatory cells and tissue edema was observed. Lymphocytes and plasma cells with a small number of leukocytes were present in the stroma. The representation of blood vessels was lower compared to the state before treatment. Collagenization of the stroma was moderately expressed. There was no histological difference between investigated groups 2 and 5. (Figs. 3 and 4)

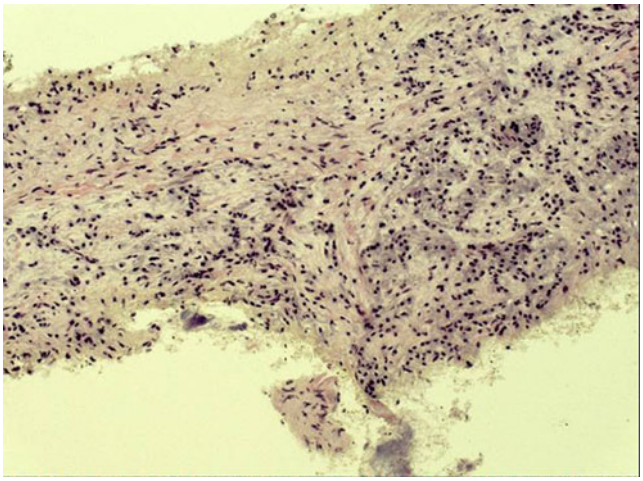

Fig. 2 Histological observation before therapy in patients with DM type 2 (H&E × 200)

Histological observation after conservative periodontal therapy and LLLT (groups 3 and 6)

Disappearance of tissue edema with a minimal number of inflammatory cells in the lamina propria was observed. The number of blood vessels was reduced, with distinguish collagenization and homogenization of the stroma. There was no histological difference between groups 3 and 6. (Figs. 5 and 6)

Discussion

It is widely accepted today that the primary etiological factor for the onset of periodontitis is dental plaque, although the exact mechanism of damage remains unknown. Also, several risk factors, such as DM play an important role. Prevention of

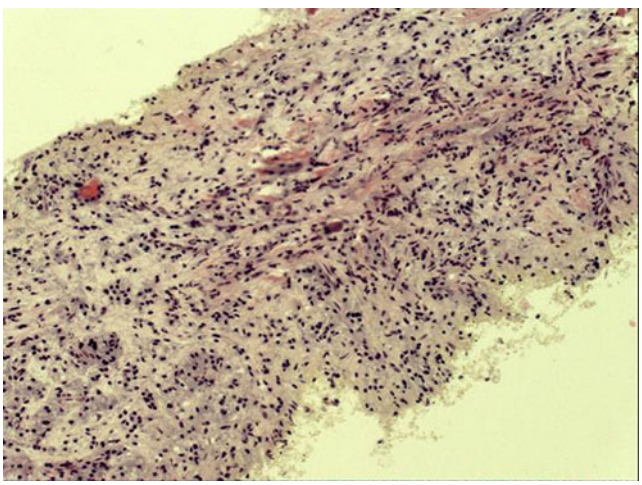

Fig. 1 Histological observation before therapy in patients with DM type 1 (H&E × 200)

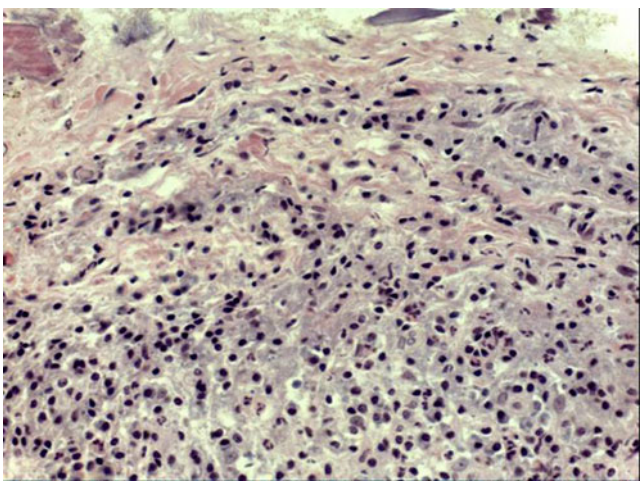

Fig. 3 Histological observation after periodontal therapy in patients with DM type 1 (H&E × 400)

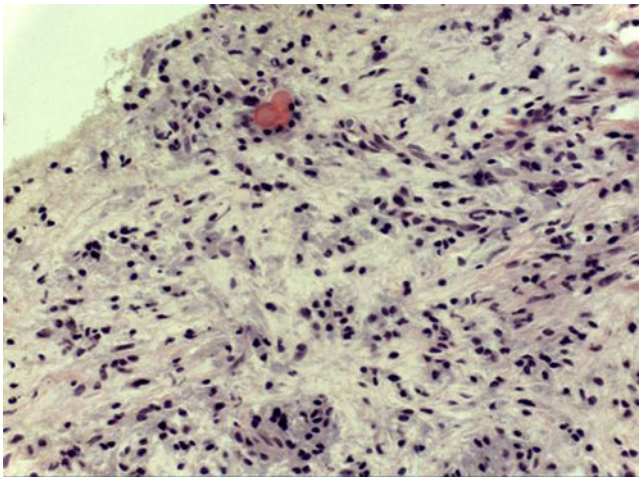

Fig. 4 Histological observation after periodontal therapy in patients with DM type 2 (H&E × 400)

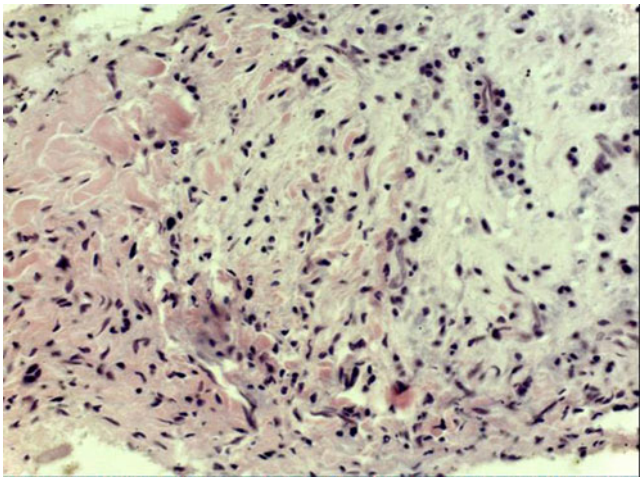

Fig. 6 Histological observation after periodontal therapy and LLLT in patients with DM type 1 (H&E × 400)

periodontitis in patients with DM is fundamentally important due to its potential adverse effect on glycemic control and promotion of diabetic complications. The dynamics of periodontal tissue destruction in patients with DM is complex and has been not clearly elucidated [21–23]. Understanding its causative pathways plays a crucial role in determining an effective treatment approach.

Periodontitis is characterized by gingival inflammation, periodontal tissue destruction, and alveolar bone loss [22, 23]. Inflammation is a basic response of periodontal tissue to damage and serves as a fast first line of defense against damage and infections. Marked inflammation affects both the epithelial and connective tissues, causing degeneration of the dermal papilla, increase in the number of inflammatory cells, destruction of reticulin fibers, and accumulation of dense collagen fibers (fibrosis). These changes are worsened by DM, apparently by hampering the inflammatory response and affecting tissue repair of the affected tissues [24, 25]. Similar changes were noticed in this study: in the gingival lamina propria inflammatory changes (tissue edema, inflammatory cell infiltrate, thickening of the basement membrane of blood vessels, collagenolysis and hyalinization of the collagen bundles) were more pronounced before therapy compared to the state observed after both therapeutic protocols.

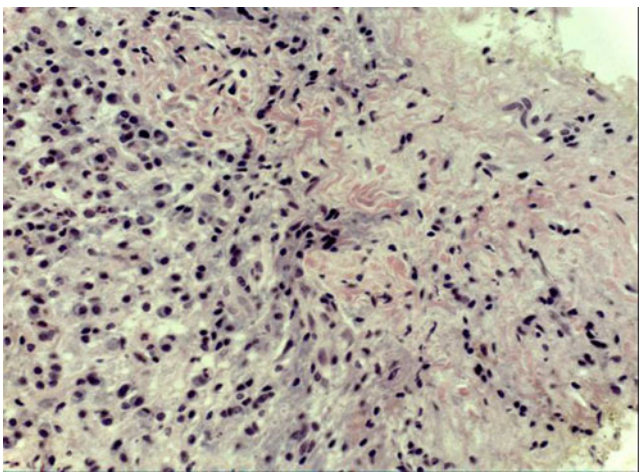

Fig. 5 Histological observation after periodontal therapy and LLLT in patients with DM type 1 (H&E × 400)

The gingival lamina propria is subject to a great range of structural patterns. Because of its vascularity, this tissue exhibits considerable lability and may be considered to reflect, in an even greater degree than lamina epithelialis, responses to both systemic and local conditions. Differences in connective tissue density and organization, the degree of polymerization of matrix, show many different patterns which further studies may correlate biochemically, physiologically, and clinically. Gingival lamina propria in this study showed leukocyte inflammation with small lymphoplasmatic infiltrate and a lot of fibroblasts and the plasmacytoma. Before the therapy, this inflammatory cell infiltrate was observed in the lamina propria between the fibrosclerotic areas and pseudocystic formations due to very pronounced lysis of collagen. Similar alterations in the gingival epithelium and connective tissue with DM have been reported by several authors [26, 27]. Atrophy and pleomorphism of the gingival epithelium, with inflammatory infiltrate and increased intercellular spaces, have been seen in patients with DM. The lamina propria in normal gingival is generally less cellular than in patients with DM, and the collagenous tissue, chiefly in papillary crests, is coarsely textured and densely packed. According to Silva et al. [24], diabetic animals presented a compromised differentiation of the epithelial cells, reduction in soluble collagen, and distortion of reticular fibres.

The treatment of gingivitis and periodontitis has gone through various stages: from the simplest, conservative

treatment methods, through improved surgical interventions, to a new era marked by laser technology. The purpose of the present study was to evaluate the efficiency of periodontal therapy and LLLT in patients with DM. After applied LLLT and conservative periodontal therapy, more pronounced signs of gingival tissue healing were observed than after applying only conservative periodontal therapy. Similar findings were observed by other investigators. Pesevska et al. noticed [28] suppression of TNF-alpha in gingival tissue after low-level laser treatment (1.875 J/cm^2) as adjunct to scaling and root planing. Data may suggest beneficial anti-inflammatory effects of the laser treatment when used as adjunctive periodontal treatment. Angelov et al. [29] assessed the effects of low-level laser treatment in combination with scaling and root planning in patients with periodontitis. The plaque index, gingival index, and sulcular bleeding index were recorded, and all three clinical parameters showed that a low-level diode laser can have a beneficial effect for treating inflammatory chronic advanced periodontitis. There are an insufficient number of studies analyzing histological changes in the inflamed human gingival tissue after LLLT [20, 26, 30, 31]. Clinical studies have shown beneficial effects of laser therapy in inflammatory pathology, such as bursitis, tendinitis, and other syndromes [32, 33]. In this study, histological findings of gingival tissue treated with LLLT showed expressed healing and pronounced collagenization as confirmed by the work of other authors. Logan et al. [34] examined the production of collagen after the application of laser radiation and noticed release of inflammatory mediators in the interstitium, and increased activity of fibroblasts after absorption of laser light in the superficial dermis. Reddy et al. [35] noted that LLLT significantly stimulates proliferation of fibroblasts, collagen production, and connective tissue stabilization. Byrnes et al. [36] investigated the effects of LLLT on cutaneous wound healing in an animal model of DM type 2. Significant improvement in wound healing histology and wound closure were found following treatment with 4 J/cm^2. The results demonstrate that LLLT at an energy density of 4 J/cm^2 is effective in improving the healing of cutaneous wounds in an animal model of DM type 2, suggesting that LLLT (632 nm, 4 J/cm^2) would be effective in treating wounds in diabetic patients [36]. Maiya et al. [37] investigated the effect of low-energy He-Ne laser treatment on wound healing dynamics (histological and biochemical) in diabetic rats and concluded that LLLT promotes tissue repair processes [37].

In order to be more certain of this hypothesis, further animal and human experiments with a larger sample size should be performed including immunohistochemical analysis of related cytokines and chemokines. Moreover, the blood glucose level in patients with DM should be further investigated to correlate glycemic level, periodontal tissue destruction, and response to therapy.

Conclusions

It can be concluded that low-power lasers have shown efficacy in the treatment of periodontitis in patients with diabetes mellitus. Because of more pronounced alterations of periodontium in patients with diabetes mellitus, the use of low-power laser is of particular importance.

LLLT together with conservative periodontal therapy may lead to better and longer-lasting therapeutic results, and the usage of LLLT as a standard form of adjuvant periodontal therapy can be recommended in patients with diabetes mellitus. Further LLLT studies are needed to demonstrate improvement in measured standard clinical periodontal parameters in these patients over time.

Acknowledgments This research was supported by a grant from the Serbian Ministry of Education and Science, No.175061.

Author disclosure statement No competing financial interests exist.

References

1. Southerland JH, Taylor GW, Offenbacher S (2005) Diabetes and periodontal infection: making the connection. Clin Diabetes 23 (4):171–178
2. Taiyeb-Ali TB, Raman RPC (2000) Vaithilingam RD (2011) relationship between periodontal disease and diabetes mellitus: an Asian perspective. Periodontology 56:258–268
3. Natto ZS, Al-Zahrani MS (2010) Periodontal bone loss and self-reported medical conditions in a dental school patient population. J Int Acad Periodontol 12(4):104–109
4. Lalla E, Park DB, Papapanou PN, Lamster IB (2008) Oral disease burden in Northern Manhattan patients with diabetes mellitus. Am J Public Health 98(Suppl 1):91–94
5. Teeuw WJ, Gerdes VEA, Loos BG (2010) Effect of periodontal treatment on glycemic control of diabetic patients. A systematic review and meta-analysis. Diabetes Care 33(2):421–427
6. Nassar H, Kantarci A, van Dyke TE (2007) Diabetic periodontitis: a model for activated innate immunity and impaired resolution of inflammation. Periodontol 2000 43(1):233–244
7. Nishimura F, Iwamoto Y, Soga Y (2007) The periodontal host response with diabetes. Periodontol 2000 43(1):245–253
8. Graves DT, Liu R, Oates TW (2007) Diabetes-enhanced inflammation and apoptosis: impact on periodontal pathosis. Periodontol 2000 45(1):128–137
9. Liu R, Bal HS, Desta T, Krothapalli N, Alyassi M, Luan Q, Graves DT (2006) Diabetes enhances periodontal bone loss through enhanced resorption and diminished bone formation. J Dent Res 85(6):510–514
10. Lappin DF, Eapen B, Robertson D, Young J, Hodge PJ (2009) Markers of bone destruction and formation and periodontitis in type 1 diabetes mellitus. J Clin Periodontol 36(8):634–641
11. Iacopino AM (2001) Periodontitis and diabetes interrelationships: the role of inflammation. Ann Periodontol 6:125–137
12. Houreld NN, Abrahamse H (2008) Laser light influences cellular viability and proliferation in diabetic-wounded fibroblast cells in a dose- and wavelength-dependent manner. Lasers Med Sci 23:11–18
13. Posten W, Wrone DA, Jeffrey SD, Arndt KA, Sirunya S, Murad A (2005) Low-level laser therapy for wound healing: mechanism and efficacy. Dermatol Surg 31(3):334–340

14. Rabelo SB, Villaverde AB, Nicolau RA, Salgado MAC, Melo MDS, Pacheco MTT (2006) Comparison between wound healing in induced diabetic and nondiabetic rats after low-level laser therapy. Photomed Laser Surg 24(4):474–479
15. Hagiwara S, Iwasaka H, Okuda K, Noguchi T (2007) GaAlAs (830 nm) Low-level laser enhances peripheral endogenous opioid analgesia in rats. Lasers Surg Med 39(10):797–802
16. Peševska S, Nakova M, Ivanovski K et al. (2010) Dentinal hypersensitivity following scaling and root planing: comparison of low-level laser and topical fluoride treatment. Lasers Med Sci 25(5):647–650
17. Reddy K (2004) Photobiological basis and clinical role of low-intensity lasers in biology and medicine. J Clin Laser Med Surg 22 (2):141–150
18. Bayat M, Abdi S, Javadieh F, Mohsenifar Z, Rashid MR (2009) The effects of low-level laser therapy on bone in diabetic and nondiabetic rats. Photomed Laser Surg 27(5):703–708
19. Obradović R, Lj K, Peševska S (2009) Influence of low-level laser therapy on biomaterial osseointegration: a mini-review. Lasers Med Sci 24(3):447–451
20. Aykol G, Baser U, Maden I et al. (2011) The effect of low-level laser therapy as an adjunct to non-surgical periodontal treatment. J Periodontol 82(3):481–488
21. Lalla E (2007) Periodontal infections and diabetes mellitus: when will the puzzle be complete? J Clin Periodontol 34:913–916
22. Matthews J, Jokstad A, Clarkson J (2009) Weak evidence suggests scaling and root planing may help people with Diabetes improve glycemic control. J Am Dent Assoc 140(9):1144–1145
23. Nanci A, Bosshardt DD (2006) Structure of periodontal tissues in health and disease. Periodontol 2000 40(1):11–28
24. Silva JAF, Lorencini M, Reis JRR, Carvalho HF, Cagnon VHA, Stach-Machado DR (2008) The influence of type I diabetes mellitus in periodontal disease induced changes of the gingival epithelium and connective tissue. Tissue Cell 40(4):283–292
25. Lee W, Aitken S, Sodek J, McCulloch CAG (2006) Evidence of a direct relationship between neutrophil collagenase activity and periodontal tissue destruction in vivo: role of active enzyme in human periodontitis. J Periodontal Res 30(1):23–33
26. Mealey BL, Rethman MP (2003) Periodontal disease and diabetes mellitus. Bidirectional relationship. Dent Today 22(4):107–113
27. Mealey BL, Oates TW (2006) Diabetes mellitus and periodontal diseases. J Periodontol 77(8):1289–1303
28. Pesevska S, Nakova M, Gjorgoski I, Angelov N, Ivanovski K, Nares S, Andreana S (2011) Effect of laser on TNF-alpha expression in inflamed human gingival tissue. Laser Med Sci. doi:10.1007/s10103-011-0898-x
29. Angelov N, Pesevska S, Nakova M, Gjorgoski I, Ivanovski K, Angelova D, Hoffmann O, Andreana S (2009) Periodontal treatment with a low-level diode laser: clinical findings. Gen Dent 57(5):510–513
30. Cobb CM (2006) Lasers in periodontics: a review of the literature. J Periodontol 77(4):545–564
31. Houreld N, Abrahamse H (2007) In vitro exposure of wounded diabetic fibroblast cells to a helium-neon laser at 5 and 16 J/cm^2. Photomed Laser Surg 25(2):78–84
32. Garcia VG, Fernandes LA, de Almeida JM, Bosco AF, Nagata MJH, Martins TM, Okamoto TO, Theodoro LH (2009) Comparison between laser therapy and non-surgical therapy for periodontitis in rats treated with dexamethasone. Laser Med Sci 25(2):197–206
33. Bingöl U, Altan L, Yurtkuran M (2005) Low-power laser treatment for shoulder pain. Photomed Laser Surg 23(5):459–464
34. Logan KM, Clements BA (2001) Low-intensity laser therapy (830 nm) in the management of minor post-surgical wounds: a controlled clinical study. Laser Surg Med 28:27–32
35. Reddy GK, Stepno-Bihel LL, Ekwemena CS (2001) Laser photostimulation accelerate wound healing in diabetic rats. Wound Repair Regen 9(3):248–255
36. Byrnes KR, Barna L, Chenault VM, Waynant RW, Ilev IK, Longo L, Miracco C, Johnson B, Anders JJ (2004) Photobiomodulation improves cutaneous wound healing in an animal model of Type II Diabetes. Photomed Laser Surg 22(4):281–290
37. Maiya GA, Kumar P, Rao L (2005) Effect of low intensity Helium-Neon (He-Ne) laser irradiation on diabetic wound healing dynamics. Photomed Laser Surg 23(2):187–190