Lasers Med Sci (2013) 28:297–301
DOI 10.1007/s10103-012-1149-5

ORIGINAL ARTICLE

Proinflammatory cytokine levels in saliva in patients with burning mouth syndrome before and after treatment with low-level laser therapy

Sonja Pezelj-Ribarić · Lumnije Kqiku · Gordana Brumini · Miranda Muhvić Urek · Robert Antonić · Davor Kuiš · Irena Glažar · Peter Städtler

Received: 8 May 2010 / Accepted: 25 June 2012 / Published online: 8 July 2012

Abstract The aim of this study was to determine the levels of proinflammatory tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) cytokines in whole unstimulated saliva in subjects with burning mouth syndrome (BMS) before and after treatment with low-level laser therapy (LLLT). BMS is characterized by a continuous, painful burning sensation in a clinically normal-appearing oral mucosa. A sample consisting of 40 consecutive subjects was selected on a voluntary basis from the pool of patients who presented for diagnosis and treatment of BMS at the Oral Medicine Unit of the Faculty of Medicine of the University of Rijeka. For determination of salivary levels of TNF-α and IL-6, ELISA (Sigma Immunochemicals, St. Louis, MO, USA) was performed to determine the salivary levels of TNF-α and IL-6. After 4 weeks of LLLT, the salivary levels of TNF-α and IL-6 in the experimental group decreased significantly ($p<0.001$). There was no significant difference in the experimental group regarding visual analogue scale.

Keywords Burning mouth · Laser · Cytokine

Introduction

Burning mouth syndrome (BMS) is reported by individuals as a sensation of pain and burning in the oral mucosa, most frequently affecting the tongue but also occurring in other regions of the mouth such as the lips, buccal mucosa, and floor of the mouth. The intensity of this sensation ranges from mild to severe. Patients with BMS frequently have

S. Pezelj-Ribarić (✉) · M. M. Urek · D. Kuiš · I. Glažar
Department of Oral Medicine and Periodontology,
School of Dentistry, Faculty of Medicine, University of Rijeka,
Braće Branchetta 20,
Rijeka, Croatia
e-mail: sonja.pezelj-ribaric@medri.hr

M. M. Urek
e-mail: miranda_um@hotmail.com

D. Kuiš
e-mail: kuis@net.hr

I. Glažar
e-mail: irena.glazar@medri.hr

L. Kqiku · P. Städtler
Department of Preventive and Operative Dentistry, Endodontics, Pedodontics, and Minimally Invasive Dentistry, University Dental Clinic,
Auenbruggerplatz 6a,
8036 Graz, Austria

L. Kqiku
e-mail: lumnije.kqiku@medunigraz.at

P. Städtler
e-mail: peter.staedtler@medunigraz.at

G. Brumini
Department of Informatics, Faculty of Medicine,
University of Rijeka,
Braće Branchetta 20,
Rijeka, Croatia
e-mail: bgord@medri.hr

R. Antonić
Department of Prosthodontics, School of Dentistry,
Faculty of Medicine, University of Rijeka,
Braće Branchetta 20,
Rijeka, Croatia
e-mail: Robert.Antonic@medri.hr

other complaints, amongst which xerostomia and loss or altered sense of taste are most frequently mentioned [1, 2]. BMS often occurs within a range of medical and dental conditions, from nutritional deficiencies and menopause to dry mouth and allergies. But, their interrelationship is still unclear, and the exact cause of burning mouth syndrome cannot always be identified with certainty.

BMS appears to be more prevalent in middle-aged and older women (mean age, 50–60 years), with a female-to-male ratio varying from 3:1 to 16:1 [3]. Its pathogenic mechanisms and etiological factors are largely unknown. Some researchers have suggested that the disorder may be a manifestation of somatization, while others have reported it to be more closely related to neuropathic pain than to somatoform chronic pain syndromes [4, 5]. The majority of studies have revealed a variety of psychosocial features and personality disorders in BMS patients, such as alexithymic traits, cancerophobia, somatization, obsession–compulsion, personal sensitivity, hostility, psychoticism, and social isolation, as well as significantly higher adverse early life experiences and a higher mean score for anxiety and depression, compared with appropriate controls. However, as with other chronic pain syndromes, these findings do not distinguish between cause and effect.

The treatment of burning mouth syndrome is usually directed at its symptoms. Studies generally support the use of low doses of clonazepam [6], chlordiazepoxide [7], and tricyclic antidepressants [8]. A potential noninvasive treatment for BMS patients is low-level laser therapy (LLLT). In recent studies, many authors have reported significant pain reduction with LLLT in painful stomatitis [9] and severe pain in patients submitted to hematopoietic stem cell transplantation [10]. In our previous investigation, we found that interleukin-6 (IL-6) levels in saliva in patients with BMS were significantly increased compared with healthy subjects [11]. This supports the assumption that IL-6 is an objective marker for diagnostics and the detection of painful condition BMS. Tumor necrosis factor-alpha (TNF-α) is a soluble mediator and is released from immunocompetent cells in inflammatory processes. The biological effects of TNF-α include the activation of leukocytes such as lymphocytes (T and B cells), macrophages, and natural killer cells; fever induction; acute-phase protein release; cytokine and chemokine gene expression; and endothelial cell activation [12].

The aim of this study was to investigate TNF-α and IL-6 levels in whole unstimulated saliva of patients with BMS before and after LLLT.

Materials and methods

The study involved 40 patients with diagnosed BMS in the tongue, who were examined at the Oral Medicine Unit of the Faculty of Medicine of the University of Rijeka. Each subject completed a questionnaire for demographic and health information. The criteria for inclusion in the case group were diagnosis of BMS and the absence of any systemic diseases or local oral factors that might be involved in burning mouth sensation. All subjects were informed of the aims and procedures of research, as well as of the fact that their medical data would be used in research. Within the research they were guaranteed respect of their basic ethical and bioethical principles—personal integrity (independence, righteousness, well-being, and safety) as regulated by the Nűrnberg codex and the most recent version of the Helsinki declaration. Only those subjects who gave written permission in the form of informed consent were included.

The individuals underwent complementary examinations (complete blood count, blood glucose level, and estrogen level), and only those with normal values took part in the study. Clinical examination was performed according to the standard clinical criteria. Each patient was evaluated according to subjective pain reporting: 0–10 visual analogue scale (VAS: 0, no pain; 10, pain as bad as could be) [13]. After initial evaluation and diagnosis, the patients were divided in two groups:

Group 1 20 patients receiving real LLLT (experimental group)
Group 2 20 patients receiving inactive/placebo laser (control group)

After informed consent had been obtained and medical, dental, and social histories collected, salivary flow rates were determined for each participant. Samples were obtained by asking the subjects to swallow first, tilt their head forward, and expectorate all saliva into 50-ml tubes for 5 min without swallowing. The final volume and flow rate of saliva were determined gravimetrically (Analytical Balance, Model WTS-6001, Sartorius Corp., Long Island, NY, USA) [14].

Afterwards, the saliva specimens were stored at −80 °C until the beginning of analysis. For determination of salivary levels of TNF-α, ELISA (Sigma Immunochemicals, St. Louis, MO, USA) was performed to determine the salivary levels of TNF-α. The assay was performed according to the manufacturer's instructions, and the results were expressed in picograms per milliliter.

The BMS patients were then treated with LLLT. Twenty subjects in the experimental group were treated 5 days in a week for four consecutive weeks with a 685-nm gallium–aluminum–arsenic diode laser (Medio LASER Combi Dental, Iskra Medical, Ljubljana, Slovenia). Twenty patients in the control group with BMS were treated with inactive (placebo) laser.

The output power of the laser was measured for 7 min and found to be practically constant. The laser output power

Table 1 Demographic data

Group	Age (year)	Gender	
	Mean±SD	Male *N*	Female *N*
Experimental	60.2±6.3	5	15
Control	61.1±2.2	8	12
Statistic	p=0.550	p=0.311	

was controlled weekly using analogue power meters provided by the manufacturer. The laser light was delivered through an optical fiber (flexible fiber bundle with a 2-mm circular opening), and the output power was measured at the fiber opening. The laser treatment was performed by holding the laser probe in light contact with the tissue. During each session, the laser treatment was delivered to the tissue by a straight optical fiber with a 2-mm spot size. The treatment areas, each one being a 1-cm^2 surface, included tongue mucosa. The laser was applied on the tongue mucosa for 10 min (685 nm, continuous wave, 30 mW output power, 3.0 J/cm^2). The treatment time (t) for each application point was calculated using the following equation: t (in seconds)= 3.0 J/cm^2×1 cm^2/0.03 (W). The laser treatment was performed in a punctual mode for 100 s per point. The average energy density delivered to the treatment areas was 3.0 J/cm^2. The effect of the laser light was evaluated after the final treatment. For the placebo group, the laser device was adjusted for the same time and applied to the same points, but without power.

Statistical analysis

Statistical analysis of data was performed by using Statistica for Windows, version 8.1 (StatSoft, Inc., Tulsa, OK, USA). The patients' age in both groups was presented as mean±standard deviation (SD). One-way analysis of variance (ANOVA) was used to test the differences in age. Depending on the normality of distribution, data on TNF-α and IL-6 were presented as mean ±standard deviation, and VAS as median and interquartile range. The results of TNF-α and IL-6 were compared using repeated measures ANOVA. The results of VAS were compared by Wilcoxon test. All statistical values were considered significant at the p level of 0.05.

Table 2 Detected values of TNF-α before and after LLLT

TNF-α (pg/ml)	Before	After	Statistic p
Experimental group	0.437±0.124	0.234±0.060	<0.001
Control group	0.303±0.090	0.271±0.167	0.359
Statistic p	<0.001	0.258	

Table 3 Detected values of IL-6 before and after LLLT

IL-6 (pg/ml)	Before	After	Statistic p
Experimental group	0.401±0.151	0.141±0.037	<0.001
Control group	0.193±0.101	0.166±0.098	0.298
Statistic p	<0.001	0.196	

Results

The sample included 40 patients, whose demographic characteristic are shown in Table 1. The levels of TNF-α and IL-6 were measured in whole saliva samples using ELISA. In the experimental group, levels of TNF-α measured before LLLT were significantly greater than TNF-α levels following therapy (p<0.001). Levels of IL-6 before LLLT were also significantly greater than after LLLT (p<0.001). In the control group, no significant difference was found in the levels of TNF-α and IL-6 before and after the application of LLLT (all p values >0.05) (Tables 2 and 3).

There were no statistically significant differences regarding VAS before and after the application of the LLLT in the experimental group (p=0.748) (Table 4).

Discussion

The present study was designed to test the application of LLLT as an alternative means for the treatment of BMS through the evaluation of the TNF-α and IL-6 levels in samples of whole saliva. The results demonstrated a statistically significant reduction in the salivary levels of proinflammatory cytokines, both TNF-α and IL-6 in patients with BMS following treatment with LLLT for the duration of 4 weeks. The treatment of burning mouth syndrome is usually directed at its symptoms and is the same as the medical management of other neuropathic pain conditions. Antidepressants, such as trazodone, did not effectively relieve BMS-associated symptoms, as studied by Tammialia-Salonen et al. [15]. Recently, topical benzydamine hydrochloride rinse was not shown to be effective in managing the pain of BMS patients [16].

In contrast, patients who received systemic capsaicin did report a decrease in pain symptoms. However, as reported by Petruzzi et al. [17], side effects, such as gastric pain, were

Table 4 VAS before and after LLLT

VAS		Statistic p
Before	After	
7 (5–8)	6 (5–8)	0.748

also experienced, thus questioning the potential benefit of this treatment. Capsaicin may not be palatable or useful in many patients [18].

LLLT is reported to improve peripheral circulation, oxygenate hypoxic cells, and help remove noxious products [19], and has been successfully applied to various painful oral mucosal diseases [20]. According to some authors, laser is a treatment modality that is becoming widely known. There are also reports on the effect of LLLT in the inhibition of inflammatory mediator secretion, such as prostaglandin E_2 and interleukin 1-β [21]. LLLT also appears to have virustatic and bacteriostatic effects [22].

While many authors have presented opposing results of laser effectiveness in the treatment of pain or inflammation, majority of the studies (about 85 %) demonstrated that the reduction of pain is effective (13). The physiological mechanisms underlying the significant decrease in pain after LLLT are unknown. Some possible mechanisms associated with LLLT include changes on the cellular level with increased ATP production by the mitochondria, increased serotonin, endorphins, decreased inflammation, and improved local blood circulation [22]. Scientific evidence shows that BMS is related to the salivary levels of proinflammatory cytokines, so these cytokines may be used as biological indicators of disease [11]. In our previous study, we examined the therapeutic response to LLLT in patients with denture stomatitis, and we found that LLLT may be an efficacious choice of therapy [23]. In this study, active LLLT improved the salivary levels of proinflammatory cytokines in comparison with the control group. This evaluation of burning mouth syndrome is subjective and not totally controlled in clinical research areas. Our results show that there was some improvement considering VAS, but it was not statistically significant. VAS is a tool widely used to measure subjective experience and pain. It has been established as valid and reliable in a range of clinical and research applications, although there is also evidence of decreased sensitivity when used in some groups of patients [24].

The results of the present study provide evidence that the reduction of the salivary TNF-α and IL-6 after LLLT corresponds to the clinical improvement. Knowledge regarding the role of cytokines in the outcome of BMS may provide the basis for future therapeutic interventions.

Acknowledgments This research was supported by a grant from the Ministry of Science and Technology (062-0650444-0442), Republic of Croatia.

References

1. Lamey PJ, Lamb AB (1988) Prospective study of etiological factors in burning mouth syndrome. Br Med J 296:1234–1236
2. Bergdahl J, Anneroth G (1993) Burning mouth syndrome: literature review and model for research and management. J Oral Pathol Med 22:433–438
3. Scala A, Checchi L, Montevecchi M, Marini I, Giamberardino MA (2003) Update on burning mouth syndrome: overview and patient management. Crit Rev Oral Biol Med 14:275–291
4. Macfarlane TV, Blinkhorn AS, Davies RM, Ryan P, Worthington HV, Macfarlane GJ (2002) Orofacial pain: just another chronic pain? Results from a population-based survey. Pain 99:453–458
5. Maina G, Albert U, Gandolfo S, Vitalucci A, Bogetto F (2005) Personality disorders in patients with burning mouth syndrome. J Pers Disord 19:84–93
6. Woda A, Navez ML, Picard GPC, Pichard-Leandri E (1998) A possible therapeutic solution for stomatodynia (burning mouth syndrome). J Orofac Pain 12:272–278
7. Gorsky M, Silverman S, Chinn H (1991) Clinical characteristics and management outcome in the burning mouth syndrome. An open study of 130 patients. Oral Surg Oral Med Oral Pathol 72:192–195
8. Gremeau-Richard C, Woda A, Navez ML, Attal N, Bouhassira D, Gagnieu MC, Laluque JF, Picard P, Pionchon P, Tubert S (2004) Topical clonazepam in stomatodynia: a randomised placebo-controlled study. Pain 108:51–57
9. Toida M, Watanabe F, Goto K, Shibata T (2003) Usefulness of low-level laser for control of painful stomatitis in patients with hand-foot and mouth disease. J Clin Laser Med Surg 21:363–367
10. Antunes HS, de Azevedo AM, da Silva Bouzas LF, Adão CA, Pinheiro CT, Mayhe R, Pinheiro LH, Azevedo R, D'Aiuto de Matos V, Rodrigues PC, Small IA, Zangaro RA, Ferreira CG (2006) Low-power laser in the prevention of induced oral mucositis in bone marrow transplantation patients: a randomized trial. Blood 109:2250–2255
11. Simčić D, Pezelj-Ribarić S, Grzić R, Horvat J, Brumini G, Muhvić-Urek M (2006) Detection of salivary interleukin 2 and interleukin 6 in patients with burning mouth syndrome. Mediat Inflamm 1:54632
12. Chen CP, Hertzberg M, Jiang Y, Graves DT (1999) Interleukin-1 and tumor necrosis factor receptor signaling is not required for bacteria induced osteoclastogenesis and bone loss but is essential for protecting the host from a mixed anaerobic infection. Am J Pathol 155:2145–2152
13. Venancio Rde A, Campari CM, Lizarelli Rde F (2005) Low intensity laser therapy in the treatment of temporomandibular disorders: a double-blind study. J Oral Rehabil 32:800–807
14. Rhodus NL, Cheng B, Myers S, Bowles W, Ho V, Ondrey F (2005) A comparison of the pro-inflammatory, NF-kappaB-dependent cytokines: TNF-alpha, IL-1-alpha, IL-6, and IL-8 in different oral fluids from oral lichen planus patients. Clin Immmunol 114:278–283
15. Tammiala-Salonen T, Forssell H (1999) Trazodone in burning mouth pain: a placebo-controlled, double-blind study. J Orofac Pain 13:83–88
16. Sardella A, Uglietti D, Demarosi F, Lodi G, Bez C, Carrassi A (1999) Benzydamine hydrochloride oral rinses in management of burning mouth syndrome a clinical trial. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 88:683–686
17. Petruzzi M, Lauritano D, De Benedittis M, Baldoni M, Serpico R (2004) Systemic capsaicin for burning mouth syndrome: short-term results of a pilot study. J Oral Pathol Med 33:111–114
18. Grushka M, Epstein JB, Gorsky M (2002) Burning mouth syndrome. Am Fam Physician 65:615–620
19. Pinheiro AJ, Cavalcanti ET, Pinheiro TI, Alves MJ, Manzi CT (1997) Low-level laser therapy in the management of disorders of the maxillofacial region. J Clin Laser Med Surg 15:181–183
20. Antunes HS, Ferreira EM, de Matos VD, Pinheiro CT, Ferreira CG (2008) The impact of low power laser in the treatment of

conditioning-induced oral mucositis: a report of 11 clinical cases and their review. Med Oral Patol Oral Cirug Bucal 13:189–192
21. Shimizu Y, Yamaguchi M, Goseki T, Shibata Y, Takiguchi H, Iwasawa T, Abiko Y (1995) Inhibition of prostaglandin E2 and interleukin 1-beta production by low-power laser irradiation in stretched human periodontal ligament cell. J Dent Res 74:1382–1388
22. Emshoff R, Bösch R, Pűmpel E, Schöning H, Strobl H (2008) Low-level laser therapy for treatment of temporomandibular joint pain: a double-blind and placebo-controlled trial. Oral Med Oral Pathol Oral Radiol Endod 105:452–456
23. Simunović-Šoškić M, Pezelj-Ribarić S, Brumini G, Glažar I, Gržić R, Miletić I (2010) Salivary levels of TNF-alpha and IL-6 in patients with denture stomatitis before and after laser phototherapy. Photomed Laser Surg 28:189–193
24. McCormack HM, Horne DJ, Sheather S (1988) Clinical applications of visual analogue scales: a critical review. Psychol Med Nov 18:1007–1019