Makara Journal of Health Research

Volume 22
Issue 1 *April*

Article 3

4-1-2018

Low-Level Laser Therapy to the Major Salivary Glands Increases Salivary Flow and MUC5B Protein Secretion in Diabetic Patients with Hyposalivation: A Preliminary Study

Awaludin Wibawa
Oral Biology Graduate Programme, Faculty of Dentistry, Chulalongkorn University, Bangkok 10310, Thailand, bowo_giggsy@yahoo.com

Jeerus Sucharitakul
Department of Biochemistry, Faculty of Dentistry, Chulalongkorn University, Bangkok 10310, Thailand

Rungthip Dansirikul
Endocrine Center, Bangkok Hospital, Bangkok 10310, Thailand

Pagaporn P. Pisarnturakit
Department of Community Dentistry, Faculty of Dentistry, Chulalongkorn University, Bangkok 10310, Thailand

Patita Bhuridej
Department of Community Dentistry, Faculty of Dentistry, Chulalongkorn University, Bangkok 10310 , Thailand

See next page for additional authors

Recommended Citation

Wibawa A, Sucharitakul J, Dansirikul R, Pisarnturakit PP, Bhuridej P, Arirachakaran P, et al. Low-Level Laser Therapy to the Major Salivary Glands Increases Salivary Flow and MUC5B Protein Secretion in Diabetic Patients with Hyposalivation: A Preliminary Study. Makara J Health Res. 2018;22.

Low-Level Laser Therapy to the Major Salivary Glands Increases Salivary Flow and MUC5B Protein Secretion in Diabetic Patients with Hyposalivation: A Preliminary Study

Authors

Awaludin Wibawa, Jeerus Sucharitakul, Rungthip Dansirikul, Pagaporn P. Pisarnturakit, Patita Bhuridej, and Pratanporn Arirachakaran

Makara J. Health Res., 2018, 22(1): 14-21
doi: 10.7454/msk.v22i1.8547

Low-Level Laser Therapy to the Major Salivary Glands Increases Salivary Flow and MUC5B Protein Secretion in Diabetic Patients with Hyposalivation: A Preliminary Study

Awaludin Wibawa[1], Jeerus Sucharitakul[2], Rungthip Dansirikul[3], Pagaporn P Pisarnturakit[4], Patita Bhuridej[4], Pratanporn Arirachakaran[5*]

1. Oral Biology Graduate Programme, Faculty of Dentistry, Chulalongkorn University, Bangkok 10310, Thailand
2. Department of Biochemistry, Faculty of Dentistry, Chulalongkorn University, Bangkok 10310, Thailand
3. Endocrine Center, Bangkok Hospital, Bangkok 10310, Thailand
4. Department of Community Dentistry, Faculty of Dentistry, Chulalongkorn University, Bangkok 10310, Thailand
5. Oral Medicine Clinic, Faculty of Dentistry, Chulalongkorn University & Dental Center, Bangkok Hospital, 2 Soi Soonvijai 7, New Petchburi Road, Bangkok 10310, Thailand

[*]*E-mail: ariracp@gmail.com*

Abstract

Background: To investigate the effect of low-level laser therapy to salivary gland function in diabetic patients with hyposalivation. **Methods:** Twelve diabetic patients were recruited. A 940-nm indium-gallium-arsenide-phosphide low-power semiconductor diode laser was used to stimulate the major salivary glands with an irradiation time of 40 s; this was done three times a week for 2 consecutive weeks. Patients were given questionnaires related to dry mouth symptoms. Salivary flow rates, questionnaire responses as well as MUC7, MUC5B and histatin 5 protein salivary concentrations were assessed at the first and sixth visits after laser therapy as well at the 6-week follow-up visit. **Results:** The unstimulated salivary flow rate and MUC5B concentration at the follow-up visit significantly increased ($p < 0.01$). Histatin 5 concentration significantly decreased at the follow-up visit compared with that at the first visit ($p < 0.05$). The mean dry mouth score revealed a significant decrease in dry mouth symptoms at the sixth visit and follow-up visit compared with those at the first visit ($p < 0.001$). The positive correlation between dry mouth score and flow rate was the strongest at the sixth visit ($r_s = 0.549$). **Conclusions:** Low-level laser therapy increased the salivary flow rate and decreased dry mouth symptoms in diabetic patients.

Keywords: *diabetes, hyposalivation, laser therapy, salivary proteins*

Introduction

Saliva is composed of approximately 99% water; the remaining 1% consists of electrolytes and several types of macromolecules, including antimicrobial factors.[1,2] Approximately 65% of unstimulated whole saliva comes from the submandibular glands, 20% from the parotid glands, 7%–8% from the sublingual glands and 5%–8% from the minor salivary glands. However, when stimulated, saliva from the parotid glands increases to approximately 50% of the whole saliva volume and the remaining 50% comes from the other salivary glands as well as the gingival crevicular fluid.[1,2] Saliva has several functions, which include lubricating the oral cavity and protecting against pathogens with defensive proteins such as mucins and histatins.[1-3]

Mucins are salivary glycoproteins that are mostly secreted by the submandibular glands.[3,4] The function of mucin in the salivary defence system is to protect oral tissues from the outer environment and to hydrate and lubricate the oral cavity. Furthermore, mucins aid in mastication, speech and swallowing and are involved in agglutinating oral microorganisms.[1,2] There are two mucin isoforms based on molecular weight (MW): high-MW (>1000 kDa) gel-forming MUC5B and low-MW (120–150 kDa) MUC7.[5] MUC5B lubricates oral surfaces due to its hydrophilic carbohydrate properties. MUC7 has a shorter oligosaccharide side chain than MUC5B; however, MUC5B binds to fewer oral microorganisms than MUC7.[2]

Histatins are histidine-rich antimicrobial peptides produced from all major salivary glands and are known for their antifungal activity.[6] A study revealed that histatin 5, a histatin subtype, has remarkable fungicidal and fungistatic activities against *Candida albicans*.[5] Salivary gland hypofunction includes subjective symptoms and objective signs of dry mouth that, in most cases, are related to systemic diseases, such as Sjögren's syndrome,

hypertension and diabetes mellitus.[7] Some studies have reported an association between diabetes mellitus and hyposalivation. Type 1 and 2 diabetic patients show a high prevalence of dry mouth.[8,9]

A study has demonstrated that the MUC5B concentration tends to decrease in patients showing hyposalivation.[10] However, another study found that MUC5B and MUC7 concentrations were not significantly different between Sjögren's syndrome patients with oral dryness and controls.[7] Moreover, a decrease in histatin 5 concentration was related to an increased susceptibility to fungal infection.[11] Thus, we speculated that mucin and histatin 5 concentrations are decreased in diabetic patients with hyposalivation.

Dry mouth symptoms can be relieved by modifying eating/drinking habits and using salivary substitutes, lubricants or sialogogues to stimulate salivary flow;[2] however, the lubricating effect lasts for only as long as these agents are used.

A recent study demonstrated that low-level laser therapy (LLLT) or biostimulation increased the salivary flow rate.[12] LLLT involves the noninvasive and safe clinical application of light at a power ranging from 50 mW to 500 mW and wavelength ranging from 630 nm to 980 nm.[13] LLLT significantly increases the salivary flow rate in xerostomia patients[12,14] and has been demonstrated to be an effective noninvasive treatment in patients with mouth dryness.[15] To the best of our knowledge, the effect of LLLT on the major salivary glands of diabetic patients with hyposalivation has not been reported. We hypothesised that use of LLLT on the major salivary glands would improve the salivary flow rate, and we evaluated the quality of saliva by measuring MUC7, MUC5B and histatin 5 levels in diabetic patients with hyposalivation.

Methods

Participant recruitment. The study protocol was approved by the Ethics Committee of Bangkok Hospital, Bangkok, Thailand, in accordance with the ethical standards of the Declaration of Helsinki.

Twelve diabetic patients who visited the diabetic clinic at Bangkok Hospital from November 2015 to April 2016 were recruited on a voluntary basis. The diabetic patients were diagnosed by a physician according to one of the following four criteria: 1) HbA_{1C} level of ≥ 6.5%, 2) fasting plasma glucose level of ≥126 mg/dL (7.0 mmol/L; fasting was defined as no caloric intake for at least 8 h), 3) 2-h plasma glucose level of ≥200 mg/dL (11.1 mmol/L) during an oral glucose tolerance test and 4) random plasma glucose level of ≥200 mg/dl (11.1 mmol/L).[16] The estimated sample size was 10 participants. Alpha was set to 0.05, power to 90%, with standard deviation referred to form our pilot study (0.14), and expected difference after LLLT. As our inclusion criteria is USFR less than 0.25 mL/min and mean of adult USFR is 0.40 mL/min.

The patients were initially approached by giving them an educational brochure on diabetes-related oral health issues. Only patients diagnosed with hyposalivation, an unstimulated salivary flow rate (USFR) of <0.25 mL/min, were recruited. The following patients were excluded: those aged <18 years, those who were pregnant, those diagnosed with oral or maxillofacial neoplasms, those who consumed >1 drink per day (women) or 2 drinks per day (men)[17] and those who used illicit drugs (long-term regular injection of opioids, amphetamines or cocaine).[18] A brief medical history of each patient was taken as supporting information.

Low-level laser therapy (LLLT). Each patient underwent an oral examination prior to laser therapy. Laser therapy was performed by a dentist at Bangkok Hospital, during which the parotid and submandibular glands were extraorally exposed and the sublingual glands were intraorally exposed. Slow circulating laser movements were performed during therapy to ensure comprehensive treatment of the gland area. The salivary glands were stimulated with a 940-nm indium-gallium-arsenide-phosphide low-power semiconductor diode laser (Epic^{TM}10, Biolase Inc, Irvine, CA, USA). Stimulation was performed three times a week for 2 consecutive weeks. The dentist and patients wore protective eyeglasses during the procedure. Each parotid, submandibular and sublingual gland was stimulated using 0.1 W output power for 40 s/cm^2 area. An energy density (ED) of 4 J/cm^2 was used based on previous studies[12,14,15,19,20] along with the following equation: ED (J/cm^2) = W × s/cm^2.

Salivary flow rate measurement. Saliva was collected three times at the first visit prior to laser therapy, at the sixth visit after laser therapy and at the 6-week follow-up visit. Unstimulated and stimulated whole saliva collection was performed from nine in the morning to noon using standard techniques as described by Navazesh and Christensen.[21] Prior to saliva collection, the patients were instructed to stop eating, drinking and smoking for 1 h. For unstimulated saliva collection, the patients were directed to lean forwards and spit their saliva for 5 min into a sterilised plastic cup that was pre-weighed using a digital scale (Denver Instrument Balance, Bohemia, NY, USA). The collection procedure was repeated two more times. The USFR was calculated using the mean weight of the three saliva samples divided by 5 mins.

To stimulate saliva flow, the patients were instructed to chew 1 g of tasteless paraffin (Parafilm, Neenah, WI,

USA) and to not swallow their saliva during chewing. Those with dentures were directed to chew the paraffin without removing their dentures. All patients were told to spit their saliva into a pre-weighed plastic cup every 30 s for 2 mins. The collection procedure was repeated two more times. The stimulated salivary flow rate (SSFR) was calculated using the mean weight of the three saliva samples divided by 2 min.

Enzyme-linked immunosorbent assay (ELISA) (MyBio-Source, San Diego, CA, USA) was performed to determine MUC7, MUC5B, and histatin 5 salivary protein levels. Unstimulated saliva was used for mucin analysis as mucins are mostly produced from the submandibular and sublingual glands,[4] whereas stimulated saliva was used for histatin 5 analysis because the parotid glands, where histatin 5 is produced, are more involved in stimulated saliva secretion.[9] ELISA was performed in triplicate following the manufacturer's instructions.

Dry mouth symptoms. A questionnaire related to xerostomia was given to each patient three times: at the first visit before laser therapy, at the sixth visit after laser therapy, and at the 6-week follow-up visit. Because dry mouth symptoms are subjective, a self-administered questionnaire was used to assess xerostomia symptoms. The 11-item questionnaire, as modified from the Xerostomia Inventory-Dutch Version[22], is shown in Table 1. A visual analogue scale was used to quantify the response of each item [not agree (0) to totally agree (10)]. The mean dry mouth score and the correlation between dry mouth score and salivary flow rate were analysed for each visit.

Statistical analysis. Statistical analysis was performed using SPSS Statistics for Windows, version 22 (IBM Corp, Armonk, NY, USA). Statistical analysis was performed using non-parametric tests because the data were not normally distributed. Salivary flow rate, salivary protein concentrations and dry mouth score for each item were assessed using the Friedman test followed by the post hoc Wilcoxon signed-rank test to determine significant differences. Mean salivary flow rates, salivary protein concentrations and dry mouth scores were presented in tables. Correlation between mean dry mouth score and salivary flow rate during the different visits were analysed using Spearman rank test. Dry mouth scores were reversed prior to analysis (i.e., 8 became 2). Mean dry mouth scores per visit and correlation between mean dry mouth scores and salivary flow rate during the different visits were presented in tables. A p-value of <0.05 was considered significant.

Table 1. **Questionnaire Items**

No.	Question
1	I sip liquids to aid in swallowing food (SIP-LIQ)
2	My mouth feels dry when eating a meal (DRY-MEL)
3	I get up at night to drink (NGT-DRK)
4	My mouth feels dry (MTH-DRY)
5	I have difficulty eating dry foods (DIF-DRY)
6	I suck sweets or cough lozenges to relieve dry mouth (SWT-DRY)
7	My lips feel dry (LIP-DRY)
8	I have a lot of dental caries (DEN-CAR)
9	I have bad breath (BAD-BRH)
10	My tongue sticks to my palate (TNG-PLT)
11	I have bleeding when brushing (BLD-BRS)

Results

Twelve diabetic patients (6 males and 6 females aged 37–86 years) were recruited. All patients participated until the sixth visit, and 10 patients returned for the 6-week follow-up visit.

Unstimulated Salivary flow rates (USFR). A trend of increased USFR was demonstrated over the study period (Table 2). There were significant increases in the USFR between the first and sixth visits ($p = 0.005$) and between the first visit and the 6-week follow-up visit ($p = 0.005$). No significant difference was found between the sixth visit and the 6-week follow-up visit ($p = 0.241$).

Stimulated Salivary Flow Rates (SSFR). The results exhibited a trend of increased SSFR over the course of the study (Table 2); however, there were no significant differences during the visits at the three different times ($p > 0.05$).

Salivary Proteins. ***MUC7,*** We found a trend of decreased MUC7 concentration over the course of the study (Table 2) Although slight decreases were noticed at the sixth visit and the 6-week follow-up visit, there were no significant differences during the visits at the three different times ($p > 0.05$).

MUC5B. The results showed a trend of increased MUC5B salivary concentration; however, a slight decrease was noted at the sixth visit (Table 2). There was no significant difference between the first and sixth visits ($p = 0.875$). In contrast, significant increases were found between the first visit and the 6-week follow-up visit ($p = 0.037$) and between the sixth visit and the 6-week follow up visit ($p = 0.028$).

Histatin 5. Although the sixth visit showed slightly increased histatin 5 salivary concentrations, the overall trend demonstrated decreased histatin 5 concentration (Table 2). Histatin 5 concentrations did not significantly differ between the first and sixth visits ($p = 0.530$). Nonetheless, significant decreases were found between the first visit and the 6-week follow up visit ($p = 0.047$) and between the sixth visit and the 6-week follow up visit ($p = 0.022$).

Table 2. Mean Salivary Flow Rates, Salivary Protein Concentrations and Dry Mouth Scores

	First visit[#]	End of therapy[##]	6-week follow-up visit	*p**
USFR (mL/min ± SD)	0.14 ± 0.08[a,b]	0.29 ± 0.16[a]	0.32 ± 0.16[b]	<0.01
SSFR (mL/min ± SD)	0.79 ± 0.47	0.92 ± 0.43	0.94 ± 0.42	0.232
MUC7 (ng/mL ± SD)	3.29 ± 5.36	2.49 ± 4.05	2.43 ± 5.04	0.519
MUC5B (ng/mL ± SD)	9.15 ± 5.15[a]	8.08 ± 2.92[b]	13.78 ± 8.65[a,b]	<0.05
Histatin 5 (ng/mL ± SD)	192.10 ± 141.52[a]	234.86 ± 245.98[b]	100.89 ± 8.65[a,b]	<0.05
Dry mouth score ($\bar{x}$ ± SD)	4.05 ± 3.25[a,b]	1.26 ± 1.18[a]	1.03 ± 1.19[b]	<0.001

[#]Baseline, [##]End of therapy
*Friedman test
[a,b]Groups with the same superscript letters are significantly different according to the Wilcoxon signed-rank test.

Table 3. Mean Dry Mouth Scores of Each Questionnaire Item per Visit

Questionnaire Items	V1 (mean ± SD)	V2 (mean ± SD)	V3 (mean ± SD)	*p**
SIP-LIQ	2.42 ± 2.87	0.92 ± 0.99	0.90 ± 0.99	0.291
DRY-MEL	3.08 ± 3.53	1.50 ± 0.79	1.10 ± 0.99	0.483
NGT-DRK	3.83 ± 3.27[a]	0.92 ± 0.79[a]	1.33 ± 1.06	0.042
MTH-DRY	4.75 ± 2.89[a,b]	1.50 ±1[a,c]	0.80 ± 0.63[b,c]	0.002
DIF-DRY	2.42 ± 2.87	1.33 ± 0.98	1.20 ± 1.13	0.965
SWT-DRY	2.67 ± 2.96	1.08 ± 1.38	0.60 ± 0.84	0.070
LIP-DRY	5.67 ± 3.14[a,b]	1.17 ± 0.83[a]	1.60 ± 1.50[b]	0.001
DEN-CAR	3.92 ± 3.50[a,b]	1.75 ± 1.91[a]	1.40 ± 1.78[b]	0.042
BAD-BRH	4.5 ± 3.87[a]	1.58 ± 1.62[a]	1.70 ± 1.83	0.016
TNG-PLT	4.00 ± 3.69	1.00 ± 0.74	0.70 ± 0.67	0.072
BLD-BRS	2.17 ± 2.17	0.83 ± 0.94	1.20 ± 1.39	0.28

V1: Baseline; V2: End of therapy; V3: 6-week follow-up visit
*Friedman test
[a,b,c] Groups with the same superscript letters are significantly different according to the Wilcoxon signed-rank test.

Table 4. Correlation between Mean the Dry Mouth Score and Salivary Flow Rate during the Different Visits

	USFR		SSFR	
	Sixth visit[##]	6-week follow-up visit	Sixth visit[##]	6-week follow-up visit
Mean dry mouth score*	0.549 (p = 0.064)	0.102 (p = 0.778)	0.387 (p = 0.215)	0.121 (p = 0.740)

[##]End of therapy
*Based on questionnaire items 4 and 7

Dry Mouth Score. The mean dry mouth scores demonstrated a decreasing trend over the course of the study (Table 2). Significant decreases in the mean dry mouth score were found between the first and sixth visits (p < 0.001) and between the first visit and the 6-week follow-up visit (p < 0.001) Although a slight

decrease was observed between the sixth visit and the 6-week follow-up visit, the difference was not significant ($p = 0.268$).

The mean dry mouth scores for each questionnaire item are seen in Table 3. Item 7 (my lips feel dry) had the highest mean score (5.67 ± 3.14) at the first visit, followed by item 4 (my mouth feels dry) (4.75 ± 2.89) and item 9 (4.5 ± 3.87) (I have bad breath). Items 4, 7, 3 (I get up at night to drink), 8 (I have a lot of dental caries) and 9 showed significant decreases from the first visit to the 6-week follow-up visit ($p < 0.05$ for items 3, 8 and 9 and $p < 0.01$ for items 4 and 7) (Table 3). However, only item 4 exhibited a significant time-dependent decrease. Only items 4 and 7 were used in the correlation analysis at the sixth visit and 6-week follow-up visit because they best represent dry mouth. The results revealed no significant differences between mean dry mouth scores and salivary flow rates at the sixth visit and 6-week follow-up visit (Table 4). However, the strongest positive correlation between these parameters was found at the sixth visit ($r_s = 0.549$).

Discussion

The present study evaluated the effect of LLLT on major salivary gland function in diabetic patients with hyposalivation. Our findings showed that LLLT to the major salivary glands significantly increased the USFR and MUC5B salivary concentration and alleviated patients' dry mouth symptoms.

We found that LLLT increased the USFR in diabetic patients; however, the elevation in SSFR was not significant. The normal USFR is at least 0.25 mL/min,[2] and the mean USFR of 0.14 mL/min found at the first visit was below this value. After LLLT, the mean USFR, but not the SSFR, increased to within the normal range. Our results are consistent with those of a previous study on subjects with hyposalivation.[21-23] These findings may result from LLLT inducing ATP production by activating the electron transport chain in mitochondria,[24] stimulating cell function. However. LLLT did not improve either the USFR or xerostomia in patients undergoing radiotherapy;[25] this may be due to acinar atrophy and chronic salivary gland inflammation, which may lead to necrosis,[26] implying that LLLT is not effective on atrophic glands and suggesting that the response of the major salivary glands to LLLT differs under physiological and pathological conditions.

The typical MUC5B concentration in unstimulated whole saliva ranges from 0.05 ng/mL to 0.78 ng/mL.[27] Surprisingly, our patients showed much higher concentrations of both mucins compared with the normal values, which could possibly be explained by the difference in salivary protein content in diabetics compared with non-diabetics. Increased MUC1 concentrations in saliva are associated with the presence of proinflammatory cytokines.[28] Diabetes is an inflammatory disease; thus, the higher mucin concentration found in our study is likely due to changes in proinflammatory cytokine levels in the salivary glands of diabetic patients.[29] LLLT-induced significant increase in MUC5B concentrations found at the 6-week follow-up visit may have resulted from the bio-modulatory effect of LLLT on the major salivary glands.

We observed a lower concentration of MUC7 than of MUC5B, which is consistent with a previous report demonstrating that MUC5B is the predominant mucin in saliva.[30] Our results showed that the MUC7 concentration was higher than normal (3.29 ± 5.36 ng/mL vs 0.06–0.32 ng/mL)[27] and did not significantly increase by LLLT. MUC7, but not MUC5B, is localised in serous acini in the sublingual, submandibular, lingual and palatine glands.[31] The slight decrease in MUC7 concentration in our study may be due to damaged serous acini in diabetic patients.

A previous study revealed lower salivary histatin concentrations in diabetic children than in controls,[32] suggesting that the antifungal and bacterial enzyme inhibition activities of histatin may not be optimal in diabetic patients. However, further investigations are necessary to resolve these issues. We found that the salivary histatin 5 concentration significantly decreased at the 6-week follow-up visit, which does not agree with the result of a previous investigation demonstrating that LLLT had a mild disinfecting effect against *C. albicans* and reduced inflammation in denture stomatitis patients.[33] Previous studies concluded that serous cells in the parotid glands of diabetic patients are prone to intracellular lipid accumulation,[29,31,32,34] this may explain the decreased histatin 5 concentrations found in our study because serous cells in the parotid glands are involved in secreting this protein.[9] Moreover, dissimilar levels of diabetes severity among the patients in our study may have resulted in different acinar cell function between them as most diabetic patients take multiple drugs, whose use is related to salivary gland hypofunction.[2] The decreased histatin 5 concentration at the follow-up visit supports the insignificant increase in the SSFR found in our study, suggesting that the parotid glands of diabetic patients are more sensitive to salivary gland impairment, given that the parotid glands contribute to stimulated salivary secretion and histatin 5 production.

As indicated by the dry mouth score results, LLLT decreased the subjective dry mouth symptoms throughout the course of our study, which is in line with previous reports that found that LLLT effectively reduced dry mouth symptoms.[12,14,15,34] Among all questionnaire items, item 4 (my mouth feels dry) demonstrated the highest mean score prior to LLLT, indicating that the major subjective sign of dry mouth is

the feeling of dryness inside the mouth, as found in a previous study.[22]

The questionnaire analysis revealed that items 4 (my mouth feels dry) and 7 (my lips feel dry) significantly decreased following LLLT, indicating that laser therapy reduces diabetes-induced dry mouth symptoms. A previous study also found an association between diabetes and dry mouth symptoms.[35] It is important to note that item 4 significantly decreased at each visit, indicating that LLLT alleviated most dominant dry mouth symptoms in the present study.

The strongest positive correlation between mean the dry mouth score and the salivary flow rate was seen at the sixth visit, implying that the maximum reduction in dry mouth symptoms was achieved after the sixth laser exposure; however, no significant differences were found among the visits. These results are similar to those of a previous study,[25] which concluded that decreased dry mouth symptoms are not always directly proportional to increased salivary flow rates and vice versa.

One aspect concerning our statistical analysis is that the SPSS Friedman test was unable to assess unequal patient numbers in the three visits due to two patients not attending the 6-week follow-up visit. To resolve this issue, the test was performed using 10 patients, excluding the data from the missing patients. However, the mean scores presented in this study represent the values from all 12 patients, except for the 6-week follow up data that were based on the 10 patients who attended the follow-up visit.

The laser parameters used in our study were 0.1 W power and 4 J/cm^2 ED, which has been suggested as the most effective ED for cell stimulation.[12,14,15,19,20] In addition, our pilot study using five study patients found that their salivary flow rates increased; thus, these parameters were used throughout the present study. Moreover, previous experimental models have demonstrated that LLL at therapeutic intensities penetrated living tissue from 2 cm to 5 cm, including the scalp and bone and reaching the bone tissue, depending on the types of tissue layers involved and the patient's metabolic status.[36-45] Although there may be a reduction in laser light energy due to tissue absorption, reflection or refraction, it is reasonable to assume that LLLT reached the major salivary glands, including both lobes of the parotid gland. Additional investigations are necessary to determine the optimum therapeutic level using a range of energy levels.

Our study showed that the LLLT-induced increase in the SSFR was not significant and that histatin 5 concentration decreased. Because the parotid glands are responsible for the SSFR and histatin 5 secretion, it may be necessary to use a higher laser ED on these glands to achieve optimal results in diabetic patients. The slight decrease in MUC7 concentrations observed in our study may be a result of damage to the sublingual, submandibular, lingual and palatine gland serous cells in the diabetic patients. Applying laser therapy over the minor glands as well as the major salivary glands may result in even higher increases in salivary flow and quality; this should be explored in future studies. To the best of our knowledge, our report is the first to use LLLT on the salivary glands in diabetic patients. However, we only evaluated a few diabetic patients due to the limited study duration and difficulty in recruiting patients.

Conclusions

LLLT is a beneficial approach to elevate the USFR and MUC5B concentration and to decrease dry mouth symptoms in diabetic patients with hyposalivation.

Acknowledgement

The authors would like to thank Dr. Kevin Tompkins for his critical review of the manuscript. This study was supported by the Bangkok Hospital Research Fund, 2016 and Thanes Pattana, Bangkok, Thailand.

Funding

None.

Conflict of Interest Statement

The authors declare that they have no conflict of interest.

References

1. Puy CL. The role of saliva in maintaining oral health and as an aid to diagnosis. *Med Oral Pathol Oral Cir Bucal*. 2006;11:449-55.
2. Sreebny LM, Vissink A. *The enigma of dry mouth. In*: *Dry mouth, the malevolent symptom*: *A clinical guide*. USA: John Wiley & Sons; 2010.
3. Veerman EC, van den Keybus PA, Valentijn-Benz M, Amerongen AN. Isolation of different high-Mr mucin species from human whole saliva. *Biochem J*. 1992;283:807-11.
4. Levine M, Reddy M, Tabak L, Loomis R, Bergey E, Jones P, et al. Structural aspects of salivary glycoproteins. *J Dent Res*. 1987;66:436-41.
5. Puri S, Edgerton M. How does it kill?: Understanding the candidacidal mechanism of salivary histatin 5. *Eukaryotic cell*. 2014;13:958-64.
6. Ahmad M, Piludu M, Oppenheim FG, Helmerhorst EJ, Hand AR. Immunocytochemical localization of histatins in human salivary glands. *J Histochem Cytochem*. 2004;52:361-70.
7. Chaudhury NM, Proctor GB, Karlsson NG, Carpenter GH, Flowers SA. Reduced Mucin-7 (Muc7) sialylation and altered saliva rheology in Sjögren's syndrome

associated oral dryness. *Mol Cell Proteomics*. 2016;15:1048-59.
8. Moore PA, Guggenheimer J, Etzel KR, Weyant RJ, Orchard T. Type 1 diabetes mellitus, xerostomia, and salivary flow rates. *Oral Surg Oral Med Oral Pathol Oral Radiol Endodont*. 2001;92:281-91.
9. Borges B, Fulco GM, Souza A, de Lima KC. Xerostomia and hyposalivation: A preliminary report of their prevalence and associated factors in Brazilian elderly diabetic patients. *Oral Health Prev Dent*. 2010;8:153-8.
10. Almståhl A, Wikström M, Groenink J. Lactoferrin, amylase and mucin MUC5B and their relation to the oral microflora in hyposalivation of different origins. *Oral Microbiol Immunol*. 2001;16:345-52.
11. Khan SA, Fidel Jr PL, Al Thunayyan A, Varlotta S, Meiller TF, Jabra-Rizk MA. Impaired histatin-5 levels and salivary antimicrobial activity against *C. albicans* in HIV infected individuals. *J AIDS Clin Res*. 2013;4:193.
12. Pezelj-Ribarić AS, Gržetić N, Urek MM, Glažar I, Kuiš D. Salivary flow rate before and after low level laser therapy. *Int Mag Laser Dent*. 2010;2:6-8.
13. Tunér J, Beck-Kristensen PH, Ross G, Ross A. *Photobiomodulation in dentistry*. *In*: *Principles and practice of laser dentistry*. 2nd ed. United States: Mosby; 2015. p.251.
14. Lončar B, Mravak Stipetić M, Baričević M, Risović D. The effect of low-level laser therapy on salivary glands in patients with xerostomia. *Photomed Laser Surg*. 2010; 29:171-5.
15. Vidović Juras D, Lukač J, Cekić-Arambašin A, Vidović A, Canjuga I, Sikora M, et al. Effects of low-level laser treatment on mouth dryness. *Col Antropol*. 2010;34:1039-43.
16. American Diabetes Association (USA). Standards of medical care in diabetes—2014. *Diabetes Care*. 2014;37:S14-80.
17. U.S Department of Health and Human Services. *Dietary Guidelines for Americans*. 7th ed. U.S Government Printing Office. Washington DC: U.S Department of Health and Human Services; 2010.
18. Degenhardt L, Hall W, Warner-Smith M, Lynskey M. *Comparative quantification of health risks*: *global and regional burden of disease attributable to selected major risk factors*. Geneva: World Health Organization; 2004. p.1109-76.
19. Kana JS, Hutschenreiter G. Effect of low power density laser radiation on healing of open skin wounds in rats. *Arch Surg*. 1981;116:293-6.
20. Simões A, Nicolau J, De Souza DN, Ferreira LS, de Paula Eduardo C, Apel C, et al. Effect of defocused infrared diode laser on salivary flow rate and some salivary parameters of rats. *Clin Oral Invest*. 2008;12:25-30.
21. Navazesh M, Christensen C. A comparison of whole mouth resting and stimulated salivary measurement procedures. *J Dent Res*. 1982;61:1158-62.
22. Van der Putten G-J, Brand HS, Schols JM, de Baat C. The diagnostic suitability of a xerostomia questionnaire and the association between xerostomia, hyposalivation and medication use in a group of nursing home residents. *Clin Oral Invest*. 2011;15:185-92.
23. Terlević Dabić D, Jurišić S, Vučićević Boras V, Gabrić D, Bago I, Vrdoljak DV. The effectiveness of low-level laser therapy in patients with drug-induced hyposalivation: A pilot study. *Photomed Laser Surg*. 2016;34:389-93.
24. Yu W, Naim JO, McGowan M, Ippolito K, Lanzafame RJ. Photomodulation of oxidative metabolism and electron chain enzymes in rat liver mitochondria. *Photochem Photobiol*. 1997;66:866-71.
25. Saleh J, Figueiredo MAZ, Cherubini K, Braga-Filho A, Salum FG. Effect of low-level laser therapy on radiotherapy-induced hyposalivation and xerostomia: a pilot study. *Photomed Laser Surg*. 2014;32:546-52.
26. Guchelaar H-J, Vermes A, Meerwaldt J. Radiation-induced xerostomia: Pathophysiology, clinical course and supportive treatment. *Support Care Cancer*. 1997;5:281-8.
27. Gabryel-Porowska H, Gornowicz A, Bielawska A, Wójcicka A, Maciorkowska E, Grabowska SZ, et al. Mucin levels in saliva of adolescents with dental caries. *Med Sci Monitor*. 2014;20:72-7.
28. Li X, Wang L, Nunes D, Troxler R, Offner G. Pro-inflammatory cytokines up-regulate MUC1 gene expression in oral epithelial cells. *J Dent Res*. 2003;82:883-7.
29. Pradhan A. Obesity, metabolic syndrome, and type 2 diabetes: inflammatory basis of glucose metabolic disorders. *Nutr Rev*. 2007;65:S152-6.
30. Thornton DJ, Khan N, Mehrotra R, Howard M, Sheehan JK, Veerman E, et al. Salivary mucin MG1 is comprised almost entirely of different glycosylated forms of the MUC5B gene product. *Glycobiol*. 1999;9:293-302.
31. Nielsen P, Mandel U, Therkildsen M, Clausen H. Differential expression of human high-molecular-weight salivary mucin (MG1) and low-molecular-weight salivary mucin (MG2). *J Dent Res*. 1996;75:1820-6.
32. Cabras T, Pisano E, Mastinu A, Denotti G, Pusceddu PP, Inzitari R, et al. Alterations of the salivary secretory peptidome profile in children affected by type 1 diabetes. *Mol Cell Proteomics.* 2010;9:2099-108.
33. Maver-Biscanin M, Mravak-Stipetic M, Jerolimov V. Effect of low-level laser therapy on Candida albicans growth in patients with denture stomatitis. *Photomed Laser Surg*. 2005;23:328-32.
34. Simoes A, de Campos L, de Souza DN, de Matos JA, Freitas PM, Nicolau J. Laser phototherapy as topical prophylaxis against radiation-induced xerostomia. *Photomed Laser Surg*. 2010;28:357-63.
35. Busato IMS, Ignácio SA, Brancher JA, Moysés ST, Azevedo-Alanis LR. Impact of clinical status and salivary conditions on xerostomia and oral health-related quality of life of adolescents with type 1 diabetes mellitus. *Community Dent Oral Epidemiol*. 2012;40:62-9.
36. Tedford CE, Delapp S, Jacques S, Anders J. Quantitative analysis of transcranial and intraparenchymal light penetration in human cadaver brain tissue. *Lasers Surg Med*. 2015;47:313-22.
37. Henderson TA, Morries LD. Near-infrared photonic energy penetration: Can infrared phototherapy effectively reach the human brain?. *Neuropsychiatr Dis Treat*. 2015;11:2091-208.
38. Bradley P, Groth E, Rajap A. Low intensity laser therapy for hard tissue problems of the oro-facial region. *Proceedings of the 6th International congress on lasers in Dentistry*; London; 1998. p.103-5.
39. Gursoy B, Bradley PE. A study of soft tissue penetration by 830 nm wavelength using radiometer and CCD camera. *Proceedings of the International Congress on Lasers in Dentistry*; London; 1994. p.105.
40. Snyder-Mackler L, Barry AJ, Perkins AL, Soucek MD. Effect of He-Ne laser irradiation on skin resistance and

pain in patientes with trigger points in the neck and back. *Phys Therapy*. 1989;69:336-41.
41. Byrne KR, Waynant RW, Ilev IK, Wu X, Bama L, Smith K, et al. Light promotes regeneration and functional recovery and alters the immune response after spinal cord injury. *Laser Surg Med.* 2005;36:171-85.
42. Di Giacomo P, Orlando S, Dell' Ariccia M, Brandimarte B. Low level laser therapy: laser radiation absorption in biological tissues. *Appl Phys A.* 2013;112:71-5.
43. Hode T, Duncan D, Kirkpatrick S, Jenkins P, Hode L. The importance of the coherence in phototherapy. *Proceeding of the SPIE 7165, Mechanisms for Low-Light Therapy* IV: 7165. United States: Spie Bios; 2009. p. 1-10.
44. Hudson DE, Hudson DO, Wininger JM, Richardson BD. Penetration of laser light at 808 and 980 nm in bovine tissue samples. *Photomed Laser Surg*. 2013;31:163-8.
45. Joensen J, Øvsthus K, Reed R, Hummelsund S, Iversen V, Lopes-Martins R, et al. Skin penetration time-profiles for continuous 810 nm and superpulsed 904 nm lasers in a rat model. *Photomed Laser Surg*. 2012;30:68-94.