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ORIGINAL ARTICLE

The impact of photobiomodulation of major salivary glands on caries risk

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Abstract

Dental caries is a complex multifactorial chronic infectious disease guided by several risk or protective factors. Saliva has an important role in caries and the remineralization process. Caries risk assessment is defined as the probability of new caries lesion development or the existing lesion progression in a given time period. Caries diagnostics and risk factor assessment are followed by targeted elimination of risk factors and less conservative but abundant preventive therapeutic measures. The aim of our prospective randomized study was to elucidate on how photobiomodulation of major salivary glands with polychromatic light or LED light affects caries risk factors in high caries-risk patients. Thirty-six patients were assigned to one of the following three experimental groups: the first, irradiated with polarized polychromatic light (40 mW/cm^2, wavelengths 480–3400 nm); the second, a continuous LED light (16 mW/cm^2, wavelengths 625, 660, 850 nm); the third, same LED light in a pulsed mode. The fourth group was the control, for which a non-therapeutic visible light was used. Light was administered extra-orally bilaterally above the parotid and submandibular glands for 10 min and intra-orally above the sublingual glands for 5 min, 3 times a week, for 4 consecutive weeks. Each patient's caries risk was assessed according to Cariogram before and after therapy. Caries risk factors were determined from samples of saliva before therapy, two weeks after it commenced, at the end of therapy, and four weeks after the end of therapy. At the end of treatment, the following findings were obtained: In the group irradiated with polarized polychromatic light and in the group irradiated with continuous LED light, the *Streptococcus mutans* and *Lactobacillus* counts decreased and salivary buffering capacity increased ($p < 0.05$). In the group irradiated with pulsed LED light, *Streptococcus mutans* counts decreased and unstimulated salivary flow and salivary buffering capacity increased ($p < 0.05$). In all three experimental groups, caries risk was lower ($p < 0.05$). In the placebo control group, there were no statistically significant differences between parameters before and after therapy. We concluded that photobiomodulation of major salivary glands in high caries-risk patients can reduce the cariogenic bacteria in saliva and improve some salivary parameters, thus reducing caries risk.

Keywords Photobiomodulation therapy · Low-level laser therapy · Dental caries susceptibility · Saliva

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Introduction

Dental caries is one of the most prevalent chronic diseases worldwide. It is a complex multifactorial chronic infectious disease that may affect general health as well as quality of life [1]. Modern caries management strategies are designed to prevent, arrest, or reverse the demineralization of dental hard tissues [2].

The dynamic process of demineralization and remineralization is guided by several risk or protective factors. The rate and the severity of caries are affected by the salivary flow, its pH and buffering capacity, oral hygiene habits, dietary habits, tooth resistance (morphology, crowding defects, restorations), the use of protective fluoride supplements and the main caries risk factors, the presence of acidogenic bacteria in the dental biofilm—plaque [3]. The quantity of *Streptococcus mutans* and *Lactobacillus* in plaque is strongly related to the start and the progression of the caries process [4].

Saliva, its quality and quantity, plays an important role in both caries development and the remineralization process.

Many conditions, diseases, and medications affect salivary production. The sequel of hyposalivation is often reduced salivary buffering ability [5]. A low salivary pH value for several hours causes an early onset of the demineralization process, thus enabling the development of new and the progression of existing carious lesions [6].

A patient's caries risk assessment is defined as the probability of new caries lesion development and/or the existing lesion progression over a given time [7]. Caries risk assessment and caries prediction can be demonstrated through an open-source program, Cariogram [8]. The program contains an algorithm that presents a "weighted" analysis of the input data, mainly biological factors. In the literature, Cariogram is described as one of the most accurate caries prediction methods [9].

Phototherapy is a treatment with different modalities of light sources, such as laser, light-emitting diode (LED) light, halogen light, or others. The authors agree that the effects of polychromatic light, LED, and laser lights are comparable, since the coherency of light source does not influence its therapeutic effect [10]. Photobiomodulation (PBM) is a type of phototherapy using low-power light. Its primary effect is a physiologic response of tissue instead of a thermal or cytotoxic effect [11]. In the irradiated tissue, the absorbed photons of light change the form or function of chromophores [12]. The main effect of PBM is the stimulation of the enzyme cytochrome C oxidase in the mitochondria, resulting in activated cell signalling pathways. The final effects are an accelerated cell metabolism, increased ATP production, and diminished oxidative stress, which result in better cell viability [13, 14]. Irradiated areas have also been associated with improved perfusion, better immune response, and faster wound healing [15–17].

Discussion on the use of different modalities of therapeutic light suggests that the effects of pulsed and continuous modes of same light are not equal [18]. The advantage of LED light irradiation is the possibility of using a specific and most appropriate wavelength—one with optimal penetration depth and therapeutic effect. It has been reported that the light can penetrate 23 cm deep into a tissue, thus allowing the irradiation of a larger area with negligible thermal effect [14, 19].

PBM also has some systemic effects, when the target tissue has not been directly reached by photons. These effects are caused by the autocrine, paracrine, and endocrine bioactive molecules released from the irradiated tissues [20].

In dental medicine, PBM has been found to be effective in relieving the side effects of cancer treatment, alleviating facial pain, relieving the symptoms of Sjögren's syndrome, and reducing the amount of periodontal pathogenic bacteria. It stimulates salivary glands, enhances the regeneration of the non-damaged glandular tissue after cancer irradiation therapy, improves the antimicrobial properties of saliva, and alleviates the harmful effects of hyposalivation on oral mucosa [20–23].

There have been some studies on the impact of photobiomodulation on caries risk factors, researching its effect on salivary glands and oral bacteria. Animal studies have pointed out the growth of salivary ductal epithelium and increased salivary flow after PBM. Increased salivary enzymatic activities of peroxidases and catalases have also been detected without evidence of improved salivary flow [24–27]. Given the positive results but relatively minimal understanding achieved by research thus far, further investigation into the impact of PBM is needed and long-term follow-up desired.

In contrast to photodynamic therapy antimicrobial effects, still little is known about the antibacterial activity of PBM, where no additional pigment is needed [28]. It has been reported that light can slow down the growth of the bacteria *Escherichia coli* and *Streptococcus aureus* in inflamed skin wounds [29]. One in vitro study demonstrated that, in contrast to photodynamic therapy, PBM did not induce direct bacteriolysis but caused damage in the bacterial cell wall [30]. However, a more recent in vitro study on single- and dual-species oral microbial biofilm found no changes in bacterial cell morphology after PBM but only disrupted *Streptococcus mutans* aggregation [31]. Despite the non-bactericidal effect on *Streptococcus mutans* and *Candida albicans* in biofilms, their suppressed growth has been observed [31].

Thus far, there has been no study on the direct effects of PBM on the occurrence and the progression of caries. Some studies have already established its favorable effect on a single caries risk factor, such as increased salivary flow, improved saliva characteristics, and the possible effect of reducing bacterial growth in plaque biofilm. These conclusions point to a possible advantage of PBM therapy in high caries-risk patients [31–33].

The aim of our study was to elucidate on how photobiomodulation of major salivary glands by use of polychromatic light or LED light affects caries risk in high caries-risk patients. We also wanted to compare the effects of one or more different wavelengths and, on the other hand, the effects of pulsed in comparison to continuous mode of the same wavelength.

Methods

Thirty-six participants were included in a prospective randomized clinical study. They were normally mobile outpatients, who used to come to our department several times per month to receive restorative treatment at students' dental clinical practice. Each participant signed an informed consent form after the course of the research was explained. Inclusion criterion was high caries risk as shown by Cariogram. The research was approved by the Republic of Slovenia National Medical Ethics Committee, approval number 0120-539/2016-2 KME 40/11/16.

For each participant, general and dental histories were taken. Dietary and oral hygiene habits were assessed, and a detailed dental clinical status was registered; dental caries was assessed by two calibrated examiners in accordance with ICDAS criteria and dental plaque in conformance with Silness and Löe [34, 35].

The impact of each participant's oral health on their quality of life (QoL) was recorded by use of the standardized modified long (49 questions) Oral Health Impact Profile (OHIP) questionnaire [36]. Each question was formulated such that the participant made a check mark by the indicated frequency he/she encountered a certain problem [37]. A summary score of the answer categories was calculated. With the OHIP, we evaluated categories, such as limited functionality, psychological issues, physical disability, psychic disability, and general disability. With two added questions, we evaluated the participant's self-perceived oral health and appearance.

Salivary parameters of stimulated and unstimulated saliva were determined. Unstimulated saliva samples were collected during a 5-min period, gathered into a labelled container. For the stimulated saliva samples, a participant chewed a pellet of medical paraffin during a 5-min gathering period. The volume of collected saliva was measured using a standard medicinal syringe, and salivary flow rate in milliliter per minute (mL/min) was calculated. Salivary pH values were measured with the Vario pH device (WTW GmbH, Weilheim, Germany), with measuring accuracy of + 0.01. Buffering capacity was determined with the CRT buffer test (Ivoclar Vivadent, Schaan, Liechtenstein). Colony densities of *Streptococcus mutans* and *Lactobacillus* bacteria were determined semi-quantitatively with the CRT bacteria test (Ivoclar Vivadent, Schaan, Liechtenstein).

With regard to unstimulated saliva, the flow rate and pH value were determined. For stimulated saliva, the flow rate, pH value, buffering capacity, and colony densities of *Streptococcus mutans* and *Lactobacillus* were determined. The collection of saliva and the test assessment were performed by the same trained person, who used the standardized protocol; all was done in accordance with the manufacturer's instructions.

At the baseline and at the end of PBM, caries risk was evaluated with Cariogram. It evaluated the following main recorded caries risk factors: eating habits, the amount of dental plaque, bacterial counts of *Streptococcus mutans* and *Lactobacillus,* salivary flow, preventive fluoride use, and past caries experiences. Cariogram identified caries risk factors as well as calculated an individual's caries risk and the possibility of avoiding new caries.

Patients were referred to the Department of Dental Diseases, Medical Faculty, University of Ljubljana, to receive caries treatment. Patients who had high caries risk determined according to Cariogram were offered to participate in the trial. Patients were randomized among groups in their sequence of entry in the trial, each by drawing enclosed envelope prepared by a neutral person and containing the number of belonging experimental group [38]. For irradiation therapy, the participants were randomly assigned into 4 groups. In the first experimental group, 9 participants were irradiated with polarized polychromatic light, Bioptron AG, Zepter, Wollerau, Switzerland.

In the second experimental group, 8 participants were irradiated with a light-emitting diode (LED) light in continuous mode using Ortholumm ML5/1, Votan d. o. o., Ljubljana, Slovenia.

In the third experimental group, 7 participants were irradiated with LED light in pulsed mode at wavelengths of 625, 660, and 850 nm and an average power density of 16 mW/cm^2 using Ortholumm ML5/1 (same device with that previously mentioned).

The fourth group of 12 participants was the control group, and they were irradiated with a placebo device using a non-therapeutic low-energy visible light. Other technical data of therapeutic lights are in Table 1. None of the PBM devices contained the ultraviolet (UV) spectrum of light.

For all patients, the light was administered extraorally and bilaterally above the parotid and submandibular glands for 10 min and intraorally above the sublingual glands for 5 min for a total of 25 min per session (Fig. 1), 3 times a week, for 4 weeks. The PBM participants wore protective eyeglasses during the procedure.

Dental caries was reassessed at the end of therapy, and salivary risk factors were re-evaluated after two weeks, at the end of therapy, and four weeks after the end of therapy. The OHIP questionnaire was completed before the commencement and after the end of therapy.

Table 1 Technical data of therapeutic lights used

	Experimental groups		
	1 Bioptron Polarized polychromatic light ($N = 9$)	2 Ortholumm Continuous LED light ($N = 8$)	3 Ortholumm Pulsed LED light ($N = 7$)
Wavelengths	480 nm to 3400 nm	625, 660, and 850 nm	625, 660, and 850 nm
Average-specific energy/density	40 mW/cm^2	16 mW/cm^2	16 mW/cm^2
Power	50 W	10 W	10 W
Light energy per minute	2.4 J/cm^2	1 J cm^2	1 J cm^2
Spot size	95 cm^2	92 cm^2	92 cm^2
Average polarization rate	Over 95%	Program 1, 292 Hz	
Other		Duty cycle 50%	Accelerated flashing frequency from 50 to 2000 Hz Pulse duration from 10 to 0.25 ms

The methods used in the research did not present any health risk to the participants. No side effects of PBM were noted during and/or after the research.

The data were analyzed using the SPSS program (IBM SPSS Statistics 22, USA). Statistical differences among the experimental groups before therapy were compared by a one-way analysis of variance (ANOVA, Dunnett's test, $p < 0.05$). The parameter values obtained after two weeks of irradiation therapy, at the end of therapy, and four weeks after the end of therapy were compared to the pre-therapy values by a one-way analysis of variance for repeated measurements (RM ANOVA; Dunnett's test, $p < 0.05$). The differences in caries risk, caries prediction, and OHIP questionnaire scores before and after the therapy were analyzed by a paired t test or Wilcoxon test. OHIP questionnaire scores for the group of all patients included in our study were compared to the general population scores [36, 37] by a t test.

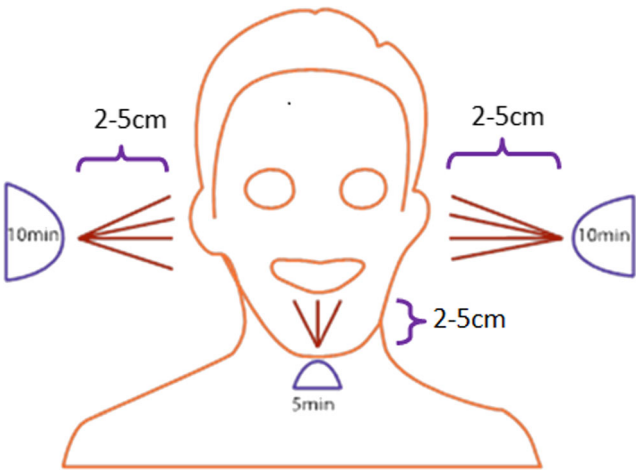

Fig. 1 Scheme of the irradiation protocol

All results were expressed as mean values and standard deviation of means, with the criterion of significance at $p < 0.05$ (Table 2).

Results

Before therapy, there were no statistically significant differences among the experimental groups and control with regard to age and gender, dental status, caries risk, salivary flow rate, salivary pH value and its buffering capacity, and counts of cariogenic bacteria *Streptococcus mutans* and *Lactobacillus* (CFU/mL) in Kruskal–Wallis ANOVA (Dunnett's test) (Tables 3 and 4). In Table 2, there is information regarding baseline diseases.

The participants and their numbers during the trial are presented in flow diagram (Fig. 2). Most prevalent reasons for not completing the study were personal or practical reasons (trial demanding too much of their time), and four patients had contraindications for PBM.

The effect of photobiomodulation therapy in major salivary glands

Experimental group irradiated with polarized polychromatic visible light

At the end of therapy and 4 weeks after, *Streptococcus mutans* and *Lactobacillus* counts were significantly reduced (RM ANOVA, Dunnett's test, $p < 0.05$). Salivary buffering capacity was significantly increased after two weeks, and the differences remained significant until four weeks after the end of therapy (Dunnett's test, $p < 0.05$).

Table 2 Group description—basic data of baseline diseases

Control	Experimental groups		
1 Placebo group ($N = 12$)	2 Bioptron Polarized polychromatic light ($N = 9$)	3 Ortholumm Continuous LED light ($N = 8$)	4 Ortholumm Pulsed LED light ($N = 7$)
Periodontal disease	Sjögren's syndrome, dermatomyositis	Periodontal disease, rheumatism, heart failure	Periodontal disease, high blood pressure, diabetes type II
Status post-OP carcinoma lingue, thyroid disorders after irradiation	Status post-OP carcinoma nasopharynx and neck (irradiation 35 times and chemotherapy), dermatomyositis	Periodontal disease	Sensitive teeth, Myelodysplastic syndrome
Sensitive teeth, varicose veins	Status post-OP squamous cell carcinoma (irradiation 30 times 64 Gy and chemotherapy)	Sensitive teeth, gastroesophageal reflux disease, xerostomia	Asthma, breast cancer
Gastroesophageal reflux disease, breast cancer, gastritis	Malignoma, thyroid disorders after irradiation (32 times with 64 Gy)	Sjögren's syndrome	[a]
Gastroesophageal reflux disease, multiple sclerosis, epilepsy	Meningioma, asthma, kidney stones	Xerostomia, epilepsy	
Osteoporosis [a]	[a]	Myasthenia gravis	
		7 years after TIA, congenital artherio-vein fistule on hand finger [a]	

[a] Other patients have no baseline diseases

At the end of therapy and compared to the values prior to it, caries risk as assessed by Cariogram was lower (Wilcoxon test, $p = 0.016$) as was also the prediction of new caries development (paired t test, $p = 0.00172$) (Table 4).

In contrast, compared to the values obtained before therapy, there were no significant differences in unstimulated and stimulated salivary flow rates and pH values after 2 weeks of therapy, at its end, and 4 weeks after its end.

Experimental group irradiated with continuous LED light

At the end of therapy and 4 weeks after that, *Streptococcus mutans* counts decreased (RM ANOVA, Dunnett's test, $p < 0.05$). Lactobacillus counts decreased after only 2 weeks of therapy and remained significantly decreased 4 weeks after the end of therapy (Dunnett's test, $p < 0.05$). Salivary buffering capacity significantly increased by the end of phototherapy (Dunnett's test, $p < 0.05$). At the end of therapy, caries risk

Table 3 Group description—basic data of clinical dental status

	Control	Experimental groups		
	Placebo group ($N = 12$)	Bioptron Polarized polychromatic light ($N = 9$)	Ortholumm Continuous LED light ($N = 8$)	Ortholumm Pulsed LED light ($N = 7$)
Number of males	3*	5	3	5
Average age (years)	48.83 ± 13.35	45.11 ± 12.58	57.88 ± 15.30	54.86 ± 14.74
Age (years)	(34–79)	(25–59)	(33–72)	(37–78)
Teeth	23.00 ± 5.81	26.56 ± 3.84	23.88 ± 5.84	24.71 ± 3.99
Prosthetic crowns	2.17 ± 2.98	3.33 ± 6.06	3.88 ± 6.17	3.29 ± 4.42
Dental bridges	0.42 ± 1.00	0.11 ± 0.33	1.38 ± 2.56	1.71 ± 2.98
Restored tooth surfaces	28.67 ± 14.67	34.78 ± 13.07	20.63 ± 14.82	22.57 ± 11.59
Carious lesions	18.25 ± 18.17	12.78 ± 7.26	10.38 ± 14.82	16.57 ± 21.28
Tooth surfaces with non-cavitated caries lesions	12.25 ± 9.24	9.89 ± 4.88	9.13 ± 12.15	13.57 ± 20.49
Tooth surfaces with cavitated caries lesions	6.00 ± 10.36	2.89 ± 3.33	1.25 ± 2.76	3.00 ± 3.61

*Statistically significant difference among groups

Table 4 Bacterial counts, buffering capacity, and caries risk according to Cariogram before therapy, after 2 weeks, at the end, and 4 weeks after the end of therapy in experimental group irradiated with polarized polychromatic visible light

Experimental group (polarized polychromatic visible light)	Before therapy	After 2 weeks of therapy	At the end of therapy	4 weeks after the end of therapy
Streptococcus mutans (CFU/mL)	3.67 ± 0.50	2.89 ± 1.05	2.00 ± 1.12*	2.56 ± 1.13*
Lactobacillus (CFU/mL)	3.78 ± 0.44	3.00 ± 0.71	1.89 ± 0.78*	2.56 ± 0.73*
Buffering capacity	1.89 ± 0.60	2.67 ± 0.50*	2.56 ± 0.53*	2.67 ± 0.50*
Caries lesions	7.00 ± 5.81	–	4.89 ± 4.43	–
Caries risk according to Cariogram	2.67 ± 0.50	–	2.33 ± 1.22*	–
Without new caries lesions according to Cariogram	0.15 ± 0.12	–	0.43 ± 0.27*	–

*Statistically significant difference in comparison to values before therapy

according to Cariogram was lower (Wilcoxon test, $p = 0.008$) as also the prediction of new caries development (paired t test, $p = 0.000239$) as compared to the values prior to therapy (Table 5).

After 2 weeks of therapy, the unstimulated saliva had a significantly higher pH value (Dunnett's test, $p = 0.031$). In contrast, compared to the values obtained prior to therapy, there were no significant differences in unstimulated or stimulated salivary flow rate or in the pH value of stimulated saliva, after 2 weeks of therapy, at its end, and 4 weeks after its end.

Experimental group irradiated with pulsed LED light

After 2 weeks of therapy, at its end, and 4 weeks after that, *Streptococcus mutans* counts decreased (RM ANOVA, Dunnett's test, $p < 0.05$). Salivary buffering capacity significantly increased at the end of therapy (Dunnett's test, $p = 0.015$). In addition, at the end of therapy, both caries risk as assessed by Cariogram (Wilcoxon test, $p = 0.031$) and the prediction of new caries development (paired t test, $p = 0.00838$) were lower, compared to the values before therapy (Table 6).

After 2 weeks of therapy, there was a significantly higher unstimulated salivary flow rate (Dunnett's test, $p = 0.005$), which also remained higher at the end of the treatment (Dunnett's test, $p = 0.016$) (Fig. 3). However, in 4 weeks, the salivary flow returned to the basal levels. In contrast, compared to the values obtained prior to therapy, there were no significant differences in the pH of unstimulated saliva or in the stimulated salivary flow rate and its pH, after 2 weeks of therapy, at its end, and 4 weeks after its end.

Control group irradiated with placebo light

In the group irradiated with placebo light, there were no differences in any observed measurements at any given time during the study. There was also no change in caries risk and the prediction of new caries development according to Cariogram (Table 7).

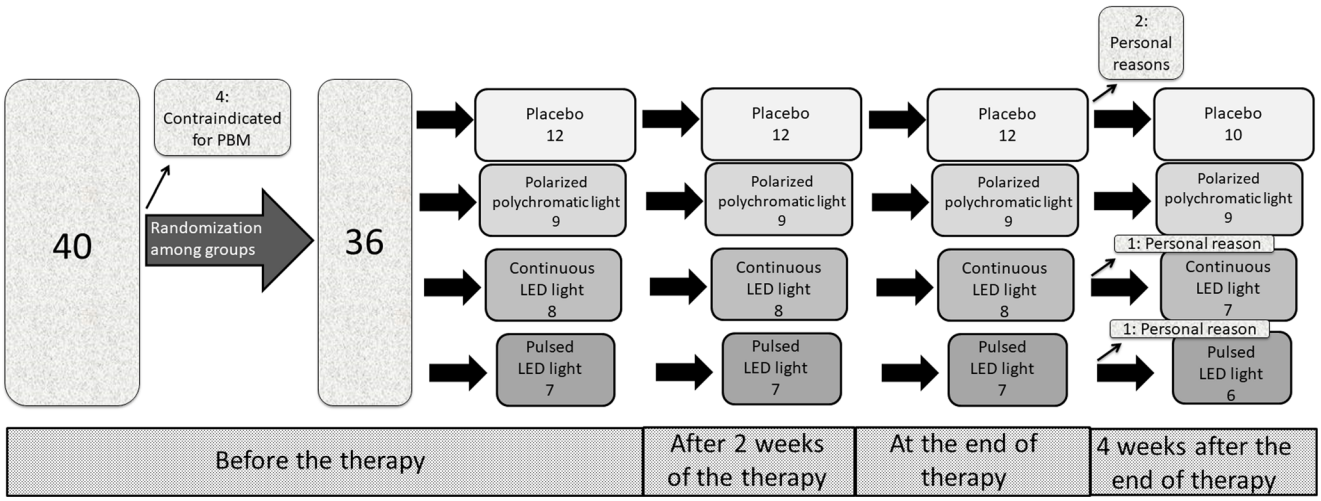

Fig. 2 Flow diagram of number of patients during the trial. *Statistically significant difference in comparison to values before therapy

Table 5 Bacterial counts, buffering capacity, and caries risk according to Cariogram before therapy, after 2 weeks, at the end, and 4 weeks after the end of therapy in experimental group irradiated with continuous LED light

Experimental group (continuous LED light)	Before therapy	After 2 weeks of therapy	At the end of therapy	4 weeks after the end of therapy
Streptococcus mutans (CFU/mL)	3.00 ± 0.93	2.13 ± 1.46	1.50 ± 1.19*	1.71 ± 1.38*
Lactobacillus (CFU/mL)	3.25 ± 0.89	1.63 ± 1.30*	1.88 ± 0.83*	2.14 ± 1.34*
Buffering capacity	2.14 ± 0.69	2.57 ± 0.79	2.86 ± 0.38*	2.67 ± 0.52
Caries lesions	8.13 ± 15.07	–	8.13 ± 14.72	–
Caries risk according to Cariogram	3.75 ± 0.46	–	2.12 ± 0.64*	–
Without new carious lesions according to Cariogram	0.19 ± 0.10	–	0.47 ± 0.12*	–

*Statistically significant difference in comparison to values before therapy

The quality of life due to OHIP questionnaire

Before therapy, there were no differences among groups with regard to subjective parameters in quality of life assessed by the OHIP questionnaire. All the patients with a high caries risk ($N = 36$) had statistically more functional limitations (t test, $p = 0.0301$), higher psychological discomfort (t test, $p = 0.000225$), more physical disability (t test, $p = 0.00808$), more psychological disability (t test, $p = 0.00893$), and more general disability (t test, $p = 0.00481$) in comparison to the general population. After therapy, there was no statistically significant improvement for any group in any parameter of quality of life per the questionnaire.

Discussion

Results of our study show that after the PBM of major salivary glands of all three therapeutic groups, the colony density of *Streptococcus mutans* was reduced and salivary buffering capacity improved. Our findings confirm the results of similar studies and support the idea that PBM could affect some caries risk factors [33, 39, 40]. Red visible light and near infrared light (NIR), regardless of its source, are known to penetrate at least a few centimeters under the skin [19]. Using transcutaneous and intraoral irradiation, we were able to reach most of the major salivary gland tissue. Results of our study are in line with research that obtained different effects of continuous versus pulsed modality of light [18]. We discovered that the continuous LED light mostly affected *Lactobacillus* counts while the pulsed LED light mostly influenced salivary flow.

Streptococcus mutans colony density is correlated with initial caries lesions [41]. In our study, it was reduced in all therapeutic groups. Significant changes were noted after 2 weeks in both groups irradiated with LED light and after 4 weeks in the group irradiated with polarized polychromatic light. It is notable that *Streptococcus mutans* counts remained lower for another month after the end of PBM. This may suggest the long-term effect of PBM on cariogenic bacteria. Our results show that time needed to achieve an effect was likely lower for LED therapeutic light compared to polarized polychromatic light.

Lactobacillus colony densities were only reduced in groups irradiated with a polarized polychromatic and a continuous LED light. Similar results have been observed in an in vitro study using 780 nm low-level laser therapy (LLLT). Low-energy light reduced the aerobic metabolism of *Streptococcus mutans* biofilm and its growth on selective agar [31]. PBM caused disaggregation of the microorganisms and disturbed their adherence to the substrate. Reduction of *Escherichia coli* and *Staphylococcus aureus* growth in vitro using a broad spectrum of therapeutic lights, 400–800 nm, and

Table 6 Bacterial counts, buffering capacity, and caries risk according to Cariogram before therapy, after 2 weeks, at the end, and 4 weeks after the end of therapy in experimental group irradiated with pulsed LED light

Experimental group (pulsed LED light)	Before therapy	After 2 weeks of therapy	At the end of therapy	4 weeks after the end of therapy
Streptococcus mutans (CFU/mL)	2.71 ± 1.70	1.43 ± 1.13*	1.86 ± 1.57*	1.50 ± 1.38*
Lactobacillus (CFU/mL)	3.14 ± 0.90	1.71 ± 0.49	2.14 ± 0.69	2.50 ± 1.22
Buffering capacity	2.28 ± 0.49	2.86 ± 0.38	3.00 ± 0.00*	2.83 ± 0.41
Caries lesions	10.00 ± 13.69	–	8.00 ± 11.17	–
Caries risk according to Cariogram	3.57 ± 0.79	–	2.00 ± 1.00*	–
Without new carious lesions according to Cariogram	0.25 ± 0.22	–	0.49 ± 0.19*	–

*Statistically significant difference in comparison to values before therapy

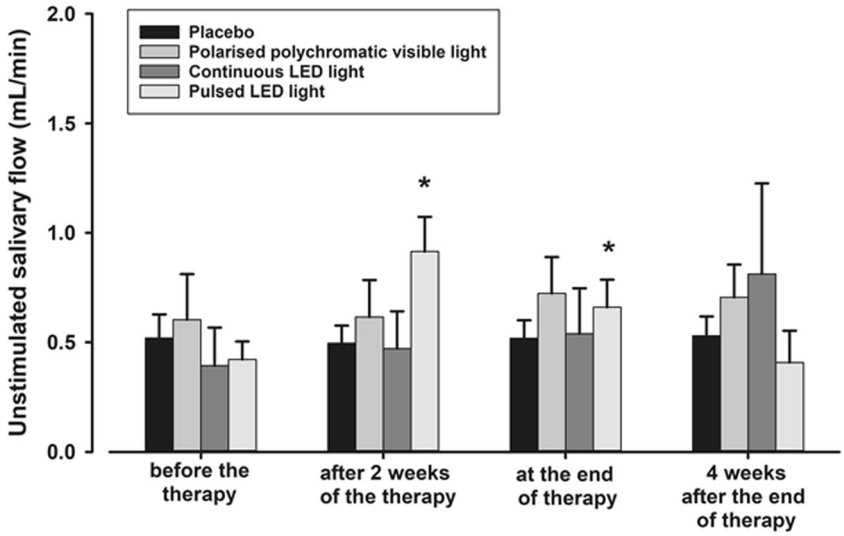

Fig. 3 Unstimulated salivary flow

LLLT with wavelengths of 660, 830, and 904 nm were also confirmed [29, 42].

Salivary buffering capacity significantly increased in all therapeutic groups. The change could be attributed to changes in salivary contents, such as higher concentrations of bicarbonate, phosphate, and protein buffers. In the placebo group, as expected, the buffering capacity remained the same.

Unstimulated salivary flow rate significantly improved in the group irradiated with a pulsed LED light. The results were short-term only. Reports in the literature on PBM improving salivary flow vary. PBM of rats' sublingual salivary glands using 660 and 780 nm laser light did not improve salivary flow [27]. However, in a clinical trial, Simoes detected increased salivary flow using a 660 and 808 nm laser [24]. There have also been encouraging reports from studies on patients with hyposalivation. PBM with low-energy laser wavelengths at 660, 685, 830, and 904 nm resulted in improved salivary flow. The possibility of partial salivary gland existence has even been mentioned [32, 33, and, 40]. Claims are founded on basic research. It has been noted that PBM was able to improve local microcirculation and oxygenation by vasodilatation and accelerated angiogenesis [11, 17, 43, and, 44] consecutively with possible increased salivary production.

In our research, the variation in salivary flow can be attributed to the high variability of salivary flow in the research groups. It is possible that the amount of atrophied acinar tissue in some of the participants was so high that additional stimulation could not bring a notable increase in salivary flow. The fact that atrophied salivary glands cannot be stimulated using PBM has been described by de Jesus [27]. On the other hand, Loncar et al. concluded that PBM could improve salivary flow in patients with xerostomia [32]. It was proposed that the effect of PBM was correlated to the amount of functional acinar salivary cells. The main differences between our research and that of Loncar are the inclusion of participants with different medical conditions and the use of a different PBM device. Loncar excluded from his research participants with xerostomia after head and neck cancer therapy, patients taking specific medications, and those with Sjögren's syndrome. All these are known to have more salivary gland atrophy than those without such characteristics. Loncar used a 904 nm low-energy laser. Higher wavelengths are known to penetrate deeper into the tissue [45].

Table 7 Bacterial counts, buffering capacity, and caries risk according to Cariogram before therapy, after 2 weeks, at the end, and 4 weeks after the end of therapy in experimental group irradiated with placebo light

Control group (placebo light)	Before therapy	After 2 weeks of therapy	At the end of therapy	4 weeks after the end of therapy
Streptococcus mutans (CFU/mL)	2.83 ± 1.34	2.91 ± 1.22	3.00 ± 1.21	3.00 ± 1.33
Lactobacillus (CFU/mL)	2.92 ± 1.08	3.00 ± 1.09	3.17 ± 0.83	3.00 ± 1.05
Buffering capacity	2.25 ± 0.62	2.27 ± 0.47	2.17 ± 0.58	2.10 ± 0.57
Caries lesions	14.25 ± 18.97	–	14.58 ± 19.37	–
Caries risk according to Cariogram	3.42 ± 0.67	–	3.50 ± 0.67	–
Without new caries lesions according to the Cariogram	0.25 ± 0.12	–	0.25 ± 0.13	–

Salivary pH values at the baseline of our research were rather high compared to research with patients who had had head and neck radiotherapy [46]. In comparison to the results of our study, the change in pH value was less pronounced, and the effect of PBM was short-term only. Our results varied among experimental groups. In the group irradiated with continuous LED light, the pH value was higher after 2 weeks of PBM. Saliva with a higher pH value is better able to protect hard tooth substances from demineralization. Prior to our research, we did not find any studies on the effect of PBM on salivary pH values. However, as noted in some research, higher pH is considered to be most likely due to a change in the composition of saliva, where higher concentrations of bicarbonate and changed enzymatic activity were found [24, 25]. A higher pH and changed salivary contents could indicate the regeneration of ductal epithelial cells in a salivary gland.

The number of active caries lesions was determined by clinical examination according to ICDAS criteria at baseline and after 4 weeks of PBM [35, 47]. There were no significant changes in the number of active carious lesions in any of the groups. This finding could be due to the relatively short duration of our research, as caries is known as a process that may take longer to develop [48]. In order to determine accurately changes in the number of active or arrested caries lesions, longer follow-up times would be appropriate.

As assessed by Cariogram at the end of PBM, caries risk and the probability of new caries lesion development decreased in all therapeutic groups. Such a result is also consistent with our finding that several separate caries risk factors improved. There are no reports of research on the effects of PBM on caries risk. There is, however, a good deal of evidence of PBM influencing separate caries risk factors, such as oral biofilm growth or salivary flow [31–33].

The gold standard of caries therapy after diagnosing status, character, and the progress of caries and determining its risk factors, first of all, includes all necessary restorative measures, such as preparation and sealing of carious cavities. Restorative therapy is followed by targeted preventive measures, such as the use of mouthwashes with chlorhexidine, topical application of fluorides, oral hygiene improvement, diet modification, and saliva supplements if necessary. The patients from our study received all the necessary standard caries therapy after the end of the study as the treatment and preventive strategies suggested by Cariogram. In causative therapy of caries, PBM could be helpful as a supplementary method and could not substitute the gold standard. PBM addresses to one of the major risk factors, which gold standard of caries management cannot—saliva quality and its secretion. Our findings are directed to patients with dry mouth syndrome, who would mostly benefit from PBM as a supplementary or a preventive measure to help in decreasing caries risk.

The weakness of this method is that the PBM should be administered frequently to achieve the effect and the long-term effect is not yet known. Next more long-term research should address the question what is the optimum protocol to achieve the best results, how many irradiations are needed for stabile improvement of low cariogenic bacterial counts and increased salivary flow, and how often the therapy needs to be repeated.

The quality of life (QoL) in our participants was found significantly lower both at the beginning and at the end of PBM compared to the general population. The result was expected; systemic diseases, such as Sjögren's syndrome, and side effects from head and neck cancer therapy contributed to individuals' high caries risk and were in themselves a major cause of a lower life quality. There was no significant improvement of QoL after PBM. The latter fact suggests that the effects of PBM are mostly physiological and not based on psychological effects.

Conclusion

We determined that PBM of major salivary glands with polychromatic polarized light or with LED light can reduce cariogenic bacteria counts and improve salivary buffering capacity and therefore may reduce overall caries risk in high caries-risk patients.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

The manuscript represents valid work; neither this manuscript nor one with substantially similar content under this authorship has been published or is being considered for publication elsewhere (except as described in the manuscript submission); and copies of any closely related manuscripts are enclosed in the manuscript submission.

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Ethical approval The study was approved by the Republic of Slovenia National Medical Ethics committee (Nr. 0120-539/2016-2 KME 40/11/16).

Informed consent Each participant signed an informed consent form after the course of the research was explained.

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