

Original Article

Running Title: Pilocarpine Mouthwash in Radiation-Induced Xerostomia

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The Effect of 1% Pilocarpine Mouthwash on Salivary Flow Rate in Patients with Radiation-Induced Xerostomia: A Double-Blind Randomized Clinical Trial

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Abstract

Background: Radiation-induced hyposalivation is a common complication of radiotherapy for head and neck cancers. The most commonly prescribed medication for hyposalivation is Pilocarpine. However, due to the numerous systemic side effects associated with Pilocarpine, there has been a proposal to use it as a mouthwash. This study aimed to evaluate the impact of 1% Pilocarpine mouthwash on salivary flow in patients with radiation-induced xerostomia.

Method: This double-blind, randomized clinical trial involved 63 patients with radiation-induced xerostomia. The patients were randomly allocated into the Pilocarpine hydrochloride 1% mouthwash group and the placebo one. Patients were instructed to use these mouthwashes four times a day, with 30 drops each time, for two minutes. Unstimulated saliva production in patients was measured using the spitting method at three stages: two weeks before the commencement of radiotherapy, two weeks after, and four weeks after the completion of radiotherapy. These measurements were then compared between the two groups. Statistical analysis included Chi-square, independent t-test, and Analysis of Variance (ANOVA) with repeated measures and the Sidak post hoc test. Statistical analysis was conducted using SPSS 17, and a significance level of $P < 0.05$ was applied.

Results: A comparison of saliva secretion between the Pilocarpine mouthwash group and the control group at various time points after radiotherapy revealed that saliva secretion in the control group significantly decreased compared to the Pilocarpine mouthwash group ($P < 0.001$).

Conclusion: 1% Pilocarpine mouthwash is recommended for managing radiation-induced xerostomia.

Keywords: Head and neck neoplasms, Salivation, Mouthwashes, Pilocarpine, Radiotherapy

Introduction

Radiation therapy is one of the most common treatment options for head and neck cancers and plays a vital role in the management of many cancers by increasing the patient's chances of survival. In addition, in some cases, it can result in a complete cure.¹ Head and neck radiotherapy, despite its apparent benefits, is associated with unavoidable side effects such as hyposalivation, which in some patients can last for a lifetime. Unfortunately, in many patients, radiation-induced xerostomia caused by the damage to acinar cells of the salivary glands is inevitable.¹⁻⁴

Decreased salivation can result in significant disorders, including severe pain, speech disorders, dysphagia, dental caries, especially cervical caries, mucosal infections such as candidiasis, atrophic papillary changes of the tongue, halitosis, nutritional and taste disorders.^{5, 6} Furthermore, reduced salivation has considerable effects on the quality of life of these patients, as it can limit their social interactions and exacerbate depression.⁷ Moreover, this state can cause or intensify mucositis, which may even limit the continuance of radiotherapy.⁸ Various methods have been proposed to prevent radiation-induced hyposalivation.⁹ Frequent consumption of fluids and the use of sugar-free chewing gum, Bethanechol, and acupuncture are some of these approaches that can stimulate the remaining salivary capacity.¹⁰ Systemic sialagogues can help to stimulate saliva as well.¹¹

Pilocarpine is one of the medications among salivary stimulants and has been suggested as the best available agent.² However, several side effects such as sweating, hot flashes, nausea, and increased airway mucus secretion have also been reported with Pilocarpine.¹²

Pilocarpine mouthwash has been proposed as an alternative to oral Pilocarpine tablets to minimize the side effects of systemic pilocarpine.^{13,14} Besides, Pilocarpine mouthwash has been proven to be safe even for the elderly.¹³ Previous clinical trials on this subject used different medication regimens, resulting in different findings.^{15,16} Therefore, this clinical trial aims to investigate the effect of Pilocarpine 1% mouthwash on salivary flow rate in patients with radiation-induced xerostomia.

Patients and Materials

Study design

This study was a double-blind, randomized clinical trial in which blinding was performed for both therapists and patients. This study was carried out according to the CONSORT statement.¹⁷

Sample size

To determine the sample size, the study of Haddad et al.³ was used, in which the average rate of salivary flow reduction in the two groups of Pilocarpine and placebo was 40.32 ± 22.04 and 57.05 ± 21.53 ml, respectively. Thus, considering the error rate of the first type equal to 0.05 and the test power of 80%, the minimum number of 28 patients in each group was calculated. Finally, ninety patients were assessed for eligibility, and 66 patients (33 samples in each group) were included in the study (Figure 1).

All sequential patients diagnosed with head and neck carcinoma who underwent radiotherapy in the radiation oncology department of Shahid Madani Hospital of Tabriz University of Medical Sciences in 2021-2022 were screened for the study.

The

<http://www.graphpad.com/quickcalcs/index.cfm> website was used by FH to create a randomization list. In the next step, sequential patients enrolled in the study were randomly assigned to intervention and

control groups by sealed and opaque envelopes with an allocation ratio of 1:1. The hospital staff assigning the patients to study groups was not aware of the allocation sequence until the moment of assignment (allocation concealment).

Inclusion and exclusion criteria

Inclusion criteria:

1. Patients who have completed radiotherapy for head and neck cancers.
2. Patients aged 18 to 60 years old.
3. Patients with complaints of xerostomia.

Exclusion criteria:

1. Patients for whom pilocarpine is contraindicated (established allergy to pilocarpine, history of cardiovascular disease, glaucoma, asthma).
2. Patients with residual or recurrent disease.
3. Patients who have received concurrent chemotherapy.
4. Patients who have undergone any xerostomia treatment.
5. Patients with autoimmune diseases such as Sjögren's syndrome.
6. Smokers.

All patients in both groups underwent radiotherapy (5000 cGy) utilizing an Elekta Synergy system (Elekta AB, Stockholm, Sweden), using an oral shield to safeguard oral structures, particularly the salivary glands.

Preparation of mouthwashes

To prepare a 1% pilocarpine mouthwash, 0.5 ml of sterile 2% Pilocarpine ocular eye drop (Glaupin 2%, Sina Darou, Iran) was combined with 9.5 ml of Irsha Kids Mouthwash (Shafa Pharmaceutical, Alborz, Iran) to achieve a final volume of 10 ml, as outlined in previous studies (39). Irsha Kids Mouthwash (Shafa Pharmaceutical, Alborz, Iran) underwent dilution with water to match the taste and color profile of placebo mouthwashes. A pharmacologist used identical containers to encode the mouthwashes as sample A for

the Pilocarpine mouthwashes and sample B for the placebo mouthwashes.

A dental nurse, unacquainted with the coding, dispensed the samples. The clinician and patients were blinded to the specific mouthwash allocated to each patient. The nature of the intervention administered to individual patients remained undisclosed until data analysis. Patients were instructed to retain 30 drops of the mouthwash four times daily, with each application lasting two minutes. They were instructed not to ingest the solution and to ensure complete expulsion by spitting out the entire volume. Any instances of discoloration led to the immediate disposal of the mouthwash.

During the initial appointment, a two-week supply of mouthwash was dispensed to the patients. They were instructed to return any remaining mouthwash at the subsequent two-week follow-up session. The weight of the mouthwash within each container was measured as a means of assessing patient compliance. New mouthwash supplies were furnished to the patients after the second week. Weekly reminder phone calls were conducted to reinforce compliance. Demographic data, including age, gender, and pertinent medical histories, such as cancer stage and site, received treatments, and treatment termination dates, were extracted from the patient's medical records.

Intervention

Two weeks prior to the first session of radiotherapy, a salivary sample was obtained and considered as a baseline, and the use of mouthwashes began the next day and continued for one month. Salivary sampling was repeated two weeks and four weeks after the completion of radiotherapy.

Sialometry

The unstimulated saliva flow rate was measured in three stages using the spitting method for all patients.¹⁹ Patients were asked to abstain from drinking or eating anything for 90 minutes before sampling. They were then asked to drain their saliva once or twice a minute for 5 minutes in a

calibrated test tube.²⁰ The salivary flow rate was recorded in milliliters per minute.

Ethical considerations

The study was conducted following the Declaration of Helsinki and received approval from the ethics committee of Tabriz University of Medical Sciences (IR.TBZMED.REC.1400.499). It has been registered with the Iranian Registry of Clinical Trials under the identifier IRCT20210830052335N1. The study protocol can be accessed at <https://en.irct.ir/trial/58396>. Following a comprehensive explanation of the study to the patients, all eligible individuals were requested to complete and provide their signatures on the informed consent form.

Statistical analysis

The SPSS version 17 was used for statistical analysis. The Kolmogorov-Smirnov test was used to investigate the normality of the data distribution. Chi-square and independent t-tests were used to compare the variables between the groups. Analysis of variance with repeated measures and the Sidak post hoc test were used to evaluate salivary secretion over time. In all cases, $P < 0.05$ was considered statistically significant.

Results

For each group, the numbers of participants who were randomly assigned, received the allocated treatment, and were included in the analysis are depicted in figure 1. Recruitment commenced in October 2021, and the final follow-up of the last patient occurred in August 2022. The age range of the Pilocarpine group was 40-62 years, with a mean age of 53.32 ± 6.08 years. In the control group, the age range was 38-65, with a mean age of 51.48 ± 8.03 . There were no significant differences in the ages of the study group participants ($P = 0.314$) (Table 1).

Regarding gender, 67.7% of participants in the Pilocarpine mouthwash group and 61.3% in the control group were males. Gender distribution between the two study groups was analyzed using the chi-square

test (Table 2). The results did not reveal a significant difference ($P = 0.596$). Laryngeal cancer was the predominant cancer type in both groups, with a prevalence of 45.2% in the Pilocarpine mouthwash group and 38.7% in the control group. Similarly, comparing cancer types between the two study groups by the chi-square test did not show any significant differences ($P = 0.826$) (Table 2). Notably, most patients in both groups were in stage three of the disease. Additionally, the comparison of cancer stages between the two study groups, evaluated using the chi-square test (Table 2), did not reveal any significant differences ($P = 0.786$).

The comparison of salivary secretion in the Pilocarpine mouthwash group at different time points after radiotherapy, conducted through repeated measures analysis of variance, demonstrated a significant difference ($P < 0.001$). The Sidak post hoc test results indicated that the salivary flow rate consistently decreased significantly at all time points ($P < 0.001$).

Similarly, the analysis of salivary secretion in the control group at different time points after radiotherapy using repeated measures analysis of variance showed a significant difference over time ($P = 0.005$). The Sidak post hoc test results revealed a consistent significant decrease in salivary secretion over time in this group ($P < 0.001$).

The comparison of basal salivary volume between the study groups, assessed by independent t-test, did not yield a significant difference ($P = 0.974$).

When comparing salivary secretion between the Pilocarpine mouthwash group and the control group during various periods after radiotherapy, as shown in figure 2, a significant difference between the two groups was observed ($P < 0.001$). The Sidak post hoc test indicated that the salivary flow rate in the control group was significantly lower than that in the Pilocarpine mouthwash group during follow-up sessions ($P < 0.001$) but not at baseline ($P = 0.076$).

Five patients in the Pilocarpine mouthwash group reported experiencing palpitations, while two mentioned excessive sweating. However, these symptoms were mild, and the patients continued the trial.

Discussion

Comparison of salivary secretion in the Pilocarpine mouthwash group at two and four-week follow-ups after radiotherapy showed that the salivary flow rate had consistently decreased significantly. The salivary flow rate in the control group was significantly lower than that of the Pilocarpine mouthwash group at follow-up sessions. Despite many advances in cancer biology and radiation therapy in recent decades, salivary gland dysfunction remains a significant and lasting problem after radiotherapy of head and neck malignancies.^{21 22} The patients in the present study in both the pilocarpine and control mouthwash groups were in their fifth decade of life, and the majority (over 60%) were male. Previous studies have reported a higher prevalence of head and neck malignancies in men than in women.^{23, 24} In the study of Haddad et al., 60% of the patients with this kind of cancer were male.³ Moreover, numerous reports have indicated that the typical age for head and neck cancers is around 50 to 70 years old.²⁵ In the present study, laryngeal cancer was the most common kind of cancer in patients. Several reports have reported that the most common head and neck cancer in Iran is laryngeal cancer^{26,27}. In the present study, 1% Pilocarpine mouthwash was used 4 times daily for 2 minutes each time. The study by Song et al. stated that the minimum effective dose for increasing the level of unstimulated saliva by Pilocarpine mouthwash is 1% after at least 1 minute of use.¹⁴ The present study examined patients' salivation at three time intervals before starting radiotherapy, two and four weeks after the beginning of the radiotherapy. Several studies have shown that salivary gland dysfunction after radiotherapy for head and neck

malignancies usually begins within the first weeks after starting treatment.^{20, 21, 28} Onset of serous acinar cell destruction can occur in a few days after radiotherapy.²⁹

Decreased salivation in patients receiving radiotherapy is due to the destruction of serous acinar cells, with the development of acute inflammation.³⁰ The histological basis for decreased salivation after radiation therapy is not fully understood. Recent molecular studies have revealed that inflammatory cytokines, such as TNF- α , reduce the release of aquaporin 5 (a group of plasma membrane proteins responsible for transporting water molecules from the membrane), thereby reducing aqueous salivary secretions.³¹ Stimulation of salivary gland function can help patients with some residual salivary gland parenchyma through sialogogue medications such as pilocarpine and cevimeline.³² The use of systemic Pilocarpine during radiation therapy has been suggested to reduce xerostomia^{3, 33,34}, and the use of Pilocarpine for treating chronic hyposalivation has been thoroughly investigated.³⁵ Pilocarpine is a parasympathomimetic drug that affects the muscarinic cholinergic receptors in the acinar cells of salivary glands, thereby improving salivary secretion.² Muscarinic acetylcholine receptors have five subtypes, M1–M5; Pilocarpine's main therapeutic effects are mediated by M3 receptors, which activate the effector enzyme phospholipase C beta, which hydrolyses phospholipid PIP₂, causing the production of the second messenger's inositol triphosphate and diacylglycerol and calcium and protein kinase. As a result, M3 cholinergic agonists can upregulate calcium and lead to smooth muscle contraction.³⁶ A systematic review by Riley et al., although linking Pilocarpine to increased salivary secretion, suggested the need for further evidence.³⁷ A systematic review concluded that the administration of systemic Pilocarpine has beneficial effects on salivary flow after radiotherapy.³⁸

In the present study, the mean salivation in Pilocarpine mouthwash patients was lower than average only in the last measurement (four weeks after radiotherapy). In contrast, in the control group, the mean salivation was lower than average two and four weeks after radiotherapy. Some studies investigate the effect of pilocarpine in topical form to reduce its adverse effects,^{13, 39}. However, few studies have studied the effects of topical pilocarpine in radiation-induced xerostomia. A clinical trial by Akhavan Karbasi et al. studied the preventive effects of pilocarpine and reported that pilocarpine mouthwash effectively prevents xerostomia.¹⁶ Besides, it can prevent the reduction of saliva. Similarly, the present study's findings indicated that the salivary secretion in the control group decreased significantly over time more than in the Pilocarpine mouthwash group. Tawechaisupapong et al. studied the efficacy of pilocarpine lozenge in patients with post-radiation xerostomia and showed that salivary production in pilocarpine treatment groups increased significantly.¹⁴ In the present study, Only 1% Pilocarpine mouthwash was used, but a study by Motamed et al. showed that pilocarpine 2 and 1% mouthwash for 2 weeks increased salivary flow in patients with radiation-induced xerostomia, and the effect was dose-dependent. No side effects were reported with higher dose mouthwash.¹⁸ The participants of this study had different types of head and neck cancer, and they were in different stages of the disease; therefore, it can be suggested that the results can be used for the reduction of the symptoms of radiation-induced xerostomia in all these situations. The limitation of this study was that it assessed only the short-term effects of the pilocarpine mouthwash on radiation-induced xerostomia; therefore, in order to achieve more accurate results regarding the effect of Pilocarpine mouthwash on saliva secretion in these patients, it is suggested to design a study with larger sample size and considering factors such as chemotherapy

and long follow-ups Another limitation of this study is that received doses of salivary glands in the participants were not estimated and matched between groups.

Conclusion

The salivary flow rate decreased over time in both groups. The reduction in salivary flow rate was notably more pronounced in the control group compared with the Pilocarpine mouthwash group. After four weeks, the salivary flow rate was significantly elevated in the Pilocarpine mouthwash group compared with the control group. In light of the findings from this current study, it is advisable to consider using 1% Pilocarpine mouthwash as a therapeutic option for radiation-induced xerostomia.

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Conflict of Interest

None declared.

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CONSORT 2010 Flow Diagram

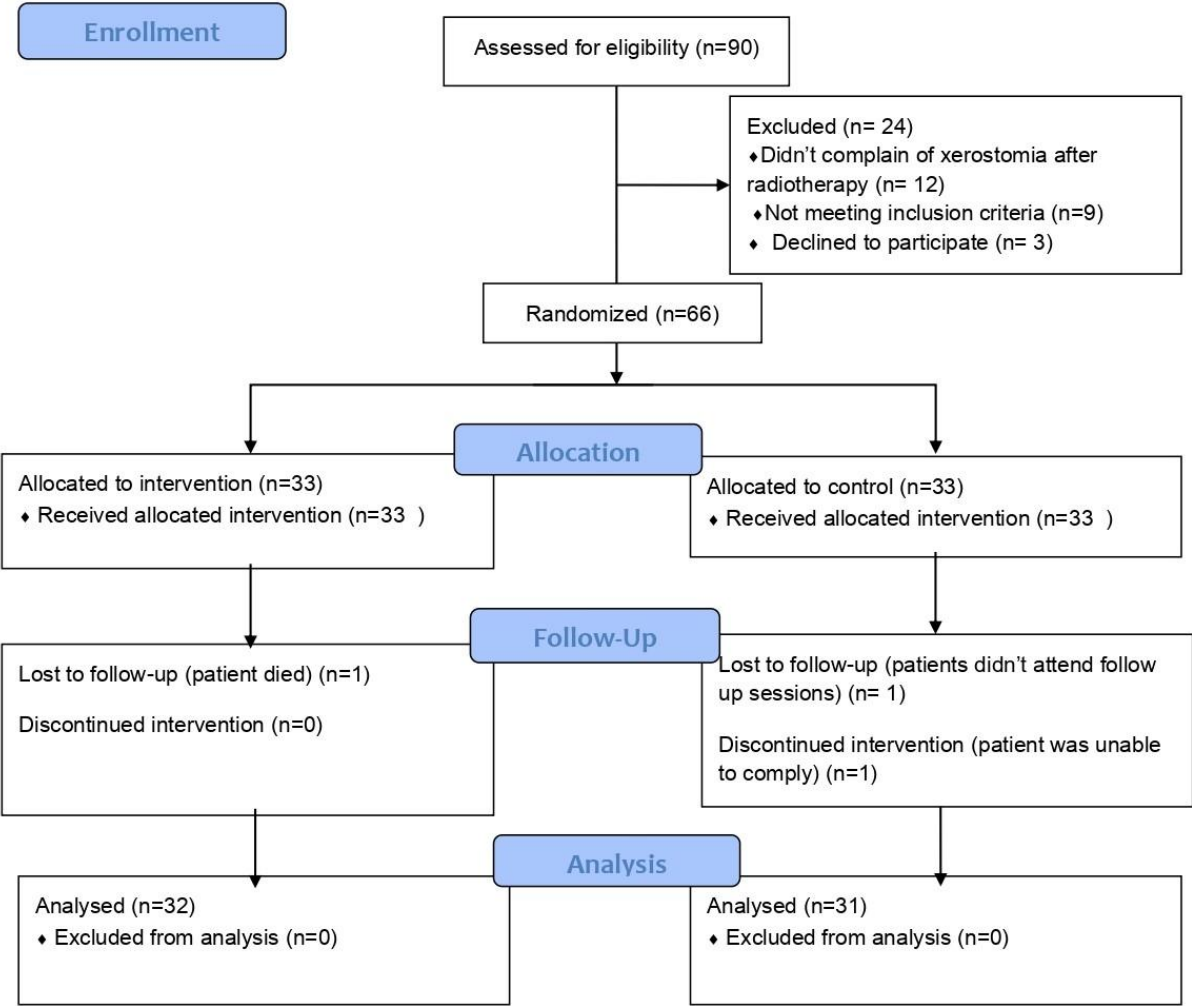


Figure 1. CONSORT 2010 flow diagram illustrates participant enrollment and follow-up procedures.

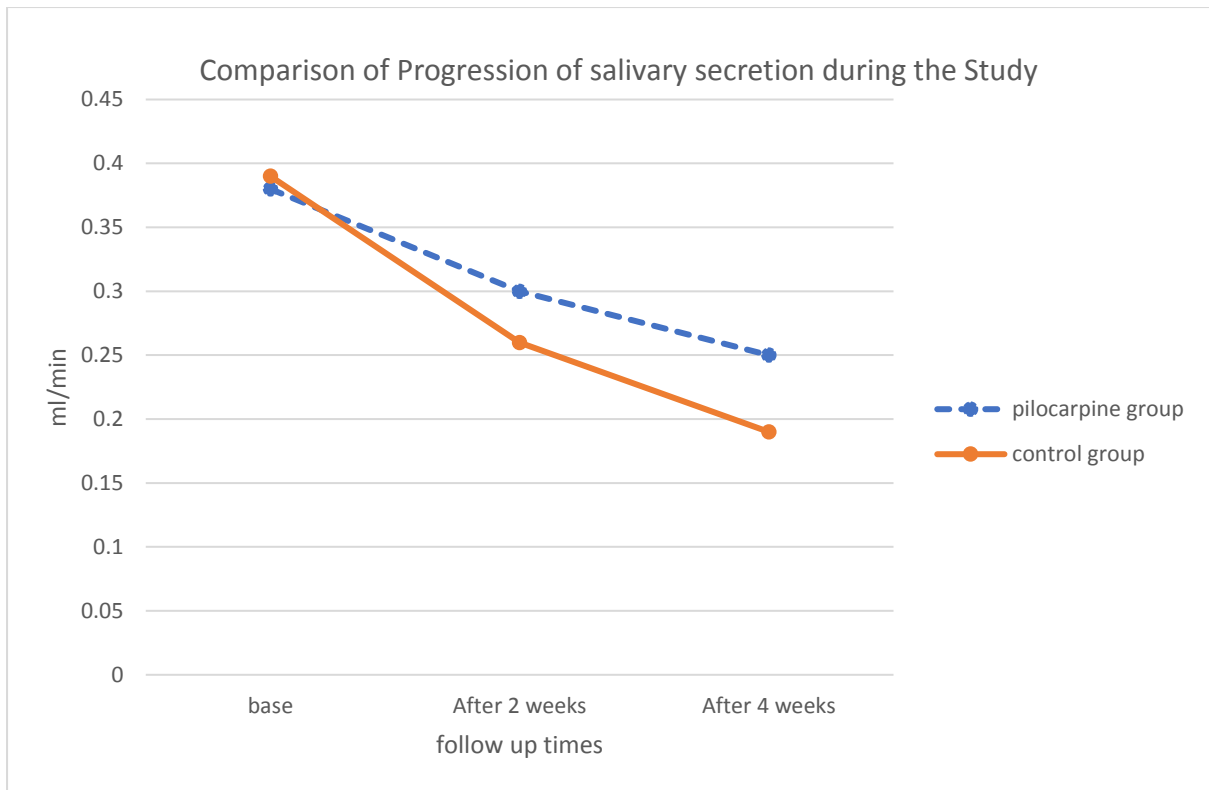


Figure 2. This figure presents a comparative analysis of salivary secretion between the Pilocarpine mouthwash and the control groups at various time intervals following radiotherapy. The results reveal a noteworthy disparity in salivary flow rates, with the control group exhibiting a significantly lower rate than the Pilocarpine mouthwash group during follow-up sessions.

Table 1. Demographic characteristics and clinical features (cancer site and stage) of the study participants in Pilocarpine and placebo mouthwash groups

Variable	Pilocarpine (n = 32) (frequency (percent))	Control (n = 31) (Frequency (percent))	P- value
Age (mean \pm SD in years)	53.32 \pm 6.08	51.48 \pm 8.03	0.314 ^a
Sex	Male	21(65.7%)	0.596 ^b
	Female	11(34.3%)	
Cancer site	Larynx	10(31.2%)	0.826 ^c
	Nasopharynx	4(12.6%)	
	Oral	9(28.1%)	
	Salivary gland	2(6.2%)	
	Nasal	6(18.8%)	
	Hypopharynx	1(3.1%)	
Cancer stage	I	3(9.4%)	0.786 ^c
	II	10(31.2%)	
	III	15(46.8%)	
	IV	4(12.6%)	

^a P-value based on an independent T-test; ^b P-value based on Chi-square; ^c P-value based on Multinomial logistic regression

Table 2. Mean Salivary flow rate in Pilocarpine and placebo mouthwash groups at different times after radiotherapy

Groups	Times	Mean \pm SD (ml/min)	Standard error	min	Max	95% confidence interval		<i>P</i> -value ^a
						Lower band	Upper band	
Intervention group (Pilocarpine mouthwash)	base	0.38 \pm 0.16	0.02	0.18	0.60	0.32	0.43	<0.001
	After 2 weeks	0.30 \pm 0.11	0.01	0.10	0.48	0.26	0.33	
	After 4 weeks	0.25 \pm 0.13	0.02	0.08	0.41	0.20	0.29	
Control group (Placebo)	base	0.39 \pm 0.20	0.03	0.19	0.69	0.32	0.46	<0.001
	After 2 weeks	0.26 \pm 0.14	0.02	0.09	0.46	0.21	0.30	
	After 4 weeks	0.19 \pm 0.11	0.01	0.06	0.34	0.15	0.22	

^a *P*-value based on Repeated Measures ANOVA