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Minor salivary glands function is decreased in hyposalivation-related diseases

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ABSTRACT

Objectives: The aim of this cross-sectional study was to investigate the relationship between minor salivary gland (MSG) flow rates and oral dryness degrees in patients with xerostomia induced by primary Sjögren's syndrome (pSS), IgG4-related sialadenitis (IgG4-RS), radiation therapy-induced dry mouth (RTDM), or Steven–Johnson syndrome (SJS).
Design: 160 patients with pSS, IgG4-RS, RTDM, or SJS and their age- and sex-matched healthy control subjects were enrolled. The whole saliva flow rates and MSG flow rates were measured in four locations, including the upper labial, lower labial, buccal, and palatal mucosae. The degree of oral dryness was assessed in patient groups using the summated xerostomia inventory (SXI).
Results: The flow rates of whole saliva and most MSGs in patient groups were significantly lower than the flow rates in healthy control groups ($P < 0.05$). The mean relative percentage of decrease in saliva flow rates was smaller in MSGs than in whole saliva in patient groups ($P < 0.05$), indicating that these disorders have less impact on MSGs. Among the four MSG locations (the upper labial, lower labial, buccal, and palatal), buccal glands showed the highest flow rates in patient groups ($P < 0.05$). SXI scores were significantly higher in pSS and RTDM patients than in IgG4-RS and SJS patients ($P < 0.05$). The degree of xerostomia varied among different patient groups ($P < 0.05$) and there was no clear correlation between MSG flow rates and SXI scores ($P > 0.05$).
Conclusions: MSG function is significantly reduced in pSS, RTDM, IgG4-RS, and SJS patients, but this reduction is more pronounced in the major salivary glands.

1. Introduction

The oral cavity has 600–1000 minor salivary glands (MSGs) scattering beneath the labial, buccal, palatal, and lingual mucosa. The saliva secreted by MSGs accounts for less than 10% of the whole saliva (Dawes & Wood, 1973). However, due to their close proximity to the oral mucosa, continuous supply of small amounts of saliva, and high concentration of protective substances such as mucins and IgA, MSGs play a significant role in oral functions, including protection and lubrication of the oral mucosa, as well as maintaining taste and oral mucosal immunity (Eliasson & Carlen, 2010). Moreover, MSGs play a major role in saliva production during sleep; therefore, a decreased MSG flow rate appears to account for night-time dry mouth (Dijkema et al., 2012). A correlation between MSG flow rates and the thickness of the residual saliva film on the oral mucosa has been observed, suggesting that MSG saliva secretion is important for the subjective sensation of dry mouth (Won, Kho, Kim, Chung, & Lee, 2001). This view is further supported by observations that local areas of dry mucosa can trigger dry sensation (Dawes, 1987). Satoh-Kuriwada et al. proposed that dry mouth is more strongly related to reduction in labial saliva flow than to decrease in stimulated whole saliva (Satoh-Kuriwada, Iikubo, Shoji, Sakamoto, & Sasano, 2012). However, hyposalivation is generally defined as an unstimulated whole saliva flow rate of <0.1 ml/min or a stimulated rate of <0.7 ml/min, and very few studies have focused on MSG flow rates in patients with dry mouth (Ericsson & Hardwick, 1978; Satoh-Kuriwada et al., 2012).

Abbreviations: pSS, primary Sjögren's syndrome; IgG4-RS, IgG4-related sialadenitis; RTDM, radiation therapy-induced dry mouth; SJS, Steven–Johnson syndrome; HNC, head and neck cancer; MSG, minor salivary gland; SXI, summated xerostomia inventory.

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Severe hyposalivation caused by Sjögren's syndrome (SS) and radiation therapy (RT) for head and neck cancer (HNC) is frequently confronted in the clinic of oral medicine. Flow rates of the labial glands in pSS and RT patients were reportedly lower than in control subjects (Eliasson, Almstahl, Lingstrom, Wikstrom, & Carlen, 2005). IgG4-related sialadenitis (IgG4-RS), which has main characteristics of enlarged salivary and lacrimal glands and decreased saliva secretion, is a component of IgG4-related diseases. Considering its close relationship with Mikulicz disease, IgG4-RS was once considered identical to primary SS, which are now recognized as separate diseases (Himi, Takano, Yamamoto, Naishiro, & Takahashi, 2012; Masaki, Sugai, & Umehara, 2010).

In 1997, Murube-del-Castillo proposed transplantation of MSGs as a treatment for severe dry eyes secondary to Steven-Johnson syndrome (SJS) (Geerling, Raus, & Murube, 2008). SJS, also known as erythema multiforme major, is a life-threatening reaction of the skin to particular types of medication. Not only skin and mucosal membranes, but also salivary and lacrimal glands are often involved, resulting in dry eye and dry mouth (Sant', Hazarbassanov, de Freitas, & Gomes, 2012; Saeed, Mantagos, & Chodosh, 2016). However, no previous study has evaluated MSG functions in IgG4-RS and SJS patients.

Although the underlying pathogenesis appears different, all four conditions exhibit certain levels of salivary gland hypofunction. Previously, we investigated the characteristics of MSG flow rates in healthy individuals (Wang, Shen, Liu, Si, & Yu, 2015). The present study was designed to further investigate the characteristics of MSG flow rates in patients with hyposalivation-related diseases and to examine the potential relationship between MSG flow rates and the degree of oral dryness.

2. Materials and methods

2.1. Subjects and study design

As the main objectives of this study is to compare flow rates of MSGs between patient groups and healthy controls, the formula for comparation between independent quantitative samples was used to caculate the sample size. Based on our preliminary study, a sample size of 26–37 patients or healthy subjects was needed (Wang et al., 2015). From June 2014 to May 2015, 160 patients with pSS, IgG4-RS, RTDM, or SJS were enrolled in this study. All patients were referred to the Department of Oral and Maxillofacial Surgery or Department of Oral Medicine at the Peking University School of Stomatology or the Department of Ophthalmology at the Beijing Tong Ren Hospital, Capital University of Medical Science. Information pertaining to age, sex, disease duration, smoking, allergies, comorbidities, drug consumption, number of remaining major salivary glands, and radiation dose received was recorded. Disease duration was defined as the period from the initial observation of dry mouth or enlargement of major salivary glands to first visit. Measurements were performed in all patients before possible submandibular/labial gland biopsy in the pSS and IgG4-RS groups. The inclusion/diagnostic criteria for each patient group were described below.

SS patients were required to meet the American-European Consensus Group criteria for pSS (Vitali et al., 2002). None of the patients were receiving treatment with steroids or immunosuppressive agents.

IgG4-RS was diagnosed according to the following criteria: (1) persistent (> 3 months) swelling in more than one major salivary glands; (2) elevated serum IgG4 level (> 135 mg/dl); and (3) infiltration of IgG4-positive plasma cells in the tissue (IgG4-positive plasma cells/IgG-positive plasma cells ratio > 0.4 according to immunostaining). In addition, it was necessary to rule out other disorders such as sarcoidosis, Castleman's disease, Wegener's granulomatosis, lymphoma, and cancer (Li et al., 2015).

RTDM was diagnosed according to the following criteria: (1) history of head and neck cancer originating in the oral cavity, oropharynx, or nasopharynx; (2) history of treatment with surgery and postoperative radiotherapy or radiotherapy alone; (3) total radiation dose received was above 40 Gy; (4) no past history of RT or malignancies; and (5) absence of local recurrence or distant metastases.

The inclusion criteria for patients in the SJS group were as follows: (1) history of SJS caused by allergy to drugs or an infectious process; (2) persisting symptom of dry eyes despite previous ophthalmologic treatment including application of artificial tears and punctal plugs, and (3) ophthalmologic evaluation: Schirmer test <2 mm, break-up time (BUT) <5 s, and fluorescence staining (+) (Qin et al., 2013).

Each disease group had the same number of patients: pSS group: 40 women(median age 55.5 years; age range, 26–71 years); IgG4-RS group: 16 women and 24 men (median age 56 years; age range, 23–81 years), RTDM group: 15 women and 25 men (median age, 55.5 years; age range, 27–83 years), and SJS group: 21 women and 19 men (median age, 25 years; age range, 7–62 years). The control group for each patient group consists of 40 age- and sex-matched individuals without dry mouth, dry eye, or clinical evidence of any systemic disease (Table 1). The study was designed and conducted in complete accordance with the World Medical Association Declaration of Helsinki (version 2002), and it was approved by the Ethics Committee of the Peking University School of Stomatology (PKUSSIRB: 201412003). All experiments were undertaken with the understanding and written consent of each subject.

2.2. Dry mouth questionnaire

The intensity of mouth dryness in patients was assessed using the summated xerostomia inventory (SXI), which included the following five questions: "my mouth feels dry," "I have difficulty in eating dry foods," "my mouth feels dry when eating a meal," "I have difficulties swallowing certain foods," and "my lips feel dry". The

Table 1
Basic information in pSS, IgG4-RS, RTDM and SJS groups and healthy controls.

		N	Sex (M/F)	Median age (years)	Age range (years)	Median duration (months)	Num	Total RT dose (Gy)
pSS	Control	40	0/40	50.5	26–74	0	6	0
	Patients	40	0/40	55.5	26–71	40	6	0
IgG4-RS	Control	40	24/16	57.5	23–83	0	6	0
	Patients	40	24/16	56	23–81	12	6	0
RTDM	Control	40	25/15	57	27–83	0	6	0
	Patients	40	25/15	55.5	27–83	10	4.9 ± 0.3	58.2 ± 10.4
SJS	Control	40	19/21	26	5–62	0	6	0
	Patients	40	19/21	25	7–62	54	6	0

N represents number of patients in each group; num represents the number of remaining major salivary glands.

respondents were required to rate each item using a three-point Likert scale, where a score of 1 indicated "never" and a score of 3 indicated "always". The total SXI score was a summation of the scores for the five individual items and ranged from 5 to 15, with higher scores indicating more severe dry mouth (He, Wang, & Li, 2013). The degree of dry mouth was classified on the basis of these scores as follows: 5, no dry mouth; 6–8, mild dry mouth; 9–12, moderate dry mouth; and 13–15, severe dry mouth.

2.3. Measurement of saliva flow rates

Whole saliva flow rates at rest and under stimulation with 2.5% citric acid and MSG flow rates from four locations, including the upper labial, lower labial, buccal, and palatal mucosa, were measured as previously reported (Wang et al., 2015). Before acid stimulation, whole saliva flow rates under chewing stimulation were measured by asking the subjects to chew on unflavored gum base (Wrigley Confectionery Limited, China) at the rate of 60/min. Patients were then instructed to rest for 15 min before acid stimulation. With the specific gravity of saliva considered as 1, the whole saliva flow rates were calculated and expressed in ml/min and MSG flow rates in $\mu l/min/cm^2$.

2.4. Statistical analysis

All statistical analyses were performed using SPSS 16.0 for Windows (SPSS Inc., Chicago, IL, USA). A *P*-value of <0.05 was considered statistically significant (one-tailed analysis for comparison between MSG flow rates in 4 conditions and age- and sex-matched healthy controls; two-tailed analysis for others). Whole saliva flow rate and MSG flow rate values are expressed as means ± standard deviations (SDs). The reduction in saliva flow rates at rest and under stimulation in each patient group relative to the flow rate in the corresponding control group was calculated as a relative percentage decrease and expressed in percentage (%); a mean relative percentage decrease for 4 MSG flow rates was calculated in each patient group. Whole saliva flow rates and MSG flow rates were compared between patient and control groups using paired *t*-tests, while they were compared within each patient group using one-way ANOVA. The relative percentage decreases in saliva flow rates were also compared within each disease group using one-way ANOVA. The same comparisons were conducted among the four disease groups using the Kruskal–Wallis test. SXI scores were expressed as medians and ranges and analyzed using the Kruskal–Wallis test. The distribution of the different degrees of dry mouth was expressed as a percentage and analyzed using chi-square tests. Possible influencing factors on SXI scores included age, sex, disease duration, whole saliva flow rates, and MSG flow rates in pSS, IgG4-RS, and SJS groups; the number of remaining major salivary glands and radiation dosage received were added for the RTDM group (Table 1). These factors were analyzed for bivariate correlations using Pearson's correlation coefficients.

3. Results

3.1. SXI scores and degree of dry mouth in patient groups

SXI scores for the four patient groups were shown in Table 2. The scores were significantly higher in pSS and RTDM groups than in IgG4-RS ($P < 0.05$) and SJS groups ($P < 0.05$). Xerostomia induced by pSS was more serious than that induced by radiation exposure; however, the difference did not reach statistical significant level ($P = 0.073$). No significant difference in SXI scores was found between IgG4-RS group and SJS group ($P = 0.93$; Table 2; Fig. 1).

Table 3 showed the distribution of different degrees of dry mouth, which was significantly different among four patient groups ($P < 0.01$). Most patients in the pSS group exhibited moderate or severe dry mouth, most patients in the RTDM group exhibited moderate dry mouth, and most patients in the IgG4-RS and SJS groups exhibited mild or moderate dry mouth.

Table 2
SXI scores in patients with pSS, IgG4-RS, RTDM, and SJS.

Patient group	No. of patients	SXI score			
		Median	Range	P_5	P_{95}
pSS	40	11	6–15	7	15
IgG4-RS	40	8*	5–13	5	13
RTDM	40	10	5–15	6	14
SJS	40	8.5*	5–15	5	13

P_5: percentile 5%; P_{95}: percentile 95%.
* $P < 0.05$ compared with SXI scores of pSS patients.

3.2. Whole saliva flow rates and MSG flow rates in patient groups and control groups

Whole saliva flow rates at rest and under stimulation and MSG flow rates in four patient groups and the corresponding control groups were shown in Tables 4 and 5.

3.2.1. Comparisons of whole saliva flow rates and MSG flow rates between patient and control groups

Both MSG flow rates and whole saliva flow rates were lower in all patient groups than in the corresponding control groups, with the exception of the palatal gland flow rate in the IgG4-RS group and upper labial gland flow rate in the SJS group. Mean MSG flow rates in 4 sites in patient groups were lower than those in healthy controls ($P < 0.01$). The relative decreases ranged from 3.67% to 74.1%. The relative decrease of flow rates varied widely among the four MSGs. Therefore, the mean relative decrease of MSG flow rates was compared with the relative decrease of whole saliva flow rates. The mean relative decrease of MSG flow rates was smaller than that of whole saliva flow rates in the pSS and RTDM groups ($P < 0.01$). In IgG4-RS group, the mean relative percentage decrease of MSG flow rates was lower than that of whole saliva flow rates at rest and under chewing-stimulated whole saliva flow rates ($P < 0.01$), no difference was found between the mean relative percentage decrease of MSG and that of the acid-stimulated whole saliva ($P > 0.05$). In SJS group, the mean relative percentage decrease of MSG flow rates was lower than that of whole saliva flow rates under acid or chewing stimulation ($P < 0.05$); however, no difference in relative decrease was observed at rest ($P > 0.05$).

3.2.2. Comparisons of whole saliva flow rates and MSG flow rates within and among the patient groups

Whole saliva flow rates under chewing or acid stimulation showed a gradual increase from the level at rest in healthy control, pSS, IgG4-RS and SJS groups. Among MSGs, the buccal glands showed the highest flow rates ($P < 0.01$), followed by the palatal and labial glands in pSS and SJS groups. There were no significant differences among the flow rates of the palatal, upper labial, and lower labial glands ($P > 0.05$). In IgG4-RS and RTDM groups, the buccal glands showed the highest flow rates ($P < 0.01$), followed by the palatal and labial glands; there were no significant differences between flow rates of the upper labial and lower labial glands ($P > 0.05$).

Next, intergroup comparisons were conducted. The SJS group showed the highest whole saliva flow rates at rest and under acid stimulation ($P < 0.01$), followed by the IgG4-RS, RTDM and pSS groups. There was no significant difference between the two latter groups ($P > 0.05$). With regard to whole saliva flow rates under chewing stimulation, the SJS and IgG4-RS groups showed

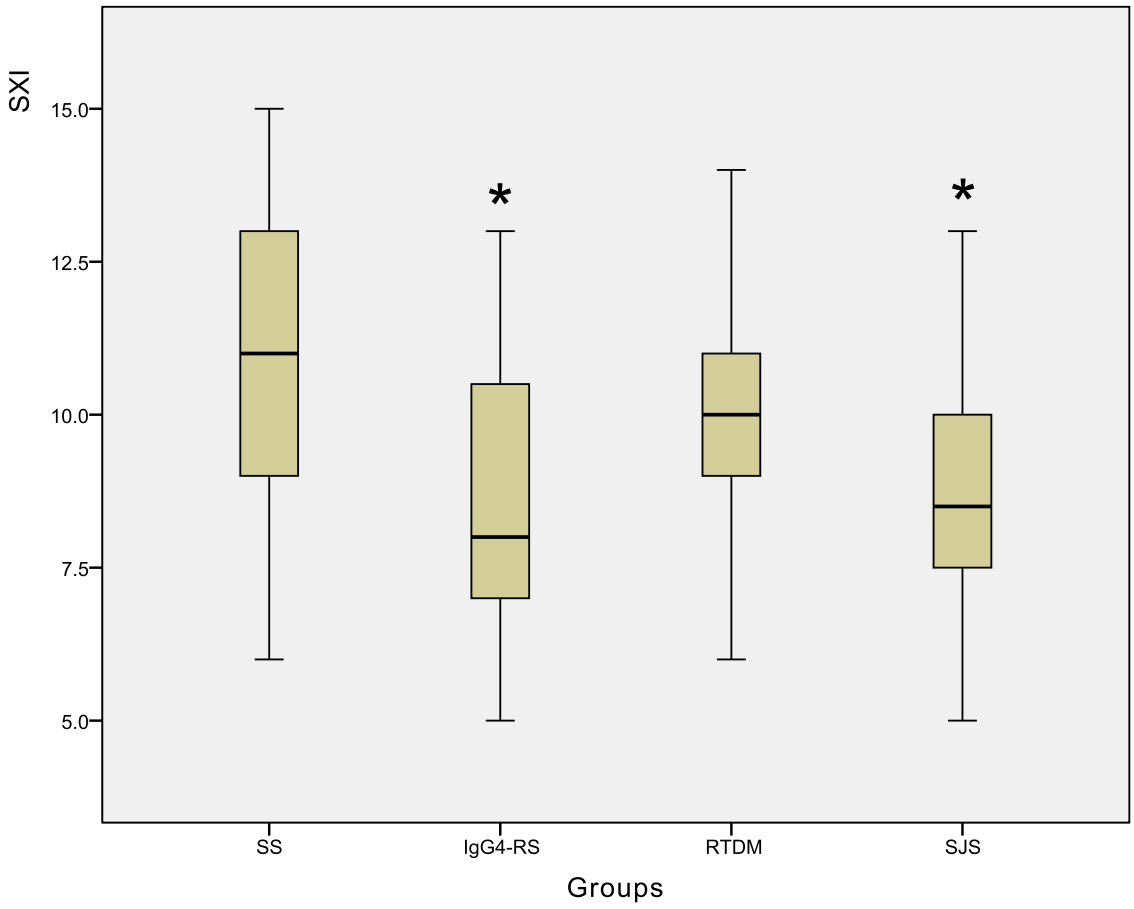

Fig. 1. SXI scores in SS, IgG4-RS, RTDM and SJS patients.
*$P < 0.05$ compared with SS group; bar presents CI 90%.

Table 3
Degree of dry mouth in patients with pSS, IgG4-RS, RTDM and SJS.

Patient group	No. of cases	Dry mouth/n (%)			
		None	Mild	Moderate	Severe
pSS	40	0 (0)	5 (12.5)	23 (57.5)	12 (30)
IgG4-RS	40	3 (7.5)	19 (47.5)	17 (42.5)	1 (2.5)
RTDM	40	2 (5)	5 (12.5)	28 (70)	5 (12.5)
SJS	40	7 (17.5)	13 (32.5)	16 (40)	4 (10)
Total	160	12 (7.5)	42 (26.25)	84 (52.5)	22 (13.75)

significantly higher values than the RTDM and pSS groups ($P < 0.01$), whereas there was no significant difference between the former two and latter two groups ($P > 0.05$). The SJS group had the highest mean flow rate of MSGs in 4 sites ($P < 0.01$), followed by the IgG4-RS, RTDM, and pSS groups. There were no significant differences among the latter three groups ($P > 0.05$, Fig. 2). The flow rates of the upper and lower labial glands were the highest in the SJS group ($P < 0.01$), followed by the IgG4-RS, RTDM, and pSS groups. There were no significant differences among the latter three groups ($P > 0.05$). The buccal glands exhibited significantly higher flow rates in the SJS, IgG4-RS, and pSS groups than in the RTDM group ($P < 0.01$), with no significant differences among the

Table 4
Whole saliva flow rates in pSS, IgG4-RS, RTDM and SJS patients and healthy controls (Mean ± SD).

		Flow rate of whole saliva at rest	Flow rate of whole saliva under chewing stimulation	Flow rate of whole saliva under acid stimulation
pSS	Control	0.40 ± 0.29	1.40 ± 0.64	2.42 ± 1.10
	Patients	0.07 ± 0.03**	0.51 ± 0.21**	0.70 ± 0.28**
	RP	71.82 ± 25.33	53.40 ± 34.48	64.00 ± 22.53
IgG4-RS	Control	0.40 ± 0.30	1.47 ± 0.81	2.43 ± 1.34
	Patients	0.13 ± 0.13**	1.07 ± 0.82**	1.50 ± 0.91**
	RP	44.28 ± 67.36	40.01 ± 31.12	19.50 ± 66.30
RTDM	Control	0.39 ± 0.35	1.70 ± 0.79	2.31 ± 1.08
	Patients	0.06 ± 0.07**	0.41 ± 0.34**	0.50 ± 0.40**
	RP	74.08 ± 37.08	68.28 ± 34.71	73.00 ± 29.73
SJS	Control	0.41 ± 0.22	2.02 ± 0.93	2.90 ± 1.34
	Patients	0.32 ± 0.20*	1.01 ± 0.59**	1.85 ± 1.16**
	RP	16.84 ± 52.26	32.63 ± 29.27	38.89 ± 40.38

RP represents the relative percentage of decrease in saliva flow rates in patient groups compared with healthy controls; l/min is the unit for flow rate of whole saliva; **$P < 0.01$ compared with healthy controls; *$P < 0.05$ compared with healthy controls.

Table 5
MSG flow rates in pSS, IgG4-RS, RTDM and SJS patients and healthy controls (Mean ± SD).

		Flow rate of lower labial glands	Flow rate of upper labial glands	Flow rate of buccal glands	Flow rate of palatal glands	Mean RP of MSG flow rates
pSS	Control	1.90 ± 0.68	1.98 ± 0.66	2.82 ± 0.63	2.08 ± 0.65	2.19 ± 0.47
	Patients	1.43 ± 0.45**	1.43 ± 0.50**	2.25 ± 0.48**	1.58 ± 0.48**	1.67 ± 0.37**
	RP	7.5 ± 65.31	20.4 ± 40.73	15.32 ± 30.13	14.82 ± 43.16	14.53 ± 36.03
IgG4-RS	Control	2.10 ± 0.57	2.17 ± 0.50	2.92 ± 0.74	2.01 ± 0.49	2.30 ± 0.40
	Patients	1.31 ± 0.54**	1.30 ± 0.55**	2.32 ± 0.58**	1.89 ± 1.12	1.70 ± 0.44**
	RP	30.96 ± 41.90	36.57 ± 31.72	12.97 ± 38.44	0.4 ± 60.94	20.10 ± 28.54
RTDM	Control	1.98 ± 0.63	2.24 ± 0.72	2.76 ± 0.83	2.20 ± 0.53	2.30 ± 0.49
	Patients	1.27 ± 0.60**	1.29 ± 0.43**	1.89 ± 0.50**	1.53 ± 0.61**	1.50 ± 0.46**
	RP	27.7 ± 42.12	36.1 ± 30.91	24.32 ± 35.40	25.76 ± 37.23	28.45 ± 29.76
SJS	Control	2.27 ± 0.62	2.05 ± 0.53	2.85 ± 0.62	2.14 ± 0.42	2.33 ± 0.40
	Patients	1.81 ± 0.62**	1.81 ± 0.58	2.53 ± 0.80*	1.80 ± 0.43**	1.99 ± 0.43**
	RP	10.03 ± 55.58	4.79 ± 39.97	8.15 ± 34.37	12.17 ± 27.50	11.44 ± 24.56

RP represents the relative percentage of decrease in saliva flow rates in patient groups compared with healthy controls; μl/min/cm^2 is the unit for MSG flow rate; **$P < 0.01$ compared with healthy controls; *$P < 0.05$ compared with healthy controls.

former three groups ($P > 0.05$), while the palatal glands showed no significant differences among the four groups ($P > 0.05$). The relative percentage decrease in whole saliva flow rates at rest and under acid stimulation was significantly higher in the pSS and RTDM groups than in the IgG4-RS and SJS groups ($P < 0.01$). The mean relative percentage decrease of MSG flow rates showed no significant differences among the four groups ($P > 0.05$).

3.3. Factors influencing SXI score

In the pSS group, there was a correlation between SXI score and age (r = 0.453, $P = 0.003$), SXI score and whole saliva flow rates under acid stimulation (r = −0.328, $P = 0.039$), and SXI score and whole saliva flow rates under chewing stimulation (r = −0.396, $P = 0.011$), while only age (r = 0.359, $P = 0.023$) showed a correlation with the SXI score in the SJS group (Table 6).

4. Discussion

In this study, patients with 4 hyposalivation-related diseases showed varied degrees of xerostomia. Whole saliva flow rates and mean MSG flow rates in patient groups were decreased compared to control groups. However, MSG function was more preserved than major salivary glands.

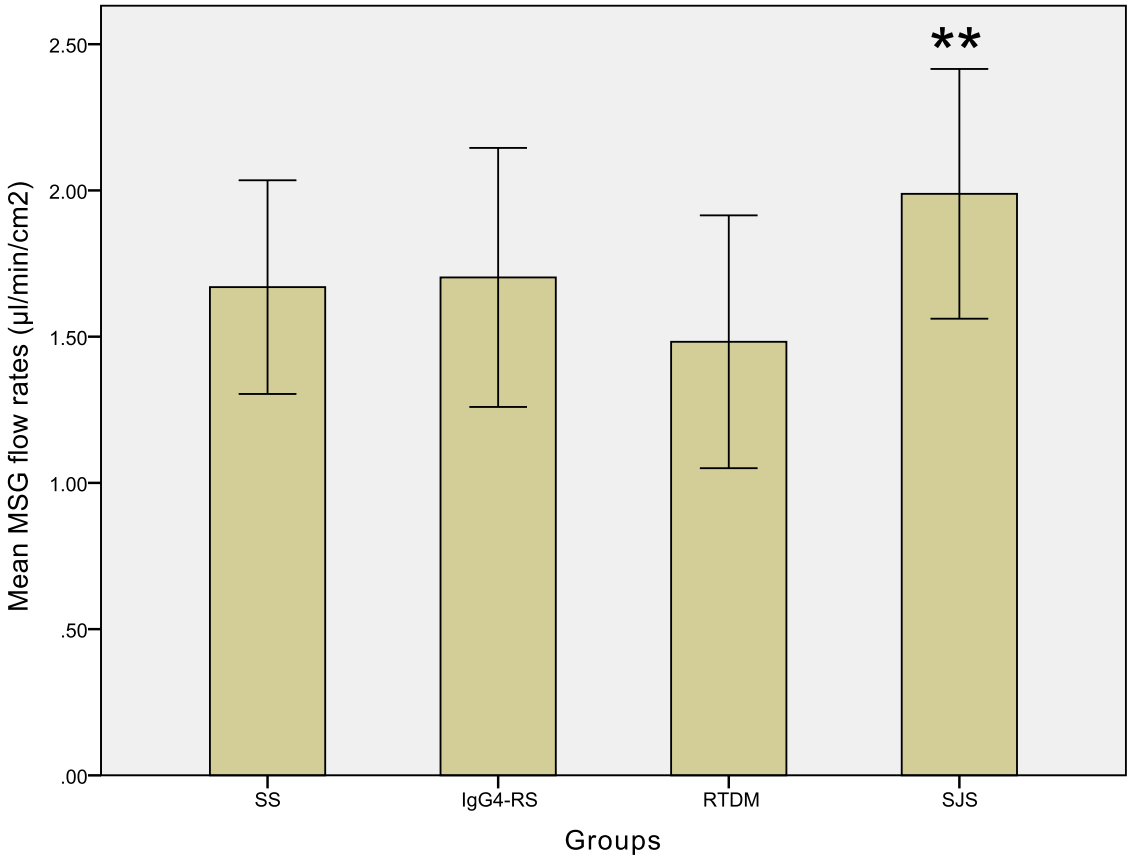

Fig. 2. Mean MSG flow rates in SS, IgG4-RS, RTDM and SJS patients.
*$P < 0.01$compared with SS group; Bar presents SD.

Table 6
Correlation between SXI scores and influencing factors.

Patient group			Sex	Age	Disease duration	WSFR at rest	WSFR under acid stimulation	WSFR under chewing stimulation	Mean MSFR	Num	Radiation dose
pSS	SXI	r		0.453**	−0.206	−0.291	−0.328*	−0.396*	−0.125		
		P		0.003	0.202	0.068	0.039	0.011	0.444		
IgG4-RS	SXI	r	0.196	−0.093	−0.004	−0.148	0.01	−0.138	−0.006		
		P	0.225	0.569	0.979	0.364	0.951	0.395	0.972		
SJS	SXI	r	−0.192	0.359*	0.158	−0.262	−0.199	−0.217	−0.067		
		P	0.235	0.023	0.331	0.103	0.219	0.179	0.679		
RTDM	SXI	r	0.09	0.037	0.253	−0.021	−0.045	−0.114	−0.029	0.141	−0.203
		P	0.58	0.821	0.115	0.899	0.785	0.483	0.858	0.386	0.21

Num represents the number of remaining major salivary glands. r represents Pearson's correlation coefficient. ** $P < 0.01$; * $P < 0.05$.

pSS, IgG4-RS, RTDM, and SJS are the most often causes of dry mouth in our hospital. Different degrees of dry mouth have been reported by patients with these diseases in different studies (Eliasson, Birkhed, Heyden, & Stromberg, 1996; Eliasson, Carlen, Laine, & Birkhed, 2003; Eliasson, Birkhed, & Carlen, 2009). The feeling of the oral dryness is usually measured with the SXI, which was developed by Thomson et al. in 2011 and displayed satisfactory psychometric properties (Thomson et al., 2011). The Chinese version of the SXI has been cross-culturally adapted in order to retain the psychometric properties of the original version (He et al., 2013). In the present study, we observed that patients with pSS, IgG4-RS, RTDM, and SJS exhibited mild, moderate, or severe dry mouth according to their SXI scores. RTDM and pSS patients had higher SXI scores than IgG4-RS and SJS patients, indicating that pSS and radiation exposure have more serious impact on salivary gland function.

In accordance with SXI scores, whole saliva flow rates and the relative percentage decrease of whole saliva flow rates at rest and under stimulation were more extensively affected in pSS and RTDM patients than in IgG4-RS and SJS patients. Decreased whole saliva flow rates have been widely reported in patients with pSS and radiation exposure (Blanco et al., 2005; Cheng, Wu, Kwong, & Ying, 2011); however, very few studies have assessed saliva secretion in patients with IgG4-RS and SJS. Our study observed that whole saliva flow rates under gum chewing or acid stimulation showed a step by step increase in pSS, IgG4-RS, and SJS patients, indicating that gustatory stimulation is stronger than mechanical stimulation in these diseases. The whole saliva flow rates were significantly higher under acid stimulation compared to under chewing stimulation, consistent with the previous observations that gustatory stimulation was stronger than mechanical stimulation in these diseases (Abelson, Barton, & Mandel, 1990; Risheim & Arneberg, 1993; Olsson, Spak, & Axell, 1991). However, there was no difference between the two types of stimulation in the RTDM group, possibly because gustatory sensations are impaired during radiation exposure. In the clinical setting, sugar-free chewing gum or throat lozenges are used to relieve dry mouth in these patients.

The functions of MSGs have not been extensively elucidated and there were very few studies focusing on MSG flow rates. Eliasson et al. studied pSS and RTDM patients and reported that flow rates of the labial glands, but not the buccal glands, were lower than those in the control groups (Eliasson et al., 2005). In our study, MSG flow rates were lower in most patients with severe (pSS and RTDM) or less severe (IgG4-RS and SJS) xerostomia than in the corresponding healthy controls. However, there were no significant differences in mean MSG flow rates between IgG4-RS, RTDM, and pSS groups. Similarly, there was no difference between mean relative decreases of MSG flow rates in four disease groups. Different cutoff points for MSG flow rates in dry mouth have been adopted. Although the available measuring techniques are simple, rapid, and noninvasive, it is difficult to establish a diagnosis index using MSG flow rates only because of large variations and low accuracy (Satoh-Kuriwada et al., 2012; Niedermeier & Huber, 1989). Furthermore, unlike the findings in a previous study, most mean relative percentage decreases in MSG flow rates were lower than the relative percentage decrease in whole saliva flow rates at rest or under stimulation (Satoh-Kuriwada et al., 2012). Similar results were reported in a previous study including patients consuming dry mouth-inducing drugs (Won et al., 2001). This may be explained by the fact that the saliva secreted by MSGs accounts for less than 10% of whole saliva (Dawes & Wood, 1973). Furthermore, there was no correlation between SXI scores and MSG flow rates in the four patient groups in our study, which indicates that MSG flow rate measurements cannot replace whole saliva flow rate measurements.

Radiation therapy for head and neck cancers generally includes co-irradiation of the major salivary glands and MSGs located beneath the oral mucosal surfaces (van de Water, Bijl, Westerlaan, & Langendijk, 2009). Previous studies reported serous cells were more sensitive to radiation damage than mucus cells (Beetz et al., 2013). Our results also showed MSG function was preserved better than major salivary gland function, indicating that improving the secretion of MSGs and decreasing the evaporation of minor gland saliva are potential measures to alleviate dry mouth (Hedner, Birkhed, Hedner, Ekstrom, & Helander, 2001; Smidt, Torpet, Nauntofte, Heegaard, & Pedersen, 2010; Rhodus, 1997; Niedermeier et al., 2000). In addition, the MSG flow rates and whole saliva flow rates are significantly stimulated by pilocarpine in patients with Sjögren's syndrome (Rhodus, 1997). This parasympathomimetic stimulation may be used to stimulate MSG secretion in patients with radiation-induced dry mouth. For severe cases of dry eye caused by SJS, when transplantation of MSGs is needed, it would be helpful to measure MSG flow rates before surgery to rule out contraindications and to choose proper donor site (Geerling et al., 2008).

In our previous study, we explored MSG flow rates in healthy individuals. The flow rates in 4 sites were recorded and the flow rate of buccal glands was the highest while there was no difference between flow rates of labial and palatal glands (Wang et al., 2015). In the present study, the same protocol was followed, and similar results were observed in the patients with pSS, RTDM, IgG4-RS or SJS, further indicating that the MSG function is preserved in these four disorders.

Dry mouth and hyposalivation represent subjective and objective aspects of salivary gland hypofunction. However, hyposalivation is not necessarily associated with dry mouth. It is widely accepted that dry mouth is a consequence of decreased whole saliva flow rates (Dawes, 1987). In pSS patients in our study, age and whole saliva flow rates under acid or chewing stimulation were correlated with SXI score, similar findings were observed in

previous studies (Suh, Lee, Chung, Kim, & Kho, 2007; Longman, McCracken, Higham, & Field, 2000). However, many patients with hyposalivation do not complain of dry mouth. In IgG4-RS, RTDM, and SJS patients, SXI scores were not related to whole saliva flow rates. Some investigators found oral dryness and salivary hypofunction are not quantitatively related to whole saliva (Hochberg et al., 1998; Narhi, Meurman, & Ainamo, 1999; Nederfors, 2000; Lofgren, Isberg, & Christersson, 2010; Spielman, Ben-Aryeh, Gutman, Szargel, & Deutsch, 1981) or major gland saliva (Fox, Busch, & Baum, 1987). Possible reasons may be large variations in patients' discomfort thresholds and tolerance or adaptation over time. In RTDM patients, a clear relationship was found between the mean oral cavity radiation dose and patient-rated dry mouth (Eisbruch et al., 2001). In contrast, the dose distributions to the oral cavity or MSGs were found to have limited significance in the development of dry mouth (Beetz et al., 2013; Jellema, Doornaert, Slotman, Leemans, & Langendijk, 2005). However, these studies reported dose distributions to MSGs, instead of MSG flow rates. These apparently conflicting results may be due to differences among studies with regard to the manner of delineating the oral cavity.

Our study has some limitations. First, although the SXI helped in differentiating the degree of dry mouth in different patient groups, additional cases are required to test its ability to differentiate different forms of dry mouth within one subscale. Moreover, the SXI does not include important questions pertaining to mouth dryness at night, speech, sleep, and quality of life, which was mostly included in the original version of XI. However, the original XI is not validated in Chinese. Further studies should modify this assessment instrument (Dijkema et al., 2012; Dirix, Nuyts, Vander, Delaere, & Van den Bogaert, 2008). Second, proteins of saliva, which play an important role in dry mouth (Nederfors, 2000), were not assessed in our study. Further studies including qualitative and quantitative assessments of salivary proteins to explore the role of salivary proteins in the saliva secreted by MSGs in the development and the sensation of dry mouth are necessary.

5. Conclusions

In conclusion, whole saliva flow rates and MSG flow rats are significantly decreased in patients with pSS, IgG4-RS, RTDM, and SJS. Compared to the reduction in whole saliva flow rates, MSG flow rates are less impacted in these conditions. Further investigations on the changes in saliva components are necessary for clarifying whether MSG saliva plays a role in moisturizing and lubricating the oral surface tissues.

Conflict of interests

There is no conflict of interest.

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Ethics approval

This study "Characterictics of flow rates of minor salivary glands in patients with hypofunction of salivary glands" was conducted with the approval of Peking University School and Hospital of Stomatology on June 18, 2014.

Author contributions

Zhen Wang did clinical work, acquisition of data, analysis and interpretation of data, drafted the article and gave final approval of the version to be published. Wei Li, Xia Hong and Jia-Zheng Su took part in collecting the patients and analysis and interpretation of data. Hong Hua, Xin Peng and Lan Lv took part in design of work and interpretation of data. Guang-Yan Yu made substantial contributions to conception and design of work and took part in interpretation of data, revised the article critically and gave final approval of the version to be published.

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