Clinical and histopathologic characteristics of submandibular gland in Stevens-Johnson syndrome: A comparative study

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Objective. The objective of this study was to investigate the clinical manifestations and pathologic appearances of the submandibular gland (SMG) in Stevens-Johnson syndrome (SJS).

Study Design. Patients with autologous transplantation of SMG for treatment of severe dry eye between March 1998 and May 2018 were divided into the SJS group (70 cases) and non-SJS group (50 cases) according to the history of SJS. The SMG weight and computed tomography volume and salivary flow rate were measured. The concentration index and secretion index were estimated using scintigraphy with technetium-99m-pertechnetate. Histopathology studies of SMG tissues were conducted, and the acini parameters were measured using a digital image analyzer.

Results. A decreased computed tomography volume and weight was observed in 48.57% the SJS group and 2% in the non-SJS group ($P < .01$). The rest whole, acid-stimulated whole, and SMG rest salivary flow rates decreased in the SJS group ($P < .05$). The normal SMG concentration index (37.5% vs 96.67%, $P < .001$) and secretion index (35% vs 96.67%, $P < .001$) rates were lower in the SJS group than in the non-SJS group. The glandular parenchyma was reduced, the acinar space was widened, and the fat content was increased in the SJS group.

Conclusion. SMG atrophic and degenerative changes occurred in the SJS group, with a decrease in salivary secretion function in more than half of the patients. (Oral Surg Oral Med Oral Pathol Oral Radiol 2022;133:326−332)

Stevens-Johnson syndrome (SJS) is a serious but rare adverse drug reaction involving the skin and mucosa.[1-3] It is a systemic disease involving multiple systems and organs, such as the liver, kidney, lacrimal gland, etc.[4]

It is reported that 40% of SJS survivors have "Sjögren-like syndrome" and experience dry mouth and dry eyes.[5,6] Lymphocytic infiltration in the lip glands of these patients indicates that the pathogenesis of toxic epidermal necrosis and dissolution may be related to an autoimmune phenomenon.[5] Some studies have shown mild acinar atrophy, lymphocyte infiltration, and fibrosis in the lacrimal glands.[7] The content of saliva may also change. Chosidow et al. reported that the acidity of saliva became stronger, the buffering capacity decreased, and the viscosity of saliva was abnormal.[8] In our previous preliminary study, we observed some extent of impairment in salivary gland function. Additionally, histologic examination showed atrophic changes in the submandibular gland (SMG).[9] However, the morphologic and functional characteristics of the involved salivary glands are still unexplored and require further elucidation.

In this study, we compared the clinical manifestations and histopathologic appearances of the SMGs in patients with SJS and patients without SJS who had

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Statement of Clinical Relevance

Dry mouth and dry eyes are common sequelae of Stevens-Johnson syndrome. Histomorphological changes and decreased salivary function occurred in the salivary glands of patients with Stevens-Johnson syndrome.

received microvascular autologous transplantation of SMG for treatment of severe dry eye.

MATERIALS AND METHODS
Patient selection
This study was approved by the ethics committee of the Peking University Health Science Center (IRB00001052-08048) and conducted in accordance with the Declaration of Helsinki guidelines for human research.

Patients who received microvascular autologous transplantation of SMG for treatment of severe dry eye at the Department of Oral and Maxillofacial Surgery, Peking University School of Stomatology between March 1998 and May 2018 were enrolled in this study. Patients were divided into 2 groups, the SJS and non-SJS groups, according to the history of SJS episodes. The inclusion criteria for the SJS group were as follows: (1) serious drug allergy symptoms that occurred within 4 to 28 days after medication and (2) extensive skin detachment as well as high fever, malaise, and a rapidly developing blistering exanthema of macules and target-like lesions accompanied by mucosal involvement, with clinical morphology fulfilling Roujeau's diagnostic criteria.[3,10,11] The control group included patients without a history of SJS in whom severe dry eye only resulted from local factors such as keratoconjunctivitis. We excluded patients with systemic diseases such as Sjögren syndrome.

All patients provided informed consent for the surgical treatment and related examinations. A total of 185 patients were enrolled in this study (Figure 1). The indications, contraindications, and surgical procedures of the techniques were the same as previously described.[12] The SJS group included 70 patients, including 37 male and 33 female patients, aged between 7 and 70 years, with a median age of 34 years. The drug-induced adverse reactions were as follows: sulfanilamide in 23 cases, cephalosporin in 8 cases, penicillin in 5 cases, nonsteroidal anti-inflammatory and analgesic drugs in 4 cases, others in 7 cases, and unclear in 23 cases. Patients with unclear etiology who could not be included as control participants were excluded. Fifty patients were included in the non-SJS group, including 19 male and 31 female patients, aged

Fig. 1. Patient with severe dry eye due to Stevens-Johnson syndrome.

between 9 and 69 years, with a median age of 32 years. The male:female ratio in the SJS and non-SJS groups was 1.12:1 and 0.61:1, respectively.

Measurement of the weight and computed tomography volume of submandibular glands
A computed tomography (CT) study was performed using an 8-slice scanner (BrightSpeed; GE Medical Systems, Piscataway, NJ). Volumes of the SMGs were reconstructed by volume rendering[13,14] and compared with normal values reported previously[15] (Table 1). Glandular atrophy was diagnosed if the volumes were smaller than the normal values. CT images were assessed by an experienced radiologist and a maxillofacial surgeon (Z-PS and Y-PW).

Evaluation of the secretory function of the SMG
Measurement of salivary flow rate. Before saliva collection, patients were instructed to rinse their mouths with water and rest for 5 minutes. Their heads were tilted slightly forward. Using the spitting method, the whole saliva at rest was collected for 5 minutes into a preweighed cup. After 5 minutes of rest, the stimulated whole saliva was collected by smearing 2.5% citric acid solution on the lateral side of the tongue with a swab every 30 seconds for another 5 minutes.[16,17] A modified Wolff T device was used to collect saliva secreted by the SMG.[18] The salivary flow was calculated from the weight of saliva assuming 1 g equal to 1 mL.

Technetium-99m scintigraphy. Before surgery, each patient underwent scintigraphy using a standardized protocol.[19] The patient was positioned supine under a γ-camera (Starcam 4000 I; GE Healthcare) and a low-energy, high-resolution, parallel-hole collimator was centered over the face. Immediately after intravenous injection of 185 MBq of technetium-99m-pertechnetate, sequential 1-minute images were acquired for 30 minutes. After 20 minutes, approximately 0.5 mL of citric acid was applied to accelerate salivary secretion. Digital data on the uptake secretion of pertechnetate were collected simultaneously using an online computer system. Time-activity curves were calculated using manually drawn oval regions of interest around both SMGs.[20]

The concentration index (CI) was calculated using the following formula:

CI = (maximum uptake value − background)/background.

The period from stimulation using 2.5% citric acid solution to the minimum value after stimulation within 30 minutes was considered to be the "secretion phase." The secretion index (SI) was calculated using the following formula:

Table 1. Quantitative measurements of the weight and the CT volumes of SMG in SJS and non-SJS groups and control participants.

	Male			Female		
Age(years)	Normal (n = 90)	Non-SJS (n = 50)	SJS (n = 70)	Normal (n = 90)	Non-SJS (n = 50)	SJS (n = 70)
CT volumes/cm^3						
<25	9.48 ± 2.42	9.38 ± 2.22	6.45 ± 2.23*	7.75 ± 1.39	7.73 ± 1.54	6.27 ± 1.25*
25-44	10.22 ± 2.13	9.15 ± 2.12	6.97 ± 1.45[†]	8.24 ± 1.21	7.86 ± 1.67	6.22 ± 1.52[†]
45-60	9.97 ± 1.40	9.81 ± 1.47	6.12 ± 1.30[†]	8.10 ± 1.48	8.77 ± 1.36	5.44 ± 2.03[†]
Weight (g)						
<25	12.29 ± 2.82	12.37 ± 2.33	9.05 ± 2.43[†]	10.86 ± 2.07	10.75 ± 1.94	8.45 ± 1.94[†]
25-44	13.25 ± 2.54	13.03 ± 2.16	9.71 ± 1.55[†]	11.31 ± 1.77	11.46 ± 1.72	8.91 ± 1.23[†]
45-60	12.88 ± 1.99	12.64 ± 1.88	8.99 ± 1.82[†]	10.72 ± 2.09	10.87 ± 1.93	7.76 ± 1.57[†]

Data presented as mean ± standard deviation.
CT, computed tomography; *SMG*, submandibular gland; *SJS*, Stevens-Johnson syndrome.
*Significantly different compared with the normal group, $P < 0.05$.
†Significantly different compared with the normal group, $P < 0.01$.

SI = (maximum value before stimulation using citric acid − minimum value after stimulation using citric acid)/(maximum value before stimulation using citric acid − background) × 100%.

The mean values of the bilateral sides were taken to be the CI and SI of the SMG.

Histopathologic studies. During transplantation of SMGs, a small piece of gland tissue with a 5 mm diameter was harvested from 19 patients in the SJS group and 17 patients in the non-SJS group for histopathologic studies. The gland specimens were fixed in neutral formalin and embedded in paraffin. Serial 5-μm slices were cut and stained with hematoxylin and eosin.

One of 5 slides from each gland was studied by randomly choosing 3 fields with squared areas of 348,657.025 μm^2. The following parameters were measured and calculated: Maximum diameter of the acinus, the ratio of glandular parenchyma to the whole area of each field, and proportion of serous acinus and mucous acinus. All variables were calculated using ImageJ (v1.8.0, American, National Institutes of Health).[21,22]

Statistical analysis
Statistical analyses were performed using the Statistical Package for the Social Sciences (v20.0; SPSS, Chicago, IL). Qualitative variables were expressed as absolute numbers and percentages and analyzed using the chi-square test. Quantitative variables were expressed as means ± standard deviation or median (interquartile range), depending on the normality of the distribution, as assessed using the Kolmogorov-Smirnov test. Differences between the 2 groups were examined using the Mann-Whitney U test or Student t test. Differences between more than 2 groups were examined using the Kruskal-Wallis test. Statistical significance was set at $P < .05$.

RESULTS
The weight and CT volume of submandibular glands
As observed during the operation, the gross appearance of the SMG size in the SJS group was smaller than that in the non-SJS group (Figure 2). The weight and CT volume of the SMG are presented in Table 1. Compared with the normal references of glandular CT volumes[15,20] and glandular weight,[23] 34 out of 70 cases (48.57%) in the SJS group showed decreased values of SMG for both, whereas only 1 out of 50 cases (2%) in the non-SJS group showed decreased values of SMG for both ($P < .01$ in both cases). However, no significant differences in glandular weight and glandular CT volumes were found between the non-SJS group and control patients.

The changes of secretory function of the SMG
Salivary flow rate. Salivary flow rates were examined in 50 patients in the SJS group and 40 patients in the non-SJS group. An obvious decrease in rest whole salivary flow rate (0.31 ± 0.21 vs 0.42 ± 0.25; $P < .05$), acid-stimulated whole salivary flow rate (1.76 ± 1.32 vs 2.54 ± 1.55; $P < .05$), and rest salivary flow rate of SMG (0.09 ± 0.04 vs 0.16 ± 0.06; $P < .001$) in the SJS group compared with the non-SJS group was
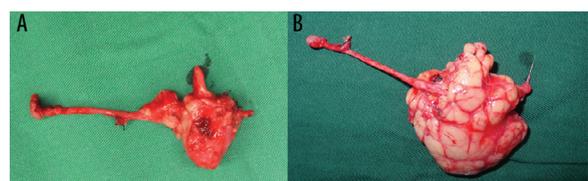

Fig. 2. The gross appearance of the submandibular gland in the (A) Stevens-Johnson syndrome group showed obvious decreased size, compared with that in the (B) non−Stevens-Johnson syndrome group.
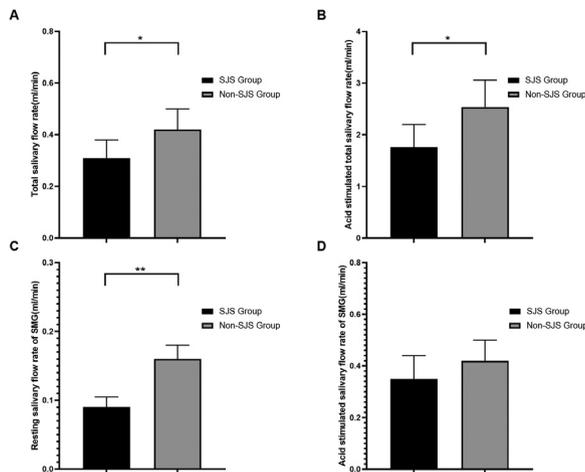
Fig. 3. Salivary flow rate of Stevens-Johnson syndrome (SJS) group and non-SJS group: (A) whole salivary flow rate; (B) acid-stimulated whole salivary flow rate; (C) rest salivary flow rate of submandibular gland; (D) acid-stimulated salivary flow rate of submandibular gland.

observed. However, no significant differences were found between the SJS and non-SJS groups in acid-stimulated salivary flow rate of SMG (0.35 ± 0.27 vs 0.42 ± 0.24; $P = .197$) (Figure 3, Table 2).

Scintigraphic results. The SMG curve in the non-SJS group showed an early slow-rising uptake followed by a sharp decline after acid stimulation (Figure 4). The percentage of patients with a normal CI rate in the SJS group (15/40, 37.5%) was much lower than that in the non-SJS group (29/30, 96.67%; $P < .001$) Similarly, the percentage of patients with a normal SI rate in the SJS group (14/40, 35%) was much lower than that in the non-SJS group (29/30 patients, 96.67%; $P < .001$) (Figure 5).

Histomorphologic changes. The histologic appearances of the SMG also showed obvious atrophic changes,

Table 2. Comparisons of various salivary flow rates between SJS and non-SJS groups.

Salivary flow rate*	SJS (n = 50)	non-SJS (n = 40)	P Value
Whole salivary flow rate	0.31 ± 0.21	0.42 ± 0.25	.029
Acid-stimulated whole salivary flow rate	1.76 ± 1.32	2.54 ± 1.55	.013
Rest salivary flow rate of SMG	0.09 ± 0.04	0.16 ± 0.06	<.001
Acid-stimulated salivary flow rate of SMG	0.35 ± 0.27	0.42 ± 0.24	.197

SJS, Stevens-Johnson syndrome; *SMG*, submandibular gland.
*Data presented as mean $\pm$ standard deviation (mL/min).

although their architecture remained intact. The glandular parenchyma was reduced, the acinar space was widened, and the fat content was increased (Figure 6). No obvious histologic changes in the SMG were observed in the non-SJS group. Furthermore, the histomorphometric study showed that the glandular parenchyma (63.76 ± 17.34 vs 73.1 ± 17.06; $P < .01$) of the SMG in the SJS group decreased, whereas no significant difference in the proportion of serous acini and mucinous acini (15.56 ± 8.20 vs 14.90 ± 7.15, $P > .05$) or the maximum diameter of acini (72.93 ± 21.93 μm vs 68.16 ± 30.32 μm, $P > .05$) was found between the 2 groups.

DISCUSSION

In the present study, we compared the clinical manifestations and pathologic appearances of the SMGs between 70 patients in the SJS group and 50 patients in the non-SJS group. The case-control study demonstrated that histomorphologic changes occurred in the SMG and that the salivary secretion function of the SMG was impaired to some extent in patients with SJS. This indicated that the SMG is one of the organs affected by SJS, and clinicians should pay more attention to this phenomenon.

SJS is a systemic disease involving multiple organs, such as the mucosa in the digestive, urogenital, and respiratory systems and skin. Most of the involved organs show acute symptoms and signs. Although the involvement of salivary glands seldomly shows symptoms and signs of acute serious adverse drug reactions, chronic long-term damage to the gland is frequently observed. Therefore, damage to the salivary gland remains unattended.

There are various types of non-tumor salivary gland diseases, such as Sjögren syndrome, IgG4-related sialadenitis, and chronic obstructive sialadenitis. Most of them are inflammatory diseases and histopathologically show lymphocytic or lymphoplasmatic infiltrate and lymphoid follicle formation. The SMGs damaged in SJS show atrophic and degenerative changes, including decreased weight and size of the gland, reduced glandular parenchyma, widened acinar space, and increased fat content. No obvious inflammatory cell infiltration was observed in the damaged SMG in SJS. This indicates that the involvement of the SMG in SJS is different than that in the inflammatory salivary gland diseases.

The SMG is an important functional organ of the body. The saliva secreted by the SMG accounts for 65% of the whole rest saliva.[24] With morphologic changes of the gland, such as atrophy and degeneration of the SMG, the salivary secretion function of the gland
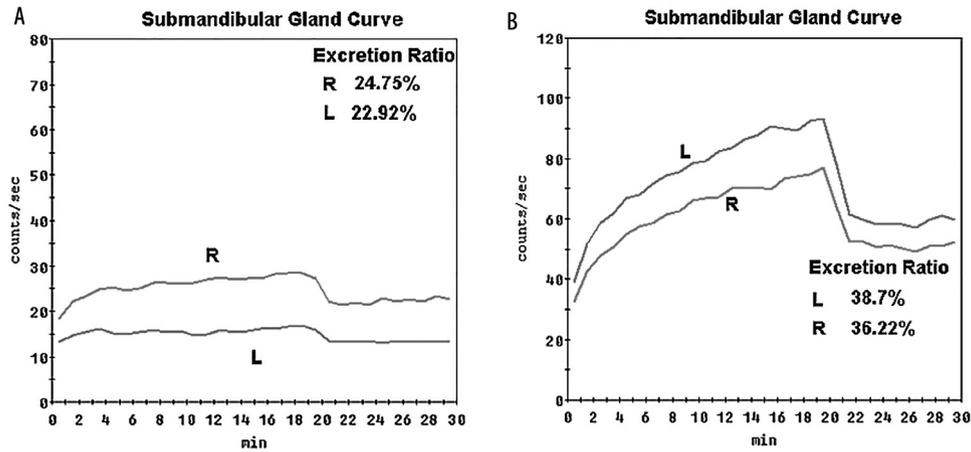
Fig. 4. Dynamic time-activity curve obtained in different patients. (A) Patient in the Stevens-Johnson syndrome group and (B) patient in the non−Stevens-Johnson syndrome group.

decreases. The results of our study using scintigraphy showed that the resting salivary flow rate, uptake, and secretion indices, the most important indices for evaluation of salivary gland functions, obviously decreased in the SJS group compared with those of the non-SJS group. The extent of the decrease in salivary secretion function varies from total loss to almost normal depending on the extent of damage to the glandular tissues.[18] In our study, the resting salivary flow rate of the SMG decreased in the SJS group, although no significant difference in the stimulated salivary flow rate of the SMG was observed between the SJS and non-SJS groups. These results are related to 2 aspects. First, all patients included in our study met the indication for SMG transplantation for the treatment of severe dry eye. Patients with severe damage to the secretion function were excluded from the study. Second, only the rest salivary flow rate decreases during the early stage of damage to the salivary glands. Both rest and stimulated salivary flow rates decreased when severe damage occurred in the salivary glands.

The results of our study deserve further attention in clinical practice. The salivary gland may be one of the most commonly involved organs in SJS. Parotid glands and SMGs should be examined in patients with SJS, when necessary, and evaluation of salivary secretion function should be performed. Damage to the salivary gland in SJS may lead to xerostomia. A history of SJS should be included during the collection of clinical data on patients with dry mouth. SJS is one of the most common causes of severe dry eye disease. Treatment for severe dry eye through SMG transplantation mandates evaluation of SMG secretion function as a critical preoperative procedure for determining whether the patient meets the indications and to select appropriate donor SMGs.

There are some limitations of our study. All patients underwent SMG transplantation for severe dry eye. Patients with severe SMG damage caused by SJS, which is a contraindication for SMG transplantation, were excluded from our study. Data on their salivary flow rate, technetium-99m scintigraphy, and gland tissues could not be obtained in this study. Most patients included in this study displayed acute allergic reactions and recovered after a certain period. The acute damage to the SMG in SJS requires further study.
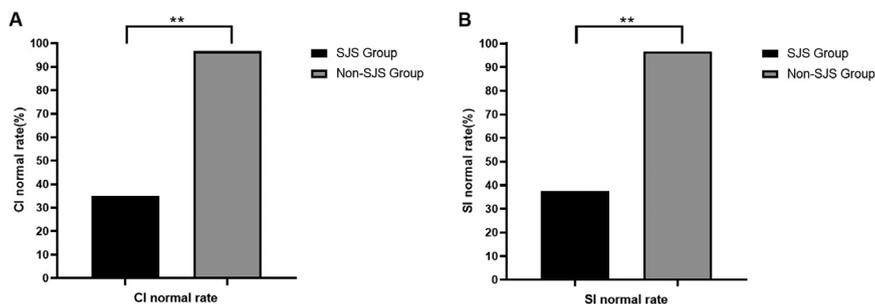

Fig. 5. The percentage of patients with normal CI (35% vs 96.67%, $P < 0.001$) and normal response to acid stimulation (37.5% vs 96.67%, $P < 0.001$) of the submandibular gland in the (A) Stevens-Johnson syndrome (SJS) group compared with the (B) non-SJS group.
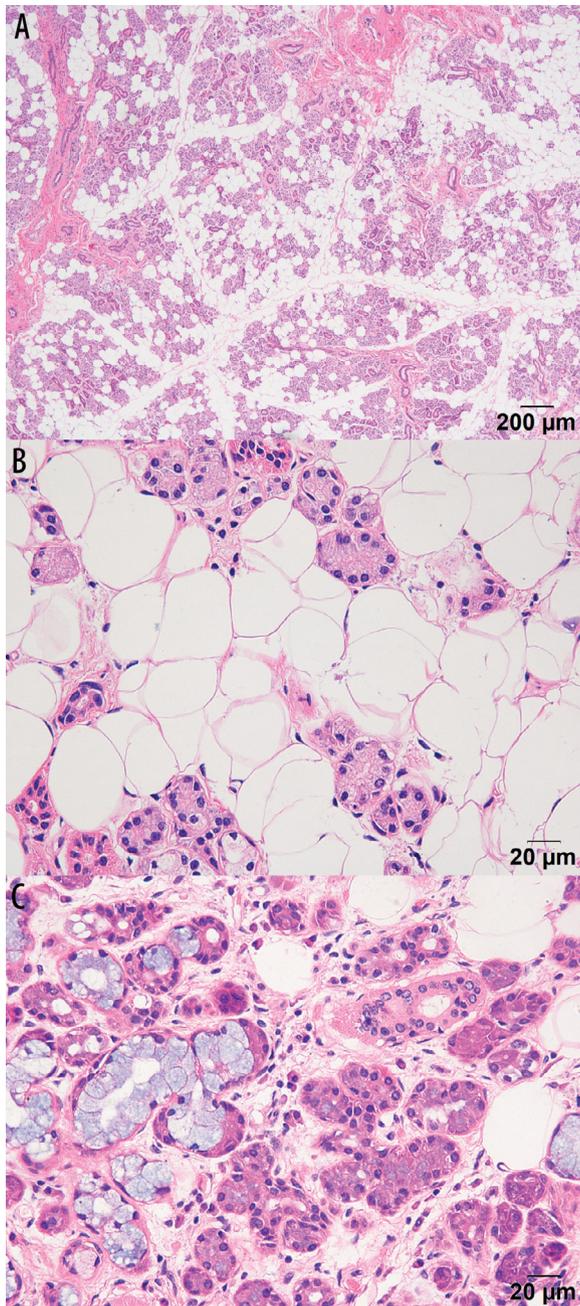
Fig. 6. Histologic appearances of the submandibular gland showed obvious atrophic changes although their architectures remained. (A) The fat content increased, (B) the glandular parenchyma was reduced, and (C) the acinar space was widened. (A) Scale bar = 200 μm; (B), (C) scale bar = 20 μm.

CONCLUSIONS

The SMG is one of the organs damaged in SJS. Atrophic and degenerative changes occur in the SMG of patients with SJS. Decreased salivary secretion function occurs in approximately half of patients.

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