A screening test for capsaicin-stimulated salivary flow using filter paper: a study for diagnosis of hyposalivation with a complaint of dry mouth

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Objective. The purpose of this study was to develop a simple screening technique for diagnosis of hyposalivation with dry mouth by estimation of capsaicin-stimulated salivary flow using filter paper.

Study design. An assay system comprising 5 spots containing starch and potassium iodide on filter paper incorporating or without capsaicin and a coloring reagent was designed. We investigated whether the number of colored spots using the filter paper incorporating capsaicin could distinguish between healthy subjects and subjects with hyposalivation and dry mouth.

Results. In the healthy group (>200 μL/min; n = 33), the capsaicin-stimulated salivary flow significantly increased as compared with the resting salivary flow, from 1.2 ± 1.4 to 2.9 ± 1.3 colored spots (P < .05). In contrast, the hyposalivation group with dry mouth (<100 μL/min; n = 32) hardly changed (4.4 ± 1.0 vs 4.9 ± 0.2), except for 3 subjects who had considerable elevated secretion on capsaicin stimulation.

Conclusion. By measuring resting and stimulated salivary flows, this method should be useful for evaluating retained functional ability of salivary glands and screening of hyposalivation with dry mouth. (Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2011;112:73-80)

It is well known that saliva plays an important role in maintaining oral health and correct oral function. Salivary hypofunction is caused by various factors, including aging and the use of drugs (e.g., diuretics, antidepressants neuroleptics), whereas other causes include diseases, mainly Sjögren’s syndrome, sarcoidosis, chronic graft-versus-host disease, and neck and head radiotherapy.1-4 On the other hand, although dry mouth is the conventional term used to denote the subjective complaint of oral dryness, it is positively correlated with a decrease in salivary flow.5,6 Decreased salivary flow is often associated with many signs and unpleasant symptoms, such as masticatory dysfunction, swallowing disorders, deterioration of dental caries and periodontal diseases, halitosis, ill-fitting dentures, taste and speech disorders, pain, and a burning sensation in the oral mucosa.7,8 Therefore, early diagnosis of decreased salivary flow is important for promotion of both oral health and overall health. In general, it is well known that the resting salivary flow rate is more important than the stimulated flow rate as a determinant of oral dryness,9,10 although the association between resting salivary flow and stimulated salivary flow was corroborated by several studies.11-13 In contrast, Valdez and Fox4 pointed out that, among patients with dry mouth with very low salivary flow rates, the stimulated salivary flow rate, which reflects the functional capacity of the salivary gland, can be a more meaningful indicator.

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of the extent of gland dysfunction. In fact, a recent study suggests that the secretion rate of stimulated saliva is correlated with the severity of dry mouth–related symptoms,14 although Smidt et al.15 reported that patients with systemic diseases who had low stimulated salivary flow rates did not always have low resting salivary flow rates. These reports suggest that it is very important to estimate both resting salivary and stimulated salivary flow at one time for screening of hyposalivation with a complaint of oral dryness. Regarding the diagnosis of decreased salivary flow, there are already several easier, validated methods, including measurement of resting salivary flow, such as the Oral Schirmer test,16 drooling method,17 and stimulated salivary flow of the Saxon test.18 However, the Saxon test is time-consuming for collecting saliva and requires a special apparatus, and the Schirmer test is now available only for resting salivary flow. Previously, we devised a novel, simple, and safe method for estimation of resting salivary flow using filter paper, based on the iodine-starch reaction and paper chromatography.19 However, this method is not available for estimation of the stimulated salivary flow. Considering this, we first attempted to improve the accuracy for estimation of resting salivary flow by changing the number of spots and distance between the spots on the filter paper reported previously, and, second, to develop a novel, simple, and safe method for evaluation of the stimulated salivary flow using filter paper incorporating chemesthetic and gustatory stimuli, such as capsaicin, nicotine, and citric acid.20 We also tried to evaluate the association of capsaicin-stimulated salivary flow and resting salivary flow at one time in a hyposalivation group with complaints of dry mouth and a healthy group, so as to clarify the usefulness for screening of hyposalivation with complaints of dry mouth.

MATERIAL AND METHODS

Subjects
A total of 180 subjects (142 women and 38 men, 19-76 years of age), including those with complaints of oral dryness and voluntary patients, were enrolled in the study and analyzed in the Department of Preventive Dentistry, Graduate School of Dental Medicine, Hokkaido University, Sapporo; Wakayama Medical University, Wakayama; and JR Sapporo Hospital, Sapporo, Japan. Among these individuals, 157 subjects were enrolled to confirm the accuracy of the relationship between the number of colored spots and resting salivary flow rate. Another 80 subjects were enrolled to analyze both resting and capsaicin-stimulated salivary flows and categorized into 3 groups by the resting whole salivary flow rate: a group with hyposalivation with dry mouth (<100 μL/min; n = 32, 26 women and 6 men, 19-75 years of age), a group with medium hyposalivation (100-200 μL/min; n = 15, 11 women and 4 men, 24-61 years of age), and a healthy group (>200 μL/min; n = 33, 26 women and 7 men, 19-76 years of age). Thirty-two subjects who had hyposalivation with dry mouth had already undergone oral examination, a hyposalivation test (resting salivary flow rate <100 μL/min), and testing for diagnosis of sjögren’s syndrome.9 They were composed of patients with sjögren’s syndrome (n = 1), systemic lupus erythematosus (n = 2), scleroderma (n = 1), and other patients (n = 18) having hyposalivation with a complaint of dry mouth. Hyposalivation was defined as a resting salivary flow rate of less than 100 μL/min, according to the criteria proposed by erisson and hardwick,21 and medium hyposalivation was defined as a resting salivary flow rate of 100 to 200 μL/min. Subjective oral dryness was also evaluated by the question “Does your mouth usually feel dry?”22 This project was approved by the Ethics Committee of the Hokkaido University Graduate School of Dental Medicine and local committees based on the Declaration of helsinki. All subjects gave their informed consent to participate.

Collection of saliva
Resting whole saliva was collected after asking the subjects to sit in a quiet room, with the head slightly down, not swallowing, but spitting the accumulated saliva into a sterile polypropylene conical tube (30 × 115 mm; Becton Dickinson, Franklin Lakes, NJ, USA) for 10 minutes, as described previously.19 The volume of saliva was estimated by weighing the tube before and after collection, assuming the specific gravity of saliva to be 1.0 g/cm³. Individual saliva was centrifuged at 10,000 rpm for 10 minutes and the supernatant was used for in vitro experiments.

Preparation of filter paper for estimation of resting salivary flow
The filter paper for estimation of the resting salivary flow was made using soluble starch, potassium iodide, and chromatography paper, except for 5 spots containing 30 μg of starch and 49.6 μg of potassium iodide per spot on the filter paper (Fig. 1, A), as described previously.19

Preparation of filter paper for estimation of stimulated salivary flow rate
Capsaicin (Wako Pure Chemical Industries, Ltd, Tokyo, Japan), nicotine (Sigma-Aldrich Co, St. Louis, MO, USA), and citric acid (Kanto Chemical Co. Inc., Tokyo, Japan) were used as chemesthetic and gustatory stimuli for estimation of the stimulated salivary flow. The tip of the filter paper that was prepared for estima-
tion of the resting salivary flow was saturated with 60 μL of capsaicin or nicotine solution dissolved in ethanol, or 40 μL of citric acid solution dissolved in distilled water. The papers were allowed to dry at room temperature for 1 hour and then tightly wrapped with aluminum foil and kept at 4°C to prevent further loss of the stimulus.

Detection of capsaicin distributed on the paper
The detection of capsaicin was carried out based on the traditional color reaction of phenol-ferric chloride. For this, 1% ferric chloride solution was sprayed on the paper and then allowed to color at room temperature, purple indicating the presence of capsaicin. The colored area was measured as the size of capsaicin distributed on the paper.

Coloring reagent
The coloring reagent was prepared from a solution of 31% hydrogen peroxide (Kanto Chemical Co. Inc.), ethyl alcohol (Kanto Chemical Co. Inc.), and distilled water at a ratio of 1:7:1, as described previously.

Measurement of the number of colored spots on the filter paper
The number of colored spots on the filter paper was measured by addition of a coloring reagent, as described previously. To simulate clinical examination at first glance, the number of colored spots was scored using a scale of a nearly entirely colored spot = 1, a partly colored spot = 0.5, and a colorless spot = 0. For the estimation of resting and stimulated salivary flow, after sampling resting saliva, subjects were asked to open their mouths and lift the tongue. The tip of the paper was put on the center of the floor of the mouth in the sublingual region. The edge of the paper was lightly held by the subject or investigator if the paper seemed to move during the 2-minute collection, except when otherwise specified. After 2 minutes, the filter paper was taken out and then the coloring reagent (approximately 100 μL) was added dropwise to the spots on the filter paper.

Statistical analysis
Spearman’s test and Kruskal-Wallis 1-way analysis of variance were used for the statistical analysis. Data are expressed as mean ± standard deviation. Regression analysis was used to determine the significance of the correlation between 2 changes. Differences were considered significant at \( P < .05 \).

RESULTS
Relationship between number of colored spots and saliva volume in vitro. To investigate the correlation between the number of colored spots and the corresponding volume of saliva, the paper was dipped into a known volume of saliva prepared from the individual subject. After 2 minutes of infiltration, addition of the

Fig. 1. Correlation between number of colored spots on the paper and saliva volume in vitro. A, For estimation of the salivary flow the filter paper was spotted in 5 places as described in the text. B, The filter paper was dipped into a known volume using individual saliva. After 2 minutes of infiltration, the colored spots produced by addition of the coloring reagent were measured on the paper (inset). The number of colored spots is expressed as mean ± SD of 10 samples from individual saliva. (\( n = 10, r = -0.981, P < .001 \)).
coloring reagent to colorless spots of the paper caused them to suddenly turn dark blue and then blue within a few minutes. As shown in Fig. 1, B, 5 colored spots were observed in less than 100 μL of saliva, whereas no colored spots were seen in excess of 500 μL. When the number of colored spots was scored using a scale of a nearly entirely colored spot = 1, a partly colored spot = 0.5, and a colorless spot = 0, a significant negative correlation was observed between the number of colored spots and the saliva volumes of 100 μL and 500 μL (n = 10, r = −0.981, P < .001; Fig. 1, B). These results showed that the assay system was reliable for quantitative determination of the saliva volume. However, a viscous saliva sample among them showed a partly colored spot in spite of being in excess of 500 μL, because viscosity seemed to interfere with infiltration of saliva by capillary action in the paper chromatography and subsequently saliva could not completely penetrate into the paper in 2 minutes.

**Correlation between number of colored spots and resting salivary flow rate.** Having shown that measurement of the number of colored spots reflected saliva volume, we next examined the relationship between the resting whole salivary flow rate and the number of colored spots at 2 minutes after insertion of the paper into the sublingual region in healthy subjects and those with complaints of decreased salivary flow. As shown in Fig. 2, when the number of colored spots was scored individually, a significant negative correlation was observed between the number of colored spots and the resting whole salivary flow rate (n = 157; r = −0.783, P < .001).

**Comparison of salivary flow after stimulation with capsaicin, nicotine, and citric acid.** We next tried to estimate the stimulated salivary flow using filter papers incorporating the chemesthetic and gustatory stimuli capsaicin, nicotine, and citric acid. When the tip of paper incorporating capsaicin was put on the center of the floor of the mouth in the sublingual region, capsaicin caused a significant transient rise in salivary secretion in a dose-dependent manner, leading to a decreased number of colored spots (Table 1). However, capsaicin also induced a burning/pricking sensation at more than 2.4 μg. The nicotine (120 μg) also induced a sensory effect similar to the capsaicin, but it elicited an unpleasant taste. On the other hand, citric acid, one of the gustatory stimuli, stimulated salivary secretion not associated with any irritant, unlike the capsaicin and nicotine. However, the stimulation-response required a high concentration of citric acid, more than 2000 μg, which interfered with the iodine-starch reaction. Consequently it made it difficult to estimate the colored spots. Based on these results, 1.4 μg of capsaicin was chosen and used as the stimulant for estimation of the stimulated salivary flow in further experiments. The area of capsaicin distributed on the tip of the paper was shown to be uniform in size (10.3 × 21.0 mm) by colorization using ferric chloride solution, as shown in Fig. 3.
Differences of capsaicin-stimulated salivary flow in the groups having hyposalivation with dry mouth, medium hyposalivation, and healthy subjects. As described previously, the assay system using filter paper, which is based on the theory of paper chromatography moving potassium iodide dependent on saliva volume, showed a correlation between the number of colored spots and salivary volume. Therefore, we further investigated the relationship between the number of colored spots and capsaicin-stimulated salivary flow using filter paper incorporating 1.4 µg of capsaicin to clarify whether the number of colored spots on the filter paper incorporating capsaicin could easily distinguish between subjects with hyposalivation with complaints of dry mouth and healthy subjects. As shown in Fig. 4, the capsaicin-stimulated salivary flow in the healthy group significantly increased as compared with the resting salivary flow, with the numbers of colored spots being 1.2 ± 1.4 for the stimulated salivary flow and 2.9 ± 1.3 for the resting salivary flow (n = 33; P < .05). Subjects with medium-hyposalivation (n = 15) also had a slight increase that reflected the functional capacity in the salivary gland (4.4 ± 0.6 for resting salivary flow vs 3.6 ± 1.0 for stimulated salivary flow; P < .05). In contrast, in the hyposalivation group with complaints of dry mouth (n = 32), there was hardly any change in the number of colored spots (4.4 ± 1.0 for the stimulated salivary flow vs 4.9 ± 0.2 for the resting salivary flow), except that 3 subjects had considerable elevation of secretion upon capsaicin stimulation. These results demonstrated that the number of colored spots using the filter paper incorporating or without capsaicin could easily evaluate salivary gland function in the subjects and could also distinguish between subjects having hyposalivation with complaints of dry mouth and healthy subjects.

**DISCUSSION**

This is the first report on the measurement of stimulated salivary flow using filter paper incorporating capsaicin comparing patients with hyposalivation with complaints of dry mouth and a healthy group. The study suggested that the capsaicin-stimulated salivary flow in the filter paper was a good indicator that could be used as a simple, objective test for screening salivary function.

**Table I. Comparison of salivary flow after stimulation with capsaicin, nicotine, and citric acid**

<table>
<thead>
<tr>
<th>Spot no.</th>
<th>No stimulation</th>
<th>Capsaicin</th>
<th>Nicotine</th>
<th>Citric acid</th>
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<tr>
<td>1</td>
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Resting and stimulated salivary flows were estimated for 1 minute after the paper was put on the center of the floor of the mouth (under the tongue). Other experimental conditions are described in the text.

+ , nearly entirely colored spot; ±, partly colored spot; –, colorless spot.

**Fig. 3.** The area of capsaicin distributed on the filter paper incorporating capsaicin. The detection of capsaicin was carried out as described in the text. A, Capsaicin-free filter paper; B, filter paper incorporating 600 µg of capsaicin.
gland function. The idea is based on reports that measurement of decreased resting salivary flow from sublingual and submandibular glands may well contribute to early diagnosis of Sjögren’s syndrome, whereas the stimulated salivary flow rate can be a more meaningful indicator of the extent of gland dysfunction in hyposalivation with complaints of dry mouth. We previously established a screening method for decreased salivary flow by estimation of the resting salivary flow using filter paper, but it is not available for estimation of the stimulated salivary flow. Therefore, we first attempted to improve the accuracy for estimation of resting salivary flow by increasing the number of spots from 3 to 5 and changing the distance between the spots on the filter paper reported previously. The assay system showed a highly significant correlation between the number of colored spots and saliva volume in vitro and the resting salivary flow rate. Therefore, we had confidence that it could also be applicable for estimation of stimulated salivary flow because it is based on the theory of paper chromatography, moving potassium iodide dependent on saliva volume. We previously established a screening method for decreased salivary flow by estimation of the resting salivary flow using filter paper, but it is not available for estimation of the stimulated salivary flow. Therefore, we first attempted to improve the accuracy for estimation of resting salivary flow by increasing the number of spots from 3 to 5 and changing the distance between the spots on the filter paper reported previously. The assay system showed a highly significant correlation between the number of colored spots and saliva volume in vitro and the resting salivary flow rate. Therefore, we had confidence that it could also be applicable for estimation of stimulated salivary flow because it is based on the theory of paper chromatography, moving potassium iodide dependent on saliva volume. Next, we attempted to make filter paper incorporating chemesthetic and gustatory stimuli and to estimate the stimulated salivary flow in patients with hyposalivation with complaints of dry mouth and a healthy group. In the selection of one stimulant from among various chemesthetic and gustatory stimuli, one examiner (W.S.) carried out all of the testing for comparison of salivary flow after stimulation with capsaicin, nicotine, and citric acid. The time of stimulation was restricted to 1 minute, because 2-minute stimulation with capsaicin led to salivary flow of more than 500 µL and subsequently we could not detect colored spots or quantitatively compare the responses to the chemesthetic and gustatory stimuli. Capsaicin caused a significant transient rise in salivary secretion at low concentrations (1.0-1.4 µg), which led to a pungent taste for a split second in subjects but did not interfere with the color reaction of the iodine starch. On the other hand, nicotine and citric acid were not available as stimulants for determining stimulated salivary flow in the subjects, because nicotine is subject to the Japanese Poisonous and Deleterious Substances Control Law and citric acid interfered with the color reaction of iodine starch. Saccharin (200-800 µg) was also assessed as a sweet stimulus in a preliminary experiment, but the sweet was a relatively ineffective gustatory stimulus in comparison with the other stimuli. Thus, capsaicin was chosen as the stimulant and used at a dose of 1.4 µg. When the tip of the paper incorporating capsaicin was inserted under the tongue, it was observed that the salivary flow significantly increased in 1 to 2 minutes in healthy subjects. Several investigators have reported that elevation of secretion upon capsaicin stimulation starts after 1 to 2 minutes via activation of capsaicin receptors in the oral cavity and ceases 2 to 6 minutes later. From these results, we decided to estimate the stimulated salivary flow for 2 minutes after the paper incorporating capsaicin was inserted under the tongue. As expected, our results revealed significant differences between healthy subjects and those with hyposalivation with complaints of dry mouth.
Dry mouth. The healthy group showed a significant reduction in the total number of colored spots after capsaicin stimulation, whereas no significant change was observed in the group with hyposalivation with complaints of dry mouth. However, 3 subjects in the hyposalivation group had elevation of secretion after capsaicin stimulation in spite of decreased resting salivary flow. Therefore, the number of colored spots after capsaicin stimulation reflects the functional capacity of the sublingual-submandibular glands, as the secretion induced by insertion of capsaicin using a cotton swab under the tongue is mainly derived from the sublingual glands. Regarding the possibility of trauma, irritation, and sensation of burning in tissue induced by capsaicin, Chinese investigators also reported that capsaicin may provide a new therapeutic strategy to improve submandibular gland hypofunction because capsaicin receptors exist in the human submandibular gland and are involved in regulating salivary secretion. These reports support the idea that the use of capsaicin does not lead to burning mouth syndrome/disorder. However, it is necessary to make this point clear, showing that the paper incorporating capsaicin induces minimal pain through activation of vanilloid receptors under the tongue. Thus, our newly developed method using filter paper incorporating capsaicin or without capsaicin can easily estimate both resting and stimulated salivary flows at 1 time. Therefore, it should be useful for evaluation of retained functional ability of sublingual-submandibular glands and screening of hyposalivation with a complaint of dry mouth by measurement of resting and stimulated salivary flows.

REFERENCES

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