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Characteristics of the saliva flow rates of minor salivary glands in healthy people

Zhen Wang^a, Ming-Ming Shen^b, Xiao-Jing Liu^a, Yan Si^c, Guang-Yan Yu^{a,*}

^a Department of Oral and Maxillofacial Surgery, Peking University School and Hospital of Stomatology, 100081, Beijing, PR China

^b Department of Oral and Maxillofacial Surgery, School of Stomatology, the Second Hospital of Hebei Medical University, 050000, Shijiazhuang, PR China

^c Department of Preventive Dentistry, Peking University School and Hospital of Stomatology, 100081, Beijing, PR China

ARTICLE INFO

Article history:

Accepted 23 November 2014

Keywords:

Minor salivary gland

Saliva

Saliva secretion

Saliva flow rate

Salivary gland

ABSTRACT

Objectives: To investigate the normal range and characteristics of saliva secretion in the minor salivary glands (MSGs).

Design: The flow rates of MSGs were measured in 4 anatomical locations of oral mucosa, and the relationship between MSG flow rates and whole saliva flow rates were assessed in 300 healthy subjects stratified by age and sex. An additional 30 young females were further evaluated for flow symmetry, effects of stimulation, circadian effects in MSGs, and the relationship with the flow rates of major salivary glands.

Results: (1) The mean saliva flow rates were 2.10 ± 0.66 (lower labial glands), 2.14 ± 0.62 (upper labial glands), 2.88 ± 0.72 (buccal glands) and 2.15 ± 0.51 (palatal glands) $\mu\text{l}/\text{min}/\text{cm}^2$, respectively. The flow rate of buccal glands was significantly higher than the rates of SMGs in other locations ($P < 0.01$). (2) 5-year-old children had the lowest MSG flow rates ($P < 0.0001$) while the 10–14-year-old group had the highest ($P < 0.001$). (3) MSG flow rates were independent of sex ($P > 0.05$), right vs. left ($P > 0.05$), and citric acid (2.5%) stimulation ($P > 0.05$). (4) Only labial MSG displayed a significant secretory circadian rhythm with the highest rate in the evening ($P < 0.05$). (5) A weak correlation was found between the flow rate of palatal glands and that of unstimulated whole saliva ($r = 0.195$, $P = 0.007$).

Conclusions: Our findings provide a reference for functional evaluation of MSGs and for donor site selection of MSG transplantation for treatment of severe dry eye syndrome.

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1. Introduction

The surface of oral tissues is covered by a thin coat of saliva. This film is mainly composed of small amounts of mucous secretions from the minor salivary glands (MSGs). MSGs are distributed throughout the oral mucosa, and according to their

locations, they can be divided into labial, buccal, palatal, lingual and retromolar glands.¹ There are 600–1000 MSGs in the human oral cavity, and they contribute to less than 10% of the volume of whole saliva.² Despite the small volume of saliva secreted, MSGs have long been considered to be of significant importance for oral tissue protection, lubrication, maintenance of local immunity and the sense of taste. These

* Corresponding author. Tel.: +86 10 82195245; fax: +86 10 62173402.

E-mail address: gyyu@263.net (G.-Y. Yu).

Abbreviations: MSGs, Minor salivary glands; SM, Submandibular gland; PG, Parotid gland.

<http://dx.doi.org/10.1016/j.archoralbio.2014.11.016>

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functions are supported by their unique anatomical distribution, their proximity to oral tissue surfaces, and the relatively large amounts of mucins and immunoglobulins compared with the major salivary glands. MSGs are thought to secrete spontaneously, which makes these functions possible when people are at rest, such as during sleep.³ However, our knowledge about the saliva secreted from MSGs is much less compared to that about whole saliva or saliva from major salivary glands. Furthermore, reported data on the saliva flow rates of MSGs are conflicting because of various uncontrollable factors.³

Saliva flow rates vary across MSGs in different locations. It is widely accepted that the flow rate of the buccal glands is relatively high, followed by the lower labial and palatal glands.⁴ However, the normal values of saliva flow rates in MSGs in different locations, which is very important to evaluate the secretory function of MSGs, are of great variation. Other factors, such as age and sex, are known to influence the flow rate of whole saliva. Stimulation with citric acid increases secretion of the major salivary glands. Saliva secretion follows the circadian rhythm, with the lowest secretion rate during sleep and the highest rate in late afternoon.⁵ However, most of these flow rates were measured for whole saliva or saliva from major salivary glands, and it is not clear whether the saliva flow rate of MSGs has the same characteristics as that of whole saliva or saliva from major salivary glands. Therefore, a well-controlled systemic study is critical to reveal the characteristics of the saliva flow rates of MSGs in healthy people.

Several features of MSGs may cause the difference in secretion from major salivary glands. Firstly, the structure of MSGs is not as complex as that of major salivary glands. MSGs consist of small clusters of secretory cells with a short excretory duct that transports the saliva to the surface of the mucosa.⁶ Secondly, apart from the lingual von Ebner's glands, which secrete serous saliva, MSGs are predominantly mucous, with a varying amount of sero-mucous cells in their structure. Thirdly, the MSGs have little or no sympathetic innervations. In addition to other innervations that include neuropeptide-containing (vasoactive intestinal peptide, substance P, neuropeptide Y) and nitric oxide synthase-positive nerve fibres, MSGs are mainly controlled via the parasympathetic nervous system with cholinergic transmission.^{7,8} It is supported by the studies that parasympathetic agonist (carbachol) activates and antagonist (atropine) blocks secretion from the cells of human labial glands.^{9,10} Whether other factors influencing the secretion of the major salivary glands are also related to the secretion of MSGs remains unclear.

The aim of this study was to investigate the normal range of saliva flow rates from MSGs and the characteristics of MSGs in different locations in healthy subjects.

2. Materials and methods

2.1. Subjects

Three hundred and thirty subjects (150 males and 180 females; aged 5–89 years) were enrolled in this study. They are divided into two groups. In the first group, 300 subjects were enrolled for measurement of the saliva flow rates from

MSGs. The subjects were further divided into five age groups: 5, 10–14, 15–44, 45–59, and 60–89 years, with 30 males and 30 females in each group. In the other group, 30 females aged 21–25 years were enrolled to observe the flow rates of MSGs at symmetric sites and after stimulation with 2.5% citric acid, and the possible circadian rhythm of saliva secretion. All subjects were in good health, had good oral hygiene, and were free of caries and salivary gland diseases. None of them had received any medication that could cause dry mouth. This project was approved by the Ethics Committee of the Peking University School of Stomatology (IRB00001052-080-48). All subjects signed their informed consent for participation.

2.2. Saliva collection

To reduce possible influence of circadian and seasonal variations on saliva secretion, saliva samples were collected at the same time of day, at 09:00 AM and 11:00 AM, in an air-conditioned room, where room temperature was kept 20–24 °C and humidity was kept 40–70%.¹¹ Subjects were asked to refrain from eating, drinking, smoking, and brushing their teeth for at least 90 min before collection. Saliva collection was performed by an experienced researcher.

2.3. Collection of whole saliva

Before collection, the subjects were instructed to rinse their mouths with water and then rest for 5 min with their eyes open and head tilted slightly forward. By using the spitting method,¹² whole saliva at rest was collected for 5 min into a pre-weighed cup. After 5 min 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 s for another 5 min. By defining specific gravity of saliva as 1, flow rates were calculated and recorded in ml/min.

2.4. Collection of saliva from MSGs

MSG saliva was collected from the following sites of oral mucosa (Fig. 1): the upper labial mucosa (left to the midline, halfway between the vermilion border and the labial fraenum), the lower labial mucosa (left to the midline, halfway between the vermilion border and the labial fraenum), the buccal mucosa (halfway between Stensen's duct and the angle of the mouth) and the palatal mucosa (5 mm left to the midline, medially at the border of the soft and hard palate). Saliva from the palatal gland was not collected in 5-year group and 10–14-year group considering their inability to cooperate.

During collection of labial gland saliva, the labial mucosa was everted gently. During collection of buccal or palatal gland saliva, the subjects were asked to keep their mouths wide open. After the mucosa was carefully dried with gauze, a strip of filter paper (Whatman No. 41, 1 × 2 cm² in size) was immediately placed onto the mucosa and then light pressure was applied. The saliva was collected for 30 s. The strip of filter paper was removed and placed in an air-tight container to protect the collected saliva from evaporation. The container and the strip were weighed before and after

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