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Gender differences in the saliva of young healthy subjects before and after citric acid stimulation

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ABSTRACT

Background: Gender differences in the function and anatomical features of salivary glands are well known. However, specific gender differences in the biochemical composition and salivary flow rate (SFR) remain uncertain. Collection methods affect the assessment of the salivary composition and SFR, which are also highly affected by acid stimulation.

Methods: In the present study, we analyzed the differences in salivary characteristics of SFR, pH and salivary α-amylase (sAA) for 28 females and 27 males before and after citric acid stimulation, as measured by 3 different collection methods sequentially.

Results: Salivary pH values were significantly lower in females than that in males, both before and after stimulation, irrespective of collection methods. Salivary pH consistently increased after acid stimulation in both genders. Mean SFR in females before acid stimulation was significantly lower than that in males in all 3 samples collected. No gender difference in sAA was evident.

Conclusion: Substantial gender differences in biochemistry and flow of saliva exist, and these findings are robust, as evidenced by reasonable consistency of the data among different saliva sampling methods.

1. Introduction

Estrogen has effects on salivary glands and contributes to gender differences in oral health, including differences in dental caries rates [1,2]. Estrogen treatment has a beneficial effect on salivary flow rate (SFR) [1]. The size and weight of salivary gland are lower in women compared with that in men [3]. There is also gender specific difference in gene expression in human parotid gland, which correlates with salivary gland function [4]. Gender-specific differences in biochemical composition of saliva and SFR have not been consistently identified. Several reports have shown that females had a significantly lower mean SFR than males in unstimulated whole saliva [3,5] as well as in parotid saliva stimulated by 2% citric acid [6]. However, other studies did not find significant differences in SFR between two genders, although the lowered salivary pH in female than male was observed [7]. Lack of a significant gender differences in salivary pH was also observed in another study [8]. Moreover, no gender differences were reported in salivary α-amylase (sAA) levels in either high- or low-stress patient groups [9], nor were differences observed in unstimulated whole saliva [7].

Saliva is secreted from the major salivary glands (parotid, submandibular and sublingual) and some minor glands through a network of salivary ducts. Half the volume of saliva stimulated is secreted from the parotid glands [10], whereas, unstimulated saliva is mainly derived from the submandibular glands [11]. Thus, salivary source, biochemical composition, and SFR are affected by stimulation status. The majority of the reported gender-specific differences in saliva have been evaluated on unstimulated condition, and very few data are available regarding gender-specific differences under a stimulated condition. Moreover, previous studies have suggested that using different collection methods would influence the salivary biomarkers and SFR [12,13].

2. Material and methods

2.1. Participants

The study was carried out at Guangzhou University of Chinese Medicine from March 2014 to June 2014. 20–40 year old healthy subjects were recruited. Exclusion criteria included alcohol ingestion in recent one week, and tobacco consumption during the previous 3 months, any oral disease (e.g. periodontal disease and gingivitis), autoimmune,

Abbreviations: SFR, salivary flow rate; sAA, salivary α-amylase; sTAPM, sAA total activity per minute; PD, Passive drooling; RS, rotating a swab from a Salivette®; CS, chewing a Salivette® swab.

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infectious, musculoskeletal, or malignant disease, and recent operation or trauma. The study was conducted according to the guidelines laid down in the declaration of Helsinki, and all procedures involving human volunteers were approved by the Academic Ethics Committee of Guangzhou University of Chinese Medicine. All the participants provided written informed consent.

2.2. Saliva collection methods

To minimize possible confounding effect of circadian rhythms in sAA activity and SFR, saliva sampling was carried out between 14:30 h and 16:00 h in a bright quiet room after resting comfortably seated for 30 min. The participants were refrained from eating, exercising, or drinking any beverages for 1 h before sampling. Half an hour before collection, participants were instructed to rinse mouth with water and then drink 200 ml of warm water.

Passive drooling (referred to here as PD) into an aseptic 10 ml collecting tube, rotating a swab from a Salivette® (RS), and gently chewing a Salivette® swab (CS) are 3 common saliva collection methods [14,15]. The 3 saliva collections were carried out on 3 consecutive days, respectively. The order in which the 3 collections were used for each subject was randomized, and the interval between each collection was 24 h to allow SFR and salivary compositions return to basal levels.

The initial collection by PD was of unstimulated saliva, which was performed as follows: head was held tilted down to pool saliva at the front of the oral cavity for 5 min, and then sample was directed into the collection tube placed adjacent to the lower lip. Citric acid stimulation was then performed by placing a square filter paper (1 cm × 1 cm, Hangzhou Special Paper Co., Ltd; Model: 102) saturated with 10% citric acid on the upper tip of the tongue for 30 s. Before collecting the acid-stimulated saliva, subjects were asked to raise their tongue tips up, which were then swabbed and dried with cotton swab in an effort to avoid citric acid remaining on the tongue tip that could interfere with salivary pH value. The acid-stimulated saliva was collected immediately with another new collecting tube by the same procedure as described above.

For RS sampling, the unstimulated and acid-stimulated samples were collected by rotating a Salivette® swab (Sarstedt), without chewing or biting the swab, at a steady rotation speed (6 times per min) for 1.5 min before and after acid stimulus, respectively. The swab was then centrifuged (4400 g, 10 min, 4 °C) to obtain the saliva sample. Collecting saliva by CS before and after stimulation involved chewing the Salivette swab for 1.5 min with a chewing frequency of 25–30 bites/min.

2.3. Determination of salivary indicators

The SFR, defined as ml/min, was measured immediately after collection using the gravimetric method [16]. The weight of tube or Salivette® before (m_1, g) and after (m_2, g) collecting whole saliva were recorded. The SFR (ml/min) = ($m_2 - m_1$) / (t), where t was collection time (min). The density of whole salivary was set at 1.0 g/ml as determined previously [17]. Salivary pH was measured immediately after saliva collection using a laboratory pH meter (FE20, METTLER TOLEDO Ltd., Switzerland). The immediate pH assessment avoids pH fluctuations due to CO_2 flux in or out of the sample. The sAA activity was determined using an enzymatic hydrolysis assay of the chromogenic substrate maltose (Sigma-Aldrich Product Number M5885) [18] with absorbance values detected by ultraviolet spectrophotometer (UVmin-1240, SHIMADZU, Kyoto Japan) at 540 nm. The RSD of intra-assay and inter-assay were 3.81% and 4.38%, respectively. Unit definition: one unit (U) of sAA liberates 1.0 mg of maltose from starch in 3 min at pH 6.9 at 37 °C. The sAA total activity per minute (sTAPM) was calculated as: sTAPM (U/min) = sAA activity per unit volume (U/ml) × SFR (ml/min).

2.4. Statistical analysis

After Schapiro-test for normality, the differences of outcome variables before and after acid stimulation as well as males and females were compared using the paired and independent *t*-test, respectively, if distributed normally, while the rank sum test was used if not distributed normally. All data were expressed as mean ± SD. Analyses were performed with SPSS 18.0 and the significance level was set at $p < 0.05$. Two-sided significance tests were used throughout.

3. Results

Fifty-five subjects were enrolled, ranging in age from 21 to 34 y: 27 males (age: 25 ± 2.80, heart rate: 72.07 ± 7.34, BMI: 20.79 ± 1.62) and 28 female (age: 25 ± 1.52, heart rate: 72.07 ± 6.58, BMI: 19.9 ± 1.53), indicating that there were not statistically significant baseline differences in age, BMI and heart rate between the genders.

As determined by PD sampling, compared to baseline, the SFR, pH, sAA activity and sTAPM increased significantly ($p < 0.05$) for both genders after the initial acid stimulation (Table 1). In the PD samples, the mean pH values of females both before and after acid stimulation were significantly lower than males ($p < 0.01$). Also, the SFR before acid stimulation was significantly lower in females ($p < 0.05$, Table 1).

For RS sampling, after stimulation, there was a modest, yet statistically significant, increase in sAA and sTAPM only in female subjects. Also after stimulation, both genders showed substantial increases in pH and SFR. Consistent with the PD findings, pH values of pre- and post-acid treatment and unstimulated SFR were significantly different between two genders (Table 1).

For CS sampling, the same pattern of significant pH value changes was likewise observed. Upon acid stimulation, the CS sampling method detected a significant increase in SFR in female subjects (but not in males). With CS sampling method, no significant difference was observed between pre- and post-acid stimulation, nor was there a gender difference in sAA and sTAPM.

The mean pre- to post-stimulation ratios for SFR, pH, sAA activity, and sTAPM were all >1.0. There were no significant differences between genders, and this held true for all 3 collection methods (Table 1).

4. Discussion

Previous studies have suggested that there are gender-related differences in the functions and anatomical features of salivary glands [1–4]. However, gender differences in biochemical composition or SFR have been inconclusive or contradictory. In the present study, we analyzed the differences in salivary characteristics for females and males before and after citric acid stimulation, utilizing 3 different sampling methods. As expected, our data revealed significant differences in salivary characteristics between resting and stimulated conditions.

Several prior studies demonstrated that the sample collections involving mechanical stimulation—for example in the case of Salivette swab—can lead to an increase in SFR and altered salivary composition [19,20]. Furthermore, gustatory stimulation, especially acid stimulation, plays a major role in elevated SFR and altered salivary composition [10,11,21,22]. So far, few studies have considered measuring the impact of acid stimulation on salivary properties between male and female using different saliva collection methods. In the present study, saliva collection with Salivette and stimulation by citric acid may provide an opportunity to determine salivary characteristics under different conditions. Our data have shown that gender was not a determining factor in the responsiveness of salivary glands to citric acid stimulation, as assessed by the pre- to post-stimulation ratio using 3 collection methods (Table 1). These data indicate both genders have a similar response pattern to citric acid stimulation. Thus, neither gender nor collection method explains our findings in the effect of citric acid on salivary glands.