Comparison of Salivary IgA Secretion Rate Collected by the Aspiration Method and Swab Method

Koji HAMURO1,a, Yoshifumi KOTANI1,a, Masamichi TOBA1, Keiji KAKUMOTO2 and Noriyuki KOHDA1*

1Otsu Nutraceuticals Research Institute, Otsuka Pharmaceutical Co., Ltd., 3–31–13 Saigawa Otsu, Shiga 520-0002, Japan
2Information Management Office, Drug Safety Research Center, Tokushima Research Institute, Otsuka Pharmaceutical Co., Ltd., 463–10 Kagasuno, Kawachi-cho, Tokushima 771-0192, Japan

These authors contributed equally to this work.

Received November 12, 2012; Accepted January 23, 2013

Salivary immunoglobulin A (IgA) is used as an immunity marker, as saliva can be easily collected, noninvasively with little stress. However, several saliva collection methods can be used. Our comparison between samples collected using different methods demonstrated that the salivary IgA secretion rate in samples collected using an aspiration method was significantly correlated with that in samples collected using a swab method. Moreover, the significant circadian variation in salivary IgA secretion rate in the aspirated saliva suggested that the aspiration method does not suppress salivary IgA secretion rate variability compared with the swab method. Therefore, the aspiration method should be considered as the preferable saliva collection method.

Key words: Saliva collection, IgA, aspiration, swab

The mucosal immune system maintains human health by forming the first line of defense in the body. Secretory immunoglobulin A (SIgA) is an antibody that is mainly secreted from the mucosal tissue of the digestive tract, lungs, respiratory tract, etc. It is the major antibody in the mucosal secretion, and it plays an important role in the prevention of disease by inhibiting the invasion of pathogenic microorganisms, allergens, etc. into the mucosal tissue [1]. Therefore, evaluation of SIgA can be used to assess the risk of infection.

SIgA is found in exocrine secretions such as saliva, nasal secretion, bronchoalveolar lavage fluids, intestinal fluids, and breast milk. Salivary IgA is generally considered as an immunity or stress marker, because saliva can be easily collected, noninvasively with little stress. Many studies have demonstrated that salivary IgA concentrations decline with intensive exercise, psychological stress, or aging [2–15]. Moreover, a correlation has been reported between the decline in salivary IgA and the incidence of upper respiratory tract infection (URTI) [16–24]. Thus, enhancement or preservation of salivary IgA secretion is considered to contribute to better health and has been reported to be influenced by lactic acid bacteria and food factors [25–29].

Studies investigating the causes of salivary IgA reduction have used various saliva collection methods. However, the spitting method for collecting unstimulated saliva has been the most commonly used collection method in studies examining the relationship between salivary IgA and the incidence of URTI. Salivary IgA is known to exhibit intraindividual and interindividual variations that are influenced by multiple factors, such as saliva collection methods, circadian changes, and lifestyle [2–15, 30]. Thus, more consistent salivary IgA measurements should be obtained for proper evaluation of salivary IgA secretion. The spitting, swab, and aspiration methods are the main saliva collection methods used [31]. Unstimulated, slightly stimulated, and stimulated saliva is collected using the spitting, aspiration, and swab methods, respectively. Michishige et al. demonstrated that, among the 3 saliva collection methods, the spitting and aspiration methods showed a significant positive correlation of salivary IgA concentrations [32]. The association between salivary IgA and URTI risk has been exhibited not only through salivary IgA concentrations but also through salivary IgA secretion rate [16–24]. Therefore, in this study, we compared the salivary IgA secretion rate in saliva samples collected using aspiration and swab methods.

Healthy male and female individuals aged 20–30 years and those aged 65 or over were included in this study, with the following exclusion criteria: (1) smoking; (2) intense exercising; (3) blood test results, and blood pressure and
pulse rate values significantly outside the normal range; 
(4) history of digestive system or immune system disease 
including pneumonia, cancer, inflammatory colitis, and 
rheumatoid arthritis; (5) periodontitis or oral cavity 
bleeding; (6) antibiotic administration within 2 weeks 
 prior to the blood test; and (7) intake of drugs that could 
 influence digestive system or immune system function 
such as antiflatulents, antidiarrhetics, drugs that promote 
digestive function, steroids, immunosuppressants, 
etc. In addition to the above listed criteria, individuals 
considered unsuitable for the study by the supervising 
doctor were excluded from the study. The study specifics 
were explained to the participants, and a written informed 
consent was obtained from each subject before the 
study was performed. Subsequently, the investigator 
interviewed and examined the candidate subjects, 
and blood biochemical and hematological tests were 
conducted. As a result, a total of 60 subjects were selected 
for the study, including 15 individuals from each of the 
4 participant groups, which consisted of young men, 
young women, elderly men, and elderly women. This 
study was approved by the Ethics Committees of HUMA 
CORP, a contract research organization, and performed 
in accordance with the Helsinki Declaration.

Subjects were asked to avoid alcoholic beverages 
and to fast from 9:00 PM on the night prior to the day of 
saliva collection. Saliva was collected from 10:00 AM to 
12:00 AM and from 2:00 PM to 4:00 PM. Subjects had an 
assigned meal between 8:30 AM and 9:00 AM, after which 
they brushed their teeth and rinsed their mouths with tap 
water. Subjects rested prior to saliva collection. At 9:55 
AM, the subject rinsed their oral cavities 3 times with 
tap water, and at 10:00 AM, they were asked to swallow 
the saliva in their oral cavities. A piece of cotton roll was 
placed on each subject’s right back teeth, and another 
was placed on his/her left back teeth. During the 1 min 
of saliva collection, the subject was asked to masticate once 
per second at the beat of a metronome. The 2 cotton rolls 
containing the saliva were then immediately separated 
and placed in 2 exclusive tubes for temporary preservation 
on ice. Then, 2 new pieces of cotton roll were similarly 
placed on the subject’s right and left back teeth, and the 
subject was also asked to masticate once per second at 
the beat of a metronome, for the 1 min duration of saliva 
collection. The 2 cotton rolls that contained the saliva 
were then immediately collected, respectively placed in 
the 2 exclusive tubes, and preserved on ice. This saliva 
collection procedure was repeated for a total of 5 times. 
Then, at 11:15 AM (saliva collection time of 11:20 AM) and 
11:35 AM (11:40 AM saliva collection time), saliva was 
collected following the same procedure used at 10:55 AM, 
and each sample was temporarily preserved on ice. For 
all 3 saliva collections conducted over a 1-hr period, the 
subject was asked to maintain a resting condition in a 
sitting position for 5 min before collection until the 
completion of saliva collection. Subjects had an assigned 
meal at 12:30 PM and then brushed their teeth and rinsed 
their mouths with tap water. Then, saliva samples were 
collected at 2:00 PM, 2:20 PM and 2:40 PM by the aspiration 
method, and at 3:00 PM, 3:20 PM, and 3:40 PM by the swab 
method.

The weight of saliva collected every minute for 
5 min using Salivette® cotton rolls was calculated as the 
difference in weight between the exclusive tube used for 
saliva collection, which held 10 pieces of saliva-containing 
cotton rolls, and the same tube empty. We assumed that 
the weight of collected saliva represented the volume of 
collected saliva. Subject recruitment, selection and saliva 
collection were carried out by Huma corp.

Subjects were included in the analysis if their collected 
samples achieved the volume required for salivary IgA 
congestion measurement, irrespective of the saliva 
collection method, collection frequency, and collection 
time point. Salivary IgA concentration was measured by 
enzyme immunoassay using a commercial kit (Salivary 
Secretory IgA Indirect Enzyme Immunoassay Kit; 
Salimeters, State College, PA, USA). Salivary IgA
secretion rate (μg/5 min) was calculated by multiplying the salivary IgA concentration (μg/ml) by the saliva secretion rate (ml/5 min). Comparison between salivary IgA secretion rate (μg/5 min) was conducted using one-way or multi-factor ANOVA, including the following factors: samplings, age, gender, sampling time point, and the number of sampling times. Correlation of corresponding salivary IgA secretion rate between samples collected by the aspiration method and swab method was analyzed using Pearson’s correlation coefficient. Statistical analyses were performed using SAS software (R9.1, SAS institute Japan, Tokyo, Japan). A two-tailed p-value <0.05 was considered significant for all tests.

We selected a total of 60 participants, who were equally divided between young men, young women, elderly men, and elderly women, such that 15 participants represented each group; however, an elderly woman withdrew from the study for personal reasons. We analyzed the samples of subjects who provided all 12 saliva samples using both the aspiration and swab methods. Accordingly, the subjects included in the analysis were categorized as: 9 young men, 8 young women, 9 elderly men and 7 elderly women. A summary of the salivary IgA secretion rate of these 33 subjects is presented in Table 1. The salivary IgA secretion rate was higher in the saliva samples collected by the swab method than in those collected by the aspiration method. This could be due to the effect of masticatory stimulation of the amount of saliva secretion (data not shown). Aufricht et al. reported that the salivary IgA concentration was inversely correlated with the salivary secretion rate in saliva collected by the spitting method [30]. By the swab method, the salivary IgA secretion rate of the first sample collected in the morning from elderly participants was significantly higher than that of the second and third samples collected. Additionally, the salivary IgA secretion rate of the first afternoon sample collected from elderly participants was significantly higher than that of the third sample collected. Although such differences were not detected in samples collected from young participants, the average values of the first samples were higher than those of the second and third samples, both in the morning and in the afternoon sample collections. On the other hand, by the aspiration method, only the first morning sample collected from elderly participants was significantly higher than that of the third samples; however, no other significant differences were observed between samples collected from young and elderly subjects.

All 396 samples collected from the 33 subjects by the swab method and aspiration method were analyzed. Comparison analysis of corresponding salivary IgA secretion rate (μg/5 min) between samples collected by the aspiration method and swab method revealed a significant correlation (p<0.01) with a Pearson’s correlation coefficient of 0.573 (Fig. 1). Michishige et al. reported that the salivary IgA concentration was significantly correlated in samples collected by
the aspiration and spitting methods [32]. Correlation between the salivary secretion rate in samples collected by aspiration and spitting methods is expected, since unstimulated and slightly stimulated saliva are collected using these methods. This observation and our results suggest that salivary IgA secretion rate in saliva samples collected by aspiration, swab, and spitting methods could be correlated.

Table 2 demonstrates the statistical analysis results based on 4 factors: age, gender, sampling time and the number of samples collected. A significant difference was detected between sampling time points when using the aspiration method but not the swab method. It has been reported by Miletic et al. that differences between morning and afternoon could be caused by circadian changes in salivary IgA [3]. Therefore, we consider that the aspiration method allows the detection of such circadian-related differences. The swab method showed a highly significant difference according to the number of samples collected. This might indicate a wide variation in salivary IgA secretion rate for samples collected by the swab method; it might also be caused by a decrease in saliva secretion volume between the first and third saliva collections among the young subjects. Our results also confirmed that, among young subjects, IgA secretion rate of saliva samples collected using the low-pressure aspiration pump was more tightly distributed than those of samples collected using the swab method (Fig. 2).

By the swab method, at least 1 out of 6 saliva samples could not be collected from 6 young men, 7 young women, 6 elderly men, and 7 elderly women. Meanwhile, saliva samples were not collected from 3 young men and 3 elderly women when using the aspiration method, and these participants were among those who were unable to provide saliva samples using the swab method as well. When using the swab method, and even if saliva secretion was stimulated by mastication, we were sometimes unable to collect saliva from cotton rolls due to a low saliva flow rate. We also could not accurately measure saliva flow when cotton rolls failed to absorb all the secreted saliva.

These show that the aspiration method can be a highly effective method for saliva sample collection. Michishige et al. recommended using the aspiration method for saliva collection because some elderly people cannot spit well [32]. Moreover, Jones et al. reported that 69% of elderly people preferred the aspiration method to the spitting method [33]. Although the swab method makes it easy to obtain saliva samples, our results showed wide variation in salivary IgA secretion rate. In addition, there are people who cannot spit saliva well due to various kinds of diseases. On the other hand, the aspiration method requires an aspiration pump but has certain strong points, such as allowing control of the aspiration period and providing high precision in saliva collection from almost all kinds of subjects.

<table>
<thead>
<tr>
<th></th>
<th>Age (Young/Elderly)</th>
<th>Gender (Male/Female)</th>
<th>Sampling time point (Morning/Afternoon)</th>
<th>Number of samples collected (1, 2, 3 times)</th>
<th>p value</th>
<th>p value</th>
<th>p value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Swab method</td>
<td>0.4293</td>
<td>0.1484</td>
<td>0.8403</td>
<td>0.0081</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aspiration Method</td>
<td>0.4668</td>
<td>0.3628</td>
<td>0.0314</td>
<td>0.1069</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

p values were obtained using multi-factor ANOVA (factors: sampling method, age, gender, sampling time point and number of samples collected). Results show correlation of corresponding salivary IgA secretion rate between samples collected by the aspiration method and swab method.

Fig. 2. Box-whisker plot of salivary IgA secretion rate
Distribution of salivary IgA secretion rate (µg/5 min) among the young and elderly participants, in samples collected by the swab and aspiration methods. The box-whisker plot displays the mean, quartiles, and the minimum and maximum values observed for each group. The plot elements and the statistics they represent are as follows: the length of the box represents the interquartile range (the distance between the 25th and 75th percentiles); the horizontal line in the box interior represents the mean; and the vertical lines issuing from the box extend to the minimum and maximum values of the analysis variable.
SALIVARY IgA SECRETION RATE AND SALIVA COLLECTION METHODS

Michishige et al. also investigated whether the concentrations of each total protein, kallikrein activity, trypsin activity and trypsin-like protease in aspirated saliva were significantly positively correlated with those in spitted saliva [32]. As described above, one of the significant benefit of the aspiration method is accurate saliva collection. Considering our salivary IgA results, the aspiration method would also be the preferable saliva collection method for examination of the secretion rate for these measurements.

In conclusion, the aspiration method leads the collected salivary IgA secretion rate to correlate with that of the swab method with high precision. Moreover, this method would prevent the failure of saliva collection and be used to collect saliva from almost all kinds of subjects under fixed conditions, such as the collection period. The aspiration method could provide a good and effective method for evaluating salivary IgA secretion rate and predicting URTI.

ACKNOWLEDGMENTS

We thank Mr. Hiroshi Okamatsu, Otsuka Pharmaceutical Co., Ltd., for considerable contribution to this study and Prof. Fumiko Michishige, School of Nursing, Kyoto Tachibana University, for providing instructions regarding saliva collection.

REFERENCES


