Evaluation of Buffering Capacity of Resting Saliva and Caries Prevalence for Caries Risk Assessment

Hiroya Gotouda,1 Chieko Taguchi,1 Akira Fukatsu,2 Hideki Makimura,3 Satoshi Hirayama,4 Masahiko Fukumoto,2 Ikuo Nasu,1 and Masaharu Makimura5

Departments of 1Preventive and Public Oral Health, 2Laboratory Medicine for Dentistry, 3Renascent Dentistry, 4Operative Dentistry, 5Social Dentistry (Dental Education), Nihon University School of Dentistry at Matsudo, Matsudo, Chiba 271-8587, Japan

Abstract
Saliva stimulated by chewing gum is often used as a sample in buffering capacity testing. However, cases are observed whereby the collection of the stimulated saliva from young children, the disabled, and the elderly is difficult, and an investigation to establish a method for saliva collection is considered necessary. Although the testing of the buffering capacity using resting saliva or residual saliva in the oral cavity as samples is considered useful, 1 mL or more of saliva is used in the measurements, and the collection of this sample is seen to be difficult. Accordingly, it is desirable to measure buffering capacity by a small volume of resting saliva. Until now, there have been few epidemiological studies investigating the association of the buffering capacity with caries prevalence in a large group in order to verify the efficacy of this parameter for risk assessment. In this research, the usefulness was investigated by means of analyzing the relationship between the buffering capacity for a small volume of resting saliva and DMF. The mean of DMF in the high buffering capacity group was significantly lower from that in the moderate buffer capacity group (p < 0.05) and the low buffer capacity group (p < 0.01). These results suggest that determination of the buffering capacity employing a small amount of resting saliva was useful and that the risk of dental caries could be assessed by measuring the salivary buffering capacity using residual saliva in the mouth as the sample.

Introduction
Caries prevalence is influenced by microbiological factors such as plaque and oral bacteria, host factors that include tooth substances and saliva, and environmental factors (1–14). The role of saliva as a host factor is especially important. The mechanisms by which saliva protects against dental caries include its buffering action, so a buffering capacity test is commonly used in the clinical setting (2, 9, 12–16). Saliva stimulated by chewing gum or paraffin is often used as a sample in buffering capacity testing (1, 3, 12, 14). However, cases are observed whereby the collection of the stimulated saliva from young children, the disabled, and the elderly is difficult, and an investigation to establish a method for saliva collection is considered necessary. Although the testing of the buffering capacity using resting saliva or residual saliva in the oral cavity as samples is considered useful, 1 mL or more of saliva is used in the measurements, and the collection of this sample is seen to be difficult (12, 14, 17). Accordingly, it is desirable to measure the buffering capacity by a small volume of resting saliva. Although reports about the buffering capacity of saliva that are based on using a small amount of saliva in a small group can be found (17), there have been few epidemiological studies investigating the association of the buffering capacity with caries prevalence in a large group in order to verify the efficacy of this parameter for risk assessment.

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capacity for a small volume of resting saliva and DMF.

**Materials and Methods**

**Subjects**

Seventy-three volunteers (average age: 22.0 years) were obtained from Nihon University School of Dentistry at Matsudo as the experimental subjects. This study was conducted with the approval (EC 02-029) of the Ethics Committee of the School of Dentistry at Matsudo, Nihon University.

**Measurement of buffer capacity**

The volume of saliva at rest was determined based on the spitting method (12, 14), and measured. After mixing 0.2 ml of saliva with 5.0 ml of distilled water for 73 subjects, 0.3 ml of 0.01 N HCl (Wako Pure Chemical Industries, Osaka, Japan) was added and mixed. The pH value of the resulting mixture was measured using a pH electrode, (Hanna Instruments, Padova, Italy) and determined as the buffer capacity of saliva. The subjects underwent a single measurement.

**Investigation of caries prevalence**

Using a mirror and probe under a bright light, caries prevalence was determined according to the standards of the Japan Association of School Dentists (8). The presence of DMF (decayed, missing and filled teeth) was calculated and recorded for each subject.

**Statistical analysis**

For the statistical analysis, Tukey-Kramer multiple comparison test was used to compare the mean values among three groups.

**Results**

The mean of the buffer capacity of saliva for 73 subjects was 4.7 ± 0.7 (mean ± SD). Otherwise, the mean of the DMF for all subjects was 7.0 ± 5.5 (Fig.1). Their subjects were classified into three groups of the buffer capacity; high-buffering capacity group (≥ pH5, n = 25, 34.2%), moderate-buffering capacity group (≥ pH4.0 and < pH5.0, n = 34, 46.6%), and low-buffering capacity group (< pH4, n = 14, 19.2%) (Fig.2).

The mean of DMF in the high-, moderate-, and low-buffering capacity groups were 4.1 ± 3.4, 7.8 ± 6.3, and 10.4 ± 4.0, respectively. The mean DMF in the high-buffering capacity group was significantly lower than that in the moderate-buffering capacity group (p < 0.05) and low-buffering capacity group (p < 0.01) (Fig.3).
Discussion

In the case of expressing the buffering capacity, the pH is often delimited and classified into units of pH 6, 5 or 4 (12, 14, 18-20). This research also used this classification. As a result, 34% of all subjects were classed as belonging to the high buffering capacity group (pH 5 or higher), 47% to the moderate buffering capacity group (pH 4 to 5), and 19% to the low buffering capacity group (pH 4 or less). The subjects in this research were adults in their 20s. Investigation of data from other age groups will be necessary hereafter. In addition, each subject underwent a single measurement. However, it is desirable to collect multiple samples, if possible, on different times and dates for more accurate measurements. In the future, analysis should be based on the mean values obtained by employing more than one salivary sample.

Gotouda et al. conducted a clinical study of the buffering capacity of saliva in order to obtain background data for the purpose of utilizing a small sample of saliva to assess the risk of dental caries and reported the usefulness of their method as a result of comparison with the conventional method (17). In this context, the correlation between the buffering capacity of a small volume (0.2ml) of the saliva to be examined in this study with the established buffering capacity of 1ml of resting saliva was investigated, and reported on the usefulness of employing a small volume of saliva to assess the buffering capacity (17). In this study, an epidemiological analysis of the association with the caries incidence rate (DMFT) was performed in order to investigate the clinical usefulness for bedside diagnosis of caries risk in daily practice. Consequently, it was suggested that the determination of the buffering capacity employing 0.2ml of resting saliva, as was done in this study, was useful and that the risk of dental caries could be assessed by measuring the salivary buffering capacity using residual saliva in the mouth (collected, for example, with a dropper) as the sample. In this study, an epidemiological investigation of the association with the development of dental caries was conducted in a large group with the purpose of achieving more effective risk assessment.

Reports concerning the relationship between dental caries and the saliva buffer capacity are common. Gotouda et al. report that a significant difference in DMFT was observed between a high buffer capacity group and a low buffer capacity group using 1 mL of resting saliva (15). Fukumoto et al. claim the buffer capacity level found by a salivary buffer capacity measurement kit using saliva when chewing was observed to significantly differ between a caries-free group and a high-caries group (21). Oncag O reports a negative correlation between the buffer capacity of saliva and DMF (2). Moreover, Ruiz Miravet A et al. report that past caries experience and the buffer capacity of the saliva are the factors included in the cariogram that showed significant correlation with the caries risk (5). Additionally, the buffering capacity rather than the pH is considered to reflect the caries risk related to host defenses in the saliva, according to previous reports (12, 14). Farsi N (22) examined the pH and buffering capacity of resting and masticating saliva, and reported that only pH showed a positive association with DMFT. The salivary flow rate has been reported to be less likely to be associated with the caries incidence rate, such as DMFT, but many studies have demonstrated a positive association between DMFT and the buffering capacity (12, 14). However, there are some reports indicating a poor association (12, 14), as with the report by Farsi N (22). In the future, an in-depth analysis will be performed using samples from more subjects and assessing other risk factors. In this research, a significant difference was observed for DMFT respectively for high, middle and low buffer capacity groups. Accordingly, it was suggested that dental caries risk analysis using a small volume of resting saliva is possible and the method is useful. The average volume of residual saliva in the mouth after swallowing is considered to be 0.8ml (range: 0.4 to 1.4ml) in a healthy adult (12, 14). If saliva is difficult to collect from a subject during mastication, the method of collecting saliva from the mouth with a dropper may be effective. Since all of the saliva in the mouth cannot be collected, use of a small volume of saliva (0.2ml) as the sample enables determination of the salivary buffering capacity without placing a burden on the patient. Hereafter, an investigation incorporating other risk factors (cariogenic bacteria levels or saliva volumes, etc.) and an increase in the number of subjects will be necessary.

References

3. Hanada N: Clinical Biology of the mutans streptococci. Tokyo:


