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Original Article

Oral health status in relation to stimulated saliva buffering capacity among Japanese adults above or below 35 years of age

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Objectives: **To evaluate dental caries and periodontal pocketing in relation to saliva buffering capacity among two groups of Japanese adults aged above 35 years old (A35) or 35 years old and below (B35).** ***Methods:*** **After measuring the initial pH of saliva, samples were titrated with 0.1N HCl to evaluate the buffer capacity. Levels of *mutans streptococci* and *Lactobacilli* in saliva, DMF and periodontal pocket were also measured.** ***Results:*** **Among B35 group, DMF of adults with High buffering capacity was significantly lower than those of adults with Medium and Low buffering capacity ($p<0.05$). Chi-square test showed the distribution of subjects with high counts of *mutans Streptococci* or *Lactobacilli* into the three buffer capacities, which was not statistically different between two age groups ($p>0.05$). There were statistical differences for the mean periodontal pocket depth on the upper right incisor of adults in A35 with High buffer capacity and those of adults with any buffer capacity in B35 group. Statistical differences were also found for the pocket depth on lower left incisor and upper right molars of adults in A35 with High buffer capacity and those of adults with low buffer capacity in B35 group ($p<0.01$).** ***Conclusion:*** **High buffering capacity of saliva might be associated with better status of oral health among Japanese adults in both age groups.**

Key words: saliva, pH, buffer capacity, caries, periodontal pocket

Introduction

There are several differences between the policy of dental care provided by the Japanese national health insurance system and that of other developed countries. While the treatment of 'diseases' such as dental caries or periodontal disease are covered by the national health insurance system in Japan, preventive measures such as oral hygiene instruction are covered only under private health insurance in this country. This strategy may have some effects on the oral health status of the whole Japanese population. It has been reported that the frequency of daily tooth brushing among Japanese adult population was related to their level of education and income[1]. Fluoridated toothpaste has been suggested as an effective measure in prevention of dental caries and is widely available in most developed countries; however, it is not as common in Japan. Moreover, according to some epidemio-

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logical studies, the prevalence of dental caries would reduce in populations where the fluoride is added to the reticulated water supply[2], drinking water in Japan is not fluoridated.

Although the interest in maintaining natural teeth is growing among the Japanese adults, knowledge about the dental disease is generally poor and many misconceptions exist regarding the causes and prevention of those diseases[3]. These indicate the need of providing oral hygiene instructions to different classes of the community as well as continuous dental health risk assessment plans and preventive measures for the public in Japan.

During the past few years, a multifactorial approach has been applied to identify high caries risk groups and individuals among child and adolescent populations that includes saliva tests[4]. Most saliva studies have focused on the relationship between saliva properties and dental diseases[5]. Decreased buffering capacity and secretion rate of saliva have been suggested as factors correlated with the occurrence of dental caries[6]. Higher numbers of *mutans Streptococci* and *Lactobacilli* in dental plaque and saliva have also been reported to be associated with a higher prevalence of caries[6]. Saliva properties reside principally in flow rate, pH and buffering capacity, and the organic and inorganic components[7]. A high correlation has been demonstrated between advancing age, reduced saliva secretion rate and root caries[8]. Further studies are required in order to determine whether a correlation exists between caries prevalence and periodontal risk in adult populations, and if clinical predictors for tooth loss can be identified via the properties of saliva. Epidemiological surveys have been conducted in many countries to determine the dental treatment need of adults aged between 35 to 44 [9].

According to a statement about the objectives of Japanese national health to be achieved over the first decade of the 21st century[10], it is important to start preventive oral care in order to slow down the progression of periodontal disease in individuals over 35 years of age. The aim of this study was to evaluate the relationship between oral health status and saliva buffering capacity among two groups of Japanese adults aged above 35 years old or 35 years old and below. The null hypothesis was that oral health status was not associated with different levels of saliva buffering capacities among the two age groups.

Methods

Study Population

The study population consisted of 111 patients (31 men and 80 women) who attended for treatment at Faculty of Dentistry Hospital of Tokyo Medical and Dental University in Tokyo, Japan. The population was classified into two age groups: above 35 years old or 35 years old and below. The main criteria for selection were general health and the ability to cooperate and to follow instructions. This study was approved by the ethics committee of Tokyo Medical and Dental University. Prior to enrolment, written informed consent was obtained from each subject based on the code of ethics of the World Medical Association (declaration of Helsinki).

DMF examination was carried out using a mouth mirror and dental explorer by two dentists from the Department of Cariology and Operative Dentistry at Tokyo Medical and Dental University. “Decayed teeth” included those teeth exhibiting enamel and/or root caries, but did not include initial white-spot enamel lesions. The criteria for a positive diagnosis of enamel caries were loss of tooth substance having reached the stage of cavitation that could be observed by clinical examination after air drying of the tooth surface and “sticking” of the explorer on the enamel surface. Third molars or the teeth extracted for orthodontic purposes were not counted as “Missing teeth”.

Saliva sampling and buffering capacity

Stimulated whole saliva was collected once at either between 9 am and 11 am or between 2 pm and 4 pm. Patients were allowed to sit in the dental chair and relax for a few minutes. A 1-gram piece of unflavoured paraffin wax was chewed for 30 sec and the saliva was collected and discarded. Then while the patient was chewing the paraffin wax, saliva was continuously collected into a container for 5 min. Saliva collection was taken at least 2 h after meals and at least 1 h after brushing to minimize effects of the diurnal variability in saliva composition[11]. Saliva pH change was measured directly using a hand-held pH meter (Twin pH B-212, Horiba Ltd. Japan). Immediately after collecting the stimulated whole saliva, 0.5 mL of each saliva sample was placed onto the pH-sensitive electrode to measure the initial pH value within 30 seconds. Then 10 μL of hydrochloric acid (HCl) was titrated into the saliva sample in the measurement chamber of the pH meter, and allowed to stabilize for a few seconds;then, pH measured again. HCl titration was repeated up to a

total of 160 μL acid was titrated, and a pH titration curve for each patient was obtained. At the point of 50 μL titrated HCl, salivary buffering capacities were ranked into one of the following three categories according to the measured pH; High buffering capacity (pH above 5.5), Medium buffering capacity (pH between 4.5 and 5.5), and Low buffering capacity (pH below 4.5)[12].

Bacteria evaluation of saliva (*mutans Streptococci* and *Lactobacilli* counts)

The levels of *mutans Streptococci* and *Lactobacilli* were measured using a commercial caries risk test, CRT® bacteria (IVOCLAR VIVADENT, Liechtenstein). The test vial had double sided agar carrier with two sides. One side of the carrier was coated with a solid selective culture medium (mitis salivarius agar enriched with sucrose) for the cultivation of *mutans Streptococci*, while the medium on the other side (Rogosa agar) was for the cultivation of *Lactobacilli*. After application of the saliva sample, the agar carries was returned to the vial according to the instructions of the manufacturer. The vials were incubated at 37 °C for 48 hours to allow the bacterial colonies to grow. The results of CRT bacteria count are expressed as low ($<10^5$ CFU) or high ($>10^5$ CFU).

Periodontal pocketing

For periodontal recordings in the clinical examination, the mouth was divided into six segments, three in the upper jaw and three in the lower jaw. The following natural teeth were evaluated; right incisor and first molars in the upper jaw, and left incisor and first molars in the lower jaw. Subjects who did not have any of the three teeth in a jaw were excluded from the study. The probing depth was measured from the gingival margin to the base of the pocket using a standard probe (Hu-Friedy, PCPUNC 15 Chicago, IL, USA), and rounded to the nearest higher millimeter, at six sites around each tooth (mesio-buccal, mesio-lingual, mid-buccal, disto-buccal, disto-lingual and mid-lingual).

Statistical Analysis

The DMF results were statistically analyzed using a two-way analysis of variance (ANOVA) at a significant level of 0.05 with the DMF as dependent variable and the age group and buffering capacity as factors. Post hoc test were also performed using Tukey's HSD test, at a confidence level of 95%. Initial saliva pH and CRT bacteria counts were the risk indicators evaluated in relation to saliva buffer capacity and age. The best cut-off points for dichotomization were ≤7.0 pH for initial saliva pH, $\geq 10^5$ for *mutans Streptococci*, $\geq 10^5$ for *Lactobacilli*[13]. The distribution of high risk individuals among the three buffering capacity levels were statistically compared between the two age groups using the Fisher's exact Chi-square test at a confidence level of 95%. For each tooth, the mean depths of periodontal pocket were statistically compared between the two age groups and buffering capacities using the Steel-Dwass' non-parametric multiple comparison test by Tukey's procedure at the 99% level of confidence[14,15]. All statistical calculations were performed using general linear modelling (SPSS Version 11.0 for Windows).

Results

Distribution of subjects into different buffering capacity groups is shown in Table 1. Two-way ANOVA revealed that DMF was influenced by both factors of age ($F=92.266$, $p<0.0001$) and buffer capacity ($F=4.921$, $p=0.009$) (Table 2). There was no interaction between the two factors ($F=0.729$, $p=0.485$). Tukey HSD test showed that there was no significant differences in DMF score between the three buffering capacities in the group of adults aged above 35 years old ($p>0.05$). However, in the other age group (adults aged 35 years old and below), mean DMF score of subjects with high buffering capacity was significantly lower than that of those with medium or low buffering capacity ($p<0.05$).

The distribution of individuals in the two age groups and three buffering capacities with regard to the initial pH is shown in Table 3. The initial pH of all subjects aged 35 years old and below were higher than 7.0. Similarly, among the adults aged over 35 years old, all the subjects with high buffering capacity had an initial saliva pH higher than 7.0; in this age group, however, the initial pH was observed to be below 6.9 for 1 subject (8%) with medium and for 5 subjects (25%) with low buffering capacities. Chi-square test showed that the distribution of individuals with high counts of *mutans Storeptococci* and *Lactobacilli* among the three buffer capacities was not statistically different between the two age groups ($p>0.05$) (Table 4).

Table 5 shows the means of periodontal pocket depths of the examined teeth among the different buffering capacity groups and different age groups. For the upper right incisor, there was a statistical difference in mean periodontal pocket depth between the subjects aged 35 or below with high buffer capacity and subjects

Table 1. The distribution of subjects in different buffering capacity groups and age groups (mean Å} s.d.)

	Age group							
	35 years old and below (age: 25.4 ± 2.5)				above 35 years old (age: 58.2 ± 11.0)			
Buffrer capacity group	n	age	male	female	n	age	male	female
High	26	25.3 ± 1.6	8	18	22	61.2 ± 11.9	6	16
Medium	10	24.7 ± 1.9	4	6	15	55.3 ± 10.0	5	10
Low	18	25.8 ± 2.6	4	14	20	56.9 ± 10.2	4	16

Table 2. The distribution of DMF in different buffering capacity groups and age groups (mean±s.d.)

	Age group			
	35 years old and below		above 35 years old	
Buffrer capacity group	n	DMF	n	DMF
High	28	7.86 ± 5.57	22	20.18 ± 4.94[b]
Medium	10	12.60 ± 4.69[a]	13	21.92 ± 6.56[b]
Low	18	12.28 ± 5.21[a]	20	22.45 ± 5.81[b]

DMF with same superscripts are not significantly different (p>0.05).

Table 3. The distribution of individuals in different initial pH in different buffering capacity groups and age groups.

Age group	Buffer capacity group	Initial pH below pH 7
35 years old and below	High	0 (0%)
	Medium	0 (0%)
	Low	0 (0%)
above 35 years old	High	0 (0%)
	Medium	1 (8%)
	Low	5 (25%)

Table 4. The distribution of individuals in *S. mutans* (>10^5 CFU) and *Lactobacilli* (>10^5 CFU) and statistics values under chi-square test in different buffering capacity groups and age groups.

	S. mutans			*Lactobacilli*		
	Buffer capacity group			Buffer capacity group		
Age group	High	Medium	Low	High	Medium	Low
35 years old and below	10	2	11	3	4	5
above 35 years old	10	8	18	6	10	17
statistics values	$\chi 2$=2.549, p>0.05			$\chi 2$= 0.405, p>0.05		

Table 5. The mean periodontal pocket depth in different buffering capacity groups and age groups.

	Buffer capacity	periodontal pocket depth (mean ± s.d.)					
Age group	group	16	46	11	31	26	36
35 years old and below	High	3.0 (0.7)	2.9 (0.5)	2.3 (0.4)[a,b,c]	2.3 (0.5)[d]	2.9 (0.6)[e]	2.9 (0.5)
	Medium	3.0 (0.2)	3.2 (0.4)	2.4 (0.5)	2.2 (0.4)	3.0 (0.2)	3.2 (0.4)
	Low	3.1 (0.5)	3.1 (0.6)	2.7 (0.8)	2.4 (0.5)	3.1 (0.7)	3.1 (0.7)
above 35 years old	High	4.2 (1.7)	3.6 (0.9)	3.3 (1.1)[a]	3.0 (0.8)	4.3 (1.5)	3.9 (1.0)
	Medium	3.8 (0.7)	3.7 (0.8)	3.6 (1.3)[b]	3.3 (0.9)	4.2 (0.9)	4.2 (1.1)
	Low	4.3 (1.7)	4.0 (1.8)	4.3 (1.8)[c]	3.8 (1.4)[d]	4.9 (1.7)[e]	3.8 (1.5)

Tooth location at periodontal pocket depth; according to FDI notation
Means with the same superscript are significantly different (p<0.01)

with any buffering capacity in the other age group of over 35 years old. The periodontal pockets on the lower left incisor of subjects aged above 35 years who had a low buffering capacity were statistically significantly deeper than those of adults with a high buffering capacity in the other age group. Likewise, the mean depth of pockets on the upper right first molar showed the same difference pattern between the groups ($p<0.01$).

Discussion

It is difficult to completely eliminate the interfering factors affecting the pH parameters of saliva samples that are collected in a dental practice setting. However, stimulated saliva is known to have little variation throughout the day.[16] Moreover, resting saliva measurements are difficult to standardize and demands a long time for the collection of samples.[17] Using the same type of hand-held pH meter as used in the current study, Moritsuka *et al*. [18] evaluated the pH change during HCl titration (10 μL of 0.1 mol/L HCl was titrated up to total of 160 μL) into resting and stimulated saliva samples to determine buffering capacity. Since, in comparison to resting saliva, stimulated saliva was more resistant to variation in pH during HCl titration, they suggested that stimulated saliva should be used rather than resting saliva for saliva buffer analysis. A more consistent correlation between salivary factors and the oral health of individuals is likely to be obtained with the use of stimulated saliva; therefore, this type of saliva was used in this study.

Ravald & Birkhed[6] suggested that the saliva buffering effect was positively correlated with root caries activity. In the current study, DMF was used as the index for the caries, since root caries was not a suitable index for the full age range of the population evaluated in this study, however, the lack of specific root caries data may be regarded as a limitation of this research work. It was found that saliva buffer capacity significantly influenced the DMF scores by the Two-way ANOVA. These results suggested that buffering capacity of saliva plays a role in the DMF score and is an important factor in maintaining good oral health. Moreover, it was found that age was also a factor that had a significant influence on the DMF score.

Ravald & Hamp[8] reported that a salivary pH below 7 seemed to affect the root surface caries activity. It has also been suggested that a salivary pH below 7 may be of risk factors for the development of caries in adults aged above 35 years old who are exhibiting a poor buffering capacity[19]. Findings of the current study indicated that the risk factor of low initial pH exists in the Japanese population, since a number of cases in the group of adults aged over 35 years old exhibited an initial pH below 6.9.

Commercial bacterial colony counting test kits have been developed and used for chair-side bacteria assessment[20]. Chi-square test showed that for individuals with high counts of *mutans Storeptococci* and *Lactobacilli*, there was no significant association between the buffer capacity level and the age group ($p>0.05$). High counts of *mutans Streptococci* were obtained in a remarkable number of cases among all groups in the current study. While the high counts of *mutans Streptococci* were expected for the low buffer capacity groups, they were unlikely in the high and medium buffer capacity group. One possible explanation for their occurrence is that *mutans Streptococci* is a minor contributor to an acidic Stephan response and to the initiation of dental caries[21]. Many in vivo studies suggest that the low final pH of plaque reflects not only the well-known emergence of *mutans Streptococci*[22] but also other numerically predominant organisms, such as non-*mutans Streptococci* and *Actinomyces*[23]. Microorganisms of interest in this regard should also include the non-*mutans Streptococci*, because they are numerous in both saliva and plaque, which can often reach a very low final pH[24], and have been shown to exhibit significant cariogenicity in rodents[25]. Moreover, saliva sample in this study were not diluted for colony counting of either *mutans Streptococci* or *Lactobacilli*. Further bacterial studies are needed to evaluate the relationship between individual saliva buffer capacity and the salivary concentration of cariogenic microorganisms and other factors such as age.

The Japanese adults aged above 35 years generally lose teeth due to periodontal disease[4], and people in this age group are more conscious of their oral health[1]. In this study, the Steel-Dwass' non-parametric multiple comparison test showed that there were statistically differences in the mean periodontal pocket depth between the groups with regard to the buffering capacity and age. For the upper right incisor, there was a statistical difference in mean periodontal pocket depth between the subjects aged 35 or below with high buffer capacity and subjects with any buffering capacity in the other age group of over 35 years old. Moreover, deep periodontal pockets were found on the mandibular incisor and maxillary molars of the adults aged above 35 years with low buffer capacity. Further

clinical longitudinal studies are needed to evaluate the relationship between individual saliva buffering capacity in relation to other periodontal parameters such as calculus formation or radiographic bone loss.

Although the number of subjects investigated in this study was limited, notable statistical relationships were found between saliva buffering capacity and oral health status of both age groups. The null hypothesis was rejected, as it seems that greater saliva buffering capacity is associated with the good dental health among Japanese adults in both age groups. It should be reemphasized here that preventive dental treatments and measures are not covered under the Japanese national health insurance system. With regard to the current status of preventive dentistry in Japan, the Japanese national health insurance system can change from a disease-depended system to the preventive care-depended system by covering the administration of simple but informative screening tests such as the saliva assessment test, described in the current study. This quantitative saliva buffering capacity test may contribute to the promotion of oral health among all adults. It is useful to identify patients with the risk factors of low buffering capacity and low initial pH, and may be useful when the preventive strategies are considered in order to reduce the progression of both the caries and periodontal diseases.

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