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Original Article

No Relationship of Salivary Flow Rate or Secretory Immunoglobulin A to Dental Caries in Children

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Abstract

To investigate the relationship between dental caries and the salivary flow rate, secretory immunoglobulin A (sIgA) or other components in children, nonstimulated whole saliva was collected and teeth status was examined in 138 boys and 134 girls aged 11-12 years. The subjects were apparently healthy. The mean salivary flow rate was faster in boys than in girls (0.29 vs 0.18 ml/min, $p<0.001$). In both sexes, secretion of salivary sIgA and three other components (total protein, calcium and amylase activity) was markedly dependent on salivary flow rates. These results suggest that basal components of resting saliva are secondarily secreted with the flow of saliva fluid. The mean erupted permanent teeth was 21.0 teeth (range: 10-28 teeth) in boys, and 23.0 teeth (13-28 teeth) in girls (sex-difference: $p<0.001$). The means of DMFT, the DMFT ratio (% of DMFT to erupted permanent teeth) and DT+dt (sum of decayed permanent and milk teeth, an index for active caries) were 3.4 DMFT (range: 0-11 DMFT), 16.0% (0-40.0%) and 0.5 DT+dt (0-7 DT+dt) in boys, and 3.8 DMFT (0-12 DMFT), 16.2% (0-44.4%) and 0.8 DT+dt (0-5 DT+dt) in girls, respectively (sex-differences: $p>0.05$ in all). The salivary flow rate or the four salivary components (either concentration or secretion rate) used here had no relationship to the DMFT ratio or to DT+dt in either sex. Variation in the flow rate or in the basal components of resting saliva may not influence caries development in healthy children.

Key words: Saliva, Flow rate, Secretory IgA, Dental caries, Children

Introduction

Dental caries of permanent teeth is very common even in children in Japan. In the Survey of Dental Disease, 1993 in Japan conducted by the Ministry of Health and Welfare[1], the mean number of decayed, missing and filled permanent teeth (DMFT) was 2.8 DMFT in children aged 10 years, and 6.6 DMFT in those aged 15 years. These findings indicate that dental caries of permanent teeth steadily increase and accumulate during these periods of growth in the Japanese population. Therefore, factors influencing caries development in children should be clarified, also, to improve the dental health of adults.

On the other hand, it is generally agreed by dental professionals that saliva and its components influence teeth status. The salivary flow rate, rather than salivary components, is thought to be a main protective factor for dental caries because of its function of dilution and clearance of cariogenic bacteria and substrates in the oral cavity[2,3]. Secretory immunoglobulin A (sIgA) in saliva may also be related to the prevention of dental caries' development[4], since sIgA is one of the humoral immune factors effective in mucosa against pathologic agents. However, the results from previous human studies on the relationship between dental caries and the salivary flow rate[5-8] or IgA[7-12] are conflicting. The effects of other components of saliva on teeth are more obscure. Thus, to investigate the rela-

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tionship between dental caries and salivary flow rate, sIgA or the levels of other components (total protein, calcium, amylase) in healthy children, resting whole saliva was collected and teeth status was determined in Japanese children aged 11-12 years.

Subjects and Methods

The subjects were 138 boys and 134 girls aged 11-12 years in the 6th grade, the last grade of a Japanese primary school. All the subjects were apparently healthy. The subjects brushed their teeth and washed their oral cavities at home just before going to school on the mornings of the sampling days, and were not allowed to eat until saliva was collected in the school. Nonstimulated whole saliva was collected by the drain method[3] at 11 a.m. on weekdays of February 1994 in the school. The method is an easy and safety method without specific procedures to collect nonstimulating saliva. Briefly, after swallowing saliva in the mouth, the subjects were seated, head slightly down, and were asked to spit the saliva spontaneously secreted into graduated plastic tubes. The collection time varied from 7 to 30 min (mean : 19.6 min) to collect an adequate sample volume for saliva analysis. The saliva was placed at 4 ℃ during the following treatment and analysis. The salivary flow rate (ml/min) was calculated from the volume of saliva collected and the collection time. After centrifugation of the saliva at 3000 rpm for 10 min, a supernatant was used as saliva samples for the analysis of its components. Within 48 hours of the sampling, salivary total protein (TP, mg/l) was determined by the Coomassie blue dye binding method using a Bio-Rad Protein Assay (Bio-Rad, California), and salivary calcium (Ca, mg/l) by the OCPC method using a Calcium C-test kit (Wako-Jyunyaku, Osaka), and salivary amylase activity (Amy, IU/ml) by the Carboxy-methyl-amylose/DEX method using an Amylase B-test kit (Wako-Jyunyaku, Osaka). Residual saliva samples were stored at -80℃ until sIgA analysis. Salivary sIgA (mg/l) was determined by the ELISA method[13] with a Purified Human Secretory IgA (Cappel, Pennsylvania) as a standard of sIgA. The interassay coefficients of variation for 10 measurements of the same sample were less than 2.0% for TP, 5.1% for Ca, 6.6% for Amy, and 7.0% for sIgA. The four salivary components were expressed also by secretion rate per minute (μg/min in TP, Ca and sIgA, IU/min in Amy).

Teeth of the subjects were examined by a school dentist (K. M.) as an annual oral health check for school children. X-rays were not used in the checkup. A DMFT ratio (% of DMFT to erupted permanent teeth) was calculated as an index for caries experience of permanent teeth, since the number of erupted permanent teeth widely varied among the subjects (10 to 28 teeth), and since DMFT was positively correlated with erupted permanent teeth in the subjects ($r=0.419$ in boys, $r=0.314$ in girls, $p<0.001$ in both). Missing permanent teeth were not found in any subjects. Children aged 11-12 years naturally have remaining milk teeth, so, the sum of decayed permanent and milk teeth (DT+dt) was used as an index for active dental caries.

Sex-differences of salivary components and teeth status were tested by the Student's t-test with or without Cochran's correction, and the Pearson's correlation coefficient test was used to test the relationship between the two variables. Spearman's correlation coefficients also calculated were not apparently different from the Pearson's, so, the Pearson's correlation coefficients are used here. In the tests, the salivary flow rate and components (both concentration and secretion rate) were used after logarithmic transformation. A commercial software for personal computers (PC-SAS, SAS Institute Inc., North Carolina) was used in the statistical analysis, and statistical significance was denoted by $p<0.05$ for all tests.

Results

Salivary components and teeth status of the subjects by sex are summarized in Table 1. Pearson's correlation coefficients among salivary flow rate and components, and the DMFT ratio or DT+dt are presented in Table 2.

The mean salivary flow rate was faster in boys than in girls (0.29 vs 0.18 ml/min $p<0.001$). The mean concentrations of salivary sIgA, TP or Amy were not different between sexes ($p>0.05$), but that of Ca was slightly lower in boys than in girls (35 vs 37 mg/l, $p<0.05$). There were inverse correlations between the salivary flow rate and the concentrations of the components in both sexes ($r=-0.010$ to -0.463, not significant in sIgA or Amy in girls, significant in the others). The mean secretion rates of salivary sIgA (20 vs 13 μg/min, $p<0.001$), TP (156 vs 103 μg/min, $p<0.001$), Ca (10.0 vs 6.8 μg/min, $p<0.001$) and Amy (126 vs 89 IU/min, $p<0.01$) were higher in boys than in girls. The secretion rates of the four components were markedly and positively dependent on the salivary flow rate in both sexes ($r=0.575$ to 0.959, $p<0.001$ for all four components).

Erupted permanent teeth ranged from 10 to 28 teeth in boys, and from 13 to 28 teeth in girls, and the mean was smaller in boys than in girls (21.0 vs 23.0 teeth, $p<0.001$). On the contrary, the mean remaining milk teeth were more in boys than in girls (3.0 vs 2.0, $p<0.01$). DMFT, DMFT ratio and DT+dt ranged from 0 to 11 DMFT, from 0 to 40.0% and 0 to 7 DT+dt in boys, and from 0 to 12 DMFT, from 0 to 44.4% and from 0 to 5 DT+dt in girls, respectively. The mean DMFT (3.4 vs 3.8 DMFT), DMFT ratio (16.0 vs 16.2%) and DT+dt (0.5 vs 0.8 DT+dt) were slightly smaller in boys than in girls, but the sex-differences were not significant.

No significant relationships between the DMFT ratio or DT+dt and the salivary flow rate or salivary components (either concentration or secretion rate) used here were found in either sex ($r=-0.159$ to 0.135, $p>0.05$ in all).

Discussion

The mean salivary flow rate found in children was less than that (0.3 to 0.4 ml/min) in adults previously reported[3,14]. The salivary gland and its secretory function may have not yet matured in the subjects aged 11-12 years. A faster flow rate in men than in women has, also, been found in adults[15]. Concentrations of the four salivary components analyzed here were slightly inversely correlated with the salivary flow rate ($r=-0.010$ to -0.463). Faster flow rates of saliva with lower concentrations of IgA and other components have been reported in previous studies[7,11,16,17]. Secretion rates of the four salivary components determined here were markedly dependent on the salivary flow rate ($r=0.575$ to 0.959). A positive correlation

between the salivary flow rate and the IgA secretion rate has been previously found[11]. The faster secretion rates of salivary components in boys than in girls found here are probably due to sex-differences in the salivary flow rate. These previous reports and our results suggest that basal components of resting saliva are secondarily secreted with the flow of saliva. The flow rate may be a more meaningful index for physiologic status of resting saliva than its components.

The use of β^2-adrenoceptor agonists for asthmatic patients aged 14 to 24 years reduced saliva secretion and increased susceptibility to dental caries[18]. Moreover, impairment of salivary secretion induced by chronic malnutrition in children[19], by irradiation in patients with tumors[20] and by other disorders[21] increased the susceptibility. These pathologic conditions could have disturbed saliva secretion to induce xerostomia, and, subsequently, may have affected teeth status. However, either in a follow-up study in teenagers[22] or in a cross-sectional study in adults[6], the salivary flow rate has not influenced the experience of dental caries in general populations. In the present study in children, neither the salivary flow rate, the concentration nor the secretion rate of salivary components analyzed here had any relationship to the DMFT ratio or DT+dt. These results from human studies suggest that variations in the saliva status of general subjects do not influence the experiences of caries or caries activities. The role of the salivary IgA antibody in dental caries development or activities has not been established[4,9]. Some studies[8,10,11] have shown inverse correlations between salivary IgA and dental caries experiences, and possible protective effects of salivary IgA against dental caries. However, other studies[7,12] failed to confirm the results. Even the salivary IgA antibody specific to Streptococcus mutans, cariogenic bacteria, had no relation to active caries or caries experiences in young adults[7]. In the previous studies related to salivary IgA, total salivary IgA was determined, but sIgA, which had more immune effects, was not analyzed separately from total IgA. In addition, the methods of saliva collection and IgA analysis varied among the studies. Salivary IgA levels could be influenced by these methods. IgA concentration was lower in saliva collected by the Salivette device than in saliva collected by suction or spitting[23]. For IgA determination, the ELISA method has much smaller coefficients of variation than the single radial immunodiffusion technique (a conventional method)[24]. These may be reasons why the previous studies had conflicting results.

In conclusion, neither flow rate, sIgA nor other components (TP, Ca or Amy) of resting whole saliva had any relationship to dental caries experiences or ativities in healthy children. Further epidemiological studies including saliva and other factors are required to establish human susceptibility to dental caries.

Table 1 Salivary components and teeth status in children aged 11-12 years.

Salivary components/ teeth status	Boys (n-138)	Girls (n=134)	Sex-difference
Salivary flow (ml/min) 1	0.29(0.26-0.32)	0.18(0.17-0.20)	***
sIgA (mg/l) 1	70(67-73)	69(66-72)	NS
(ug/min) 1	20(18-22)	13 (11-14)	***
TP (mg/l) 1	545(512-580)	563(530-598)	NS
(ug/min) 1	156(140-174)	103(93-114)	***
Ca (mg/l) 1	35(34-36)	37(36-39)	*
(ug/min) 1	10.0(8.9-11.3)	6.8(6.3-7.4)	***
Amy (IU/ml) 1	442(389-501)	488(434-548)	NS
(IU/min) 1	126(109-146)	89(77-103)	**
Erupted permanent teeth 2	21.0(4.4)	23.0(3.6)	***
DMFT 2	3.4(2.1)	3.8(2.1)	NS
DMFT ratio (%) 2	16.0(9.2)	16.2(9.0)	NS
Remaining milk teeth 2	3.0(3.3)	2.0(2.6)	**
DT+dt 2	0.5(1.0)	0.8(1.2)	NS

1 : geometric mean (95% CI), 2 : arithmetic mean (SD)
* : $p<0.05$, ** : $p<0.01$, *** : $p<0.001$, NS : not significant by Student's t-test.

Table 2 Pearson's correlation coefficients among salivary components and dental caries in children aged 11-12 years.

Salivary components	Boys (n=138)			Girls (n=134)		
	logFlow (ml/min)	DMFT ratio (%)	DT+dt	logFlow (ml/min)	DMFT ratio (%)	DT+dt
logFlow (ml/min)	—	-0.146NS	0.034NS	—	-0.084NS	0.033NS
logsIgA (mg/l)	-0.330***	-0.013NS	-0.106NS	-0.142NS	0.063NS	0.045NS
(ug/min)	0.915***	-0.159NS	-0.011NS	0.873***	-0.045NS	0.051NS
logTP (mg/l)	-0.446***	0.109NS	-0.044NS	-0.208*	-0.125NS	-0.080NS
(ug/min)	0.854***	-0.098NS	0.011NS	0.798***	-0.153NS	-0.021NS
logCa (mg/l)	-0.203*	0.091NS	0.008NS	-0.463***	0.003NS	0.004NS
(ug/min)	0.959***	-0.123NS	0.036NS	0.886***	-0.090NS	0.037NS
logAmy (IU/ml)	-0.271**	0.135NS	0.035NS	-0.010NS	-0.117NS	-0.029NS
(IU/min)	0.575***	-0.002NS	0.056NS	0.611***	-0.143NS	-0.004NS

* : $p<0.05$, ** : $p<0.01$, *** : $p<0.001$, NS : not significant

References

1) Anonymous. Report on the Survey of Dental Disease 1993. Health Policy Bureau Ministry of Health and Welfare Japan, ed. Tokyo. Oral Health Association, 1995. (in Japanese)
2) Lagerlof F, Oliveby A. Caries-protective factors in saleva. Adv Dent Res 1994 ; 8 : 229-38.
3) FDI working group. Saliva : its role in health and disease. Int Dent J 1992 ; 42 : 291-304.
4) Taubman MA, Smith DJ. Significance of salivary antibody in dental desease. Ann NY Acad Sci 1993 ; 694 : 202-15.
5) Edgar WM, Higham SM, Manning RH. Saliva stimulation and

caries prevention. Adv Dent Res 1994 ; 8 : 239-45.
6) Billings R. An epidemiologic perspective of saliva flow rates as indicatiors of susceptibility to oral disease. Crit Rev Oral Bilo Med 1993 ; 4 : 351-6.
7) Grahn E, Tenovuo J, Lehtonen O-P *et al.* Antimicrobial systems of human whole saliva in relation to dental caries, cariogenic bacteria, and gingival inflammation in young adults. Acta Odontol Scand 1988 ; 46 : 67-74.
8) Everhart DL, Klapper B, Carter Jr WH *et al.* Evaluation of dental caries experiences and salivary IgA in children aged 3-7. Caries Res 1977 ; 11 : 211-5.
9) Brandtzaeg P. Role of antibodies in saleva : facts and extrapolations. In : Guggenheim B, ed. Cariology today. Basel : Karger, 1984 : 89-97.
10) Challacombe SJ. Immunoglobulins in parotid saliva and serum in relation to dental caries in man. Caries Res 1976 ; 10 : 165-77.
11) Orstavik D, Brandtzaeg P. Secretion of parotid IgA in relation to gingival inflammation and dental caries experiece in man. Arch Oral Biol 1975 ; 20 : 701-4.
12) Everhart DL, Grigsby WR, Carter Jr WH. Evaluation of dental caries experience and salivary immunoglobulins in whole saliva. J Dent Res 1972 ; 51 : 1487-91.
13) Wood GM, Trejdosiewicz LK, Losowsky MS. ELISA for measurement of secretory IgA distinct from IgA. J Immunol Methods 1987 ; 97 : 269-74.
14) Edgar WM. Saliva. its secretion, composition and functions. Br Dent J 1992 ; 172 : 305-12.
15) Heintze U, Birkhed D, Bjorn H. Secretion rate and buffer effect of resting and stimulated whole saliva as a function of age and sex. Swed Dent J 1983 ; 7 : 227-38.
16) Shannon IL, Feller RP. Parctid saliva flow rate, calcium, phosphorus, and magnesium concentrations in relation to dental cries in children. Pediat Dent 1979 ; 1 : 16-20.
17) Kugler J, Hess M, Haake D. Secretion of salivary immunoglobulin A in relation to age, saliva flow, mood sates, secretion of albumin, cortisol, and catecholamines in saliva. J Clin Immunol 1992 ; 12 : 45-9.
18) Ryberg M, Moller C, Ericson T. Saliva composition and caries development in asthmatic patients treated with β^2-adrenoceptor agonists : a 4-year follow-up study. Scand J Dent Res 1990 ; 99 : 212-8.
19) Johannson I, Saellstrom A-K, Rajan BP *et al.* Salivary flow and dental caries in Indian children suffering from chronic malnutrition. Caries Res 1992 ; 26 : 38-43.
20) Spak CJ, Johnson G, Ekstrand J. Caries incidence, salivary flow rate and efficacy of fluoride gel treatent in irradiated patients. Caries Res 1994 ; 28 : 388-93.
21) Sreebny LM. Salivary flow and dental caries. In Guggenheim B, ed. Cariology today. Basel : Karger, 1984 : 56-69.
22) Alaluusua S, Kleemola-Kujala E, Gronroos *L et al.* Salivary caries-related tests as predictors of future caries increment in teenagers. A three-year longitudinal study. Oral Microbiol Immunol 1990 ; 5 : 77-81.
23) Aufricht C, Tenner W, Salzer HR *et al.* Salivary IgA concentration is influenced by the saliva collection method. Eur J Clin Chem Clin Biochem 1992 ; 30 : 81-3.
24) Sato K. Enzyme-linked immunosorbent assay of SIgA in whole saliva of healthy subjects and patients with oral diseases. Bull Tokyo Med Dent Univ 1991 ; 38 : 9-18.