SALIVARY SECRETORY IgA, pH, FLOW RATES, MUTANS STREPTOCOCCI AND *CANDIDA* IN CHILDREN WITH RAMPANT CARIES

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Abstract. The aim of this study was to determine the levels of secretory IgA (SIgA), pH, flow rates, mutans streptococci (MS) and *Candida* in saliva of children with rampant caries compared to those caries-free. Thirty children (age 62-123 months) were enrolled and divided into two groups: Group I, children with rampant caries, Group II, caries-free children. The average salivary flow rate was measured from the volume yielded within 5 minutes and the pH was determined using a pH-electrode. Measurement of SIgA was performed using an immunoassay kit. The levels of MS and *Candida* were determined by culture on Mitis-Salivarius Bacitracin agar and Sabouraud dextrose agar. It was found that children with rampant caries presented with significantly higher levels of salivary SIgA, MS and *Candida*. However, the mean values for salivary flow rates and pH were similar between the groups. The results reveal that children with rampant caries had significantly higher levels of SIgA, MS and *Candida* in their oral cavities. This finding tends to support the hypothesis that higher levels of salivary SIgA may reflect a past exposure of the host to cariogenic microorganisms.

INTRODUCTION

Saliva composition is an important factor in determining the prevalence of caries (Yarat *et al*, 1999). Previous studies have demonstrated that salivary secretory IgA (SIgA), pH and flow rates play important roles in the oral mucosal defense (Barr-Agholme *et al*, 1998; Benderli *et al*, 2000). SIgA is the prominent immunoglobulin in whole saliva and is considered to be the main specific defense mechanism in the oral cavity. In conjunction with several antimicrobial substances, including lysozyme, lactoferrin, salivary peroxidase, and mucins, SIgA may help maintain the oral cavity disease free by limiting microbial adherence to epithelial and tooth surfaces by neutralizing virulence factors. SIgA may also prevent the penetration of antigens into the oral mucosa. SIgA has a parabolic relationship with age. At birth, levels of SIgA are undetectable, but there is a consistent increase in the levels with age. By age 7 years the levels of SIgA reach their peak. SIgA levels remain consistently high during mid-life and then decline during old age. No gender differences in SIgA levels have been reported. SIgA is not directly related to serum levels of SIgA (Smith *et al*, 1987; Ben-Aryeh *et al*, 1990; Kugler *et al*, 1992). A lower concentration of SIgA has been conceptualized as a risk factor not only for upper respiratory infection in children and the elderly (Smith *et al*, 1987; Ben-Aryeh *et al*, 1990) but also for periodontal disease and caries (Gregory *et al*, 1992).

Dental caries is a multifactorial disease associated with cariogenic microorganisms, a high frequency carbohydrate consumption,

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poor oral hygiene, malnutrition and low socio-economic status (Tinanoff and O' Sullivan, 1997; Petersen, 2005). Despite the general global reduction of dental caries in past years, the disease in children is still a problem in many developing countries (Petersen, 2005). The current concept of dental caries centers on the fermentation of carbohydrates by cariogenic plaque bacteria, producing organic acids that act on a susceptible tooth. Mutans streptococci (MS), aciduric and acidogenic microorganisms colonizing the oral cavity, are considered to be the main causes of dental caries. They possess a wide range of cariogenic properties. For example, they synthesize water-insoluble glucans which mediates irreversible adhesion and colonization of the tooth surfaces. In addition, during periods of low concentration of exogenous substrate, they metabolize intracellular polysaccharide, which supports continual acid production (Tinanoff and O' Sullivan, 1997). Apart from MS, *Candida* species, an opportunistic dimorphic fungi have increased in medical importance over the past few decades. *Candida albicans* has recently been reported to have the potential to produce acid and suggesting a cariogenic potential (Nikawa *et al*, 2003). Many studies have demonstrated the importance of the presence of *Candida* in the oral cavity and the incidence of caries in adults and children (Scheinin *et al*, 1992; Raitio *et al*, 1996).

In studies of host defense against dental caries, salivary IgA has been found to have several properties, including microbe-aggregation, bacterial enzyme neutralization and inhibition of both MS and *Candida* adherence to buccal epithelial cells (Vudhichamnong *et al*, 1982; Hajishengallis *et al*, 1992). However, little evidence concerning the protective role of IgA antibodies against dental caries has been revealed. The protective role of salivary SIgA against dental caries investigated in both children and adults has been reported in many studies. Previous studies attempted to correlate total salivary SIgA concentrations with caries susceptibility, as recorded by an index of decayed, missing, or filled teeth (DMFT) or surfaces (DMFS) (Brandtzaeg, 1983). However, the results of these studies are variable: either positive, negative, or no correlation between total salivary IgA and dental caries could be found (Brandtzaeg, 1983). The aim of the present study was to determine the levels of salivary SIgA, salivary pH, flow rates, MS- and *Candida*-levels in children with rampant caries and in caries-free children.

MATERIALS AND METHODS

The investigation was conducted at the Pediatric Dental Clinic, Faculty of Dentistry, Mahidol University, Thailand after approval by the Ethics Committee of Mahidol University. The aims and procedures were explained to the parents of the subjects involved, and informed written consent was obtained prior to investigation.

Thirty children, aged 62 to 123 months, were selected and classified into two groups of 15 each: Group I, children with rampant caries; Group II, caries-free children. There were no statistical differences between the groups in regard to age and gender (Mann-Whitney *U* test, $p>0.05$ and chi-square test, $p>0.05$). All selected children were required to have: co-operative behavior, normal growth and development, the absence of congenital or systemic disease, the absence of dental abscesses, the absence of any medication therapy, no history of local infection in the 3 months prior to the study or prior dental treatment at the time of the examination. Each child was instructed not to eat or drink anything for 2 hours before the appointment. The dental examination was performed in a dental chair, using a dental mirror and an explorer.

In Group I, children were considered to have rampant caries if they had tooth decay of

more than 5 teeth and tooth surface decay of at least 10 surfaces. In Group II, children were considered caries-free if they did not show clinical signs of dental caries. Whole saliva was stimulated by chewing paraffin (1.0 g) for 30 seconds and was then collected for 5 minutes in a sterile plastic container. The average salivary flow rate was obtained from the total volume collected in the study time. Salivary pH was determined using a pH micro-electrode (IQ Scientific, USA). All samples were collected between 8:00 and 11:00 A.M. to minimize the circadian rhythm effects and the time spent on the procedure did not exceed 25 minutes. These saliva samples were transported immediately to the laboratory where the immunological and microbiological assays were performed. The total concentration of salivary SIgA was determined using a commercially available enzyme immunoassay kit (Salimetrics LLC, USA). The levels of MS and *Candida* in the saliva samples were determined by culture using Mitis-Salivarius bacitracin agar and Sabouraud dextrose agar, respectively, at 37ºC in 5% CO_2 for 48 hours. The identification of MS and *Candida* species was based on the colony morphology and Gram staining. The levels of microorganisms were expressed as CFU/ml. Before statistical analysis, the number of these microorganisms was converted logarithmically. Comparison of the mean SIgA, pH, flow rates, MS and *Candida* values between the groups was carried out using the non-parametric test of *U* Mann-Whitney. The level of significance was set at $p<0.05$.

RESULTS

Mean values and standard deviations for age, salivary SIgA concentration and salivary pH for the studied groups are shown in Table 1. The total salivary concentration of SIgA was significantly higher in the group of children with rampant caries (Group I) than in the caries-

Table 1
SIgA concentration, salivary pH and age of children in each group.

	Rampant caries			Caries free		
	Age (Months)	IgA (µg/ml)	pH	Age (Months)	IgA (µg/ml)	pH
1	62	105.67	6.27	65	62.65	7.05
2	67	142.70	6.80	69	60.01	7.20
3	68	109.21	7.00	70	46.65	7.01
4	72	71.04	7.10	75	31.59	6.80
5	84	120.34	6.30	78	42.09	7.04
6	88	96.43	6.50	82	47.98	7.20
7	90	102.59	6.67	84	36.14	7.15
8	92	116.09	7.10	91	27.45	6.95
9	97	87.46	7.20	93	30.75	7.17
10	101	99.36	6.90	103	133.93	7.00
11	102	135.63	6.70	110	33.28	7.05
12	113	167.90	6.74	115	103.11	6.93
13	113	190.83	6.18	115	52.56	7.30
14	115	119.49	6.53	118	98.13	7.25
15	123	59.52	6.40	123	63.81	7.20
Mean	92.46	114.964	6.59	92.73	86.473	7.08
SD	±19.19	± 34.24	±0.32	± 19.86	± 43.23	± 0.13

Table 2
Levels of MS and *Candida* detected for each studied group.

	Rampant caries		Caries free	
	MS(CFU/ml)	*Candida*(CFU/ml)	MS(CFU/ml))	*Candida*(CFU/ml)
1	$3.2x10^5$	$4.0x10^3$	$2.0x10^3$	0
2	$7.6x10^6$	0	$1.0x10^4$	0
3	$4.4x10^5$	$3.1x10^3$	$1.2x10^5$	0
4	$1.7x10^5$	0	$1.8x10^4$	0
5	$1.3x10^6$	$1.4x10^2$	$2.0x10^4$	0
6	$3.4x10^5$	$6.8x10^2$	$2.0x10^4$	0
7	$3.8x10^5$	$1.6x10^4$	$4.4x10^5$	0
8	$8.0x10^5$	0	$3.1x10^4$	0
9	$2.1x10^6$	0	$2.0x10^5$	0
10	$1.5x10^5$	2x10	$6.0x10^3$	0
11	$1.0x10^4$	$4.4x10^2$	$2.4x10^4$	0
12	$4.7x10^5$	0	$1.6x10^3$	0
13	$2.7x10^5$	0	$1.1x10^5$	0
14	$2.3x10^6$	0	$1.2x10^5$	0
15	$2.2x10^6$	$6.0x10^2$	$1.5x10^3$	0

MS = Mutans streptococci

free group (Group II) ($p<0.05$). Children with rampant caries had a salivary pH of 6.59 ± 0.32 whereas those without caries had a mean value of 7.08 ± 0.13. Salivary flow rates were similar for both groups ($p>0.05$) (1.02 ± 0.13 ml/minute in Group I and 1.03 ± 0.09 ml/minute in Group II). Table 2 shows the levels of MS and *Candida* in both groups. Significantly higher levels of MS and *Candida* were observed in Group I than in Group II ($p< 0.05$).-No *Candida* was detected in caries-free children.

DISCUSSION

It has been suggested that salivary SIgA antibodies generated by the mucosal immune system play an important role in the immune response against dental caries (Benderli *et al*, 2000). These antibodies may not only reduce the adherence of bacteria to saliva-coated tooth surfaces but may also neutralize extracellular enzymes (Hajishengallis *et al*, 1992). It has been reported that caries-free patients have significantly higher levels of naturally induced SIgA compared with caries-active subjects (Benderli *et al*, 2000). Evidence from previous studies has resulted in the perception that there should be a correlation between SIgA and dental caries. Nevertheless, a discrepancy between SIgA levels and caries prevalence still remains. It is due to this that studies have found either a positive (Bolton and Hlava, 1982) or a negative correlation (Benderli *et al*, 2000).

The normal range for SIgA is usually given as 0.1-0.3 mg/ml for adults (Brandtzaeg, 1989). Based on several Korean studies in children, the level of SIgA in normal subjects ranged from 0.025 to 0.186 mg/ml (Kim and Lee, 1982; Nam, 1985). The level of SIgA detected in our study also lies within this range. Results of this study indicate a significant positive association between SIgA and the presence of rampant caries. Similar findings were observed in other studies involving children

and young adults (Farias and Bezerra, 2003; Koga-Ito *et al*, 2004). In these studies, dental caries were significantly related to high titers of SIgA and the presence of *Streptococcus mutans* as well as large numbers of microorganisms. In contrast, Camling *et al* (1987) and Bolton and Hlava (1982) observed higher anti-streptococcus mutans IgA antibodies in caries-resistant children than in caries-susceptible ones. Our results are consistent with the view that children with rampant caries are not immunologically compromised, and that inadequate diet, host and microbial related factors may be mainly responsible for their clinical condition.

Challacombe (1980) stated salivary IgA is not directly related to protection against dental caries, but reflects a past exposure of the host to cariogenic microorganisms. In the absence of continuous stimulation, which does not happen with dental caries, the immunological titers tend to decline, which is a characteristic of the immunological system. The above interpretation, however, is not supported by other studies where high levels of salivary antibodies have been found to be related to dental caries (Rose *et al*, 1994; Benderli *et al*, 2000).

In the present study, children with rampant caries presented with higher MS and *Candida* levels in their oral cavities. Conversely, low MS level and no *Candida* were detected in caries-free children. Sziegaleit *et al* (1999) found the oral cavity of children with healthy teeth was almost devoid of *Candida* while 82% of children with carious teeth harbored *Candida* in their oral cavity. *Candida* species are common residents of mucosal surfaces in human oral cavity. They are capable of adherence to tooth surfaces, biofilm formation and carbohydrate fermentation. The presence of *Candida* in 53% (8/15) of children with rampant caries in our study (Table 2) may be evidence that carious teeth constitute an ecologic niche for these opportunistic pathogens. In addition, the acidic values of salivary pH found in this group of children favor the growth of *Candida* or are a consequence of Candidal fermentation. Patients with dental caries show high amounts of acidogenic microorganisms, such as MS (Grindefjord *et al*, 1991) and *Candida* (Nikawa *et al*, 2003) in their oral cavities. The presence of caries lesions can lead to more retentive areas for dental plaque accumulation and more difficulty in carrying out good oral hygiene. This may be the reason for the high levels of MS and *Candida* detected in their saliva. Furthermore, higher levels of microbial antigenic loads present in the oral cavity of these children probably increases the immune reaction which leads to high levels of antibody production.

Saliva is a complex mixture secreted from the parotid, submandibular, sublingual and many minor glands. These glands provide the most important source of SIgA in the upper tracts. Many factors can influence the concentration of SIgA (Kugler *et al*, 1992). A very important factor in determining the concentration of SIgA is salivary flow. Salivary flow is controlled by a variety of factors, including food ingestion, sensory stimulation, drugs, smoking, body positioning, stress, and degree of hydration (Dawes, 1993). Dietary factors, daily mood, and intense physical activity may also influence SIgA concentration (Watson *et al*, 1985; Stone *et al*, 1987). After analyzing the data obtained for salivary flow evaluation in this study, the mean values were almost identical for both groups of children (1.02±0.13 ml/minute in children with rampant caries and 1.03±0.09 ml/minute in caries-free children). This is in accordance with the finding of Dodds *et al* (1997) who showed no difference in salivary flow rates in caries active versus caries-free young adults.

Many cross-sectional studies have attempted to relate salivary flow rates and caries, however, a fundamental flaw in such studies is the use of the DMF index as a measure

of caries activity. The DMF index is a life-time cumulative index of dental disease and treatment, and may have little bearing on caries activity at a specific point in time. Similarly, a one-time determination of stimulated whole saliva flow rate may not be a comprehensive evaluation of salivary function. Furthermore, clinical studies of oral diseases in relation to salivary constituents are usually complicated by difficulties in standardizing sampling methods and laboratorial tests, which contribute to a diversity of findings.

In conclusion, the presence of rampant caries was associated with increased SIgA, MS and *Candida* levels in the oral cavity. The salivary flow rate did not influence the presence of rampant caries.

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