A Comparative Study of the Secretory IgA immunoglobulins (s.IgA) in Mothers and Children with SECC versus a Caries Free Group Children and their Mothers

Najlaa Al Amoudi*/ Haneen Al Shukairy**/Azza Hanno***

Early childhood caries (ECC) is recognized as an infectious disease. The first step in its development is primary infection by the bacterium S. mutans which has been identified as the primary etiologic factors in dental caries. Lactobacilli were also found to play a role in the progression of disease. However, the underlying mechanism of immune response to caries is unclear. The association between secretory IgA (s.IgA) and cariogenic microorganisms is still controversial.

The purpose of this study was to assess the level of salivary IgA in caries free children, and children with SECC and their corresponding mothers. The study also aims at correlating the children's levels to their mothers'.

Sixty children and their mothers attending the dental clinic in King Abdulaziz University participated in our study. Their age ranged from 3 – 5 years. The study groups consisted of thirty children with SECC and a control group consisting of thirty caries free children.

Children together with their mothers were examined and their caries level was recorded. Stimulated saliva was collected from each participant for immunological assessment. The secretory IgA (s. IgA) level was assessed by ELISA test.

Our study has shown that children with SECC and their mothers had higher levels of s. IgA than the caries free children and their mothers. A positive high correlation was found between secretory IgA of mothers and children in both groups.

Key words: *secretory IgA, SECC, s. mutans, saliva, cariogenic bacteria, primary molars, teeth.*

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INTRODUCTION

Streptococcus mutans is the most important organism involved in the initiation of dental caries. Secretory IgA against steptococci can be detected in saliva of children as young as 6 weeks of age. These antibodies are directed against the first wave of streptococcal invaders "*S. mitis and S. salivarius.*"[1] No antibodies to *S. mutans* are detected in predentate infants. As teeth erupt into the oral cavity, the microbiota undergoes a second wave of change, and tooth colonizers such as *S. sanguinis* and *S. mutans* begin to establish. IgA antibodies against *S. mutans* are usually observed in 1 year old children. Within 10 years, the child exhibits levels of IgA which are comparable to those of an adult.[2]

The role of the immune response in humans resistance to dental caries is complex and controversial. Limited studies have evaluated the association between salivary IgA and oral microorganisms in caries-free and caries-active children. The reported data are also controversial. While one group of studies have shown a positive correlation between salivary IgA and caries susceptibility,[3,4] another study has shown an inverse relationship[5] and a third group of studies have shown no correlation.[6,7] Challacombe[4] reported that high IgA levels are associated with caries lesions, Everhart *et al*[5] noted that high salivary IgA was associated with low caries activity suggesting an indirect correlation between *S. mutans* salivary IgA and caries activity in children. On the other hand Bamman and Gibbons[7] found that salivary IgA level is not correlated with dental caries, however, the study sample included a small number of children. Regarding IgA specific against *S. mutans*, Bolton and Hlava[8] reported higher levels of *S. mutans* salivary IgA in children with low caries activ-

* Najlaa Al Amoudi, BDS, MSc, D.Sc., Department of Preventive Dental Sciences, Faculty of Dentistry King Abdulaziz University

** Haneen Al Shukairy, BDS, Ms, Department of Preventive Dental Sciences, Faculty of Dentistry King Abdulaziz University

*** Azza Hanno, BDS, MSc, PhD., Department of Preventive Dental Sciences, Faculty of Dentistry King Abdulaziz University

Send all correspondence to: Prof. Najlaa Al Amoudi at Department of Preventive Dental Sciences, Faculty of Dentistry, King Abdulaziz University, P.O. Box 80209, Jeddah 21089, Saudi Arabia

Phone: (966-2) 640 1000 ext. 23626
Fax: (966-2) 640 4048

E-mail: Naj_alam@yahoo.com

ity. In a more recent study Chia *et al*[9] noted a positive correlation between *S. mutans,* salivary IgA and caries activity.

Genetic diversity of the population may further complicate the analysis of humoral responses to long term exposure to *S. mutans* antigens. A study of different racial groups has shown that Asian Thai children exhibited different salivary IgA antibody profile to *S. mutans* infection from Swedish children with similar age group.[10] In a more recent study, salivary samples from Thai children showed weaker response to *S. mutans* antigens than samples from Swedish children in terms of bands number and intensity judged on immunoblots.[11]

Another interesting aspect in the evaluation of immunological aspect of caries is the correlation between maternal and children salivary and serum antibodies. Few studies have noted a positive correlation between salivary *S. mutans* in children and their mothers. However, no studies have evaluated the association between maternal and children's *S. mutans* antibodies in serum or saliva. This observation calls for further investigations of the salivary IgA antibodies in caries free and caries active child in relation to their mothers levels to clarify the possible correlation of salivary IgA to caries pathogenesis in the mother and child pair. This study will assess the level of salivary IgA in a group mothers and children with SECC and a control group of caries free children and their mothers. Also it will correlate the levels of salivary IgA in children with SECC and caries free children to their mother's levels.

MATERIALS AND METHODS

Subject selection

Sixty children and their mothers (total of 120 subjects) attending the dental clinic in King Abdulaziz University Hospital (KAUH) participated in our study.

The age of the children ranged from 3-5 years. All subjects with systemic disorder or on regular medication were excluded from the study. Mothers were given informed consents after they were provided with verbal and written information concerning the importance and the procedures of the study. Children attending the clinic without their mothers were not included in our study. The study group (group IC) consisted of thirty children with SECC. Children were selected according to criteria provided by the guideline of the AAPD.[12] The mothers (group IM) were also examined to assess their caries level and their DMFT score was recorded. The control group (group IIC) included thirty children diagnosed as caries free. Their mothers (group II M) were also included and examined for their caries level.

Clinical examination

A comprehensive dental examination of each subject was carried out by one calibrated examiner in optimal light using mouth mirror and explorer. The diagnosis of dental caries was based on the modified WHO criteria.[13] Bitewing radiographs for each subject were taken after clinical examination. Dental caries level was expressed using the DMFT index for permanent teeth and dmft for primary teeth.[13]

Saliva sample

Stimulated saliva samples were collected from each participant for bacterial and immunological assessment. Each subject was given a piece of Paraffin wax (1g) and a calibrated cylinder (15 ml). All the participants were asked to chew the paraffin wax and to expectorate the stimulated saliva into the container. The process was carried out in 15 minutes. Saliva sampling was performed before the clinical examination between 9- 11 am and the subjects were asked not to eat or drink or chew gum for 1 hour before sampling. Subjects were also asked to refrain from tooth brushing in the morning before saliva sampling. Saliva was not taken if the child had received antibiotics within 1 month prior examination.

Immunological assay

Saliva samples were transferred on ice and clarified immediately by centrifugation at 6500×g for 30 minutes. Sediment was discarded. Clarified saliva sample were aliquoted and stored frozen at -40ºC until assayed for antibody activity by enzyme-linked immunosorbant assay (ELISA) [ALPCO Diagnostic, Secretary IgA ELISA, American Laboratory Products Company]. The collected samples were processed to detect secretory salivary IgA level. The saliva was suspended in diluted ELISA wash buffer. Then, 100ml of the sample was incubated for 60 minutes at room temperature. Peroxides-labeled anti-s IgA was added to the sample and then incubated for 60 minutes and followed by the addition of 100ml of TMB (tetramethybenzidine). Finally 50ml of acid was added to the sample to stop the reaction. The color converts from blue to yellow. The intensity of the yellow color is directly proportional to the concentration of IgA. The antibody level is expressed as arbitrary units as suggested by Lethonen *et al*.[14]

Statistical analysis

The collected data were entered in database file using D Base IV and cleaned and checked for outliners. Statistical analysis was carried out using SPSS. A group comparison regarding the total IgA antibodies was carried out using t-test. Pearson test was used for the correlation between group. Intra observer reliability was (98%, 99%, 100%) using Kappa for dmft, bitewing radiograph and *s. mutans* reading respectively.

RESULTS

Table 1 shows the frequency distribution of children and mothers by age, sex and nationality. The age of children ranged from 3 to 5 years with a mean age of 4.23 ± 0.65. Fifty three percent of the samples were males and 46.78 were females, while ninety percent of children were Saudi. The age of mothers ranged from 24 to 36 years with a mean age of 29.37 ± 2.72. Saudi mothers represented 86% of the sample.

Table 1. Frequency distribution of the sample by age, gender, and nationality

Age group (years) of children	Number	Percentage %
3 - < 4	12	20
4 - < 5	30	50
⩾6	18	30
Gender of children		
Male	32	53.3
Female	28	46.7
Nationality of children		
Saudi	54	90
Non-Saudi	6	10
Total	**60**	**100**
Age group (years) mother		
⩽30	41	68.30
>30	19	31.87
Nationality mother		
Saudi	52	86.7
Non-Saudi	8	13.3
Total	**60**	**100**

Table 2. Frequency distribution of the study (IC, IM) and control groups (II C, II M) by caries status and s. IgA

	Minimum	Maximum	Mean	SD
dmft (IC group)	4	17	8.83	3.37
dmft (II C group)	0	0	0	0
Salivary IgA (I C group)	10	210	82	67.6
Salivary IgA (II C group)	10	180	29.8	33.9
Salivary IgA (I M group)	10	600	118.1	116.4
Salivary IgA (II M group)	13	200	49.93	39.74
DMFT (I M group)	0	19	10.23	4.51
DMFT (II M group)	0	13	6.07	3.69
D - component of the dmft score (I M group)	0	9	2.97	2.59
D- component of the DMFT score (II M group)	0	7	1.33	1.92

IC = study group of children
IIC = Control group of children
IM = Study group of mother
IIM = Control group of mother

The level of salivary IgA in caries free children and children with SECC

The level of salivary IgA was higher in children with SECC compared to the caries free group. The mean value of salivary IgA was 29.8µg ±33.9 in caries free children and 82 µg ±67.6 in SECC children. The difference between the two groups was statistically significant (p-value <0.0001) (*table 3).*

The level of salivary IgA in mothers of caries free children and children with SECC

The mothers of children with SECC had higher salivary IgA level than the mothers of caries free children. The mean value was found to be 49.93 µg ± 39.74 for the caries free group and 118 µg ± 116.4 for the SECC group. The difference between the two values was statistically significant *(p-value 0.005) (Table 4).*

Table 3. Salivary IgA level among SECC children (I C) and caries free children (II C)

Caries status	n	Minimum µg	Maximum µg	Mean	SD	p-value
Caries free group(II C)	30	10	180	29.8	33.9	<0.0001
SECC group(I C)	30	10	210	82.0	67.6	

* Using T- test.

Table 4. Salivary IgA level among mothers of SECC children (I M) and mothers of caries free children (II M)

Caries status of children	n	Minimum µg	Maximum µg	Mean	SD	p-value
Caries free Group (II M)	29	13	200	49.93	39.74	**0.005**
SECC group (IM)29	30	10	600	118.1	116.4	

* Using T- test.

Correlation of salivary IgA levels in caries free children and children with SECC to their mothers' levels

Correlation test of salivary IgA levels of mothers with their children in both caries free and SECC groups was performed. There was a positive correlation between salivary IgA levels of mothers and their children in both groups *(p-value <0.0001) (Table 5*). In the SECC, as the mothers' level of IgA increased, the level of IgA in children also increased. Similarly, in the caries free group, as the mothers' level of IgA decreased the level of IgA in their children followed.

Table 5. Correlation of salivary IgA of mothers with salivary IgA of their children in the 'caries free' and SECC groups

		Salivary IgA of children	
		Correlation	p-value *
Caries free (group II)	Salivary IgA of mother	0.638	< 0.0001*
SECC (Group I)	Salivary IgA of mother	0.646	< 0.0001*

* Using Pearson test.

DISCUSSION

In the present study, the mean level of salivary IgA found in children with SECC was higher than that of caries free children. Our result supports the study of Grahn *et al*[15] who concluded that high concentration of salivary IgA in children with ECC can be attributed to the increased antigenic load, which leads to high production of antibodies. Since early childhood caries is considered an infectious disease which induces an immune response, antibody titres in children with carious lesions present with different concentrations compared to children without caries. Similar findings were also obtained by De Farias and Bezerra,[16] who investigated the total salivary IgA using nephrometric technique in two

groups of 20 children varying in age from 12 to 47 months. They reported that children with ECC presented with significantly higher level of total salivary IgA than the caries free group. The same trend was also observed in another study involving young adults with dental caries. It was reported that, dental caries was significantly related to large amount of microorganisms and high titre of total salivary immunoglobulins.[17]

On the other hand, other studies have correlated high amount of salivary antibodies to a low risk of dental caries and concluded that s. IgA has a protective role against caries.[18,19]

In an attempt to assess the immunological mother-child profile, our study measured the level of salivary IgA in the mothers of the caries free children and children with SECC and correlated the mothers' level to their children's values. The mothers of SECC children had a higher level of salivary IgA than the mothers of the caries free group children. This can be attributed to the high level of *S. mutans* among these mothers which triggered such an immune response. A high positive correlation has been found between the mothers and their children in both groups. As mentioned previously, mothers are the primary source of *S. mutans* found in children. An increase in bacterial count in the mothers is associated with an increase in the bacterial count in their children, triggering an immune response and increased titres of total salivary immunoglobulins. The present work represents the first attempt to compare the mother's level to her children's, and it highlights the role of mothers as the primary target for intervention of SECC among their siblings.

CONCLUSIONS

- A significant high level of salivary IgA was found in children with SECC.
- A significant high level of salivary IgA was found in mothers of children with SECC.
- A positive high correlation was found between salivary IgA of mothers and children in both the SECC and caries free groups.
- Salivary IgA titer in children and mothers might be an indicator of caries risk assessment.

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REFERENCES

1. Smith DJ, Taubman M.A Crit. Rev. oral biol. Med. 3: 109–133, 1992.
2. Hajishengallis et al, Infect Immun 60: 5057–5064, 1992.
3. Huis in't Veld J, van Palentein Helderman W, Backer-Dirks O. Streptococcus mutans and dental caries in humans: a bacteriological and immunological study. Antonie van Leeuwenhoek 45: 25–33, 1997.
4. Challacombe SJ. Serum and salivary antibodies to streptococcus mutans in relation to the development and treatment of human dental caries. Arch Oral Biol 25: 495–502, 1980.
5. Everhart DL, Klapper B, Carter WH, Moss S. Evaluation of dental caries experience and salivary IgA in children age of 3-7. Caries Res 11: 211–215, 1977.
6. Brandtzaeg P. The oral secretory immune system with special emphasis on its relation to dental caries. Proc Finn Dent Soc 79: 71–84, 1983.
7. Bamman LL, Gibbons RJ. Immunoglobulin A antibodies reactive with streptococcus mutans in saliva of adults, children and predentate infants. J Clin Microbiol 10: 538–543, 1979.
8. Bolton RW, Hlava GL. Evaluation of salivary IgA antibodies to cariogenic microorganisms in children. Correlation with dental caries activity. J Dent Res 61: 1225–8, 1982.
9. Chia J-S, Chang W-C, Yang C-S, Chen J-Y. Salivary and serum antibody response to streptococcus mutans antigen in humans. Oral Microbiol Immunol 15: 131–138, 2000.
10. Bratthall D, Serinirach R, Hamberg K, Widerstom L. Immunoglobulins A reaction to oral streptococci in saliva of subjects with different combinations of caries and levels of mutans streptococci. Oral Microbiol Immunol 12: 212–21, 1997.
11. Vachirarojpisan T, Shinada K, Kawaguchi Y, Laungwechakan P, Somkote T, Detsomboonrat P. Early childhood caries in children aged 6-19 months. Community Dent oral Epidemiol 32: 133–42, 2004.
12. American Academy of Pediatric Dentistry Guideline. Policy on early childhood caries (ECC): unique challenges and treatment options. 2003.
13. WHO (1987). Oral Health Surveys. Basic Methods. 3rd Edn. Geneva: World Health Organization.
14. Lehtonen OP, Grahn EM, Stahlberg TH, Laitinen LA. Amount and avidity of salivary and serum antibodies against Streptococcus mutans in two groups of human subjects with different dental caries susceptibility. Infect Immun Jan 43(1): 308–13, 1984.
15. Grahn E, TenovuoJ, LehtonenOP et al. Antimicrobial system of human whole saliva in relation to dental caries, cariogenic bacteria and gingival inflammation in young adults. Acta Odontol Scand 46: 67–74, 1988.
16. De Farias DG, Bezerra AC (2003). Salivary antibodies, amylase and protein from children with early childhood caries. Clin Oral Invest 7: 154–157.
17. Huis in't Veld J, van Palentein Helderman W, Backer-Dirks O.Streptococcus mutans and dental caries in humans: a bacteriological and immunological study. Antonie van Leeuwenhoek 45: 25–33, 1997.
18. Twetman S, Lindner A, Modeer T. Lysozyme and salivary immunoglobulin A in caries-free and caries susceptible pre-school children. Swed Dent J 5: 9–14, 1981.
19. Rose P, Gregory K, Linda E. IgA antibodies to Streptococcus mutans in caries resistant and susceptible children. Pediat Dent 16: 272–275, 1994.