

Original Research Article

## The Relationship between Salivary IgA Level and Dental Caries in Healthy School-Aged Children in Makkah Al-Mukarramah

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### ABSTRACT

**Introduction:** Dental caries is considered to be a multifactorial disease, and it affects 60-90% of school-aged children worldwide. Salivary IgA is considered the first line of defense against pathogenic bacteria and their secretions.

Controversial conclusions have been reported regarding the relationship between salivary IgA and dental caries activity; some studies demonstrated high concentrations of salivary IgA in a lower caries activity. Other studies showed high levels of salivary IgA with an increase in caries activity. Some authors did not observe any correlation.

**Aim:** The aim of the present study is to assess the relation between the level of salivary IgA and dental caries in healthy children living in Makkah city.

**Materials and Methods:** The study included a total of 90 children of both genders, age range from 7- to 12-year-old. Dental caries was assessed. Stimulated whole salivary samples were collected after dental examination in the morning (from 9 to 11 A.M.) and Saliva flow rates of the subjects were measured. The samples were transported then stored in saliva collection tubes. The salivary IgA levels were measured using Human IgA ELISA kit.

**Results and Conclusion:** There is a negative correlation between the increase in the mean levels of salivary IgA with the reduction in dental caries activity in children. This would reflect the salivary IgA protective mechanism against dental caries and streptococcus mutans in the whole saliva of low caries-active children.

**Keywords:** Dental caries, DMFT, deft, salivary IgA, ELISA.

### INTRODUCTION

Dental caries is defined as a localized, post-eruptive, pathological process of external origin that involves dissolving of the hard tooth tissue and progressing to the formation of a cavity. <sup>(1)</sup>

Dental caries is considered the most common childhood chronic disease in children aged from 5- to 17-years old and is five times more common than asthma and seven times more common than hay fever.

<sup>(2)</sup> Dental caries is a multifactorial disease,

and it affects 60-90% of school-aged children worldwide. <sup>(3)</sup>

Saliva is a dilute fluid composed of more than 99% water, therefore considered a unique biological fluid. <sup>(4)</sup> Various components of saliva both organic (peptides and proteins) and inorganic (electrolytes and water) may have a role in protecting teeth from dental caries. <sup>(5)</sup> Salivary immunoglobulin's include IgA, IgG, and IgM. Salivary IgA contributes almost 60% of the total immunoglobulin count in the saliva. <sup>(6)</sup>

Salivary IgA is considered the first line of defense against pathogenic bacteria and their secretions. <sup>(7)</sup> Salivary IgA prevents adherence of the bacteria to the tooth surface by blockage of bacterial adhesions. Salivary IgA prevents both nonspecific and stereo chemical interactions and therefore interferes with bacterial adhesion to host surfaces. <sup>(8)</sup> Salivary IgA has a role in neutralizing the bacterial toxins and enzymes by blocking their binding to cell receptors. <sup>(9)</sup>

Controversial conclusions have been reported regarding the relationship between salivary IgA and dental caries activity; some studies demonstrated high concentrations of salivary IgA in a lower caries activity. <sup>(10-13)</sup> On the other hand, other studies showed high levels of salivary IgA with an increase in caries activity. <sup>(6,14-15)</sup> However, other authors did not observe any correlation between salivary IgA and dental caries. <sup>(16-19)</sup>

Scanty studies focused on revealing the role of salivary IgA on dental caries status and its further progression particularly in children living in Saudi Arabia. Therefore the study is aimed to assess the relation between the level of salivary IgA and dental caries experience in healthy children living in Makkah city.

The significance of the study: The study will aid in early detection of dental caries and will help in providing necessary oral health preventive services and specific programs for children to protect against dental caries.

## **MATERIALS AND METHODS**

### **Study Sample**

A total of 90 children of both genders were included in this study with age range from 7- to 12-year-old. The subjects were randomly selected from different primary schools in Makkah as well as from the pediatric patients' pool attending the UQU Dental clinics seeking dental intervention or regular follow-up. Children who found to be affected by upper respiratory tract infection during the last

week prior data collection, or having a history of antibiotics intake for the last six months, or suffering from any medical illness that could affect saliva flow rate or saliva composition, were prohibited from joining this study.

### **Ethical Consideration**

Data collection was initiated only after obtaining ethical approval from the UQUDENT-IRB dated 1-11-2016 with IRB No. -40-16, where subjects which their guardians accepted the assent form and agreed to participate in the study were included. The consent forms were sent to the participants' guardians one day prior the clinical dental examination.

### **Dental Examination**

Full clinical examination of each participant's oral cavity conducted by the researcher while the child seated on a chair with a high backrest using lightweight, portable examination light touch within the blue-white spectrum according to the basic methods of Oral Health Survey. <sup>(20)</sup> The Intraoral examination was performed using disposable dental examination kit (sterile mouth mirror and dental explorer) per each participant, as in figure 1.

Dental caries was assessed for every child, the deft and DMFT indices were calculated separately. Where "def" stands for: decayed, indicated for extraction and filled primary tooth due to caries. The DMFT score describes the dental status of the permanent dentition and stands for Decayed-Missing-Filled permanent. <sup>(21)</sup> The total sum of both deft and DMFT was considered in children with mixed dentition. <sup>(22)</sup>

Participants in the study were divided according to their dental caries experience into the following groups; Group A consisted of low caries- active children with DMFT/deft < 3 and group B composed of caries-active children with DMFT/deft ≥ 3. <sup>(23)</sup> All patients' data were registered immediately into the data collection form.

### **Saliva Sample Collection**

Stimulated whole salivary samples were collected from the participating

children after dental examination in the morning (from 9 to 11 A.M.), under standard temperature and humidity conditions. The participants were instructed not to ingest anything by mouth for a minimum of 1 hour before saliva collection. (24) The saliva collection was performed by the same examiner. (25)

A graduated cylindrical plastic tube 50 ml in volume, was used for saliva collection then was appropriately labeled using a permanent marker, as in figure 2.

Stimulated whole saliva samples were collected according to the following method, (26) as shown in figure 3:

1. Instruct the patient to sit motionlessly.
2. Tell the patient to lean the head forward.
3. Tell the subject to swallow to void the mouth of saliva (starting time).
4. Instruct the subject to chew a piece of paraffin wax.
5. Every one minute, the child encouraged to spit saliva into the collection tube. Tell subject, "Spit out, keep chewing" (after the first minute), "Spit out, keep chewing" (after the second minute). Discard the first two-minute collection and proceed with another three-minute collection.

Saliva flow rates of the subjects were measured using the following equation:

$$\text{Stimulated saliva Flow rate} = \frac{\text{Total saliva volume}}{\text{Collection Period}}$$

The samples were transported with ice packs to protect salivary proteins from bacterial degradation until further processing, the box was then closed and sealed and ready for transportation. Then, the samples were stored in saliva collection tubes at 4oC for 2 hours (SANYO pharmaceutical refrigerator, MPR-414FS, Japan), then transferred to below 20o C (Thermo Fisher Scientific (Asheville) LLC Model No. UXF40086D62Asheville, NC USA) to avoid further bacterial growth and to protect unstable analyses of the saliva samples, as in figure 4. (27)



Figure 1: A photograph showing disposable dental examination kit.



Figure 2: A photograph showing a graduated cylindrical plastic tube used for saliva sample collection.

Figure 3: A photograph showing proper patient position for saliva sample collection.



Figure 4:  
A) A photograph showing the refrigerator used for saliva sample storage at 4°C  
B) A photograph showing the refrigerator used for saliva sample storage below 20°C

The Centrifuge machine (Multifuge X1R Centrifuge, USA) was set at 4oC so that the proper temperature for processing was reached before processing. The saliva samples were centrifuged following the manufacturer's instructions, at 2000 RPM

for 10 minutes to decrease the saliva turbidity and to remove cellular debris that can impact negatively on the accuracy of analysis, as in figure 5. All samples supernatant were transferred into a 1.5 ml sterile Eppendorf tubes using a micropipette (volume 1000  $\mu$ l) and appropriately labeled with a permanent marker to preserve the sample identity. <sup>(27)</sup>



Figure 5: A photograph showing saliva sample centrifuge machine.

#### Measurement of salivary IgA level

The salivary IgA levels were measured using Human IgA ELISA Kit (ab196263 – IgA Human Simple Step ELISA Kit, Made in the UK) according to the following manufacturer instruction:

1. Prepare all reagents, working standards, and saliva samples, as in figure 6.
2. Remove excess strips from the microplate frame, return them to the foil pouch including the desiccant pack, then reseal and return to 4°C storage.

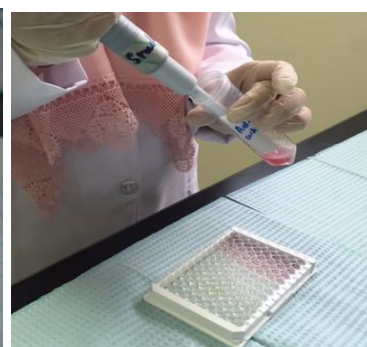
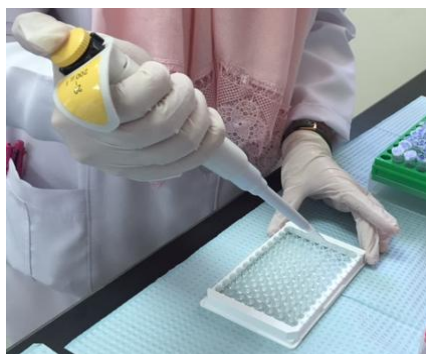


Figure 6: A photograph showing preparation of different reagents used for ELISA test, working standards.

Figure 7: A photograph showing saliva sample transfer to appropriate wells.

Figure 8: A photograph showing addition of the antibody cocktail to each well.

3. Add 50  $\mu$ L of all sample or standard to appropriate wells, as in figure 7.

4. Insert 50  $\mu$ L of the Antibody Cocktail to each well. Add antibody mixture to replicates at the same time to avoid well to well variation, as in figure 8.

5. Seal the microplate and incubate for 1 hour at room temperature on a plate shaker (Thermo Scientific MaxQ Shaker, Model no. 4334, USA) set to 400 rpm, as in figure 9.

6. Wash each well in the microplate with 3 x 350  $\mu$ L 1X, then Wash Buffer PT. The technique for washing includes aspirating or decanting from wells then distributing 350  $\mu$ L 1X Wash Buffer PT into all wells. Complete removal of liquid in every step is fundamental for good performance. Invert the microplate and blot it against clean paper towels to remove excess liquid, as in figure 10.

7. Insert 100  $\mu$ L of TMB substrate to every well in the microplate and incubate for 10 minutes on a plate shaker set at 400 rpm. Add TMB substrate to replicates at the same time to avoid well to well variation, as in figure 11.

8. Insert 100  $\mu$ L of Stop solution to each well in the same order as the TMB was added to avoid well to well variation. Shake plate on a plate shaker for 1 minute to mix. Record the OD (SPECTROstar Nano microplate reader) at 450 nm, as in figure 12.



Figure 9: A photograph showing placement of the ELISA plate on a plate shaker for 1 hour.



Figure 10: A photograph showing ELISA plate washing with a multichannel pipette.

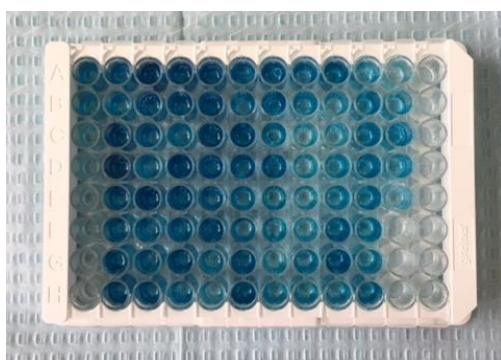


Figure 11: A photograph showing addition of TMB substrate to each well.



Figure 12: A photograph showing recording the OD on the microplate reader.

### Statistical Analysis

A t-test was used to compare the cases and control groups as regard participant's age, dental caries experience, salivary flow rate measurement and salivary IgA measurement. However, Chi square was used to compare between the participant's adherence to oral hygiene measures and dietary habits. The significance was tested at  $p \leq 0.05$ . The collected data was tabulated and statistically analyzed using (SPSS, version 22, USA).

### RESULTS

A total of 90 children were included in the present study. The participants were equally divided according to dental caries activity into two groups: Group A consisted of 45 low caries-active children and group B composed of 45 caries-active children.

The mean age value for the whole participants was  $8.662 \pm 1.315$  with a range of 7- to 10-year-old. The mean age values of group A showed a statistically insignificant difference from that of group B, as shown in Table 1.

### Adherence to oral hygiene measures

The results of the present study pointed that 38/45 (84.4%) of group A performed regular tooth brushing, while only 7/45 (15.6%) didn't adhere to regular tooth brushing. On the other hand, group B presented with only 13/45 (28.9%) children who reported that they performed regular tooth brushing, while up to 32/45 (71.1%) conveyed irregular adherence to tooth brushing, as shown in Table 2.

### Dietary habits

The results of this study revealed that in group A, up to 42/45 (93.3%) of the participants maintained healthy snacks

between the main meals, while only 3/45 (6.7%) reported ingestion of unhealthy snacks. On the other hand, group B presented with only 5/45 (11.1%) children who reported consumption of healthy diet, while up to 40/45 (88.9%) conveyed unhealthy dietary habits, as in Table 3.

### Dental caries experience

Were the def score of group B presented statistically significant higher mean value compared to the def of group A, as shown in Table 1. Similarly, the assessment of DMF score of group B showed statistically significant higher mean value in contrast to group A, as in Table 1.

### Salivary flow rate measurement

The results revealed that the mean salivary flow rate value of the whole participants equals  $1.5426 \pm 0.5456$  with the range of 0.9 to 2.1 ml/min. Group A showed statistically insignificant mean value compared to group B, as in Table 1.

### Salivary IgA measurement

The results of the current study pointed that the sIgA measurement of group A presented statistically significant higher mean value compared to the sIgA level of group B, as in Table 1.

**Table 1: Mean and SD values of different variables among the groups (n=45).**

Variable	Group A	Group B
Age	8.681± 1.410 <sup>a</sup>	8.643± 1.232 <sup>a</sup>
def	0.772± 0.8030 <sup>a</sup>	7.739± 2.314 <sup>b</sup>
DMF	0.1590± 0.4795 <sup>a</sup>	1.6304± 1.356 <sup>b</sup>
Flow rate	1.585± 0.5303 <sup>a</sup>	1.5021 ± 0.5626 <sup>a</sup>
sIgA (IU/ml)	2.6332± 0.2233 <sup>a</sup>	2.3411± 0.3851 <sup>b</sup>

Different lower case superscripts in the same row indicate significance.

**Table 2: Comparison between group A and group B as regards the adherence to tooth brushing.**

Groups	Tooth brushing		Total
	Regular	Irregular	
Group A	38 84.4%	7 15.6%	45 100.0%
Group B	13 28.9%	32 71.1%	45 100.0%
Total	51	39	90

$\chi^2 = 60.527, P < 0.0001$ \*\*\*

**Table 3: Comparison between group A and group B as regards dietary habits.**

Groups	Dietary habits		Total
	Healthy	Unhealthy	
Group A	42 93.3%	3 6.7%	45 100.0%
Group B	5 11.1%	40 88.9%	45 100.0%
Total	47	43	90

$\chi^2 = 131.43, P < 0.0001$

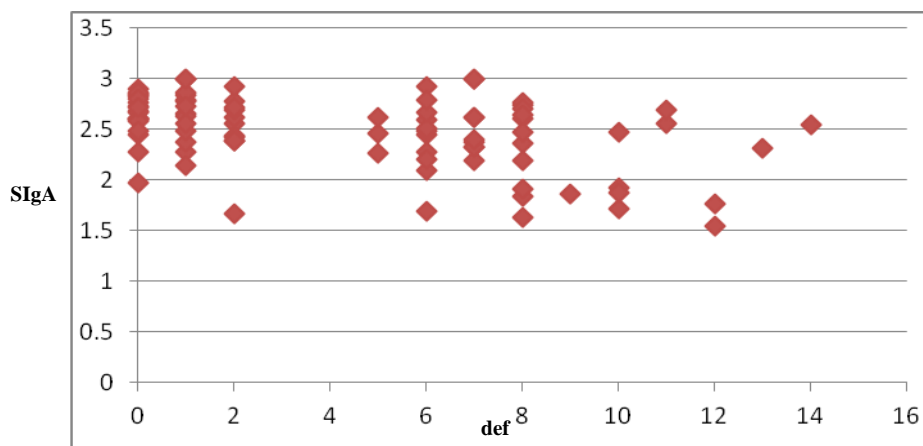


Figure 13: Scattered diagram depicting negative correlation between salivary IgA level (IU/ml) and def mean score of the primary dentition.

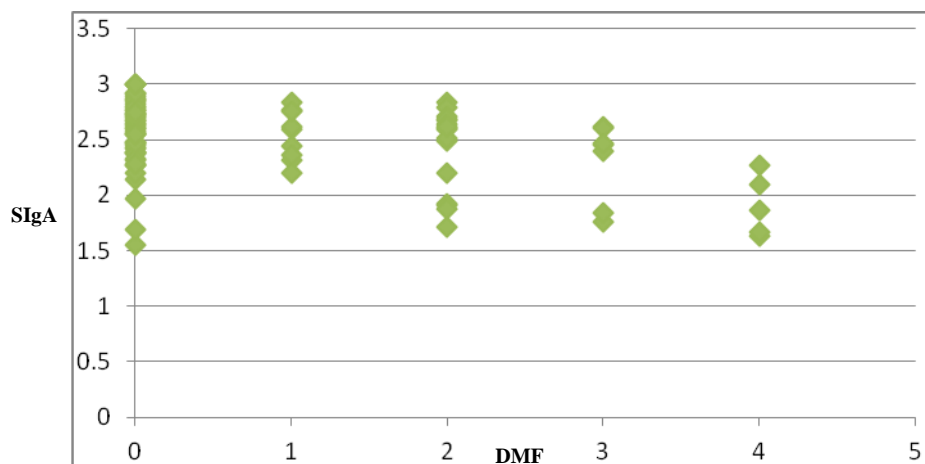


Figure 14: Scattered diagram depicting negative correlation between salivary IgA level (IU/ml) and DMF mean score.

Furthermore, the results revealed a very high statistically significant negative correlation between sIgA level and the def mean score of primary dentition at  $r = -0.4372$  and  $p < 0.0001$ , as in Figure 15. Also, a very high statistically significant negative correlation has been found between sIgA and DMF mean score at  $r = -0.4272$  and  $p < 0.0001$ , as in Figure 14.

## DISCUSSION

Streptococcus mutans represent the primary criminal of dental caries progression in human. (28) Many constituents of the human saliva, both immunoglobulins and non-immunoglobulins contribute to its antibacterial effect. (10) Salivary IgA has a significant role in fighting dental caries via inhibiting the adherence of oral bacteria to the tooth surface. (8) Salivary IgA also neutralizes the enzymes and exotoxins produced by bacteria that contribute to disease process. (9)

In the present study, dental caries was assessed using the def/DMF indices, which shows excellent Intra and inter-examiner reproducibility and reliability. The def/DMF indices give an overall view of total dental caries experience both past and present caries, the oral health status can be estimated indirectly. (21) Dental caries is known to be a multifactorial disease with the primary causal factors of sugar-concentrated diet, susceptible tooth surface, as well as microorganisms. Other local risk

factors have been identified including salivary flow, individual's adherence to oral hygiene measures, in addition to the form and arrangement of teeth. (29) Stimulated whole salivary samples were collected from the participating children in the morning (from 9 to 11 A.M.), to prevent the effect of circadian rhythm on the concentration of saliva samples. (24,30)

Regular tooth brushing was found mostly in children with less dental caries activity as compared with caries active children indicating that adherence to oral hygiene measures has a preservative effect against dental caries initiation and progression. Also, consumption of healthy snacks between the main meals was detected more in children with less dental caries activity as compared with caries active children indicating that healthy diet affects the dental caries activity significantly. (31)

Dental caries experience was seen in schoolchildren aged 7- to 12 years old in the present study and showed def values of  $(7.739 \pm 2.314)$  which are in consistent with the mean def of (8.06) found in schoolchildren aged 6-7 years old in Makkah region particularly in Jeddah city. (32)

Regarding salivary flow rate measurement, the study showed a range of 0.9 to 2.1 ml/min with no differences in salivary flow rate values between caries free and caries active children since all selected

sample selected to be clinically healthy free from any medical condition that could affect their salivary flow rate.

The results of the running study supported the rejection of null hypothesis and demonstrated an association between the increase in the mean levels of salivary IgA with the reduction in dental caries activity. The increased levels of salivary IgA would reflect the IgA protective mechanism against dental caries in the whole saliva of caries-free children. Salivary IgA protects the tooth against dental caries by interfering with bacterial adhesions to the host surface; <sup>(8)</sup> salivary IgA prevents the binding of bacterial enzymes and toxins to their corresponding receptors on tooth surface. <sup>(9)</sup>

Some studies reported the presence of a relationship between reduced salivary IgA levels and increased dental caries experience. On the other hand, other researchers revealed increased sIgA level with increased dental caries progression or couldn't reach significant association between sIgA and dental caries experience.

Similar to the results of the present study, results obtained by Soledad et al., showed a negative correlation between IgA levels and dental caries activity, the induction of IgA immunoglobulin revealed a protective role against dental caries. Similar results conducted reported an increase in the levels of s-IgA with a decrease in caries activity. <sup>(33-36,10-12)</sup> Contrary, other studies reported an increase in IgA level with an increase in caries activity. Defarias and Bezerra found that children with ECC had an increase in total salivary IgA. <sup>(15)</sup> Studies by Bruno et.al and Newbrun concluded with similar findings. <sup>(14,6)</sup> This difference may be explained by the adsorption of a greater amount of whole salivary IgA antibodies by the large number of Streptococcus mutans in caries active children in contrast to the lower Streptococcus mutans number in caries free saliva. Also, the caries free children secrete greater amount of salivary IgA antibodies to

Streptococcus mutans in their salivary glands than caries active children. <sup>(12)</sup>

Interestingly, some studies showed no correlation between dental caries and IgA levels. A study by Camling and Kohler demonstrated no clear evidence for the protective role of salivary IgA antibodies against Streptococcus mutans colonization. <sup>(17)</sup> Studies conducted by Everhart et.al., Koga et.al. and Shifa et.al. supported this hypothesis. <sup>(16,18,19)</sup>

This controversial difference in studies conclusion would be attributed to sampling size variations, different environmental factors affecting saliva sample collection, time of measurement of saliva samples, different criteria for participant selection, oral hygiene, and diet. Furthermore, the variation in the salivary immunoglobulin concentration in different studies depends upon the salivary flow rate, hormonal factors, physical activity and emotional status. <sup>(37)</sup>

## RECOMMENDATIONS

Limited literature is available on the relationship between salivary IgA levels and dental caries in children in Saudi Arabia. Thus, further expanded sample size and inclusion of remote areas should be accomplished.

Providing oral health preventive programs to children aiding in early detection of dental caries, also to educate the children regarding regular oral hygiene measures and healthy dietary habits, therefore helping in early detection of dental caries.

## CONCLUSIONS

Under the limitation of the present study, we can conclude the following:

1. There is a negative correlation between the levels of salivary IgA and dental caries activity in children. The increase of sIgA accompanied with reduction in dental caries activity. This would reflect the salivary IgA protective mechanism against dental caries in the whole saliva of children with low caries-activity.



2. Regular tooth brushing was found mostly in children with low dental caries activity as compared with caries active children indicating that adherence to oral hygiene measures has a preventive effect against dental caries initiation and progression.
3. Consumption of healthy snacks between the main meals was detected more in children with less dental caries activity as compared with caries active children.

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