

# Salivary Immunoglobulin A and *Streptococcus mutans* Levels among Lebanese Preschool Children with Early Childhood Caries

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## ABSTRACT

**Aim and objective:** We checked in this study the correlation between total immunoglobulin A (IgA) and *Streptococcus mutans* (SM) levels in saliva derived from Lebanese children with inappropriate eating habits and showing early childhood caries (ECC).

**Materials and methods:** Sixty Lebanese preschool children with similar alimentation were included in this study and divided into two groups. Group I included children having 0 cavities where group II contained children having ECC. We measured the SM and IgA levels collected from saliva and dental plaque.

**Results:** We observed a significant difference in SM levels between the two groups ( $p < 0.001$ ). There was a marginal correlation between salivary total IgA and SM collected from dental plaque ( $r = 0.33$ ,  $p = 0.077$ ). However, no significant correlation was detected between total salivary IgA and salivary SM ( $p = 0.35$ ).

**Conclusion:** This study suggests the absence of significant relationship between salivary markers of immune system and the development of ECC.

**Clinical significance:** Early childhood caries is a public dental health problem that has been affecting preschool children all over the world. Its prevention must be a priority for all professionals in the medical and dental community. This study highlights the absence of correlation between total IgA and SM collected from the saliva. This does not completely exclude the preventive role of salivary immune components, but further studies are required to better understand this relationship.

**Keywords:** Early childhood caries, Immunoglobulin A, Prevention, *Streptococcus mutans*.

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## INTRODUCTION

Saliva plays an essential role in maintaining a good oral health. It controls metabolism, adhesion, and proliferation of local microorganisms, improves the elimination of residues of carbohydrates, ensures the remineralization of teeth, and neutralizes the organic acids produced by cariogenic microorganisms causing the demineralization of tooth enamel.<sup>1</sup> Recently, and despite the worldwide effort to control its prevalence, tooth decay remains a major problem in many developing countries,<sup>2-4</sup> especially among young children where it is known as early childhood caries (ECC).<sup>5,6</sup> In Lebanon, and according to a study conducted by Chedid et al., 2011,<sup>7</sup> more than half of the children aged between 1 years and 4 years present a high risk of caries with 74.7% having at least one decay.

Early childhood caries is a special form of carious lesions.<sup>8</sup> It is encountered in patients where the food mode is characterized by bottle and breastfeeding in an inappropriate way.<sup>3,5,9</sup> In 2008, Caplan et al.<sup>10</sup> defined "inappropriate" as the frequent use of bottle or glass containing milk or other sweet drink before and after meals or sleep. The infectious nature of caries in general and ECC, in particular, implies a potential involvement of salivary antimicrobial components in the phenomena of pathogenesis.<sup>11-13</sup> However, studies about the correlation between these immunological factors (immunoglobulin G, M, A) and tooth decay in children are inconclusive, with a variety of results based on different methods of sampling and assays used.<sup>14-16</sup> The American Academy of Pediatric Dentistry considered, in a report published in 2008, that the presence of any decayed deciduous tooth, missing or filled teeth in children aged <71 months, is a sign of ECC.<sup>17,18</sup> The answer to the

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question "what causes this condition?" is important and complex. The risk factors involved in ECC have been widely described in the literature.<sup>6,8,9,19-23</sup>

The objective of our study was to clarify the role of the salivary immune system in the declaration of ECC, and its possible correlation with the level of salivary *Streptococcus mutans* (SM) and the number of cavities in a sample of young Lebanese preschoolers. In other words, we tried to provide an answer to the question: "why for a group of children from the same environment with the same eating habits, some present early childhood caries, while others do not?"

## MATERIALS AND METHODS

The studied sample included 60 children aged between 18 months and 49 months (31 boys and 29 girls) (Table 1). Regarding the gender

of the children, the choice was made arbitrarily. We obtained 13 girls and 17 boys in the “control” group. On the other hand, 16 girls and 14 boys were included in the “cavities” group (Table 1).

The inclusion criteria (Appendix 1) were absence of antibiotic intake for at least 2 weeks, good health, no previous dental treatment, and inappropriate diet consisting of a frequent use of bottle or glass containing milk or other sweetened drink before and after meals or bedtime. The exclusion criteria were (1) age different from 18 to 48 months; (2) antibiotic usage for at least 2 weeks before sampling; (3) presence of systemic disorders; and (4) exposure to previous dental treatment. The hygiene habits of the participants are summarized in Appendix 2. Before any dental and immunological examinations, an informed consent was signed by the parents.

A six-part questionnaire (Appendix 3) was completed for each child by one of the parents concerning his/her identity, siblings, socioeconomic background, medical, and dental history.

The dental clinical examination was performed by a single operator and was limited to a visual examination of the teeth using

a mirror and a probe at daylight. Decayed teeth were recorded. No radiological examination was performed.

After dental examination, children were divided into two groups depending on whether they have ECC (caries group) or not (no caries group).

The SM Dentocult Strip Mutans test<sup>24,25</sup> was used to detect SM in the saliva and plaque. This method is based on the ability of the microorganisms to adhere and grow on strips delivered with the kit after incubation. Using a sterile probe, the plaque was removed from the interdental surfaces and gently spread over the ribbed strip. Plaque collections were denoted as P1, P2, P3, and P4 corresponding to the teeth number 51, 61, 64, and 84, respectively. Saliva was collected by impregnation. After an incubation period of 48 hours at a temperature of 37°C, the scores of SM were recorded by comparing the culture media to a charter issued with the microbiological test kit.

The N-latex immunoglobulin A (IgA) assay was carried out in the laboratory of chemistry at the American University of Beirut Medical Center. After the collection of all the samples, the tubes were analyzed in a machine called “nephelometry” (the nephelometry technique is a test quickly and accurately measures the serum concentration of antibodies).

A major difficulty that we encountered when carrying out this work was being able to take saliva and plaque samples at the same time for all children. We opted for the morning between 9 a.m. and 11 a.m. to be sure that we could guarantee the 2 hours of abstention from any ingestion of food or drink (other than water), because according to previous studies,<sup>26,27</sup> the level of SM varies depending on the nature and duration of ingestion of the last meal. Thus, the samples of unstimulated saliva were taken using a sterile spoon in which we ask the child to spit or using a pipette if the child’s cooperation is negative, to remove saliva under the tongue (this technique was previously described).<sup>28</sup> Finally, if during salivary samples, the child becomes non-cooperating and begins to cry, the test was postponed because stress modifies the salivary components, which risks distorting the microbiological and immunological results.<sup>29</sup>

For each variable (P1 to P4 and S), the children were classified into two categories: “low score” category if the value of the variable is between 0 and 1; “high score” category if the variable is equal to 2 or 3.<sup>30</sup>

### Data Analysis

Descriptive statistics consisted of means and standard deviations of the variables: age, IgA levels, number of caries, and proportions of the variables: gender, saliva (S), and plaque (P1 to P4). Since IgA levels and age did not follow a normal distribution, the test rank sum Wilcoxon test was used to compare these variables between both groups. In addition, the Spearman correlation coefficient was applied to the variables in each group. The chi-square test or Fisher’s exact test was used to determine the relationship between the different categories (gender, P1 to P4, S). All analyzes were performed using SPSS version 16. A 5% level was used to declare significance and a 10% level was used to declare marginal significance.

### RESULTS

This study included 60 participants who were divided into two groups. Group I (control group) included 30 participants [13 (43.3%) girls and 17 (56.7%) boys] who have no caries. Group II (caries group) contained 30 participants [16 (53.3%) girls and 14 (46.7%) boys]

**Table 1:** Comparison of variables: gender, age, total IgA level, rate of *S. mutans* in saliva (S) and plaque (P1, P2, P3, P4, mean of P) between the two groups

Variables	Control group (n = 30)	Caries group (n = 30)	p value
Gender			0.438
[n (%) Girls]	13 (43.3)	16 (53.3)	
[n (%) Boys]	17 (56.7)	14 (46.7)	
Age (in months)	29.3 (5.4)	39.7 (7.0)	<0.001
IgA (mg/L)	23.6 (17.9)	30.4 (47.8)	0.170
S (/Children)			<0.001
0	21 (70.0%)	0 (0.0%)	
1	2 (6.7%)	1 (3.3%)	
2	6 (20.0%)	19 (63.3%)	
3	1 (3.3%)	10 (33.3%)	
P1 (/Children)			<0.001
0	10 (33.3%)	1 (3.3%)	
1	8 (26.7%)	2 (6.7%)	
2	3 (10.0%)	2 (6.7%)	
3	9 (30.0%)	25 (83.3%)	
P2 (/Children)			<0.001
0	8 (26.7%)	0 (0.0%)	
1	7 (23.3%)	1 (3.3%)	
2	8 (26.7%)	6 (20.0%)	
3	7 (23.3%)	23 (76.7%)	
P3 (/Children)			<0.001
0	16 (53.3%)	0 (0.0%)	
1	4 (13.3%)	2 (6.7%)	
2	4 (13.3%)	8 (26.7%)	
3	6 (20.0%)	20 (66.7%)	
P4 (/Children)			<0.001
0	20 (66.7%)	0 (0.0%)	
1	4 (13.3%)	6 (20.0%)	
2	4 (13.3%)	12 (40.0%)	
3	2 (6.7%)	12 (40.0%)	
Average of P	1.1 (0.9)	2.6 (0.4)	<0.001

who have caries (Table 1). Gender difference was not statistically significant ( $p = 0.438$ ) between the two groups. The average age (29.3 months) in the control group was significantly lower than that (39.7) in the “caries” group ( $p < 0.001$ ) (Table 1).

Table 1 contains summary statistics about the distribution of SM levels among members for each group along with the comparisons between the groups.

Although the “caries” group had a higher average IgA than that of the control group (23.6 vs 30.4), the difference was not statistically significant ( $p = 0.170$ ). As for S and P1–P4, we found that most people scored in the lower range (0 or 1) on those variables in the control group and in the higher range (2 and 3) in the “caries” group. The differences between the two groups were significant for all those variables. Moreover, the average P was significantly higher ( $p < 0.001$ ) in the “caries” group (average = 2.6) as compared to the control group (average = 1.1).

The results of comparing IgA among children with low vs high P1–P4 in the “caries” group are summarized in Table 2. It is clear that for all the variables, the mean IgA was always higher in the high category as compared to the low category. However, marginal significance was only seen for P1 ( $p = 0.085$ ) and P4 ( $p = 0.095$ ). A similar comparison was performed for the control group and none of the variables reached statistical or marginal significance (Table 3). As for S, there was no significant difference in IgA levels between

**Table 2:** Different levels of the average of the total IgA (mg/L) corresponding to low and high scores for the variables: P1 to P4 and saliva (S) in the “caries” group

Variable	Group	Total IgA (mg/L)	
		Mean (SD)	p value
P1	Low score	14.4 (8.5)	0.085
	High score	33.6 (10.3)	
P2	Low score	22.6 (9.3)	0.924
	High score	32.8 (11.1)	
P3	Low score	24.4 (7.4)	0.983
	High score	33.4 (12.7)	
P4	Low score	18.9 (5.0)	0.095
	High score	47.6 (20.0)	
S	Low score	34.9 (12.4)	0.350
	High score	21.4 (8.5)	

**Table 3:** Different levels of the average of the total IgA (mg/L) corresponding to high and low scores for the variables: P1 to P4 and saliva (S) in the “control” group

Variable	Group	Total IgA (mg/L)	
		Mean (SD)	p value
P1	Low score	21.5 (3.1)	0.618
	High score	28.2 (8.4)	
P2	Low score	20.6 (3.0)	0.161
	High score	33.3 (9.7)	
P3	Low score	22.4 (2.7)	0.601
	High score	28.0 (13.0)	
P4	Low score	24.4 (3.4)	0.255
	High score	11.8 (13.0)	
S	Low score	23.64 (3.4)	0.933
	High score	21.00 (–)	

the “low” and the “high” S categories in both of the groups ( $p = 0.350$  for the “caries” group and  $p = 0.933$  for the “control” group).

We further studied the correlation between IgA and each of age, average P, and number of caries for each of the two groups. In both groups, there was no significant correlation between IgA and age ( $p = 0.619$  for the control group and  $p = 0.849$  for the “caries” groups). In the control group, there was no significant correlation ( $p = 0.940$ ) between IgA and average P; however, in the “caries” groups, the correlation was positive ( $\rho = 0.33$ ) and marginally significant ( $p = 0.077$ ). The number of caries was not significantly correlated ( $p = 0.633$ ) with total IgA levels. It is worthy to note that there was also a marginally significant ( $p = 0.064$ ) positive correlation ( $\rho = 0.343$ ) between the number of caries and age.

## DISCUSSION

Till date, comparison between two populations with similar inappropriate diet habits but with different dental status (one population is free from decay while the second is affected by the ECC) has never been described in the literature. Performing such comparison was therefore the aim of the present study. The originality of our work lies in the choice of the sample and particularly the control group. This study compares for the first time, two populations with a similar diet but one of which is free from caries while the second has ECC.

According to the primary statistical analysis, the difference between genders was not statistically significant, similar to what was previously reported by Gonçalves de Farias and Barreto Bezerra in 2003.<sup>13</sup> On the other hand, there was a statistically significant difference in age between the control group (29.3 months) and the ECC group (39.7 months) with  $p < 0.001$ . We can explain this by the fact that for most parents, caries lesions at the initial stage go unnoticed, and therefore children with leukoma are almost never treated at this stage. In the literature, the average age of recruited children affected by ECC varies according to the authors: for Gonçalves de Farias and Barreto Bezerra (2003) the average age is 28 months for both groups (free or affected by caries) while it is 36.8 months for van Everdingen et al.,<sup>31</sup> 23.6 months for Febres et al.,<sup>32</sup> and 53 months for Elarabi et al.<sup>33</sup>

Regarding our first objective to evaluate and compare SM levels taken from the saliva and plaque, we obtained a significant difference between the two groups for all variables ( $p < 0.001$ ). This allows us to confirm the direct relationship between the level of SM and dental caries. These results are similar to those of Seki et al.<sup>24</sup>

In this study, we compared the total IgA levels between the two groups of children. We found out a higher level of total IgA in the caries group compared to the control group (30.6 mg/L against 23.6 mg/L). Nevertheless, the individual values that we obtained were very variable: one of the children had a level of 251 mg/L whereas the average was 89.6 in the caries group. His score of SM was 3 as he was a premature infant (this factor is considered promoting decay disease).<sup>34</sup> However, the difference was not significant ( $p = 0.17$ ). Some studies<sup>13,35</sup> confirm the positive correlation between these two variables (IgA and caries), while others have found conflicting results.<sup>36</sup>

The lack of correlation between the level of IgA and the saliva should not exclude the role of the immune system as our results could be explained by the following factors:

- The assessment was limited to the rate of total IgA and not that of the SM-specific IgA.<sup>37,38</sup>



- The lack of standardization and reproducibility problems.
- The effect of salivary flow on the composition of saliva that was not considered in our study.<sup>38</sup>
- The immaturity of the immune system in children.<sup>39</sup>
- High levels of salivary IgA may be the reflection of past exposure to SM.<sup>40,41</sup>

However, the marginal correlation obtained by comparing the level of total IgA and scores of SM taken from the plaque, opens up new research perspectives.

## CONCLUSION

The particularity of our work lies in the choice of the population. The direct connection between the decay in general and ECC in particular and the high levels of SM was evident. Thus, the lack of correlation between IgA and SM collected from the saliva does not eliminate the importance of the preventive role of salivary immune system. The marginal correlation between IgA and SM collected in the plaque shows a boundary link and needs further research. Prevention, by means commonly recognized as dental hygiene tools and control of diet, stays after all, the basic of all therapeutic measures.

## CLINICAL SIGNIFICANCE

Early childhood caries is a public dental health problem that has been affecting preschool children all over the world. Its prevention must be a priority for all professionals in the medical and dental community. This study highlights the absence of correlation between total IgA and SM collected from the saliva. This does not completely exclude the preventive role of salivary immune components, but further studies are required to better understand this relationship.

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### APPENDIX 1: INCLUSION CRITERIA

Date: -----

#### Inclusion Criteria

- |                                   |                              |                             |
|-----------------------------------|------------------------------|-----------------------------|
| 1. Age (18–48 months)             | <input type="checkbox"/> Yes | <input type="checkbox"/> No |
| 2. Antibiotic therapy for 2 weeks | <input type="checkbox"/> Yes | <input type="checkbox"/> No |
| 3. Early childhood decay          | <input type="checkbox"/> Yes | <input type="checkbox"/> No |
| 4. Systemic disorders             | <input type="checkbox"/> Yes | <input type="checkbox"/> No |
| 5. Previous dental treatment      | <input type="checkbox"/> Yes | <input type="checkbox"/> No |
| 6. Inappropriate breastfeeding    | <input type="checkbox"/> Yes | <input type="checkbox"/> No |

### APPENDIX 2: HYGIENE HABITS AND NUTRITION MODE

Patient's code: \_\_\_\_\_ Date: \_\_\_\_\_

#### Dental Hygiene and Food

- |   |  |                             |
|---|--|-----------------------------|
| 1. Does your child brush his teeth?   | <input type="checkbox"/> Yes                             | <input type="checkbox"/> No |
| 2. Is the toothpaste fluoridated?   | <input type="checkbox"/> Yes                             | <input type="checkbox"/> No |
| 3. Does your child rinse his mouth after brushing?  | <input type="checkbox"/> Yes                             | <input type="checkbox"/> No |
| 4. At what age did your child start brushing his teeth? _____   |  |                             |
| 5. Do you assist your child?  | <input type="checkbox"/> Yes                             | <input type="checkbox"/> No |
| 6. How often does he brush his teeth?   | <input type="checkbox"/> Yes                             | <input type="checkbox"/> No |
| 7. Does he still take the bottle?   | <input type="checkbox"/> Yes                             | <input type="checkbox"/> No |
| 8. If yes, how many times? _____  |  |                             |
| 9. What are his main meals? _____   |  |                             |
| 10. Does your child fall asleep with the bottle in the mouth?   | <input type="checkbox"/> Yes <input type="checkbox"/> No |                             |
| 11. Does he demand the bottle during the night?   | <input type="checkbox"/> Yes <input type="checkbox"/> No |                             |
| If your answer to number 10 is "no", do not continue. If yes:   |  |                             |
| 12. Until what age did he continue to take the bottle? _____  |  |                             |
| 13. What is the content of the bottle? _____  |  |                             |
| 14. What situation corresponds more to your child?  |  |                             |
| • Your child finishes his bottle, throws it and falls asleep?   |  |                             |
| • Your child falls asleep with the bottle in the mouth. Is the bottle kept in the mouth for several minutes or removed immediately? |  |                             |
| • Other? _____  |  |                             |

### APPENDIX 3: QUESTIONNAIRE

Date of the exam: \_\_\_\_\_

Family name: \_\_\_\_\_

First name: \_\_\_\_\_

Patient's code: \_\_\_\_\_

Date of birth: \_\_\_\_\_

Age: \_\_\_\_\_

Gender: \_\_\_\_\_

Weight: \_\_\_\_\_

Size: \_\_\_\_\_

Father's Profession: \_\_\_\_\_

Mother's Profession: \_\_\_\_\_

Brothers and Sisters:

Yes  No

Number: \_\_\_\_\_

Age: \_\_\_\_\_

Medical History:

- |                            |                              |                             |
|----------------------------|------------------------------|-----------------------------|
| • Allergy:                 | <input type="checkbox"/> Yes | <input type="checkbox"/> No |
| • Medications in progress: | <input type="checkbox"/> Yes | <input type="checkbox"/> No |
| • Previous surgeries:      | <input type="checkbox"/> Yes | <input type="checkbox"/> No |
| • General diseases:        | <input type="checkbox"/> Yes | <input type="checkbox"/> No |
| • Sleep disturbances:      | <input type="checkbox"/> Yes | <input type="checkbox"/> No |

Remarks: \_\_\_\_\_

Dental History:

At what age did he (she) have his first tooth? \_\_\_\_\_

- |                            |                              |                             |
|----------------------------|------------------------------|-----------------------------|
| • Compromised of fluoride: | <input type="checkbox"/> Yes | <input type="checkbox"/> No |
| • Dental trauma:           | <input type="checkbox"/> Yes | <input type="checkbox"/> No |