# Comparison of Immunoglobulin IgA Level in the Stimulated Saliva of Caries-Free and Caries-Active Children Aged 7-10 Years

Heba N. Yassin, B.D.S., M.Sc.<sup>(1)</sup>

# ABSTRACT

Background: Salivary immunoglobulin IgA plays an essential role in the immune response against dental caries. This studywas conducted to compare the salivary IgA levels and flow rate of stimulated saliva in caries active and caries free children.

Materials and methods: The present study included sixty healthy children age 7-10 yearswho were divided into two groups. They were caries free and caries active children (30 children in each group). Assessment and recording of caries – experience were through the application of Decayed, Missing and Filled Tooth Index (DMFT) and (dmft) index, for permanent and deciduous teeth respectively. After dental examination, stimulated saliva samples were collected from the subjects and performed under standard condition following instruction cited by Tenovuo and Lagerlof, and chemically analyzed for the detection of salivary immunoglobulin (IgA).In addition, salivary flow rate for the children were evaluated. Data was then statistically analyzed using SPSSversion 18.

Results: Salivary IgA levels were significantly higher in caries free children than caries active children and the flow rate were lower in caries active children in both gender as compared to caries free children.

Conclusions: Within the limitation of this study, it can be concluded that that the flow rate and salivary IgA levels of the whole stimulated saliva have some role in protection against dental caries.

Key words: Caries active, caries free, salivary IgA. (J Bagh Coll Dentistry 2016; 28(3):155-158).

## **INTRODUCTION**

Immunoglobulin A (IgA) is predominantly released by common mucosal immune system in human body secretions including saliva. Naturally occurring salivary IgA antibodies against different streptococcal antigens are present in saliva and constitute major defensive actions against dental caries <sup>(1,2)</sup>.

The role of salivary immunoglobulin in the protection against dental caries has been investigated in several studies <sup>(3,4)</sup>, but making this association would be complicated since there are different sampling methods, different criteria for patient group and different laboratory tests between the studies <sup>(5)</sup> and their control is impossible in any study.

It has been claimed that the imbalances in levels of salivary immunoglobulinand physicochemical properties, may play an important role in the onset and development of dental caries <sup>(6, 7)</sup>. There for, this studywas conducted with an aim to evaluation of the stimulated human whole salivary flow rate, and salivary IgA levels in relation to dental caries among children with age group of 7-10 years.

## MATERIALS AND METHODS

This study was conducted in the Department of Pedodontics and Preventive Dentistry, College of Dentistry, University of Baghdad.Sample collection was started at beginning of April 2015 till beginning of June 2015.

(1) Assist. Lecturer. Department of Pedodontics and Preventive Dentistry, College of Dentistry, University of Baghdad The study group includes 30 subjects (Group I), matching with control group (Group II) by age. Diagnosis and recording of dental caries were carried out according to the criteria of WHO<sup>(8)</sup>.

- A total of 60 subjects were divided equally into two groups:
- Group II Caries-free children having no caries, dmft/DMFT = 0.

#### **Exclusion criteria**

- Patients who are physically and medically compromised and having history of antibiotics intake since past 1 month.
- Patients who have arrested carious lesions

Dental examination of tooth surfaces was carried out by using dental explorer and plane mouth mirror. After oral examination for children, stimulated saliva samples were collected from the children, and performedunder standard condition following instruction cited by Tenovuo and Lagerlof<sup>(9)</sup>.

Immediately after collection of saliva, through five minutes and disappearance of the salivary foam, the salivary flow rate was expressed as ml/min. The salivary sample was centrifuged then the clear supernatants will be separated by micropipette and stored in a deep freeze till the time of salivary analysis for the detection of salivary IgA levels for both groups.

The salivary IgA levels were determinedby radial immunodiffusion <sup>(10)</sup> method using immunodiffusion Plate (REA Milano, Italy).Five

microliter of sample was placed in each well of immune diffusion plates. These plates were incubated at room temperature for 72 hours. The salivary IgA levels were calculated by using reference table given in the Diffu-Plate kit in which each diameter of the halo is associated a concentration.

## RESULTS

The percentage of caries - free and caries active children by gender are manifested in Table (1), this table demonstrates that girlsdisplayed the high percentage of caries - freeand caries active compared to boys.

Table (2) represents the comparison of salivary IgA level measured in mg/dl and salivary flow rate measured in ml\min, among gender in both caries group, the results show the mean salivary IgA level in girls in caries-free group was more than caries-active groups with statically not significant difference (P> 0.05) also mean Salivary IgA levelin boys among caries-free group was more than caries-active groups but difference was statically significant (P<0.05). Concerning salivary flow rate the result demonstrates that caries-free group was higher than caries-active groups in both gender and difference was statically highly significant (P=0.00).

The level of salivary IgA in the caries free group was in higher mean value than the caries active group with statistical significant differences at p<0.05. In regarding salivary flow rate the result shows that statistically highly significant differences (p<0.01)between caries active and caries free with higher mean value for caries free group compared to caries active groupAs showed in Table (3).

| Caries group     | Gender | No. | %     |
|------------------|--------|-----|-------|
| Carries          | Boys   | 12  | 40.0  |
| Caries<br>free   | Girls  | 18  | 60.0  |
| nee              | Total  | 30  | 100.0 |
| Carias           | Boys   | 11  | 36.7  |
| Caries<br>active | Girls  | 19  | 63.3  |
| active           | Total  | 30  | 100.0 |

Table 2: Salivary IgA levels (mg/dl)and flow rate(ml/min)ofcaries free andcaries active childrenby gender

|        | <i>J</i> 8 |                      |    |        |       |        |         |
|--------|------------|----------------------|----|--------|-------|--------|---------|
| Gender | Variables  | Caries group         | Ν  | Mean   | ±SD   | t-test | P-value |
| Girls^ | Salivary   | <b>Caries free</b>   | 18 | 127.29 | 28.14 | 1.54   | 0.13#   |
|        | IgA        | <b>Caries</b> active | 19 | 112.22 | 31.33 | 1.54   |         |
|        | Salivary   | Caries free          | 18 | 2.71   | 0.46  | 4.23   | 0.00**  |
|        | Flow rate  | <b>Caries</b> active | 19 | 2.02   | 0.53  |        |         |
|        | Salivary   | <b>Caries free</b>   | 12 | 130.52 | 29.17 |        | 0.03*   |
|        | IgA        | <b>Caries</b> active | 11 | 103.77 | 24.20 |        |         |
|        | Salivary   | Caries free          | 12 | 2.95   | 0.54  |        | 0.00**  |
|        | Flow rate  | <b>Caries active</b> | 11 | 1.83   | 0.55  |        |         |

^=DF=35, ^^=DF=21, #=not significant at P>0.05, \*=significant at P<0.05, \*\*=highly significant at P<0.01.

| Table 3: Comparison for total sample of salivary IgA mg/dl, salivary flow rate ml/min between |
|---|
| caries active and caries free groups  |

| Variables          | s                    | Caries group  | Ν      | Mean               | ±SD    | T-test^  | P-value |
|--------------------|----------------------|---------------|--------|--------------------|--------|----------|---------|
| Salivary IgA       | <b>Caries free</b>   |               |        |                    |        | 0.01*    |         |
|                    | Caries active        | 30            | 109.12 | 28.78              |        |          |         |
| Salivary Flow rate | Caries free          | 30            | 2.81   | 0.50               | 6 41   | 0.00**   |         |
|                    | <b>Caries</b> active | 30            | 1.95   | 0.54               | 6.41   |          |         |
| -DF=58 *=sig       | mific                | ant at P<0.05 | *:     | <sup>∗</sup> =high | lv sig | nificant | at P<0. |

\*=highly significant at P<0.01 ^=DF=58, \*=significant at P<0.05,

## DISCUSSION

Dental caries is infectious disease and accepts the hypothesis that some form of host immunity can regulate caries activity. If immunity can regulate caries activity then salivary IgA might provide a clear correlation <sup>(11)</sup>.

In this study stimulated whole saliva was collected to determine the role of salivary IgA in protection from dental caries.

This study revealed thatsalivary IgA levels were significantly higher in caries free children as compared to caries active children which is in agreement with study of Cogul et al <sup>(12)</sup>. This difference may be because greater number of cariogenic bacteria in whole saliva of caries active children which may due to low immune response among those children. The caries free children produced a greater amount of salivary IgA antibodies to cariogenic bacteria in minor, submandibular, or sublingual salivary glands than caries active children<sup>(13)</sup>. Thus, it can be suggested that the secretory immune system provides local immune protection against cariogenic organisms in the oral environment and ultimately prevents dental caries (11).

In contrast to our study, Thaweboon et al. <sup>(14)</sup> found that the presence of dental caries was associated with an increase of total salivary IgA. However, Shifa et al. <sup>(4)</sup> found no correlation between dental caries and salivary IgA levels. Thisopposing results may be due to different criteria for patient selection, different sampling methods, and differentlaboratory tests used between the studies. Also genetic cause may related with these different result. Moreover, the level of salivary immunoglobulin may change depending upon the salivary flow rate, hormonal factors, emotional states, and physical activity, etc. <sup>(5)</sup>.

Saliva effects caries attack mainly by its rate of flow. In this study, significant difference between caries activity and salivary flow rate were established. The salivary flow rate was decreased in caries-active children compared to caries-free children and statistically highly significant difference. A significant cariespreventive function of saliva is to dilute bacterial substrate <sup>(15)</sup>.Normal salivary flow rate (hydration status and stimulated saliva flow rate) imparts a strong protective influence against dental caries <sup>(16)</sup>. Similar results was seen in the study conducted by Al-Zahawi <sup>(17)</sup> and Sakeenabi and Hiremath <sup>(18)</sup>. The result of this study is in contrast to studies conducted previously by Sulliuan<sup>(19)</sup> and Russel et al. <sup>(20)</sup> which showed no significant difference between the salivary flow rate and caries activity.

The obtained results of this study show that salivary IgA and flow ratehas a convinced relation with caries activity in children and act as markers of caries activity.Physical activity of saliva (as flow rate) play an important role in oral health maintenance. Also this study shows that any change in salivary IgA level may affect in change in oral ecosystem which may lead to dental caries development.

### REFERENCES

- Hu S, Xie Y, Ramachandran P, Ogorzalek Loo RR, Li Y, Loo JA, et al. Large scale identification of proteins in human salivary proteome. Proteomics 2005; 5:1714–28.
- Brandtzaeg P. Do salivary antibodies reliably reflect both mucosal and systemic immunity? Ann N Y Acad Sci 2007; 1098: 288-311.
- 3. Radhi NJ, El-Samarrai SK, Alkhafaji JT. Dental caries in relation to salivary parameters and immunoglobulins among Down's syndrome children in comparison to normal children. J Bagh Coll Dentistry 2009; 21(3):118-24.
- Shifa S, Muthu MS, Amarlal D, Ratna P, Prabhu V. Quantitative assessment of IgA levels in the unstimulated whole saliva of caries-free and cariesactive children. J Indian Soc Pedod Prevent Dent 2008; 26:158-61.
- 5. Michalek SM, Katz J, Childers NK, Martin M, Ballovetz DF. Microbial / host interaction: mechanism involved in host response to microbial antigens. Immunologic Res 2002; 26(1-3): 223-34.
- Parslow T, Staties D, Terr A, Imboden J. Medical immunology. 10<sup>th</sup> ed. Lange medical book/ McGraw-Hill Medical Publishing division; 2001.
- 7. Holmgren J, Czerkinsky C. Mucosal immunity and vaccines. Nature Med 2005; 11(4 Suppl): S45-53.
- 8. World Health Organization (WHO). Basic methods of the oral health survey.3<sup>rd</sup> ed. Geneva, 1987.
- Tenovuo J, Legerlof F. Saliva In: Thylstup A, Fejerskov O (eds). Textbook of clinical cariology. 2<sup>nd</sup> ed. Copenhagen: Munksgaard; 1996.
- Mancini G, Carbonara AO, Heremans JF. Immunochemical quantitation of antigens by single radial immunodiffusion. Immunochemistry 1965; 2: 235-39.
- 11. Chawda JG, Chaduvula N, Patel HR, Jain SS,Lala AK. Salivary SIgA and dental caries activity. Indian Pediatr 2011; 48(9): 719-21.
- 12. Cogulu D, Sabah E, Kutukculer N, Ozkinay F. Evaluation of the relationship between caries indices and salivary secretory IgA, salivary pH, buffering capacity and flow rate in children with Down's syndrome. Arch Oral Biol 2006; 51: 23-8
- 13. Doifode D, Damle SG.Comparison of salivary IgA levels in caries free and caries active children. IJCDS 2011; 2(1): 10-14.
- 14. Thaweboon S, Thaweboon B, Nakornchai S, Jitmaitree S. Salivary secretory IgA, pH, flow rates, mutansStreptococci and Candida in children with rampant caries.Southeast Asian J Trop Med Public Health 2008; 39(5): 893-9.
- 15. Lagerlof F, Oliveby A, Ekstrand J. Physiological factors influencing salivary clearance of sugar and fluoride. J Dent Res 1987; 66:430-5.
- 16. Chaudhary CP, Pandey P, Rao V, Reddy NV, Saxena A. Estimation of salivary flow rate, pH, buffer capacity, calcium, total protein content and total antioxidant capacity in relation to dental caries severity, age and gender. Contemporary Clin Dentistry 2015; 6(5): 65-71.
- 17. Al-Zahawi ShM. The association between some salivary factors and dental caries in group of school children and adolescents in Erbil city. Zanco J Med Sci 2011; 15(2): 64-70.
- 18. Sakeenabi B, Hiremath SS. Dental caries experience and salivary streptococcus mutans, lactobacilli scores,

salivary flow rate and salivary buffering capacity among 6-year old Indian school children.J Intsoc Prev Community Dent 2011; 1(2): 45-51.

 Sulliuan A. Correlation between caries incidence and secretion rate, buffer capacity of stimulated whole saliva in 5-7 years old children matched for lactobacillus count and gingival state. Swed Dent J 1990; 14:131-5.

20. Russel JI. Macfarlance TW, Aitchison TC, Stephen KW, Burchell CK. Caries prevalence and microbiological and salivary caries activity test in Scottish adolescent. Community Dent Oral Epidemiol 1990; 18:120-5.