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Original Research Article

Assessment of Salivary Immunoglobulin A, α-amylase, pH and Flow-rate Effects on Dental Caries Experience of Down's Syndrome Children in Makkah, Saudi Arabia

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ABSTRACT

Objective: To assess the association between dental caries experience and salivary Immunoglobulin A, α-amylase, pH and flow-rate in Down's syndrome children.
Materials and Methods: One-Hundred Down's syndrome participants and one-hundred three healthy controls aged from 4 to15 years were examined to evaluate the caries experience using deft and DMFT. Unstimulated saliva was collected to assess the flow-rate, pH, sIgA and α-amylase. The total sIgA and α-amylase concentrations were measured using ELISA. Data analysis was performed using SPSS® v.20.0 software. Any p value ≤0.05 considered as significant.
Results: The deft of deciduous dentition presented significantly lower mean values in Down's syndrome children compared to the control. While the DMFT index of permanent dentition presented significantly higher mean values in Down's syndrome children compared to control group. Moreover, the salivary flow-rate and IgA were significantly higher in the Down's syndrome children. While the Salivary pH and α-amylase showed significantly diminished mean values in Down's syndrome compared to the controls.
Conclusion: In children with Down's syndrome, the enhanced levels of salivary IgA, pH and flow-rate yielded a protective effect against dental caries.

Keywords: Salivary factors, Dental health, Down's Syndrome, sIgA.

INTRODUCTION

Down's syndrome (DS) is a genetic disorder in which a person has 47 chromosomes instead of the normal 46. In most of the cases, three copies of chromosome 21 are present, so the name "trisomy 21" is being used to characterize such syndrome. [1]

World-widely, the overall incidence of DS is reported to be 1:600 to 1:800 live births. Particularly, the incidence of DS in Saudi Arabia is 1:554 live births. [2]

All in all, studies of dental caries in DS patients are controversial. In many studies [3,4] DS individuals have a lower experience of dental caries than the general population. In spite of this, other studies either higher [5,6] or did not observe any difference [7] in the experience of dental caries.

Basically, saliva is the most valuable oral fluid; it has an important role in determining the prevalence of dental caries. Specifically, Previous reports have been alleged that salivary IgA (sIgA), pH,

buffering capacity and flow-rate have critical roles in the oral mucosal defense. [8] Some differences in the saliva composition of DS children compared with healthy controls have been reported. [9]

Even though, up to now still there have been a controversy regarding the association between low dental caries experience and DS children to which a variety factors could be correlated to, in terms of buffering capacity of the saliva, salivary flow-rate, sIgA and specific factors in saliva composition.

In Saudi Arabia, scanty studies [10] assessing the association between dental caries experience and specific salivary factors of DS children have been reported. Therefore, the present study aimed to assess the relationship between dental caries experience and salivary Immunoglobulin A, α-amylase, pH and flow-rate in DS children.

MATERIALS AND METHODS

Approval from the UQUDENT-IRB ethical committee for the research at the Faculty of Dentistry, Umm Al-Qura University has been obtained before the conduction of this study with IRB number: 08-2015. Informed assent approval was obtained from the guardians of both cases and control groups.

- ***Study Group***

A total of 100 DS children and 103 healthy children as a control group of equated age range and gender in Makkah region were included in this study. The DS children were selected from special needs centers while the control group was selected from elementary and kindergarten schools with age range from 4- 15-year-old.

- ***Clinical Examination***

All children were examined clinically while sitting on an ordinary chair under light originating from a hand torch using a complete set of disposable sterile diagnostic instruments for each child then the data were recorded in the assessment chart.

For every child, the deft and DMFT indices were calculated separately:

- **DMFT index**

The DMFT score describes the dental condition of the permanent teeth. According to "WHO recommendations 1987", [11] a tooth scores D (decayed), when being a carious tooth, recurrent decay, defective filling, temporary filling. A tooth classified as M (Missing), if it was extracted due to caries. Lastly, tooth marked as F (Filled), if it had a permanent restoration.

- **deft index**

The deft index describes the dental condition of the deciduous teeth. Where "deft" stands for: decayed, indicated for extraction due to caries and permanent filling of primary tooth.

- **Saliva investigations**

➢ *Assessment of Salivary flow-rate:*

Testing of un-stimulated salivary flow-rate was performed in the morning (from 9 to 11 A.M). All the participants refrained from eating, drinking for a minimum of 2 hours before the saliva collection. The participants were comfortably seated and they were asked to avoid swallowing the saliva and lean forward to expectorate all saliva accumulated in the mouth for 3 minutes into a graduated test tube. [12] The average salivary flow-rate was measured from the total volume and expressed as milliliters per minute (ml/min).

An average of un-stimulated salivary flow-rate is considered to be more than 0.25 ml/min. If the unstimulated salivary flow-rate is less than 0.1 ml/min it was considered as hypo-salivation and if it is more than 0.6 ml/min it was considered as hyper-salivation. [13]

➢ *Assessment of salivary pH:*

Immediately after saliva collection, the pH was determined using pH indicator strips (Panpeha system, Schleicher -Schull, Germany). It uses a multi-color special and universal indicator paper with pH range from 0 up to 14 (figure 1). Normal salivary pH runs between 7.0 and 7.5, below 7.0 was considered as acidic while above 7.5 was considered as alkaline. [14]

- *Assessment of salivary Immunoglobulin A (sIgA) α-amylase:*

Salivary sIgA and α-amylase (mg/l) was determined using DRG® IgA and α-amylase salivary ELISA kits (DRG International, Inc. USA). The concentrations of sIgA and α-amylase have been read via SPECTROstar Nano microplate reader, Germany (figure 2). The data of both tests were displayed on the computer via MARS data analysis software in a clear and concise format.

- Statistical Analysis

Collected data have been tabulated and statistically analyzed using SPSS (Statistical Package for Social Sciences, SPSS Inc., Chicago, U.S.A. v.20). The continuous variables were described using mean ± standard deviation (SD). When appropriate student *t-test*, One-way ANOVA and Tukey's *post hoc* test for multiple comparisons and Pearson's correlation coefficient test were used to test the association of various collected data. A level of 0.05 was considered significant.

Figure 1: A Photograph showing pH indicator strips.

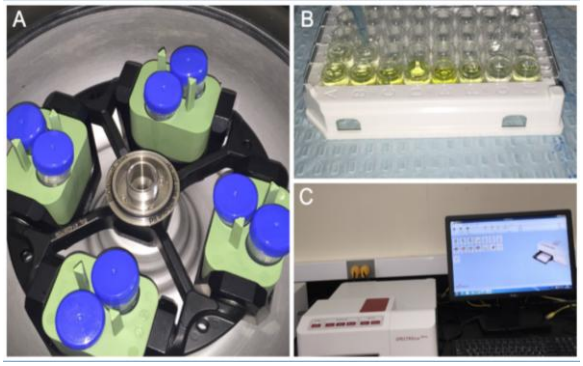

Figure 2: Photographs showing some steps of ELISA test procedure:
A: Saliva sample in the centrifuging machine.
B: the washing step of saliva samples.
C: SPECTROstar Nano microplate reader.

RESULTS

The results of the present study are summarized in Table 1. The comparison between dental caries experience revealed statistical significant difference between the mean value of deft and DMFT in DS children and control group at p=0.03 and p=0.02 respectively. The entire group of DS presented statistically significant higher mean value of salivary flow-rate compared to that of the control at p= 0.04. As illustrated in figure 3, the results of this study revealed that 59% of DS children had high salivary flow-rate while only 8.7% of the control group had high salivary flow-rate. Moreover, Table 2 shows that a statistical significant difference has been found between deft and DMFT mean values upon different salivary flow-rate groups of DS participant at p= 0.0001 and p= 0.0001 respectively. Comparing the deft mean values of the different groups the post hoc comparison Tukey's test revealed that DS children with high salivary flow-rate presented a statistically significant lesser mean value of deft and DMFT compared to DS children with normal and low salivary flow-rate.

The salivary pH of DS showed a very high statistical significant lower mean values compared to that of the control group at p=0.0007. Additionally, the results of present study show that 72% of DS children had acidic salivary pH while 54.4% of the control group had acidic salivary pH as depicted in figure 4.

The salivary IgA showed a very high statistical significant higher mean values compared to the control group at p=0.0009. In addition, the sIgA presented statistically significant negative correlation with deft at p=0.02 (figure 5). On the other hand, the negative correlation between DMFT and sIgA did not reach the significant level with r=-0.12 and p=0.6.

In contrast to healthy children, the salivary alpha-amylase level presented statistically significant lesser mean value in DS children at p= 0.0001. Although a positive correlation has been found between

alpha-amylase and dental caries experience (DMFT and deft), yet it did not reach the significant level at p=0.5 and p=0.9 respectively.

Table 1: Comparison between the DS and the control groups as regards to the deft, DMFT, salivary flow-rate, pH, sIgA and α-amylase.

Variable	Down's Syndrome (mean ± SD)	Control (mean ± SD)
deft	2.72 ± 4.01 [a]	3.88 ± 3.65 [b]
DMFT	2.27 ± 3.91 [a]	1.21 ± 2.08 [b]
Salivary Flow-rate	0.80 ± 0.66 [a]	0.64 ± 0.45 [b]
Salivary pH	6.22 ± 0.73 [a]	6.53 ± 0.54 [b]
sIgA	89.35 ± 85.87 [a]	48.09 ± 88.58 [b]
α-amylase	12.70 ± 6.80 [a]	40.38 ± 16.48 [b]

SD: Standard Deviation. Significant if P ≤ 0.05.
Different lower case superscripts indicate significance between groups at the same row.

Table 2: Comparison between Down syndrome participants as regard to the effect of salivary flow-rate (SFR) upon deft and DMFT score.

Variables	Low SFR	Normal SFR	High SFR
deft (mean ± SD)	5.20 ± 2.80 [a]	3.40 ± 1.70 [b]	1.70 ± 0.80 [c]
DMFT (mean ± SD)	3.18 ± 1.32 [a]	2.83 ± 1.20 [a]	1.00 ± 0.52 [b]

SD: Standard Deviation. Significant if P ≤ 0.05.
Different lower case superscripts indicate significance between groups at the same row.

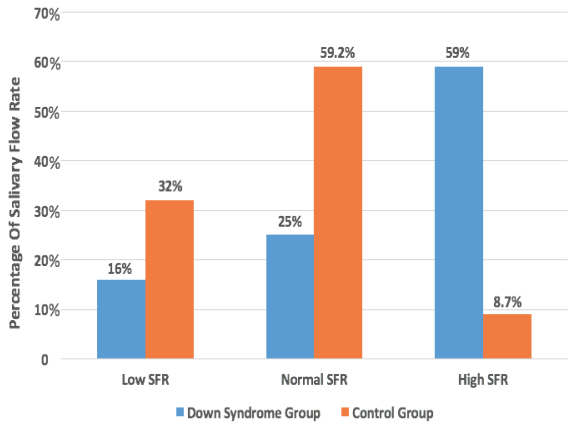

Figure 3: A clustered-column graph depicting the percentage of salivary flow-rate of DS children and control group.

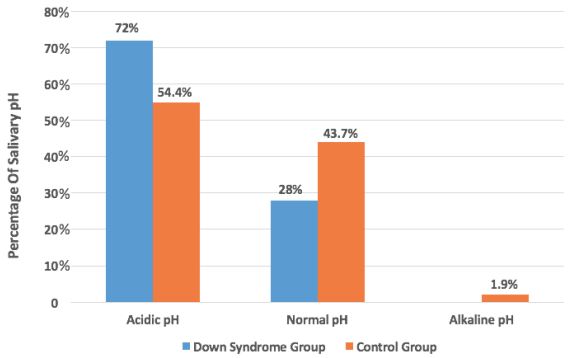

Figure 4: A clustered-column graph depicting the percentage of salivary pH of DS children and control group.

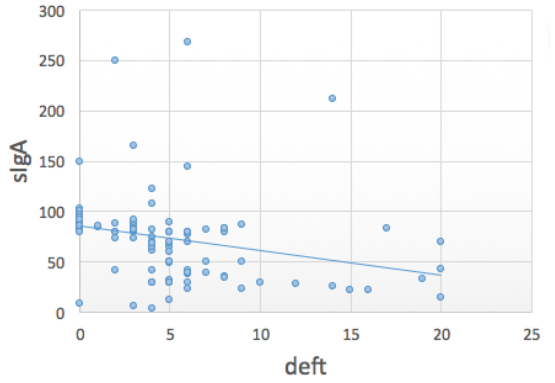

Figure 5: a scatter graph depicting the relationship between sIgA and deft score of DS children.

DISCUSSION

Many studies found a lower experience of caries in subjects with DS compared to people without DS. In the same way, *Stabholz et al.* examined the prevalence of dental caries in 32 DS children, he found 84% of them to be caries-free. [15] Other studies however, found a higher decay rate in people with DS. [16] In contrast of previous studies, the present study revealed higher prevalence of caries in permanent dentition and lower caries prevalence in primary dentition of DS children in comparison with the control group. The increase of caries incidence could be attributed to the lack of awareness about dental visits, irregular dietary habits, inadequate oral hygiene measures, parental

neglect, lack of initiative toward prevention, acidity in the oral cavity and the saliva buffering capacity. [6]

In DS individuals, most researches, [15,17] although not all, [18] have found differences in Salivary flow-rate between healthy and DS subjects. The present study showed a statistically significant increase in salivary flow-rate of DS children compared to control group. This study supports previous study [19] that found a negative relationship between salivary flow-rate and caries prevalence; increased salivary flow-rate result in decreased dental caries experience.

Salivary pH plays important roles in oral health. In particular, there are conflicting results in the salivary pH of individuals with DS and healthy individuals; no difference, [7] a higher pH [20] and a lower pH [21] have been observed. The present study supports previous reports, [22] that revealed an inverse relationship between the saliva PH and dental caries.

Significantly, salivary IgA (sIgA) plays an important role in protecting oral surfaces and preventing infectious conditions such as dental caries. [23]

Studies about sIgA level of DS children compared to healthy controls are controversial. In many studies [8,23] DS individuals have a higher level of sIgA than the general population. In spite of this, some studies [24,25] did not observe any difference of sIgA level between DS children and control group.

In accordance with previous reports, the present study revealed that the IgA levels were significantly higher in DS children than the control group. In addition, the elevated sIgA level was associated with lower dental caries experience in DS children which confirms the previous reports. [8]

The present study showed a statistically significant decrease in alpha-amylase level in DS in comparison to the control group. As well as, in accordance with previous report published by *Ahmdi et al*, [26] the present study found a positive correlation between alpha-amylase and caries prevalence in which the salivary alpha-amylase hydrolyzes starch to glucose and maltose; giving rise to products transformed into acids that might lead to dental caries.

CONCLUSION

Under the limitations of the present study we can conclude the following:

- DS participants presented lower caries experience in primary dentition in comparison to control group.
- The saliva of DS children showed elevated level of sIgA and decreased alpha-amylase level compared to healthy children
- The elevated sIgA level and reduced alpha-amylase level was associated with reduced dental caries experience in DS participants.
- The enhanced levels of salivary pH and flow-rate yielded a protective effect against dental caries.

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Conflict of interest

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

Contribution of Authors

The authors would like to declare that this manuscript is original, has not been published before and is not currently being considered for publication elsewhere.

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

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