

The relationship of salivary immunoglobulin-A with dental caries and oral hygiene status in Down's syndrome children

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ABSTRACT

A positive correlation between salivary IgA antibody levels to *Streptococcus mutans* and caries resistance in adults has been reported in literature. Such a correlation is also observed in Down's syndrome population but lacks sufficient data support. The present study was conducted to assess the relationship of salivary IgA with dental caries and oral hygiene status in Down's syndrome (DS) children compared to control group of normal subjects (NS) of school children of Chidambaram. The study population consisted of 80 subjects aged 8-14 years who were divided into four groups: Group 1 – DS subjects with DMFS= 0, Group 2 - NS with DMFS=0, Group 3 - DS subjects with DMFS= 3 and above and Group 4- NS with DMFS= 3 and above. Clinical examination was done and the study population was examined for the assessment of dental caries status (WHO 1987) and oral hygiene status (OHI -S Index). Unstimulated total saliva samples were collected and s-IgA concentration was evaluated by ELISA. Scheffe test (Intergroup comparison) and Pearson test (correlation analysis) were used to analyze the data. In DS subjects, a negative correlation of s-IgA with DMFS and OHI-S scores was found; the s-IgA levels were significantly higher, prevalence of dental caries was very low and oral hygiene status was not properly maintained when compared to the normal subjects. In the control group, a positive correlation of s-IgA with DMFS and OHI-S scores was found.

KEY WORDS: Down's syndrome, Salivary IgA, DMFS, OHI-S, ELISA.

INTRODUCTION

Down's syndrome is the best-known chromosomal syndrome involving Trisomy in 21st chromosome. Down's syndrome occurs very early in embryonic development; possibly during the first cell division.¹³ Benda CE² reported the frequency of this syndrome to be approximately 1.5 per 1000 births of mothers in the 18 to 29 year old age group. The incidence of dental decay in persons with Down's syndrome has been reported to be extremely low. Lack of oral hygiene maintenance and high prevalence of periodontal diseases in Down's syndrome population is well documented in literature.

Previous studies have shown a positive correlation between salivary IgA antibody levels to *Streptococcus mutans* and caries resistance in adults. Such a correlation is also observed in Down's syndrome population but lacks sufficient

data support.¹¹ The present study was conducted to evaluate and correlate the salivary IgA levels, dental caries status and oral hygiene status of Down's syndrome children.

MATERIALS AND METHODS

STUDY POPULATION

Down's syndrome children (DS) were selected from Down's syndrome association of Tamilnadu, Abhiramapuram, Chennai (Fig.3). Normal subjects (NS) were selected from Chidambaram school children. Children who had suffered from upper respiratory tract infection in the past one week were excluded from the study (due to development of IgA and lysozyme). Eighty subjects in the age group of 8-14 years were selected after screening the dentition for DMFS. The study population was divided into four groups;

Group 1 – DS subjects with DMFS= 0, Group 2- NS with DMFS=0, Group 3- DS subjects with DMFS= 3 and above and Group 4- NS with DMFS= 3 and above.

ARMAMENTARIUM

1. Plane mouth mirrors
2. Explorer
3. Soft pipette
4. 10ml sterile glass vales
5. Kidney tray
6. Enamel bowls
7. Tweezers
8. Cotton holders
9. Chip blowers
10. Disposable mouth masks
11. Disposable gloves
12. Sterilized cotton
13. Dettol
14. Towels
15. Dettol Soap
16. Human IgA ELISA Quantitation Kit (Catalog No. (E80-102), Bethyl Laboratories Inc. U.S.A.
17. ELISA Starter Accessory Package, used with ELISA Quantitation Kits (Catalog No. E101), Bethyl Laboratories Inc. U.S.A.

METHODS

The instruments were kept in Dettol solution for disinfection and sterilized using a water bath. Dettol was diluted by adding potable water in the ratio of 1:9 dilutions. The subjects were examined in their own settlements either in the classroom or in the corridor of the school. The subjects were allowed to sit on a chair or stool, where sufficient natural daylight was available.

The type III clinical examination was carried out during the survey by the investigator himself. This type of examination was proposed by ADA in 1970, using plane mouth mirror and explorer. An organizing clerk at the examination site maintained a constant flow of subjects to the examiner and also entered general descriptive information on the survey form, which included the name, age, sex, diet, the oral cleaning habits, materials used for cleaning the tooth, the frequency of cleaning, snacks taken in between meals and the socio-economic status.

The examination of dental caries was made with the help of WHO (1987) instruction and oral hygiene index for primary and permanent teeth

respectively. The data were entered on a standard proforma prepared with the help of the 'WHO' Oral Health Assessment Form (1987). Clinical examination was done and the study subjects were examined for the assessment of dental caries status (WHO 1987)²⁰ and assessment of oral hygiene status (OHI -S Index).⁶

Unstimulated total saliva samples (2-3ml) were collected in the wide mouthed sterile test tubes (Fig 1, 2). Subjects were informed in advance not to eat or drink (except for water) one hour before saliva collection to minimize possible food debris and stimulation of salivation. The samples were frozen and kept at -70°C until analyzed (Fig-4). The samples were then thawed and centrifuged for 15min at 12000g at 4°C to remove the mucin and debris and the supernatant was examined by ELISA.¹⁰ The s-IgA concentration was evaluated by ELISA. The ELISA protocol was performed as described in Appendix-1³. Average duplicate readings from each standard, control, and sample were taken. Intergroup comparison of various values was done with Scheffe test. Pearson correlation test was employed for correlation analysis. A standard curve was created by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. The software used was SOFTMAX.

RESULTS

In the present study, highest mean s-IgA level was recorded in DS with DMFS = 3 and above (Group III) [13.9 ±6.2mg/100ml] followed by DS with DMFS = 0 (Group I) [13.3 ±5.3mg/100ml]. Normal

Subjects with DMFS= 3 and above (Group IV) recorded 11.7 ±1.8mg/100ml followed by NS with DMFS=0 (Group II)[7.5 ±2.4mg/100ml].

Intergroup comparisons showed that the mean s-IgA levels in DS with DMFS = 0 (Group I) was significantly higher than NS with DMFS=0 (Group II) ($p = \leq 0.05$); no significant difference between mean s-IgA levels in DS with DMFS = 3 (Group III) and above and NS with DMFS= 3 and above (Group IV) ($p=0.46$); no significant difference in mean s-IgA levels in DS with DMFS = 0(Group I) and DS with DMFS= 3 and above (Group III) ($p=0.97$); the mean s-IgA levels in NSwith DMFS = 3 and above (Group IV) was significantly higher than NSwith DMFS= 0 ($p = \leq 0.05$) (Group II).



Collection of saliva from DS subjects

Fig. 1



Collection of saliva from NS subjects

Fig. 2



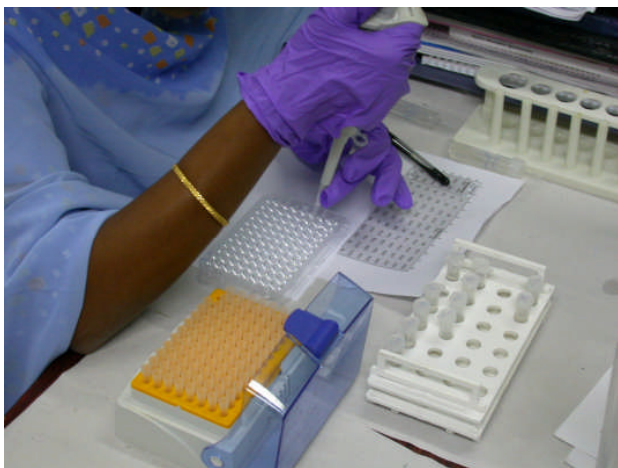
Address of Down's syndrome association in TN

Fig. 3



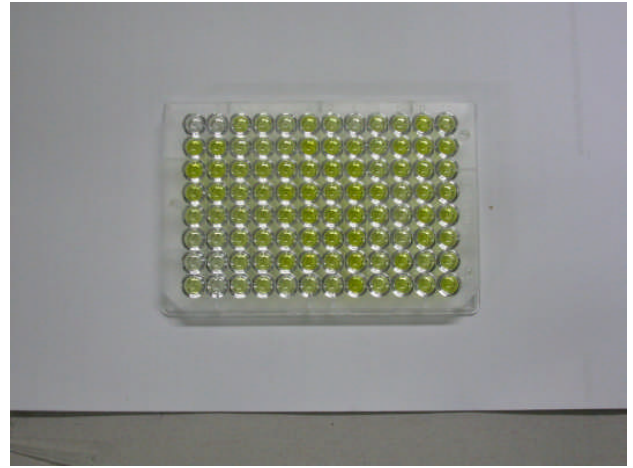
Saliva samples stored at -70°C (cryoscientific Inc)

Fig. 4



Adding saliva samples to the wells coated with IgA antibody

Fig. 5



H₂SO₄ added to TMB reaction

Fig. 6

APPENDIX-1

STEP-BY-STEP METHOD FOR HUMAN S-IgA QUANTITATION

A1. Coat with Capture Antibody: Standards, samples, blanks and /or controls were analyzed in duplicate; 96µl of capture antibody (A80-102A) was diluted to 9600µl Coating Buffer and coated on each well; Coated plate was incubated for 60 minutes; After incubation, the capture antibody, was aspirated from the solution from each well with the SKAN WASHER- 300 version – B Model- 12010 (Skatron Instruments, Norway. U.S.A). Each well was washed with wash solution with SKAN WASHER- 300 version – B Model- 12010 (Skatron Instruments, Norway. U.S.A) as follows: Each well

was filled with wash solution; Wash solution was removed by aspiration; This was repeated for 3 washes.

A2. Blocking (Post coat) solution: 200 µl of Blocking (Post coat) Solution was added to each well; Incubated for 30 minutes at RT.; After incubation, the Blocking (Post coat) Solution was removed from each well and washed three times as in step A1.

A3. Standards and Samples: Standards were diluted in sample diluents according to the chart below

Step	ng/ml	Calibrator RS10-110-3	Sample Diluents
1	500	4µl	9.6ml
2	250	1 ml from step 1	1 ml
3	125	1 ml from step 2	1 ml
4	62.5	1 ml from step 3	1 ml
5	31.2	1 ml from step 4	1 ml
6	15.6	1 ml from step 5	1 ml
7	7.8	1 ml from step 6	1 ml

Since the samples of the present had got more sIgA that the standards range given by the company. So, samples were diluted in sample diluents. 1µl of saliva samples were diluted to 100µl in sample diluents. 100 µl of samples were transferred to assigned wells and incubated for 60 minutes at RT. After incubation, samples and standards were removed and each well was washed 5 times as in Step A1.

A4. HRP Detection Antibody: HRP Conjugate (A80-102P) was diluted in Conjugate Diluents. Dilution range was 1:10,000. 100 µl was transferred to each well; Incubated for 60 minutes. After incubation, HRP Conjugate was removed and each well was washed 5 times as in Step A1.

A5. Enzyme Substrate Reaction: Substrate solution was prepared according to the manufacturer's recommendation. 9.6 ml of TMB Peroxidase substrate was mixed with and 9.6 ml of Peroxidase solution in equal volumes. 100 µl of substrate solution was transferred to each well and incubated for 15 minutes at room temperature. To stop the TMB reaction, 100 µl of 2M H₂SO₄ was added to each well. Using a Micro titer plate reader, VERSAMAX, MOLECULAR DEVICES CALIFORNIA (U.S.A) the plate was read at the wavelength of 450nm that is appropriate for the TMB Substrate solution used.

Table 1 shows the comparison of s-IgA levels in Down's syndrome group with DMFS= 0 (Group I) and control group with DMFS=0 (Group II)

Group	N	Mean s-IgA levels (mg/100ml)	Std Deviation	'p'- value (sig)
Group I	20	13.336	5.329	0.05 (p≤ 0.05)
Group II	20	7.585	2.488	

(Significant at p≤ 0.05)

Table 2 shows Comparison of s-IgA levels in Down's syndrome group with DMFS= 0 (Group I) and DMFS= 3 & above (Group III)

Group	N	Mean s-IgA levels (mg/100ml)	Std Deviation	'p'- value (sig)
Group I	20	13.336	5.329	0.97 (NS)*
Group III	20	13.980	6.243	

*Not significant

Table 3 Comparison of s-IgA levels in control group with DMFS=0 (Group II) and control group with DMFS= 3 & above (Group IV)

Group	N	Mean s-IgA levels (mg/100ml)	Std Deviation	'p'- value (sig)
Group II	20	7.585	2.488	0.05 (p≤ 0.05)†
Group IV	20	11.760	1.859	

†Significant at p≤ 0.05

Table 4 Comparison of OHI-S score in Down's syndrome group with DMFS= 0 (Group I) and with DMFS= 3 and above (Group III)

Group	N	Mean OHI-S score	Std Deviation	'p'- value (sig)
Group I	20	2.100	.854	0.986 (NS)*
Group III	20	2.185	.836	

*Not Significant

Table 5 Comparison of OHI-S score in control group with DMFS=0 (Group II) and with DMFS= 3 & above (Group IV)

Group	N	Mean OHI-S Score	Std Deviation	'p'- value (sig)
Group II	20	1.010	0.53	0.10 (NS) †
Group IV	20	1.580	0.55	

†Not Significant

Table 6 Relationship of s-IgA levels with DMFS score in Down's syndrome group

Pearson correlation sig (2-tailed)	DMFS score	s-IgA levels (mg/100ml)
DMFS score	1.000	-0.075
s-IgA levels (mg/100ml)	-0.075	1.000

(Negative correlation)

Table 7 Relationship of s-IgA levels with OHI-S score in Down's syndrome group

Pearson correlation sig (2-tailed)	s-IgA levels (mg/100ml)	OHI-S score
s-IgA levels (mg/100ml)	1.000	.084
OHI-S score	.084	1.000

(Positive correlation)

Highest mean OHI-I score was recorded in DS with DMFS = 3 and above (Group III) [2.1 ±0.8] and DS with DMFS = 0 (Group I) [2.1 ±0.8]. Normal subjects with DMFS= 3 and above (Group IV) recorded 1.5 ±0.5 followed by NS with DMFS=0 (Group II)[1.0 ±0.5].

Intergroup comparison showed that the mean OHI-S score in DS with DMFS = 0(Group I) was significantly higher than NS with DMFS=0 (Group II) ($p = 0.001$); no significant difference in mean OHI-S score between DS with DMFS = 3 and above (Group III) and control group with DMFS= 3 and above(Group IV) ($p=0.07$); no significant difference in mean OHI-S score between DS with DMFS = 0(Group I) and DS with DMFS= 3 and above (Group III) ($p=0.98$); no significant difference in mean OHI-S score between NS with DMFS = 0(Group II) and NS with DMFS= 3 and above (Group IV) ($p=0.10$).

Pearson correlation test (2-tailed significance) showed a statistically non-significant negative correlation of s-IgA levels with DMFS score in Down's syndrome group; a statistically non-significant positive correlation of s-IgA with OHI-S score in Down's syndrome group; a statistically significant positive correlation of s-IgA with OHI-S score in control group; a statistically non-significant positive correlation of s-IgA with OHI-S score in control group

DISCUSSION

It is generally accepted that *S. mutans* is the primary causative agent of dental caries in man.^{7,18} But, the relationship between s-IgA and dental caries in children with Down's syndrome has not been well established. Hence, the present study was aimed at assessing the relationship of salivary immunoglobulin-A with dental caries and oral hygiene status in Down's syndrome children.

The studies on dental caries prevalence in Down's syndrome have been less clear-cut.^{4,12,16,17} A number of studies have revealed significantly less caries prevalence in Down's syndrome children when compared to controls,^{5,17} whereas others showed no difference.⁴

The caries prevalence of group I and III in the present study are consistent with numerous studies that have suggested that the prevalence of dental caries in Down's syndrome children is very low.^{8,12,16,20} The majority of the reported studies on

the prevalence dental caries have been performed on relatively young patient populations, with mean ages in the adolescent range. Due to delayed eruption of teeth in Down's syndrome children, there is a difference in exposure times of teeth to the oral environment when compared to control patients, which has been cited as a factor to be considered in interpreting prevalence data.

Barnett M.L, Press K.P, Friedman.D and Sonnenburg. E.M(1986)¹ demonstrated that there was considerable difference in caries prevalence, most noticeable with respect to interproximal lesions. Vigild (1986)¹⁹ reported that the prevalence of approximal caries in Down's syndrome was low. In the present study, the dental caries was found to be more in the occlusal surfaces and low in the approximal and smooth surfaces. Steinberg and Zimmermen (1978)¹⁶ reported that the caries lesion Down's syndrome appeared to be limited to occlusal surfaces. Findings in the present study do not support this data.

Moriniushi T, Lopatin DE and Tanaka H (1995)¹¹ reported significant findings in which the severity level based on the DMFT score tends to be bipolar. That is, the subjects tend to be either caries-free or have a high caries experience (a DMFT score over 20). In the present study, no such findings were observed. However, the average DMFS score in the present study was in between 3 and 5.

At the other end of the spectrum from caries, is the high rate of periodontal disease seen in Down's syndrome.¹⁴ Early, severe periodontal disease is often seen with onset in the mid to late teen years. Some studies report an incidence of periodontal disease to be between 90 and 96% of adults with Down's syndrome. This was thought to be related to a lowered host immune response due to the compromised immune system with a corresponding decrease in number of T cells in Down's syndrome.¹⁵ The amount of plaque and calculus seen on the teeth is not proportionate to the severity of the disease.²¹

Chronic mouth breathing may cause decrease in salivary secretion as this reduces the natural cleansing that occurs in the oral cavity and may contribute to the development of caries. The eruption of the teeth in persons with Down's syndrome is usually delayed and may occur in an

unusual order.⁵ Kroll R.G, Bodmick J and Kobren A (1970)⁸ demonstrated that poor oral hygiene directly associated with plaque score, contributes to the high prevalence of dental caries in Down's syndrome children. Morinushi T, Lopatin DE and Tanaka H (1995)¹¹ reported a significant positive correlation between plaque score and the severity of dental caries (as measured by OCSS) in all the subjects. The results supported the concept that the prevalence of dental caries in Down's syndrome is related to microorganisms such as *s. mutans*.

In the present study, there was a significantly positive correlation of s-IgA levels with DMFS and OHI-S scores in the control group. Therefore, from these results it can be suggested that as DMFS and OHI-S scores increased, there was an increase in s-IgA levels. This increase in s-IgA levels may give protection against dental caries.

In Down's syndrome subjects, a negative correlation was observed with respect to s-IgA levels with DMFS and OHI-S scores, but the negative correlation was not statistically significant.

There was a significant increase in s-IgA levels in Down's syndrome subjects when compared to the control group. These findings suggested that though the oral hygiene in Down's syndrome subjects was not maintained properly, caries prevalence was low; and this appeared to be due to the possible immune protection conferred by the elevated salivary IgA levels.

Lee SR, Kwon H.K, Song K.B and Choi Y.H (2004)⁹ demonstrated that there was an increased s-IgA level in Down's syndrome subjects when compared with normal children. The findings of the present study also confirmed the relationship between s-IgA levels and dental caries in Down's syndrome subjects.

Recent years have witnessed a trend towards deinstitutionalizing mentally retarded patients and placing them in the community in either group homes or private homes with natural or foster parents. As a consequence of this, it is essential that disease patterns in these patients be researched upon and understood, so that appropriate treatment and preventive regimes can be implemented.

CONCLUSION

From the results of the present study, the following conclusions were drawn: The prevalence of dental caries in Down's syndrome subjects was very low when compared to the normal healthy subjects. Even though the oral hygiene status was not properly maintained in Down's syndrome they had very low caries rate. The s-IgA levels in Down's syndrome were significantly higher than the normal healthy subjects. It appeared that the increased level of s-IgA gave immune protection against dental caries. Negative correlation of s-IgA with DMFS and OHI-S scores but the negative correlation was statistically non-significant in Down's syndrome group. A statistically significant positive correlation of s-IgA with DMFS in control group was present. Positive correlation of s-IgA with OHI-S scores was evident but the positive correlation was not statistically significant in control group. Further, long-term studies should be carried out in the field of s-IgA and dental caries with different DMFS scores.

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