Comparative Assessment of Salivary Flow Rate, Buffering Capacity, Resting PH and Dental Caries In Children With Autism

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Abstract. The present study aimed to assess and compare the salivary flow rate, buffering capacity, and resting pH of autistic children with healthy children and to compare the proportion of dental caries in autistic children with healthy children. A total of 100 children were included in the study, the study group consisted of 50 autistic children, and the control group consisted of 50 healthy children who visited the Department of Pediatric and Preventive Dentistry, V S Dental College, Bangalore for a routine dental check-up. Salivary parameters like the flow rate, buffering capacity, and resting pH were measured using a saliva check buffer kit (GC co. Japan) and dental caries were recorded according to WHO criteria 1997. Salivary flow rate, buffering capacity, and resting pH were lower in autistic children when compared to that of healthy children and the results were statistically significant. There was a positive correlation between salivary parameters and dental caries. Increased incidence of dental caries in children with autism could be due to their behavioral characteristics and tendency to pouch food inside the mouth. In the present study reduced salivary flow rate, low buffering capacity, and reduced resting pH have been observed in autistic children, which could be the causative factors for the increased incidence of dental caries in these children.

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1. Introduction

Autism, also referred to as autism spectrum disorder−constitutes a diverse group of conditions related to brain development. It was first explained by Leo Kanner in the year 1943.

In the World Health Organization's definition of autism, it is defined as a neurological disability that affects social interaction, communication, and the selection and repetition of interest and activity areas (Ikram & Obaid, 2022).

The Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5) adopted the term ASD with a dyadic definition of core symptoms: early-onset of difficulties in social interaction and communication, and repetitive, restricted behaviors, interests, or activities. Atypical language development, which had been included in the triad of ASD, is now regarded as a co-occurring condition (Kim et al., 2016).

The signs of this highly variable brain disorder appear during infancy or childhood, normally before the child is three years old. As per the reports released by the Centre for Disease Control and Prevention (CDC), U.S.A, this condition is identified as 1 in 54 children. Among boys aged eight years, the prevalence was 29.7 per 1,000, 4.3 times higher than the 6.9 per 1,000 prevalence among girls aged eight years. In India itself, there are more than 1 million cases of autism, diagnosed each year (Maenner et al., 2021)

Children with autism have low frustration levels and lesser attention span; hence they may portray frequent temper tantrums. They are extremely hypersensitive to touch and noise.

The triad of impairment in social interaction, communication, and hyperactive repeated body moments is distinguishable in an autistic child. Some may also express abnormal emotional and linguistic development, visual and hearing impairment, and co-existing disabilities such as mental retardation and epilepsy; all of which might complicate dental care. Children with autism are also prone to periodontal diseases and damaging oral habits like lip biting, grinding, tongue thrusting, picking at the gingival margin, etc (Babu and Roy, 2022)

It is important to note that diagnosing ASD is difficult since there is no definite medical test like a biopsy or blood test. Clinically, it can only be diagnosed by screening a child's development or behavior. As a result, many children are not diagnosed until a much later age. An early diagnosis of the disability will facilitate early support, guidance, and necessary behavior interventions for the associated medical condition (Babu and Roy, 2022).

Saliva is an important exocrine solution, consisting of 99% water and 1% electrolytes, proteins, and enzymes secreted by the salivary glands. This fluid has immense immunological and enzymatic functions and plays a major role in the body's defense mechanisms. Besides its importance as a protective agent, saliva also plays a key role in preventing dental caries. The flow of saliva decreases the plaque accumulation on the tooth surface and also increases the clearance of carbohydrates from the mouth. Any PH below 5.5 in the oral environment promotes demineralization from the tooth surface since the plaque remains unsaturated; but a plaque PH above 5.5 helps in the super-saturation of the saliva with Calcium Phosphate ions which results in re-mineralization of the tooth surface (Bassoukou et al., 2009)

The flow rate and buffering capacity of saliva are important protective factors in oral health.

In saliva, three major systems are contributing to the buffer capacity: bicarbonate, phosphate, and protein buffer system. An inter-relationship between pH, buffer capacity, and the flow rate has been reported (Bassoukou et al., 2009 Ali Hadi et al, 2017). Regarding the low dental caries experience reported in autistic individuals, the etiopathology investigation into the role of salivary components in this illness is fundamental.

Very few studies have been published in the literature evaluating the salivary flow rate, pH, and buffer capacity of saliva from individuals with autism. Therefore, this study aimed to determine the flow rate, pH, and buffer capacity of whole saliva and the dental caries experience in children with autism, aged 6 - 14, compared to a matched control group.

2. Materials and Methods

A total of 100 children were included in the study, the study group consisted of 50 children with Autism, from various Autism institutions in Bangalore and the control group consisted of 50 healthy children who visited the Department of Pedodontics and Preventive Dentistry, V S dental college for a routine dental check-up. Ethical clearance was obtained from the Institutional ethical committee. Signed written informed consent was obtained from the parents/guardians of the children participating in the study.

Examination of the children of both the autistic group and the healthy group was done on a dental chair under natural light.

The child was draped and made to sit on the dental chair for intraoral examination such that the floor of the mouth is maintained parallel to the ground. Salivary parameters were measured using Saliva- check buffer kit (GC co. Japan) for both groups.

The salivary flow rate of unstimulated saliva was noted by visual inspection of the level of hydration, by everting the lower lip gently, blotting the labial mucosa with a small piece of gauze, and observing the mucosa under good light for droplets of saliva at the orifice of the

minor salivary glands. Time was assessed for the visible production of saliva. If the time noted was greater than 60 seconds unstimulated salivary flow rate was considered to be low.

Patients were instructed to chew the piece of wax provided in the kit for 5 minutes to measure the stimulated salivary flow rate. Saliva was collected into the collection cup at regular intervals. The quantity of saliva was measured by checking the ml marking on the side of the

cup. If the quantity of saliva at 5 minutes was <3.5ml then it was considered as very low, between 3.5-5.0ml then it was considered low, and >5.0ml was considered as normal salivary flow rate. pH strips were placed in the collected salivary sample for 10sec and then the color change was compared with the testing chart provided in the Saliva-check buffer kit, and the values were assigned accordingly. the pH of 5.0- 5.8 was considered highly acidic, 6.0-6.6 was moderately acidic, and 6.8-7.8 was considered healthy saliva.

The buffering capacity of saliva was measured using buffer test strips, sufficient saliva was drawn from the collection cup and then dispensed one drop onto each of the three test pads on the buffer strip. The test pads began to change color immediately and after 2 minutes the final results were calculated by adding the points according to the final color of each pad. A total of 0-5 was considered as a very low buffering ability of saliva, 6-9 was considered as the low buffering ability of saliva, and 10-12 was considered as the normal buffering ability of saliva.

Caries evaluation was performed under the standardized condition, using optimal artificial light, mouth mirror, and probe. Dental caries was recorded according to World Health Organization (1997) criteria.

3. Statistical Analysis

Data were analyzed using the statistical package SPSS 22.0 (SPSS Inc., Chicago, IL) and the level of significance was set at $p<0.05$. Descriptive statistics were performed to assess the mean and standard deviation of the respective groups. The normality of the data was assessed using the Shapiro-Wilkinson test. Inferential statistics to find out the difference between the groups was done using the Independent t Test (between groups) to find out the difference between the groups. The Chi-square test was used to detect the difference in proportion between the two groups.

4. Results

The present study was conducted to compare the salivary flow rate, buffering capacity, resting pH, and dental caries in healthy children and children with autism.

A total of 100 children participated in the study (50 autistic children and 50 healthy children) with the mean age and S.D being 9.38 ± 2.11 years in the control group

and 8.94 ± 2.28 years in the autistic group out of which 56% were males and 44% were females. (Table A, B)

Table A. Comparison of Age

Variable	*Normal*	*Autistic*	*P-Value (Independent t-test)*
Age	9.38±2.11	8.94±2.28	0.31

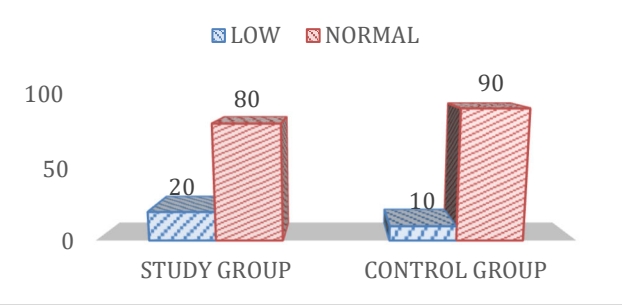
P < 0.05 is Statistically Significant*

Table B. Comparison of Gender

Variable	*Normal*	*Autistic*	*P-Value (Chi-square test)*
Male	**29(58)**	**29(58)**	1
Female	21(42)	21(42)	

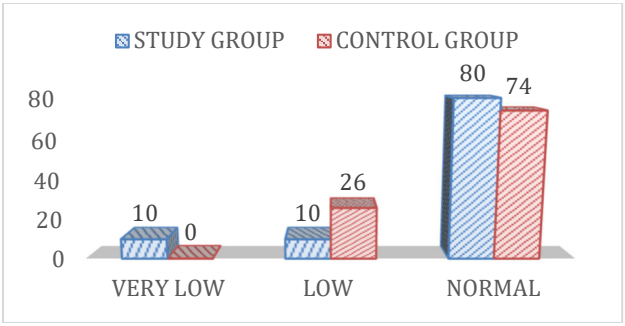
P < 0.05 is Statistically Significant*

After assessing the salivary parameters, it was noted that the unstimulated salivary flow rate was normal in 40 (80%) of the autistic children and 45 (90%) of the healthy children and was low in 10 (20%) of the autistic children and 5 (10%) of the healthy children. The normal unstimulated salivary flow rate ranges from 0.25-0.35 ml/min, and low ranges from 0.1 to 0.25 ml/min. The chi-square test reported a significant difference in the proportion of unstimulated salivary flow categories in the study group compared to the control group (p<0.05). The unstimulated salivary flow rate was observed to be low in autistic children as compared to healthy children. (Graph A)



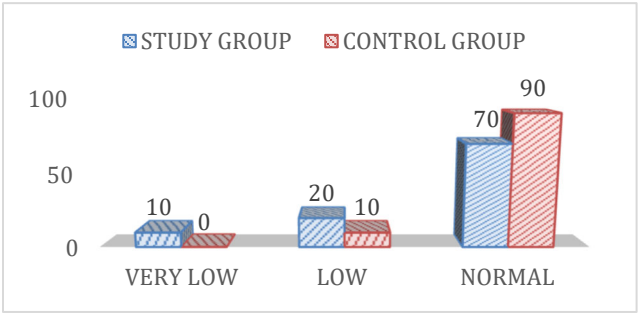
GRAPH A. Unstimulated Salivary Flow Comparison

The stimulated salivary flow rate was found to be normal in 40 (80%) of the autistic children and 37 (74%) of the healthy children, whereas stimulated salivary flow was low in 5 (10%) of the autistic children and 13 (26%) of the healthy children. A very low stimulated salivary flow rate was found in 5 (10%) of the autistic children and none of the healthy children. Normal stimulated salivary flow ranges from 1-3 ml/min, low ranges from 0.7-1 ml/min and if less than 0.7ml/min, it is considered a very low stimulated salivary flow rate. The chi-square test reported a significant difference in the proportion of stimulated salivary flow categories in the study group compared to the control group (p<0.05). (Graph B)



GRAPH B. Stimulated Salivary Flow Comparison

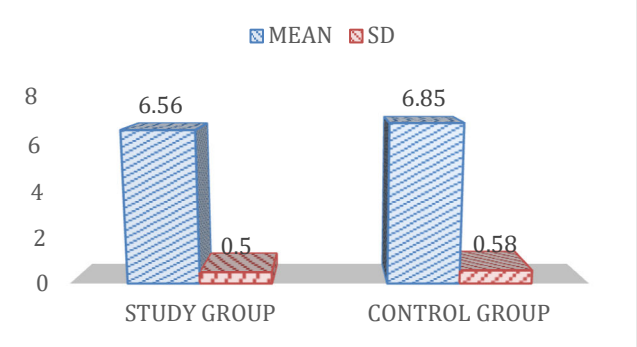
The buffering capacity of saliva was found to be normal in 35 (70%) of autistic children and 45 (90%) of healthy children. Low buffering capacity was found in 10(20%) of the autistic children and 5 (10%) of the healthy children. Very low buffering capacity was found in 5 (!0%) of the autistic children and none of the healthy children. According to Neil, 1978, the normal range of buffer capacity in the saliva is 3–30 mg/100 ml. The chi-square test reported a significant difference in the proportion of buffering capacity categories in the study group compared to the control group (p<0.05). (Graph C)



GRAPH C. Buffering Capacity Comparison

On comparison of salivary resting pH between the groups, the results were found to be statistically significant (p<0.05). Normal salivary pH ranges from 7.1 to 7.5. The mean salivary pH in the healthy group was found to be 6.85 whereas, in the autistic group, it was found to be 6.56. Salivary resting Ph was found to be higher in healthy children than in autistic children. (Graph D)

Dental caries was recorded according to World Health Organisation (2013) proforma. Decayed, missing, and filled teeth were evaluated using the DMFT/dmft index for permanent and primary dentition respectively.



GRAPH D. Resting pH Comparison

The mean dmft in the autistic children was found to be 2.28 ± 1.93 and 0.8 ± 1.27 in the healthy children. The results were found to be statistically significant (p=0.005). The filling component (f) of the dmft was found to be higher in healthy children (mean ± S.D=0.32 ± 0.84) as compared to that of autistic children (mean ± S.D =0.04 ± 0.19), with a statistically significant difference (p=0.02). (Table C)

Table C. Comparison of d, m, f & dmft

Variable	*Normal*	*Autistic*	*P-VALUE (Independent t-test)*
D	0.8±1.27	2.04±1.94	**0.003***
M	0.16±0.54	0.2±0.40	0.67
F	0.32±0.84	0.04±0.19	**0.02***
Dmft	1.28±1.60	2.28±1.93	**0.005***

P<0.05 Is Statistically Significant*

The mean DMFT in the autistic children was found to be 2.32 ± 1.73 and 0.78 ± 1.29 in the healthy children, which was statistically significant (p=0.0001). The decayed component (D) of the DMFT was found to be higher in the autistic group (mean ± S. D=1.96 ± 1.39) as compared to that of the healthy group (mean ± S.D=0.74 ± 1.17) and the difference was statistically significant (p= 0.0001). The missing component (M) of the DMFT was found to be higher in the autistic group (mean ± S. D= 0.34 ± 0.65) as compared to that of the healthy group (mean ± S.D=0) and the difference was statistically significant (p= 0.0005). (Table D)

Table D. Comparison of D, M, F & DMFT

Variable	*Normal*	*Autistic*	*P-VALUE (Independent t-test)*
D	0.74±1.17	1.96±1.39	0.0001*
M	0±0	0.34±0.65	0.0005*
F	0.04±0.197	0.16±0.37	0.04*
DMFT	0.78±1.29	2.32±1.73	0.0001*

P<0.05 is Statistically Significant

5. Discussion

The study aimed to determine the flow rate, pH, and buffer capacity of the whole saliva and the dental caries experience in children with autism, aged 6 – 14 years, compared to a matched control group.

Autistic patients constitute a small percentage of the pediatric population and require unique management because of their behavioral characteristics. Most of the autistic children exhibit bruxism (20-25%), non-nutritive chewing, tongue thrusting, and self-injury (picking at gingiva, lip biting) creating ulcerations. Oral hygiene status is poor since home care measures are exceedingly difficult for many children/parents. Children with autism have poor tongue coordination so they prefer soft and sweetened foods and tend to pouch food inside the mouth for long durations instead of swallowing it. Due to the presence of food within the oral cavity for long durations and difficulties in brushing and flossing due to a lack of motor coordination and high sensitivity to the taste of toothpaste, there is an increased propensity to caries (Babu and Roy, 2022).

Saliva is an important component in determining the progression or inhibition of dental diseases. The flow rate, buffering capacity, and resting Ph are important protective factors in oral health (Bassoukou et al., 2009).

Examination of oral hygiene and dental treatment can be difficult for children with autism due to their behavior. Saliva has been used as a mode of examination in this present study as it is a non-invasive method.

If any changes are found in the salivary flow rate, buffering capacity, and resting pH, then it can be used as a biomarker for the diagnosis of autism and to design an appropriate preventive treatment regimen for the same.

In the present study, it was reported that the unstimulated and stimulated salivary flow rate was lesser in 20% of the autistic children when compared to healthy children (p=0.04) This contrasts with the study done by Bassoukou et al. (2009) where the mean salivary flow rate between both the groups was not statistically significant(p=0.2004). Decreased saliva flow increases plaque accumulation on tooth surfaces and decreases carbohydrate clearance and this may be one of the reasons why autistic children have an increased caries prevalence.

The buffering capacity of saliva in autistic children was reported to have a statistically significant difference when compared to the healthy children where 10% of the autistic children had very low buffering capacity, 20% had a low buffering capacity and 70% of the autistic children

had normal buffering capacity as compared to 90% of the healthy children having the normal buffering capacity (p=0.0001). Testing the buffering capacity indicates the effectiveness of the saliva in neutralizing acids in the mouth, which may come from the diet, dental plaque, or from internal sources (such as gastric reflux). Bicarbonate is the most important buffering system in saliva. While unstimulated saliva has very low levels of bicarbonate, stimulated saliva has levels of bicarbonate more than 60 times higher. Bhandary and Hari, (2017) conducted a study where they found a statistically significant difference ($p<0.05$) in the buffering capacity between autistic children and their siblings which matches the findings of the current study.

The mean resting Ph in autistic children was lesser compared to healthy children (p=0.006) which indicates that the oral environment of autistic children was more acidic than healthy children which has a direct correlation with the dental caries experience in them. Diab, (2016) and Morales-Chávez MC et al. (2018) did not find a statistically significant difference (p=0.49) between the resting pH in both groups unlike the results obtained in our study.

The DMFT/dmft in permanent and primary dentitions in patients with autism is significantly higher than in healthy children, demonstrating that dental caries are more prevalent in patients with autism. These results were statistically significant and were concurrent to the results put forth by Piraneh et al., (2022), and Babu and Roy (2022) who found that autistic individuals were more prone to caries than normal individuals. The present study also showed that autistic children had a significantly greater D-component of the DMFT index when compared to healthy children, thus reiterating the fact that autistic children are in greater need of restorative treatment.

Considering the findings of this study, a comprehensive preventive educational intervention emphasizing healthy eating habits and teaching an easy, proper, and effective tooth brushing technique may be beneficial to improve oral hygiene and dental caries experience in children with an autism spectrum disorder.

6. Conclusion

In this study, autistic children had reduced salivary flow rate, resting Ph, and buffering capacity. The prevalence of dental caries was observed to be higher in autistic children as compared to healthy children. A positive correlation was observed between the salivary parameters and the prevalence of dental caries in autistic children. Children with autism prefer soft and sweetened foods and tend to pouch food inside the mouth for a long duration instead of swallowing it.

Added difficulties in brushing and flossing due to lack of motor coordination, leads to an increased propensity to caries for which a comprehensive, preventive educational approach needs to be implemented.

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