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ORIGINAL ARTICLE

Saliva flow rate, buffer capacity, and pH of autistic individuals

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Abstract The objective of the study was to evaluate saliva flow rate, buffer capacity, pH levels, and dental caries experience (DCE) in autistic individuals, comparing the results with a control group (CG). The study was performed on 25 noninstitutionalized autistic boys, divided in two groups. G1 composed of ten children, ages 3–8. G2 composed of 15 adolescents ages 9–13. The CG was composed of 25 healthy boys, randomly selected and also divided in two groups: CG3 composed of 14 children ages 4–8, and CG4 composed of 11 adolescents ages 9–14. Whole saliva was collected under slight suction, and pH and buffer capacity were determined using a digital pHmeter. Buffer capacity was measured by titration using 0.01 N HCl, and the flow rate expressed in ml/min, and the DCE was expressed by decayed, missing, and filled teeth (permanent dentition [DMFT] and primary dentition [dmft]). Data were plotted and submitted to nonparametric (Kruskal–Wallis) and parametric (Student's *t* test) statistical tests with a significance level less than 0.05. When comparing G1 and CG3, groups did not differ in flow rate, pH levels, buffer capacity, or DMFT. Groups G2 and CG4 differ significantly in pH (p=0.007) and pHi=7.0 (p=0.001), with lower scores for G2. In autistic individuals aged 3–8 and 9–13, medicated or not, there was no significant statistical difference in flow rate, pH, and buffer capacity. The comparison of DCE among autistic children and CG children with deciduous (dmft) and mixed/permanent decayed, missing, and filled teeth (DMFT) did not show statistical difference (p=0.743). Data suggest that autistic individuals have neither a higher flow rate nor a better buffer capacity. Similar DCE was observed in both groups studied.

Keywords Saliva · Flow rate · Buffer capacity · pH · Autistic disorder

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Introduction

Autism, also known as autistic disorder, childhood autism, infantile autism, and early infantile autism, is better known as pervasive developmental disorder. Under this condition, there is marked and sustained impairment in social interaction, deviance in communication, and restricted or stereotyped patterns of behaviors and interests. Abnormalities in functioning in each of theses areas are present by age 3 [10]. The reported prevalence is 5.8 to 14.9 cases per 10,000 live births [4], although recent reports have suggested that the prevalence of autism and related spectrum disorders are substantially higher than previously recognized being 38.9 per 10,000 [2]. There is a higher prevalence of autism in boys than in girls, with ratios reported averaging 3.5 or 4.0 boys to 1 girl [21]. When the disorder appears in a girl, it is

often associated with a more severe degree of mental retardation [28].

Medication is often used to minimize the behavioral symptoms that interfere in the individual's ability to participate in educational interventions [25].

Concerning the oral manifestations of autism, some authors have reported similar dental caries prevalence comparing to control groups (CGs) [6, 9, 29] with low caries activity [20], decreasing along time [26].

The flow rate and buffering capacity of saliva are important protective factors in oral health [8]. In saliva, there are three major systems contributing to the buffer capacity: bicarbonate, phosphate, and protein buffer system [32]. An inter-relationship between pH, buffer capacity, and flow rate has been reported [3].

Abnormalities in hypothalamic–pituitary–adrenal axis function were observed in some children with attention-deficit hyperactivity disorder, especially those exhibiting severe hyperactivity [16]. The abnormalities are related to the individual's stress. These individuals are extremely sensitive to outside stimulus. These factors can influence saliva flow rate and other dependent functions. In regard to the low dental caries experience reported in autistic individuals, the etiopatology investigation in to the role of salivary components in this illness is fundamental [20].

To our knowledge, there are no studies in the literature evaluating pH and buffer capacity of saliva from individuals with autism. Therefore, the aim of this study was to determine the flow rate, pH, and buffer capacity of whole saliva and the dental caries experience in individuals with autism, ages 3 to 13, compared to a matched CG.

Materials and methods

The Ethics Committee of the Universidade Cruzeiro do Sul, Brazil, approved this study (protocol number 005/05). After being informed of the objectives of the study, each parent or guardian provided a written consent for the individual to participate. This research was performed on 25 noninstitutionalized autistic boys, attending the "Associação de Amigos do Autista," AMA—São Paulo and "Centro Terapêutico Educacional" LUMI—São Paulo, reporting a medical diagnosis of autism. They were divided in two groups. The first group (G1) was composed of ten children ages 3–8, mean age (SD) 6.1 (1.5), six of which were regularly taking medication since diagnosis (methylphenidate, two; risperidone four). The second group (G2) was composed of 15 adolescents, ages 9–13, mean age (SD) 10.5 (1.2), from which 11 of which were regularly taking medication (risperidone, six; valproate, three; sertraline two). The CG was composed of 25 healthy boys, randomly selected, from individuals attending the Pediatric Dental Clinic at the Unicsul School of Dentistry, a private dental clinic, and individuals attending the Fé Cristã Church, all located in the city of São Paulo. They were also divided in two groups: The first group (CG3) had 14 children ages 4–8, mean age (SD) 6.8 (1.2), and the second group (CG4) had 11 adolescents, ages 9–14, mean age (SD) 10.8 (1.4). None of them had any systemic diseases and were not taking any medication for at least 15 days before saliva collection.

Prior to the collection of saliva, the individuals were conditioned by allowing them to touch materials to be used and by getting used to the sound of the suction pump. This was done to minimize nervousness or excitement.

Saliva samples were collected between 9:00 and 10:00 A.M. to minimize the circadian rhythms effects, 2 h after the last meal, after brushing, under slight suction through a soft plastic catheter. No stimulation was used, although the presence of the soft catheter might have provided a slight stimulation. The saliva collected during the first 10 s was discarded; saliva was then collected for 2 min, so that the flow rate could be calculated. After this period, the sampling continued until 3.5 ml saliva was collected. During the collection period, all individuals remained seated in a chair in a well-ventilated and well-lit room. If a child did not allow the saliva collection, he/she was excluded. Immediately after saliva collection, pH and buffer capacity were determined using a portable pHmeter (Digimed DU-2). The buffer capacity was measured by titration using 1 ml saliva, adding 0.2 ml of 0.01 N HCl. The process of adding 0.2 ml of 0.01 N HCl was repeated, and pH was recorded until a pH level of 4.0 or less was reached.

After saliva collection, all individuals were evaluated for caries experience. Teeth were dried and examined under an artificial light. The caries cavity diagnosis was performed according to standard procedures [33]. The number of decayed, missing, and filled teeth were recorded (dmft for primary dentition or DMFT for permanent dentition). For children with mixed dentition, dmft and DMFT were summed. No radiographs were taken.

For statistical analysis, data are presented as a mean ± SD. Student's t test was used to determine differences between the mean salivary scores of case groups and CGs; Kruskal–Wallis test was used for DMFT. The significance level was set at $p<0.05$.

Results

The data of this study were expressed in Tables 1, 2, 3, and 4. The buffer capacity of whole saliva was recorded in intervals of pH. The volume of acid added to the saliva was calculated for each considered interval.

Table 1 Mean (±SD) values for flow rate, pH, and volume of acid 0.01 N HCl used in pH ranges of whole saliva and DMFT from controls and autistic children aged 3–8 years

Variables		Controls (n=14)	Autistic (n=10)	p value
Flow rate (ml/min)		0.67±0.36	0.74±0.35	0.645[a]
pH		7.79±0.38	7.69±0.40	0.547[a]
Buffer capacity (ml acid/ml saliva)	pHi–7.0	0.64±0.40	0.45±0.26	0.202[a]
	pH 6.9–6.0	0.58±0.28	0.62±0.31	0.723[a]
	pH 5.9–5.0	0.29±0.11	0.38±0.20	0.149[a]
	pH 4.9–4.0	0.27±0.14	0.38±0.25	0.171[a]
DMFT		1.79±3.07	2.00±2.83	0.823[b]

[a] The data were compared by Student's t test
[b] The data were compared by Mann–Whitey tests

Table 1 did not show a significant difference in flow rate, pH, and buffer capacity of whole saliva or DMFT, when comparing CG to the autistic children group, ages 3–8.

Table 2 compared the results obtained from CG and the autistic children group, ages 9–13. The flow rate and DMFT did not show significant differences. The pH and buffer capacity of saliva in the range pHi–pH 7.0, were lower in autistic children compared to the CG.

Table 3 shows the results of the comparison among autistic children, ages 3–8, who regularly took the different medications prescribed to minimize the behavioral symptoms since diagnosis and those who did not take medication. No statistically significant difference was observed between flow rate (p=0.2004), pH (p=0.8314), and buffer capacity of saliva in the range pHi–pH 7.0 (p=0.1035), 6.9–6.0 (p=0.1544), 5.9–5.0 (p=0.6705), and 4.9–4.0 (p=0.8155). The same comparison was done with autistic children ages 9–13. The results showed no statistically significant difference for flow rate (p=0.5205), pH (p=0.7115), and buffer capacity of saliva in the range pHi–pH 7.0 (p=0.8484), 6.9–6.0 (p=0.5049), 5.9–5.0 (p=0.4081), and 4.9–4.0 (p=0.0792).

Table 4 showed the results of the caries experience comparison among autistic children and CG. No statistically significant difference was observed (p=0.743).

Discussion

In this study, we have examined some salivary components of autistic children compared to a CG.

Autistic disorder is represented by a pervasive developmental disorder, and nonspecific biological markers are known. The clinical picture of autism varies in severity and is modified by many factors including education, ability, and temperament [11]. Thus, we can observe individuals with the same medical diagnosis but who exhibit extremely diverse levels of functional independence represented by self-care, cognition, social interaction, and self-injurious behavior, pertinent to this condition [12].

The incidence of autism is about 14.9 in 10,000 live births [4]. This is less when compared to other syndromes or disorders, for instance Down syndrome, which is 1 in 600 [30].

The initial group was composed of 37 autistic boys. However, due to difficulties imposed by caregivers, children, or by the school, 12 children did not participate in the study.

The administration of multiple medications helped the affected individual to participate effectively in the educational and rehabilitative process. Medication was used by 17 of the 25 autistic individuals to ameliorate behavioral

Table 2 Mean (±SD) values for flow rate, pH, and volume of acid 0.01 N HCl used in pH ranges of whole saliva and DMFT from controls and autistic children aged 9–13 years

Variables		Controls (n=11)	Autistic (n=15)	p value
Flow rate (ml/min)		0.92±0.33	0.74±0.51	0.320[a]
pH		7.97±0.28	7.53±0.44	0.007[a]*
Buffer capacity (ml acid/ml saliva)	pHi–7.0	0.74±0.29	0.35±0.25	0.001[a]*
	pH 6.9–6.0	0.56±0.17	0.61±0.34	0.619[a]
	pH 5.9–5.0	0.28±0.11	0.35±0.14	0.180[a]
	pH 4.9–4.0	0.26±0.13	0.31±0.17	0.461[a]
DMFT		3.00±3,10	2.00±2.20	0.579[b]

*p<0.005
[a] The data were compared by Student's t test
[b] The data were compared by Mann–Whitey tests

Table 3 Mean (±SD) values for flow rate, pH, and volume of acid 0.01 N HCl used in pH ranges of whole saliva of autistic individuals aged 3–8 and 9–13 years using and not using medication

Variables		Autistic 3–8 using, n=6	Autistic 3–8 not, n=4	Autistic 9–13 using, n=11	Autistic 9–13 not, n=4
Flow rate (ml/min)		0.86±0.37	0.56±0.26	0.69±0.42	0.89±0.76
pH		7.67±0.36	7.73±0.51	7.50±0.51	7.60±0.15
Buffer capacity (ml acid/ml saliva)	pHi–7.0	0.56±0.22	0.28±0.24	0.34±0.24	0.37±0.33
	pH 6.9–6.0	0.74±0.22	0.45±0.37	0.65±0.34	0.51±0.38
	pH 5.9–5.0	0.40±0.13	0.35±0.30	0.37±0.13	0.30±0.17
	pH 4.9–4.0	0.37±0.12	0.41±0.40	0.35±0.17	0.18±0.07

The data were compared by Student's t test
*p<0.005

symptoms. The most frequently used drugs by the autistic children were risperidone (Risperdal), methylfenidate (Ritalin), and valproic acid (Depakene).

After conditioning, the individuals that did not allow saliva collection were excluded from the study. This factor was anticipated as autistic individuals react unexpectedly.

A negative correlation between the buffer capacity of saliva and dental caries has been reported in children with Down syndrome [1, 5, 15]. Several methods to determine the buffer capacity of saliva are available, including colorimetric and electrometric methods. To analyze the buffer capacity at different pH intervals, the titration method was used with 0.01 N HCl solution and monitoring the changes of pH at each acid addition. The present investigation used the Van Slyke formula, $\beta = \Delta Ca/\Delta pH$, where β is the buffer capacity, ΔCa denotes the amount equivalent per liter of acid added to the saliva at each pH interval, and ΔpH is the change in pH induced by the addition of acid. For practical purposes, we express the buffer capacity in volume (ml) of acid added to 1 ml of saliva in the pH range considered instead of equivalents of H^+.

Information on the effect of diseases upon the buffer capacity of saliva is absent on autistic individuals. In subjects with cystic fibrosis, the salivary buffer capacity was reported to be higher than in saliva from the CG [18], possibly due to higher phosphate levels [23]. In saliva from individuals with insulin-dependent diabetes, the buffer capacity was described to be higher than the control [19], which was not confirmed [32]. The buffer capacity of saliva from individuals with cerebral palsy determined by Dentobuff Strip kit showed no significant difference between cerebral palsy and control individuals [31]. Buffer capacity and flow rate are not hormone dependent in nonpregnant women [7], and the salivary flow rates and constituents did not practically change in patients who underwent coronary artery bypass graft surgery [27].

Previous research has suggested that children with autism may exhibit dysfunction of the hypothalamic–pituitary–adrenocortical system. Cortisol circadian rhythm, cortisol daily secretion, and its suppression response to dexamethasone had been measured from saliva or urine samples of the autistic children and their parents [24].

It must be pointed out that the saliva research literature on autistic individuals just refers to salivary cortisol levels to evaluate psychosocial stress [13, 14]. There has been no research regarding flow rate, buffer capacity, and pH. For this reason, this study is fundamental to understanding the importance of saliva and its flow-dependent components in maintaining oral health.

In this study, no difference was observed in flow rate, pH, and buffer capacity. The buffer capacity was determined using the titration method with 0.01 N HCl solution analyzing intervals of pH. Considering the interval from the initial pH to pH 7.0, we have found that both groups (control and study) ages 3–8 presented initial pH higher than pH 7.0. The volume of acid consumed in the intervals of pH 6.9–6.0, 5.9–5.0, and 4.9–4.0 differed, but significantly statistical differences were not found. The saliva parameters studied suggest that autistic disorder did not

Table 4 Mean (±SD) values for dental caries experience (DMFT and dmft) for study and control groups

	Group	dmft(n)	DMFT(n)	Mean (±SD) dmft	Mean (±SD) DMFT	p value
Dental caries experience	Study	3	22	1.67 (±2.89)	2.77 (±3.25)	0.743
	Control	4	21	1.75 (±2.87)	2.33 (±2.89)	

The data were compared by Kruskal–Wallis test
*p<0.005

interfere with either quantity or quality. Also observed in these results were similar dental caries experience in control or study groups, according to the results found by De Moor and Martens [6] and Fahlvik-Planefeldt and Herrström [9] in relation to the autistic disorder oral manifestations, which have reported similar dental caries experience when compared with CGs, with a low caries activity [20], decreasing with time [26]. In general, autistic individuals do not exhibit any specific dental findings and have a low caries rate [17, 22]. Shapira et al. [29] reported that patients with autism had severe periodontal problems but lower rates of caries than healthy persons. The low caries activity showed through this study were probably due to normal salivary parameters.

Conclusions

The present data suggest that autistic individuals demonstrate neither a higher flow rate nor a better buffer capacity. Similar dental caries experience were observed in both groups studied.

Conflict of interest declaration The authors declare that they have no conflict of interest, financial or otherwise.

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