

Salivary flow and salivary proteins in male and female children with autism spectrum disorder: pilot study

Fluxo salivar e proteínas salivares em crianças dos gênero masculino e feminino com transtorno do espectro do autismo: estudo piloto

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Resumo

Introdução: o transtorno do espectro autista é uma condição do neurodesenvolvimento que afeta o estabelecimento de vínculos e a comunicação. Cuidados odontológicos são mais difíceis em portadores desse transtorno, pois além da dificuldade de comunicação são comuns a não cooperação na higiene bucal e uso contínuo de medicamentos. Maior predisposição à cárie, alterações no fluxo e na concentração de proteínas salivares foram relatadas nesses indivíduos. **Objetivo:** considerando que o gênero pode afetar fluxo salivar e concentração proteica, nosso objetivo foi analisar esses parâmetros na saliva de crianças com autismo. **Material e método:** saliva total não estimulada foi obtida de 12 meninos e 12 meninas com idades entre 5 e 15 anos, com auxílio de um cateter, após 2 horas de jejum e higienização da cavidade bucal. O fluxo salivar foi determinado estimando-se a massa de saliva e o total de proteínas foi determinado no sobrenadante obtido após centrifugação a 10.000 x g, por 10 minutos, pelo método de Lowry, com albumina bovina como padrão. Os resultados foram expressos como média e desvio padrão, sendo submetidos aos testes de Shapiro-Wilk e Mann Whitney, com nível de significância em 5%. **Resultado:** os valores de fluxo salivar não foram significativamente diferentes quando comparados meninos (0.3555 ± 0.24 ml/min) e meninas (0.2522 ± 0.1727 mL/min), bem como os valores de proteínas (meninos: 1.430 ± 0.7480 mg/mL; meninas $1,075 \pm 0,3702$ mg/mL). **Conclusão:** em crianças com transtorno do espectro autista o gênero não influencia os valores de fluxo não estimulado e proteínas salivares.

Descritores: Transtorno do espectro autista; gênero; saliva; proteínas salivares.

Abstract

Introduction: autism spectrum disorder is a neurodevelopmental condition that affects the establishment of bonds and communication. Dental care is more difficult for people with this disorder, because in addition to communication difficulties, non-cooperation with respect to oral hygiene and continuous use of medication are common. Greater predisposition to caries, as well as alterations in the flow and concentration of salivary proteins were reported in these individuals. **Objective:** considering that sex can affect salivary flow and protein concentration, our objective was to analyze these parameters in the saliva of children with autism. **Material and method:** total unstimulated saliva was obtained from 12 boys and 12 girls aged between 5 and 15 years, with the aid of a catheter, after 2 hours of fasting and oral hygiene. Salivary flow was determined by estimating the mass of saliva. Total protein was determined in the supernatant obtained after centrifugation at 10,000 x g, for 10 minutes, by the Lowry method, with bovine albumin as standard. The results are expressed as mean and standard deviation. The data were submitted



to the Shapiro-Wilk and Mann Whitney tests, with a significance level of 5%. **Result:** salivary flow values for boys (0.3555 ± 0.24 ml/min) and girls (0.2522 ± 0.1727 ml/min), and protein values for boys (1.430 ± 0.7480 mg/mL) and girls (1.075 ± 0.3702 mg/mL) were not significantly different between sexes. **Conclusion:** in children with autism spectrum disorder, sex does not influence unstimulated flow and salivary protein values.

Descriptors: Autism spectrum disorder; gender; saliva; salivary proteins.

INTRODUCTION

Autism Spectrum Disorder (ASD) is a neurodevelopmental condition that affects 1 in 36 children, with boys having a 4-fold higher risk of its development compared to girls. Children with this disorder show deficits in social interaction, communication, and repetitive/restricted behaviors. Depression, anxiety, Tourette's syndrome, tic and bipolar disorders, and schizophrenia are frequently seen in ASD¹. Oral health and dental care of children with autism can be complicated by these behaviors, as well as by eating habits, chewing difficulties, continuous use of medication, poor oral hygiene, and damaging oral habits, such as bruxism, tongue thrusting, picking at the gingiva, and lip biting². Furthermore, these children have difficulties in tooth brushing³ and communicating oral health needs⁴.

Saliva is a fluid composed of the secretion of parotid, submandibular, sublingual, and tubal gland pairs, in addition to the secretions of hundreds of minor salivary glands⁵. The volume of saliva produced as a function of time, in minutes, is called salivary flow, which is controlled by the central and autonomic nervous systems⁶. A reduced salivary flow increases the accumulation of plaque on the surface of the teeth and reduces the rate of carbohydrate clearance in the mouth, compromising oral health⁷. Macromolecules are part of the composition of saliva, including proteins of different types, which can perform multiple, redundant, and even ambiguous functions. These proteins are involved in the processes of cleaning the mouth, aggregation and/or binding of microorganisms, plaque metabolism, and control of mineralization, among other functions⁸. An increase in the total concentration of salivary proteins has been associated with the presence of caries⁹.

Neurological alterations, sex, and age are some of the factors that can alter salivary flow and composition, thus compromising oral health¹⁰. People with ASD have a greater predisposition to caries, and alterations in total concentrations of proteins in saliva, with no apparent change in flow^{7,11-15}, however, the influence of sex on these salivary parameters needs to be further investigated. Therefore, the objective of this pilot study was to compare the unstimulated flow and the concentration of total proteins in the saliva of male and female subjects with ASD, aged between 5 and 15 years. Our hypothesis was that differences would be observed between the sexes.

MATERIAL AND METHOD

Ethics committee and sample selection

This preliminary cross-sectional observational study is part of a larger project entitled Evaluation of the oral condition of patients with autism spectrum disorder, approved by the Research Ethics Committee of the Araçatuba School of Dentistry FOA-UNESP (CAAE: 24597019.5.0000.5420).

We invited 12 female and 12 male patients with ASD from the Dental Assistance Center for People with Disabilities (CAOE) of the São Paulo University (UNESP), School of Dentistry, Araçatuba, aged between 5 and 15 years, to participate in the study. The exclusion criteria were non-collaborating patients, the need for sedation, unstable health, carriers of blood disorders, edentulous, and patients supplemented with vitamins in the two months prior to the saliva collection¹⁶.

Saliva collection

Saliva was collected by aspiration between 8:00 and 11:00 am, on a pre-scheduled date for the patient follow-up appointment at the CAO. Two hours prior to collection, the patient was requested to avoid food intake, and toothpaste or rinsing solutions. Mouth cleaning was performed two hours before the procedure using water and a brush. During sample collection, patients remained seated in a room with a pleasant temperature and light while the fluid was aspirated from the mouth floor into a sterile, sealed bottle in an ice bath, with the aid of a suction device, for a maximum of 10 minutes. Saliva was centrifuged at 10,000 x g (Centrifuge 5810 R, Eppendorf, Hamburg, Germany) at 4°C for 10 min to remove cells and reduce turbidity. The supernatant was fractionated and stored at -80°C until protein analysis¹⁶.

Saliva analysis

The collection time was recorded to calculate the salivary flow rate, expressed in mL/min. Total protein was measured using the Lowry method¹⁷.

Statistical analysis

Data are expressed as group means with standard deviation and GraphPad Prism (version 8.0) software was used. Shapiro-Wilk and Mann Whitney tests were applied, and the level of significance was 5%.

RESULTS

Participants

This study included 24 children with ASD. 12 girls and 12 boys, with average ages of 9.08 ± 2.84 and 9.17 ± 2.98 respectively. The use of drug associations was frequent among the patients, 50% in girls and 58% in boys, and anticonvulsants were the most frequent drug class recorded 31.7%¹⁶.

Saliva analysis

There were no significant differences ($p = 0.1978$) in unstimulated salivary flow values (boys: 0.3555 ± 0.24 ; girls: 0.2522 ± 0.1727). Protein values also did not differ significantly ($p = 0.3254$) when comparing the group of boys (1.430 ± 0.7480) with the group of girls (1.075 ± 0.3702), as can be seen in Figure 1.

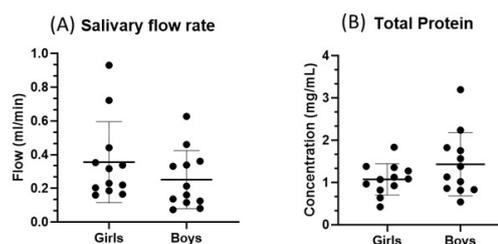


Figure 1. Salivary flow rate (A) and total protein (B) on unstimulated saliva of the girls and boys with autism spectrum disorders.

DISCUSSION

In this pilot study we concluded that there is not influence of sex on salivary flow rate and total protein in children with ASD in the age group studied. Therefore, our hypothesis was not confirmed.

ASD compromises social interaction and communication, making general and oral health care difficult¹¹. Children with ASD present a higher prevalence of caries than those without the syndrome, in addition to worse gingival health, conditions often resulting from difficulties in brushing their teeth, the impossibility of communicating about discomforts and oral alterations, inadequate habits, such as bruxism, preference for certain types and textures of food, and consumption of medicines²⁻⁴. In recent decades, there has been an increase in professional skills for diagnosing ASD cases, which is performed at increasingly early ages¹⁸. However, further access to specialized dental treatment is needed for these individuals, which depends on knowledge not only of alterations in oral structures and professional approaches, but also on a broad knowledge of the salivary secretion and composition of these individuals.

Normal salivary flow is important for the maintenance of oral health⁷. When produced in the presence of physical or chemical stimuli, salivary flow is called stimulated, while in the absence of external interference and with the individual conscious, it is called basal, resting, or unstimulated, being controlled by minor reflex activities of the central nervous system. Unstimulated saliva is produced by the submandibular, sublingual, parotid, and minor glands and is influenced by the circadian rhythm⁶. Females have less unstimulated salivary flow compared to males due to a smaller size of the major salivary glands¹⁹⁻²², however, this difference was not observed in the current study in children with ASD. Other studies carried out with ASD patients did not find differences between salivary flow when comparing children with or without the syndrome^{7,13,14}, however, analyses were not performed considering the sex of the children.

Protein secretion in saliva is influenced by circadian rhythm, hormonal changes, stress, and reduced protein intake, among other factors²³. The influence of circadian rhythm was eliminated from this work, since all saliva samples were obtained between 8 and 11 am. In addition to these factors, gender can influence the protein concentration in saliva. Studies in the literature indicate that the secretion of these components in unstimulated saliva is influenced by the size of the salivary glands, which would result in a difference between sexes, since these glands are smaller in women²⁴. In the unstimulated saliva of ASD carriers, a higher concentration of proteins was described²⁵, however, the authors did not perform a subdivision of donors according to sex. In this pilot study, the number of participants was reduced, 12 in each group, and it was not possible to consider the reproductive maturity of the girls, which we consider a limitation of the study and which may justify the fact that we did not observe a difference in the concentration of proteins between the sexes.

CONCLUSION

We conclude that in children with ASD in the age group of 5 – 15 years, there are no differences in the salivary flow and in the total concentration of proteins in relation to sex.

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CONFLICTS OF INTERESTS

The authors declare no conflicts of interest.

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