Oral Hygiene and Oral Microbiota in Children with Neurological Impairment and Oropharyngeal Dysphagia: A Study

Luiz Fernando Fregatto  
Universidade de Marília

Isabela Bazzo Costa  
Universidade de Marília

Daniel De Bortoli Teixeira  
Universidade de Marília

Janaina Costa Marangon Duarte  
Universidade de Marília

Aline Maria Noli Mascarín  
Universidade de Marília

Salum Bueno da Silveira Junior  
Universidade de Marília

Bianca Eduarda Baptistella Mesquita Serva  
Universidade de Marília

Roberta Gonçalves da Silva  
São Paulo State University

Francisco Agostinho Junior  
Universidade de Marília

Paula Cristina Cola (✉ paccola@hotmail.com)  
Universidade de Marília

Research Article

Keywords: Oral Hygiene, Bacteria, Deglutition Disorder, Neurological Disorder

DOI: https://doi.org/10.21203/rs.3.rs-689999/v1

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Abstract

Objective

This study compared the oral hygiene and oral microbiota in children with neurological impairment and oropharyngeal dysphagia with and without gastrostomy. Method: Forty children and young people participated in this study: 19 females and 21 males, aged 2 to 22 years (mean age 8.6 years). Participants were divided into two groups: group I (GI = 20) with gastrostomy and group II (GII = 20) without gastrostomy (with oral feeding). Oral hygiene was assessed using the Simplified Oral Hygiene Index (SOHI). Analysis of two bacteria, *Streptococcus mutans* and *Streptococcus sobrinus*, was performed by collecting saliva using an oral swab, then mRNA expression was evaluated using the polymerase chain reaction (PCR) technique.

Results

The oral hygiene index had a general median of 2.2, and the two groups were statistically different (Group I: median 2.9 and Group II: median 2.0) (p = 0.01751). Bacterial analysis indicated 13 individuals with *S. mutans* and none with *S. sobrinus*. Of the 13 individuals with *S. mutans*, 6 were from Group I and 7 from Group II.

Conclusions

Those with gastrostomy had worse oral hygiene, and both groups harbored the bacterium *Streptococcus mutans*.

Introduction

Children and young people with neurological impairment have a high prevalence of oropharyngeal dysphagia that can reach 99%, depending on the assessment method and the population studied. A study involving children diagnosed with severe cerebral palsy and cognitive impairment found a 99% prevalence of dysphagia. In another study, the authors found a prevalence of dysphagia in 83% of children with cerebral palsy. Both studies used clinical evaluation methods. Another study, using a clinical and instrumental method to evaluate children with quadriplastic cerebral palsy, found changes in the oral and pharyngeal phases of swallowing, with rates that reached up to 93.7% of research participants. Children and young people with neurological impairment also have a greater predisposition to develop bacterial plaque due to the difficulty of maintaining oral hygiene themselves or to inability on the part of those responsible for monitoring this care. In addition, dysphagia itself is a factor that contributes to the formation of dental plaques or stones, since it has a close relationship with oral hygiene, both in children and young people with gastrostomy, as well as those with oral feeding.
There are few studies in the literature that analyze oral hygiene in children with neurological impairment using an alternative route of food intake such as gastrostomy, and the available studies are not recent. So far, the evidence suggests that there is a greater decline in oral hygiene in children using gastrostomy; this can be explained by the inefficient oral motor control seen in severely compromised children, which has a negative influence on oral conditions, particularly oral hygiene. In another study, the authors examined the effect of food consumption on subgingival bacteria levels in children with gastrostomy and healthy children, finding that the two groups exhibited similar subgingival bacteria.

The presence of bacteria in the oral cavity has frequently been analyzed, but there are few studies involving children and young people with neurological impairment and the use of gastrostomy. It is clear that children with gastrostomy have significantly more plaques and calculus on their teeth, and more Haemophilus influenzae, with additional tendencies to more gram-negative, Pseudomonas, and Streptococcus pneumonia bacteria. For this reason, children with gastrostomy are more associated with aspiration pneumonia than children without gastrostomy.

Among the most studied bacteria in relation to the presence of plaque and dental calculus is Streptococcus mutans. In the population of children with neurological impairment and use of gastrostomy, one study compared children with and without the use of gastrostomy with healthy children, and the results pointed to a lower prevalence of S. mutans in the oral cavity of children using gastrostomy. A more recent study analyzed periodontal status in children with cerebral palsy; the authors concluded that the state of oral hygiene and the severity of periodontitis worsen as the stiffness and movement of muscle tone increases in individuals with cerebral palsy.

S. mutans has been identified as the main bacterium in the etiology of caries, and Streptococcus sobrinus has a cariogenic potential in humans. An altered state of oral health negatively affects general health and quality of life, contributing to increased rates of dental caries and periodontal disease. Difficulty in accessing dental care and lack of awareness of caregivers are real problems for the care of this population.

The hypothesis of this study is that children and young people with neurological impairment with oropharyngeal dysphagia and using alternative food (gastrostomy) present more changes in oral hygiene and oral microbiota than children and young adults with oral feeding.

Considering the scarcity of studies on children with neurological involvement and use of gastrostomy, this study aims to compare oral hygiene and oral microbiota in children and young people with neurological involvement and oropharyngeal dysphagia with and without the use of gastrostomy.

**Methods**

Forty children and young people participated in this study: 19 males and 21 females, aged 2 to 22 years old (mean age 8.6 years). All had neurologiacal impairment (cerebral palsy and genetic syndrome) and
neurogenic oropharyngeal dysphagia confirmed in their medical records. Participants were divided into two groups: Group I (GI, n = 20), composed of children and young people using gastrostomy, and Group II (GII, n = 20), composed of children and young people with oral feeding. Exclusion criteria were: children and young people with physical conditions that prevented the collection of clinical specimens, with an unstable general clinical picture, with the absence or incomplete eruption of dental elements number 11, 31, 16, 26, 36, and 46 (permanent dentition) or 51, 71, 55, 65, 75, and 85 (primary dentition) necessary for evaluation and continuous antibiotic use.

The research project was approved by the Research Ethics Committee of the institution under the number 4.391.196. All those responsible for the individuals included in the study protocol gave informed consent for participation. The selection of children and young people occurred according to their presence in outpatient care and previous analysis of medical records confirming the diagnosis of oropharyngeal dysphagia. They were invited to participate in the research and received explanations about the objectives and the method of collection. Such children and young people are followed up at the Specialties Clinic of Hospital XXX.

Assessment of oral hygiene and saliva collection were always performed by the same two professionals, one from the dentistry area and the other from the nursing area, both trained and experienced in the field. The child or young person was positioned in the Kavo dental chair, model Unik, or even in their own wheelchair, according to the positioning need. During evaluation and collection, sterile gloves, dental oral clinical mirror, dental explorer probe, disposable plastic sucker, triple syringe attached to the dental chair with an air and water jet, reflector light from the dental chair, and an oral swab were used.

**Simplified Oral Hygiene Index (SOHI)**

In order to qualify oral hygiene, quantification of plaque deposit and dental calculus was performed in the entire sample population involved. Evaluation was carried out by the same professional specializing in the field of pediatric dentistry, with seven years of experience in serving this population. For this, the Simplified Oral Hygiene Index (SOHI) proposed by Greene and Vermillion (1964) was applied, in which the existence of a plaque or calculus was verified on the buccal surface of the number 11 dental element (upper right central incisor), 31 (lower left central incisor), 16 (upper right first molar) or 26 (upper left first molar), or on the lingual surface of element 36 (lower left first molar) or 46 (lower right first molar). In the absence of one element, we replaced it with another from the same group; that is, we used the same dental arch and the same quadrant. Only fully erupted elements were considered. In deciduous or mixed dentition, the buccal surface of dental elements number 51 (upper right central incisor), 71 (lower left central incisor), 55 (upper right first molar), 65 (upper left first molar) was evaluated, as well as the lingual surface of elements 75 (lower left first molar) and 85 (lower right first molar).

**Saliva collection**

After cleaning the mouth with 100 mL of water, saliva was collected by scraping the inside of the cheeks with sterile swabs, making circular movements approximately 30 times. These swabs were cut and
placed in 2mL microtubes with gel inside. The collected samples were stored in a refrigerator at 4°C for a period of up to seven days before the extraction of genomic DNA.

**Bacterial analysis**

The saliva collected by oral swab allowed the investigation of two bacteria, Streptococcus mutans and Streptococcus sobrinus, through the expression of mRNA as measured by polymerase chain reaction (PCR). The analysis process is described below:

DNA was extracted with the commercial DNA isolation kit (Puregene, Gentra Systems, Minneapolis/EUA). In the microtubes containing the swabs, 300 µL of lysis solution was added. Then, 1.5 µL of proteinase K (20 mg/mL) and 100 µL of precipitation solution were added. Then, 300 µL of 100% isopropanol and 0.5 µL pf glycogen (20 mg/mL) were added, and the tubes were centrifuged at 1500 rpm for 3 min. The supernatant was discarded, and the tube was inverted onto absorbent paper. Then, 300 µL of 70% ethanol was used to wash the DNA. The tubes remained open 15 min for evaporation of residual ethanol, and the DNA was dissolved in 20 µL of DNA elution solution.

Extracted DNA samples were subjected to electrophoresis in 1.5% agarose gel in TBE (tris, boric acid and EDTA 0.001 M, pH 8.0) containing ethidium bromide at a concentration of 0.5 ug/mL of gel and observed in a Hoefer transluminator (model Macro-Vue UV-20) to check its integrity. The concentration of the DNA samples obtained were measured in a spectrophotometer (Ultraspex III, Pharmacia LKB Biochrom Ltd, Cambridge, England), at 260 nm. The 260/280 ratio equal to 1.8 was used to characterize the purity of the material. The samples were stored at 4 ºC until use.

In PCR, amplification of the constitutively expressed gene glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was carried out as an internal control for the reactions to confirm whether the DNA extraction process was successful. The Invitrogen protocol was adopted: in a sterile tube was added 2.5 µL of 10× PCR buffer, 0.75 µL of MgCl2 (50 mM), 0.5 µL of dNTP mix (10 mM), 1.0 µL of oligonucleotide primer forward (2 mM), 1.0 µl of oligonucleotide primer reverse (2 mM), 0.5 µL of DNA sample, 0.2 µL of Taq DNA polymerase (5 units/µL) and distilled and autoclaved water to complete the final volume of 25 µL.

The reaction was performed on the Perkin Elmer GeneAmp PCR System 2400 thermal cycler, under the conditions of 94°C for 45 s (denaturation), 60°C for 45 s (annealing), 70°C for 1 minute (extension), and 70°C for 15 min (final extension), for 25 cycles.

The FIREpol protocol was adopted for the amplification of S. mutans and S. sobrinus by PCR. In a sterile tube, 4 µL of 5× Master Mix, 0.6 µL of forward primer oligonucleotide (10 µM), 0.6 µL for reverse primer oligonucleotide (10 µM), 1.0 µL of the DNA sample and distilled, autoclaved water was added to give the final volume of 20 µL. This reaction was also performed on the Perkin Elmer GeneAmp PCR System 2400 thermal cycler, under conditions of 95°C for 30 s (denaturation), 59°C for 30 s (annealing), 72°C for 1 min (extension) and 72°C for 5 min (final extension).
The sequences of the oligonucleotide primers for the amplification of GAPDH and specific bacteria are shown in Table 1.

**Table 1.** Sequences of oligonucleotide primers used for the amplification of GAPDH and bacteria Streptococcus mutans and Streptococcus sobrinus.

| Target gene | Sequence | Fragment |
|-------------|----------|----------|
| GAPDH       | F 5' TGT TCC AGT ATG ATT CCA CC 3' | 850 bp |
|             | R 5' TCC ACC ACC CTG TTG CTG 3'   |         |
| S. mutans   | F 5' ACT ACA CTT TCG GGT GGC TTG G 3' | 517 bp |
|             | R 5' CAG TAT AAG CGC CAG TTT CAT C 3' |         |
| S. sobrinus | F 5' GAT AAC TAC CTG ACA GCT GAC T 3' | 712 bp |
|             | R 5' AAG CTG CCT TAA GGT AAT CAC T C 3' |         |

The amplified DNA samples were subjected to electrophoresis on 1.5% agarose gel in TBE containing ethidium bromide at a concentration of 1.0 µg/mL of gel and observed in a Hoefer transilluminator model Macro-Vue UV-20 to verify the expression of the analyzed genes.

**Statistical analysis**

The data were previously analyzed for normality using the Kolmogorov-Smirnov test at 5% probability. Once the presence of the normal distribution of the analyzed variables was not confirmed, it was decided to use non-parametric statistics. Thus, the Mann-Whitney test was applied to compare groups in relation to oral hygiene, with the median and interquartile range (IQR) values being presented together for each group. For the analysis of bacteria, the chi-square test was used. For both tests, the level of 5% probability was used. All statistical analyses were conducted using the R software package.

**Results**

**Table 2** - Values of medians and interquartile range (IQR - first and third quartiles) of the oral hygiene index in relation to groups I and II and the total number of children and young people.

| GROUPS   | MEDIAN | IQR       |
|----------|--------|-----------|
| GI (n=20)| 2.9    | 2.0 – 4.15|
| GII (n=20)| 2.0   | 1.53 – 2.53|
| TOTAL (n=40)| 2.2 | 1.6 – 3.08|
Table 2 shows that both groups had an oral hygiene index with values considered inadequate. In the comparison between GI and GII, it was observed that children and young people with gastrostomy had a more altered oral hygiene index, with a statistically significant difference in relation to GII (p = 0.01751).

Table 3 indicates the presence of two bacteria, S. mutans and S. sobrinus, in the oral cavity of 37 children and young people (data from three patients was not considered due to technical error in the collection of the material). The presence of S. mutans can be observed in 13 (35.2%) children and young people, six (31.6%) GI and seven (38.9%) GII, without statistically significant difference (p = 0.9037) between the two groups. S. sobrinus was not found in any of the members of either group.

Conclusion
The results of this study allow us to conclude that children and young people with neurological impairment and oropharyngeal dysphagia using gastrostomy had worse oral hygiene, and that both groups had the bacterium Streptococcus mutans in the oral cavity.

Declarations

Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

IRB approval

The study was approved by the Research Ethics Committee of the institution under the number 4.391.196. November 04, 2019. A non-opposed consent was obtained.

Consent for publication

A non-opposed consent was obtained for all participants.

Informed consent

Informed consent was obtained from all those responsible for the individuals participants included in the study.

Author Contributions: The responsibility of LFF was to conceptualization, methodology, investigation and writing. The responsibility of IBC was to methodology, investigation and writing. The responsibility of DDBT was to formal analysis. The responsibility of JCMD was to methodology. The responsibility of AMNM was to validation. The responsibility of SBSJ was to validation. The responsibility of BEBMS was to validation. The responsibility of FAJ was to investigation, writing and project administration. The responsibility of PCC was to conceptualization, investigation, writing, supervision and project administration.

Funding: This research received no external funding.

Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Institutional Review Board (or Ethics Committee) of Marilia University (protocol code 4.391.196).

Conflicts of Interest: All of the authors declare that they have no conflict of interest.

Author agreement: All authors have read and agreed to the published version of the manuscript.
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