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Research Paper

Evaluation of fecal microorganisms of children with cleft palate before and after palatoplasty

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Abstract

This study isolated and quantified intestinal bacteria of children with cleft palate before and after palatoplasty. A prospective study was conducted from May 2007 to September 2008 on 18 children with cleft palate, aged one to four years, of both genders, attending a tertiary cleft center in Brazil for palatoplasty, to analyze the effect of surgical palate repair on the concentration of anaerobes Bacteroides sp, Bifidobacterium sp and microaerophiles Lactobacillus sp in feces of infants with cleft palate before and 24 hours after treatment with cefazolin for palatoplasty. There was significant reduction of Lactobacillus sp ($p < 0.002$), Bacteroides sp ($p < 0.001$) and Bifidobacterium sp ($p = 0.021$) after palatoplasty, revealing that surgery and utilization of cefazolin significantly influenced the fecal microbiota comparing collections before and after surgery. However, due to study limitations, it was not possible to conclude that other isolated factors, such as surgical stress, anesthetics and other medications used in palatoplasty might have a significant influence on the microbiota. Considering the important participation of the intestinal microbiota on both local and systemic metabolic and immunological activities of the host, professionals should be attentive to the possible influence of these changes in patients submitted to cleft repair.

Key words: cleft palate, cleft palate/surgery, environmental microbiology, feces, surgery/plastic.

Introduction

Since birth, the gastrointestinal tract is colonized by many microorganisms ingested through the food and contact with the environment (Monreal *et al.*, 2005; Norin *et al.*, 1985; Simon and Gorbach, 1984, 1986; Sullivan *et al.*, 2001). These microorganisms constitute an ecologically dynamic community (microbiota) and play metabolic activities both in local (intestinal mucosa) and systemic levels (Hattori and Taylor, 2009; Kurokawa *et al.*, 2007; Mai and Draganov, 2009; Mitsou *et al.*, 2008; Mueller *et al.*, 2006). Anaerobes and microaerophiles predominate in the colon, with nearly 10 organisms per gram of feces (Haenel, 1970). Growth inhibition of harmful microorganisms, improvement in digestion, absorption of essential nutrients and vitamin synthesis, stimulation of the immune system and secretion of anti-inflammatory cytokines are examples of the protective effects of the intestinal microbiota, especially the Bifidobacterium sp and Lactobacillus sp. Even though the intestinal microbiota is relatively stable throughout life, factors as diseases and some drugs may affect this balance, especially during the use of antimicrobial drugs(Haenel, 1970; Monreal *et al.*, 2005). Cleft lip and/or palate are the most frequent craniofacial anomalies in human beings. In general, a cleft palate complicates the breastfeeding and impair the clinical and nutritional development of the child. The cleft palate also promotes communication between the oral and nasal cavities, further impairing the child's feeding, with reflux of food through the nose or aspiration, which also causes respiratory infections and otitis. For

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these patients, surgery is the main point of treatment. Prophylactic antibiotic therapy in surgery is indicated for patients without evidence of established infection to prevent the occurrence of systemic infection or of the surgical wound (Piltcher and Scarton, 2005). At the Hospital for Rehabilitation of Craniofacial Anomalies of the University of São Paulo, the Hospital Infection Control Committee of the Hospital for Rehabilitation of Craniofacial Anomalies of University of São Paulo advocates the use of a single dose of cefazolin upon anesthetic induction. This beta-lactam antibiotic belongs to the group of first-generation cephalosporins because they are active against Gram-positive and Gram-negative bacteria. The Lactobacillus sp are usually sensitive to penicillin G, ampicillin, cephalosporins and clindamycin. Considering the importance of the intestinal microbiota for human health and the lack of specific studies on children with cleft palate, this study analyzed the effect of surgical palate repair on the concentration of anaerobes Bacteroides sp, Bifidobacterium sp and microaerophiles Lactobacillus sp in feces of infants with cleft palate before and 24 hours after treatment with cefazolin for palatoplasty.

Materials and Methods

This study was revised and approved by the Institutional Review Board of HRAC/USP and was conducted in accordance with the World Medical Association Declaration of Helsinki.

A prospective study was conducted in the period from May 2007 to September 2008, involving 18 children with cleft palate, aged one to four years, of both genders, attending a tertiary cleft center in Brazil for surgical palate repair (palatoplasty).

The intestinal anaerobes of genders Bacteroides sp, Bifidobacterium sp and microaerophiles Lactobacillus sp were isolated from feces of children with cleft palate (CP) and quantified in two moments, before palatoplasty without use of antibiotic drugs in the past 30 days (M1), and 24 hours after treatment with cefazolin for palatoplasty (M2). Children aged more than four years or who had used antimicrobial drugs in the past 30 days were excluded from the sample.

All children were clinically evaluated by medical professionals at the institution. The children's parents or caretakers were interviewed by a single professional in individual rooms, for collection of data regarding gender, age, place of origin and history of pathologies. The parents or caretakers received information on the study, signed an informed consent form and received two sterile flasks for feces collection, being the first before surgical treatment and the second 24 hours after prophylactic treatment with cefazolin for palatoplasty. After collection, the feces were immediately sent to the Clinical Pathology Laboratory of the institution, where the intestinal microbiota of the samples was analyzed by the same professional conducting the interviews.

All children received prophylactic treatment with cefazolin before palatoplasty, which was prescribed by the surgeons. Cefazolin was used in a single dose of 25 mg/kg of body weight, by intravenous administration during anesthetic induction.

For microbiological evaluation of the fecal microbiota, 1 g of feces was weighed and added to 9 mL of phosphate buffer pH 7.2, achieving an initial dilution of 10^{-1}. After successive dilutions up to 10^{-9}, each dilution was plated in triplicate on selective media Kanamycin-vancomycin blood Agar, Bifidobacterium medium and Lactobacillus selective medium, which were incubated at 37 ºC/48 hours in an anaerobic environment. The colony forming units (cfu) present in the greatest dilution were analyzed and observe concerning the morphology of colonies, catalase test and Gram staining for the bacteriological analysis. The concentration of microorganisms was calculated in Log_{10} cfu/g of feces.

For the counting of Bacteroides sp, Bifidobacterium sp and Lactobacillus sp, medians were calculated with logarithmic transformation of data. Comparison between moments M1 and M2 was performed by the non-parametric Wilcoxon signed-rank and Mann-Whitney tests. In all analyses, the statistics calculated were considered significant when $p \leq 0.05$ (p is the probability of erroneous conclusion by the significance).

Results

Among the 18 children analyzed, there was homogeneous distribution of males (44.4%) and females (55.6%). The median age was 1.15 years, ranging from 1.05 to 2.22 in the entire group.

There was statistically significant reduction in the number of CFUs of Bacteroides sp ($p = 0.006$), Lactobacillus sp ($p = 0.000$) and Bifidobacterium sp ($p = 0.026$) in children treated with cefazolin for palatoplasty (Table 1).

Discussion

The occurrence of orofacial clefts, as well as their influence on infant feeding and the frequent utilization of antimicrobial drugs contribute to changes in the intestinal microbiota.

The study sample included children with cleft palate aged one to four years, because at the institution where the study was conducted palatoplasty is only indicated after 12 months of age.

The use of antimicrobial drugs is an important factor associated to changes in the intestinal microbiota. Several studies have demonstrated that antimicrobial therapies are able to induce fast and important changes in the intestinal microbiota (Monreal *et al.*, 2005). The prophylactic antibiotic therapy with cefazolin, indicated in palate surgeries at

Table 1 - Median of logarithmic counting of Lactobacillus sp, Bacteroides sp and Bifidobacterium sp in the feces of 18 children with cleft palate at the moments M1 and M2. Bauru-SP, 2009.

Moments	Fecal microbiota	M1 (Log10/g feces)	M2 (Log10/g feces)	Statistics (Wilcoxon)
Lactobacillus sp		6	4	p = 0.001
Bacteroides sp		9	8	p = 0.006
Bifidobacterium sp		7	6	p = 0.026

M1 = Moment before treatment with cefazolin in palatoplasty and without use of antimicrobial drugs in the past 30 days.
M2 = Moment 24 hours after treatment with cefazolin in palatoplasty.

the institution, aims at preventing infection of the surgical wound and/or systemic infection, which eventually may occur. Chuo *et al.* (2005) analyzed the ear, nose and throat microbiota of children with cleft lip and palate aged 1 to 26 months and concluded that they presented significant risk of infection by *Staphylococcus aureus* in primary lip and/or palate surgeries. A recent study conducted by Cocco *et al.* (2010) evidenced the need to conduct bacteriological examinations preoperatively to identify pathogenic microorganisms. Fernandes *et al.* (2009) compared the efficacy of intravenous cefazolin used prophylactically in patients with mandibular fractures in the anesthetic induction or for 24 hours postoperatively and did not find difference between groups. Antibiotics should be carefully prescribed because severe complications may occur, such as toxic reactions, stimulation of antibody production, high financial cost, laxity of a good surgical technique and alterations in the intestinal microbiota. According to Wise *et al.* (1998), it is estimated that 20 to 50% of antibiotics prescribed for humans are unnecessary.

This study revealed significant reduction in the quantity of microorganisms of genders Bifidobacterium sp, Lactobacillus sp and Bacteroides sp 24 hours after prophylactic treatment with cefazolin in palatoplasty. The reduction of these microorganisms may cause several harmful effects for the host, especially reduction in the resistance to colonization by pathogenic microorganisms. Once identified, these may be minimized by the health team by the adoption of measures related to therapeutic and diet management, aiming to reduce the harmful influence on the gastrointestinal microbiota of the patient.

Considering the importance of the intestinal microbiota for the host health, it was demonstrated that the utilization of cefazolin considerably influenced the ecological balance of microorganisms when the moments before and after surgery were compared. However, due to the limitations of this study, it was not possible to conclude that other isolated factors, such as surgical stress, anesthetics and other medications used in the palatoplasty had a significant influence on the microbiota. Therefore, professionals should be attentive to the possible influence of these changes in patients with cleft palate submitted to surgical cleft repair. Since the intestinal microbiota reaches stability between the first and second years of life, further studies should be conducted to better elucidate the complex interactions with the host, especially in individuals with craniofacial malformations.

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