Archives of Oral Biology 60 (2015) 1650–1654

Contents lists available at ScienceDirect

Archives of Oral Biology

journal homepage: www.elsevier.com/locate/aob

Salivary flow rate and biochemical composition analysis in stimulated whole saliva of children with cystic fibrosis

Karine Barros da Silva Modesto[a], Jéssica Bueno de Godói Simões[a], Amanda Ferreira de Souza[a], Neiva Damaceno[b], Danilo Antonio Duarte[a], Mariana Ferreira Leite[a,*], Eliete Rodrigues de Almeida[a]

[a] Department of Pediatric Dentistry at the University Cruzeiro do Sul, São Paulo, SP 08060-070, Brazil
[b] Department of Pediatrics/Pulmonology, School of Medical Sciences of Santa Casa de São Paulo, SP 01221-020, Brazil

ARTICLE INFO

Article history:
Received 6 April 2015
Received in revised form 3 August 2015
Accepted 10 August 2015

Keywords:
Cystic fibrosis
Saliva
Buffer capacity
Total protein
Sialic acid
α-Amylase
Salivary peroxidase

ABSTRACT

Objective: It is recognized that cystic fibrosis (CF) patients present a risk for oral diseases, since it affects exocrine glands, and the treatment consists of a carbohydrate-rich diet. Recognizing the protective function of saliva on maintaining oral health, the aim of the study was to evaluate salivary parameters in stimulated whole saliva from children with CF.
Methods: A case-control study was conducted comparing stimulated whole saliva of healthy ($n = 28$; control group) and CF children ($n = 21$; experimental group). Salivary flow rate, initial pH, buffer capacity (total and in each range of pH), total protein and sialic acid (total, free, and conjugated) concentration, α-amylase and salivary peroxidase activities were evaluated. Data were compared by two-tailed Student *t* test (95% CI; $p \leq 0.05$).
Results: CF patients presented a significant reduction in salivary parameters compared with the control group ($p \leq 0.05$): salivary flow rate (36%), buffer capacity (pH range from 6.9 to 6.0), sialic acid concentration (total 75%, free 61%, and conjugated 83%); α-amylase and salivary peroxidase activities (55%). Additionally, a significant increase in total protein concentration (180%) of stimulated whole saliva from CF patients was verified compared with the control group ($p \leq 0.05$).
Conclusion: Children with CF presented significant changes in salivary composition, including salivary flow rate, buffering capacity and protective proteins of the oral cavity, compared with children without CF.

1. Introduction

Cystic fibrosis (CF) is a life-limiting autosomal recessive disorder caused by a variant in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene. The degree of CFTR dysfunction correlates with clinical features and can create phenotypes other than cystic fibrosis (Cutting, 2015). Individuals with cystic fibrosis show a high degree of variability in disease severity, complications and survival (Dodge, Lewis, Stanton, & Wilsher, 2007). The organs in which clinical abnormalities have been documented most in CF are the airways, pancreas and exocrine glands (Grody, 1999). A defective electrolyte transport in epithelial cells and viscous mucus secretions from glands and epithelia characterize this disorder, considered as a disease of fluid transport in epithelia that normally require passive, electroconductive transport of Cl^{-1} (Quinton, 1990).

Patients with cystic fibrosis can present a risk for oral diseases, since the treatment consists of a carbohydrate-rich diet, use of sugary drugs, mucolytics, and more frequent meals and food supplements. On the other hand, Narang, Maguire, Nunn and Bush, (2003) argued that the use of long term antibiotics and pancreatic enzymes may grant some protection against the development and progression of dental caries in these patients. Kinirons (1983) observed a reduction in dental caries prevalence among CF patients, suggesting that there was a link between altered saliva properties and low caries experience, but he reported no relation between the changes in saliva and the severity of the disease process. Enamel defects, particularly enamel opacities, which can be disfiguring, are more common in CF patients, but the occurrence of opacities may also be related to other conditions, such as

* Corresponding author at: Pediatric Dentistry Ph.D. Program – CBS – Universidade Cruzeiro do Sul. Av. Ussiel Cirilo, 225—São Miguel Paulista, São Paulo, SP CEP 08060-070, Brazil.
E-mail address: mariana.leite@cruzeirodosul.edu.br (M.F. Leite).

http://dx.doi.org/10.1016/j.archoralbio.2015.08.007
0003-9969/

prematurity, low birth weight, and ingestion of fluoridated toothpaste (Azevedo, Feijó, & Bezerra, 2006).

These identified risk factors, added to the lack of studies dealing with the impact of cystic fibrosis on children's oral health, suggested the evaluation of certain biochemical parameters of stimulated whole saliva that present protective properties. This is most frequently studied substance for evaluating systemic disorders, as it is considered a noninvasive method that can be collected from individuals with modest training, using simple equipment, and hence provides a cost-effective approach for population screening (Deepa & Thirrunavukkarasu, 2010).

The saliva of CF affected patients contains increased levels of calcium and proteins, resulting in the formation of insoluble calcium-protein complexes that could compromise the function of salivary enzymes (Blomfield, Rush, Allars, & Brown, 1976), as well as increases in the salivary concentrations of sodium, phosphate, chloride, lipid and PGE2 (Rigas, Korenberg, Merrill, & Levine, 1989). A study showed changes in inorganic parameters of whole saliva from patients with CF, particularly increased chlorine, potassium and sodium, and reduced salivary flow rate (Gonçalves et al., 2013). However, there is a lack of reports concerning organic salivary parameters with protective, antioxidant, antimicrobial and digestive properties.

Previous studies on salivary aspects of patients with cystic fibrosis focused the inorganic composition of saliva (Blomfield et al., 1976; Gonçalves et al., 2013; Rigas et al., 1989). There is a lack of studies showing also organic aspects of saliva with different protective functions in the oral cavity. So, this study was planned with the aim to evaluate salivary parameters in stimulated whole saliva of children with cystic fibrosis (case) compared with healthy children (control), including salivary flow rate, buffer capacity, sialic acid concentration, α-amylase and salivary peroxidase activities, and total protein concentration.

2. Material and methods

2.1. Participants

This case-control study was conducted among CF children from the Public Hospital 'Santa Casa de Misericórdia de São Paulo', SP, Brazil (case group). All the children in attendance were invited to participate of the study (convenience sample). Considering ethics and exclusion criterias, final sample comprised 21CF children. Control group was matched by sociodemographics characteristics, with 28 children enrolled at a Public School, "Colégio Arnoso Costa', located in a neighborhood near to the hospital.

The study was approved by the Research Ethics Committee of Cruzeiro do Sul University, under protocol no. 205-442. All the participants were examined only after the consent form was read and signed by their parents. Patients were referred for treatment at the university, whenever necessary. The exclusion criteria used was the existence of diseases or medication use (only in control group) that could interfere with the findings of the study.

The diagnosis of cystic fibrosis was detected by examination of the equivalent NaCl molarity in sweat samples (Siryani et al., 2015). The pulmonary function (FEV1—forced expiratory volume in FEV1-1 s) were performed in patients aged more than 7 years ($n = 18$) and the values for percentage of predicted FEV1 were calculated ($n = 18$).

2.2. Saliva collection

At least 2 h after their last meal, mechanically stimulated whole saliva was collected by chewing Parafilm® between 2 and 4 p.m., to minimize the effects of circadian rhythm. Saliva produced in the first 10 s was discarded and the subsequent saliva was collected for exactly 5 min in a graduated tube to calculate the initial flow rate (mL/min). The graduated tube was kept on ice for saliva collection. During the collection period, all the children remained comfortably seated in a ventilated and illuminated room. The collected saliva was transported in a container with ice and stored at −80° until the laboratory analyses (Feres de Melo, Ferreira de Souza, de Oliveira Perestrelo, & Leite, 2014).

2.3. Salivary evaluation

Immediately after saliva collection, both initial pH and buffer capacity were determined using a portable pH meter (Ultrabasic, Denver Instrument, USA). The buffer capacity of the whole saliva was determined by titration using 1 mL of saliva and the addition of 0.2 mL of 0.001N HCl solution. The process of adding acid was repeated and the pH recorded after each addition up to a pH value of 5.5 to assist in the interpretation of the results of pH changes. The buffer capacity of whole saliva was analyzed using pH intervals as described: initial pH −7.0; followed by pH of 6.9–6.0 and then pH of 5.9–5.5. For practical purposes, buffer capacity was expressed as the volume (mL) of acid added to 1 mL of saliva (Feres de Melo et al., 2014).

Salivary total protein concentration was estimated by the Bradford method (Bradford, 1976), using bovine serum albumin as standard (Pearson's correlation coefficient: r2 = 0.9977. The colorimetric Bradford method is based on the binding of proteins present in the supernatant to the Coomassie Blue reagent (1 mL of solution: 0.01% Coomassie Blue R-250, 8.5% phosphoric acid, 4.7% ethanol).

Amylase activity was determined by the method described by Fisher and Stein (1961), using maltose as standard (Pearson's correlation coefficient: r2 = 0.9883). Samples were incubated with 1% starch solution in 20 mM phosphate buffer, pH value of 7.0 for 5 min at 30 °C. The reaction was interrupted by the addition of an alkaline solution of dinitrosalicylic acid and the mixture was maintained in boiling water for 5 min. After diluting the mixture with distilled

water, the development of color absorbance was determined at 530 nm with a spectrophotometer (Beckman DU-68, USA). One unit of enzyme activity (U) corresponded to the amount of enzyme that produced 1 μmol of the product per min.

Peroxidase activity was examined by the Chandra et al. method, modified by Anderson (1986), using lactoperoxidase as standard (Pearson's correlation coefficient: r2 = 0.9653). Interference of pseudoperoxidase activity was eliminated by performing duplicate assays in the presence of 10 mM 3-amino-1,2-triazole, an inhibitor of peroxidase activity. Peroxidase activity was measured in a medium containing 8 mM phosphate buffer, pH value of 6.0, 1 mM *O*-dianisidine and the saliva sample. The reaction was initiated by adding hydrogen peroxide to a final concentration of 0.02 mM. The development of color absorbance was determined at 460 nm in a spectrophotometer (Beckman DU-68, USA).

Free and total sialic acid concentrations were measured using the thiobarbituric acid assay, as described by Skoza and Mohos (1976), using *N*-acetylneuraminic acid solution as standard (Pearson's correlation coefficient: r2 = 0.9945). Briefly, total sialic acid concentration was determined after hydrolysis in 0.1 N sulfuric acid at 80 °C for 1 h. The samples were then treated with periodate reagent (25 mM periodic acid in 0.125 N sulfuric acid) and incubated at 37 °C for 30 min. The reaction was terminated by adding sodium arsenite (2% sodium arsenite in 0.22 mM hydrochloric acid). After the iodine color disappeared, a solution of thiobarbituric acid (0.1 M, pH 9.0) was added. The solution was heated in boiling water for 7.5 min and then cooled. Dimethyl sulfoxide was added and the intensity of the development of color was measured at 549 nm in a Spectrophotometer (Beckman DU-68, USA). The levels of free sialic acid in the samples were