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Increased activity of the antioxidants systems modulate the oxidative stress in saliva of toddlers with early childhood caries

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ABSTRACT

Objective: This study aimed to evaluate the oxidative stress levels and the enzymatic and non-enzymatic antioxidant systems in saliva of toddlers with severe early childhood caries (S-ECC).
Design: Unstimulated saliva samples were collected at the morning from 0 to 3 year-old S-ECC (n = 30) or caries-free (CF) children (n = 30/group) for evaluation of oxidative stress (OS) and total antioxidant capacity (TAC), which were measured by the ferric reducing antioxidant power (FRAP) assay, as well as to assess the activity of enzymatic (superoxide dismutase, SOD) and non-enzymatic (uric acid, UA) antioxidant systems, respectively. Data were analyzed by Student's *t*-test ($p < 0.05$).
Results: Significantly higher protein levels were observed in saliva of S-ECC children (0.083 mg/mL) than in the CF group (0.070 mg/mL). Oxidative damage was significantly lower in saliva of S-ECC children (0.0019 μmol/L/mg protein) than in CF children (0.0039 μmol/L/mg protein), while salivary TAC (61.5 μmol/L), SOD activity (36.6 UE/mL) and uric acid (7.05 mg/mL) were significantly higher in saliva of S-ECC when compared to the CF group (49.1 μmol/L, 26.8 UE/mL and 5.02 mg/mL, respectively for TAC, SOD and UA).
Conclusion: Oxidative stress levels were significantly lower in saliva of S-ECC children, what might be associated with the increased activity of salivary enzymatic (SOD) and non-enzymatic (uric acid) antioxidant systems.

1. Introduction

Severe early childhood caries (S-ECC) is one of the most common oral diseases in children. S-ECC is defined by the European Academy of Pediatric Dentistry as the presence of one or more primary decayed tooth (cavitated lesions or not), missing (due to caries) or restored tooth surfaces before 71 months of age (Vadiakas, 2008), while the American Academy of Pediatric Dentistry defines as severe S-ECC as any sign of smooth-surface caries in children younger than three years of age (American Academy of Pediatric Dentistry, 2014). High prevalence rates of S-ECC have been reported in developed and in developing countries, ranging from 46 to 96% in 3–7-year-old children, reaching levels up to 17% in 0–3-year-old children from low-income communities or where the access to dental services is difficult by political, economic and social factors(Grund, Goddon, Schüler, Lehmann, & Heinrich-Weltzien, 2015; Warren et al., 2016).

Biomarkers of oxidative damage are found in saliva as 8-hydroxy-desoxguanosine (8-Hodgkins) and malondialdehyde (MDA). Enzymatic antioxidants – glutathione peroxidase (GPx) and superoxide dismutase (SOD) – can be measured in saliva and other biological fluids, similarly as for the non-enzymatic antioxidant systems, which include uric acid (UA) and glutathione (GSH) and comprise the non-enzymatic total antioxidant capacity (TAC) (Battino, Ferreiro, Gallardo, Newman, & Bullon, 2002). Oxidative

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stress is attributed to an imbalance between free radical production, as reactive oxygen species (ROS), and the activity of enzymatic and non-enzymatic antioxidant systems, which are a powerful defense of body against damages caused by free radicals (Kirschvink, De Moffarts, & Lekeux, 2008; Tunez et al., 2007).

Increased oxidative damage biomarkers have been observed in saliva of individuals presenting periodontal disease or dental caries (Battino et al., 2002; Tóthová, Celecová, & Celec, 2003). Conversely, TAC of stimulated saliva has been shown to be significantly decreased in patients with periodontal disease (Diab-Ladki, Pellat, & Chahine, 2003), and in saliva of patients with peri-implant desease (Liskmann et al., 2007). However, unlike what would be expected, the levels of TAC were significantly higher in saliva of children, adolescents and adults presenting carious lesions in comparison with caries-free subjects (Ahmadi-Motamayel, Goodarzi, Hendi, Kasraei, & Moghimbeigi, 2013; Hegde, Rai, & Padmanabhan, 2009; Kumar, Pandey, & Agrawal, 2011; Mahjoup, Ghasempour, Gharage, Bijani, & Masrourroudsari, 2014; Hegde, Hegde, Ashok, & Shetty, 2013). These results indicate that the relationship between oxidative stress and the antioxidant systems in saliva of individuals with caries activity is not fully understood. In fact, the association between TAC and oxidative damage has not yet been evaluated in S-ECC children. In addition, no data is available on the role of non-enzymatic antioxidant system, such as uric acid, in saliva of patients with caries. Finally, and most importantly, while studies have reported isolated data on the effects of oxidative damage and TAC in S-ECC children, these aspects have not been collectively evaluated in the same group of children, so that the relationship between these variables could not be determined.

Therefore, this study aimed to evaluate the oxidative stress levels and the enzymatic (SOD) and non-enzymatic (uric acid) antioxidant systems in saliva of children in early childhood (0–3 years old) presenting S-ECC. The study's hypothesis was that oxidative stress levels and enzymatic and non-enzymatic antioxidant systems would be increased in saliva of children with caries lesions.

2. Materials and methods

2.1. Patient selection

The research protocol was approved by the Human Ethics Committee of Araçatuba Dental School, UNESP- Univ. Estadual Paulista (Permission Number CAAE 36416414.5.0000.542). Children at the age range of 0–3 years were selected from public kindergartens in the city of Araçatuba, State of São Paulo, Brazil. An initial meeting was conducted with directors of kindergartens and parents of the children to explain in details the study protocol and to answer to possible questions raised by the attendants. Following, free and informed consent forms were distributed to all parents/caregivers, and those who were unsigned entered as exclusion criteria, along with children presenting systemic diseases. From the signed informed consent forms returned to the researchers, clinical examinations were performed in 100 children, for the determination of dmfs index based on World Health Organization recommendation (WHO, 1997), performed by a calibrated dentist (PVS). Subsequently, sixty children were randomly enrolled in the study (blocking stratification), comprising 30 subjects with severe early childhood caries (S-ECC group) and 30 caries-free (CF group) (Vadiakas, 2008).

2.2. Saliva collection

To minimize possible variation due to circadian rhythm, unstimulated whole saliva was collected between 7:00 am to 8:30 am, 2 h after fasting and oral hygiene with water and toothbrush without fluoride products. All salivary samples were collected by the same investigator within kindergartens during 5 min, using a Salivette® (Sarstedt, Germany). Samples were kept on ice during collection and then were centrifuged at 5500*g* for 10 min as previously described (Cunha-Correia, Neto, Pereira, Aguiar, & Nakamune, 2014). The supernatants were fractionated and kept at −80 °C until analysis.

2.3. Determination of total protein concentration

Protein was measured by the method of Lowry, Rosebrough, Farr, and Randall (1951) with bovine serum albumin used as standard. The absorbance was determined at 660 nm. The results were expressed in mg/mL.

2.4. Measurement of malondialdehyde (MDA)

MDA is one of the products of lipid peroxidation evaluated by the method thiobarbituric acid-reactive substances (TBARS), which has been considered a biomarker of oxidative stress. MDA was determined as described by Buege and Aust (Buege and Aust, 1978). Trichloroacetic acid (10% w/v) was added to the saliva samples (125 μL) to precipitate proteins and to acidify the reaction solution. This mixture was then centrifuged (1000*g*, 3 min) and thiobarbituric acid (TBARS, 0.67% w/v) was added to the reaction medium. The sample was placed in a water bath (100 °C, 15 min). The absorbance was read at 535 nm, the molar absorption coefficient used was $1.56 \times 105\,M^{-1}\,cm^{-1}$. The results are expressed μmol/L/mg protein.

2.5. Salivary total antioxidant capacity

Salivary total antioxidant capacity was assessed by ferric reducing antioxidant power (FRAP) assay (Benzie & Strain, 1996). This method is based on reducing the ferric complex tripiridil triazine (Fe^{3+} TPTZ) to form Fe^{2+} in acidic medium. An aliquot of saliva (15 μL) was used and the absorbance was determined at 595 nm, using a standard curve of ferrous sulfate. The results are expressed in μmol/L $FeSO_4$.

2.6. Superoxide dismutase (SOD) activity

SOD activity was determined in saliva by the method of Maklund (1985) based on the inhibition of the pyrogallol autoxidation, using an aliquot of saliva (20 μL) previously diluted in tris (1:10 v/v). Absorbance was detected at 420 nm. The amount of enzyme required to inhibit 50% of the autoxidation of pyrogallol was considered as a unit of enzyme activity. Results are expressed as UE/mL.

2.7. Uric acid

Uric acid was determined in saliva using a commercial kit (Labtest Diadgnóstica SA, MG, Brazil) based on enzymatic Trinder method, following the manufacturer's instructions. The results are expressed in mg/ml.

2.8. Statistical analysis

Data are expressed as mean ± SD (Standard Deviation). Statistical analysis of the results was performed using independent Student *t*-test (Graph Pad Prism, 5.0 version). Values of $p < 0.05$ were considered as statistically significant.