Priscila Vieira da Silva

Aumento da atividade dos sistemas antioxidantes modula o estresse oxidativo na saliva de crianças com cárie precoce severa

Araçatuba – SP

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Dissertação apresentada à Faculdade de Odontologia da Universidade Estadual Paulista "Júlio de Mesquita Filho", Campus de Araçatuba, para obtenção do título de Mestre em Ciência Odontológica, área de concentração Saúde Bucal da Criança.

Orientadora: Profa. Dra. Cristina Antoniali Silva

Coorientadora: Profa. Dra Ana Cláudia M. S. Nakamune

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Dedicatória

Dedico este trabalho

A **Deus**,

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Agradecimentos

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Epígrafe

Mas, como está escrito: As coisas que o olho não viu, e o ouvido não ouviu, e não subiram ao coração do homem, são as que Deus preparou para os que o amam.

1 Coríntios 2:9

Resumo Geral

SILVA, P.V. **Aumento da atividade dos sistemas antioxidantes modula o estresse oxidativo na saliva de crianças com cárie precoce severa.** Dissertação (Mestrado) – Faculdade de Odontologia, Universidade Estadual Paulista, Araçatuba, 2016.

Resumo Geral

O estresse oxidativo é atribuído a um desequilíbrio entre a ação de sistemas antioxidantes com a produção exacerbada de radicais livres, como espécies reativas de oxigênio. A atividade dos sistemas antioxidantes enzimáticos e não enzimáticos, são uma poderosa defesa do corpo contra danos causados pelos radicais livres. Biomarcadores do estresse oxidativo podem ser observados na saliva de adultos e crianças. O objetivo deste trabalho foi avaliar os níveis de estresse oxidativo e a atividade de sistema antioxidante enzimático, como a superóxido dismutase (SOD) e não-enzimático como o ácido úrico (AU) na saliva de crianças na primeira infância (0-3 anos de idade) que apresentaram cárie precoce severa da infância (S-ECC do inglês, severe early childhood caries). Amostras de saliva não estimuladas foram coletadas pela manhã, durante 5 minutos, usando o Salivette® em crianças de 0-3 anos de idade, com cárie precoce severa na infância (n = 30) e em crianças livres de cárie (n = 30) de escolas públicas de Araçatuba – SP. Foram feitas as avaliações de estresse oxidativo (EO), pela medida da peroxidação lipídica, da capacidade antioxidante total (CAT), pelo método FRAP, bem como de sistema antioxidante enzimático, avaliando a atividade da SOD e não enzimático pela avaliação do UA, salivares. Os dados foram analisados por programa estatístico Graph Pad Prism, versão 5.0 e comparados pelo teste t de Student ($p < 0,05$). Níveis de proteína elevados foram observados na saliva de crianças S-ECC quando comparados ao grupo livre de cárie. O dano oxidativo foi menor na saliva de S-ECC, enquanto a CAT salivar, atividade da SOD e ácido úrico salivares foram mais elevados em S-ECC quando comparados ao grupo livre de cárie. Nosso estudo demonstrou que o menor dano oxidativo observado na saliva de S-ECC estaria associado ao aumento da atividade de sistemas antioxidantes enzimático e não enzimático.

Palavras-chave: cárie dentária, criança, saliva, estresse oxidativo, superóxido dismutase, ácido úrico.

General Abstract

SILVA, P.V. **Increased activity of antioxidant systems modulates oxidative stress in saliva of toddlers with severe early childhood caries.** Dissertação (Mestrado) – Faculdade de Odontologia, Universidade Estadual Paulista, Araçatuba, 2016.

Oxidative stress is attributed to an imbalance between the antioxidant systems activity and the increased production of free radicals such as reactive oxygen species. The activity of enzymatic and non-enzymatic antioxidant systems are a powerful defense of the body against damage caused by free radicals. Oxidative stress biomarkers can be observed in the saliva of adults and children. The objective of this study was to evaluate the levels of oxidative stress and antioxidant enzyme system activity, such as superoxide dismutase (SOD) and non-enzymatic as uric acid (UA) in the saliva of toddlers (0-3 years old) with severe early childhood caries (S-ECC). Unstimulated saliva samples were collected in the morning during 5 minutes using Salivette® in S-ECC children (n = 30) and in caries-free children (n = 30) of public schools in Araçatuba - SP. We evaluated the salivary protein level by Lowry method, and the oxidative stress (OS) by lipid peroxidation. The total antioxidant capacity (TAC) was analyzed by FRAP method. The activity of salivary SOD and salivary UA were assessed as enzymatic and non-enzymatic antioxidant systems, respectively. Data were analyzed by statistical program Graphpad Prism version 5.0 and the results were compared between groups by Student's t test ($p < 0.05$). High protein levels were observed in the saliva of S-ECC children when compared to caries-free group. Oxidative damage was lower in S-ECC group, while the salivary TAC, SOD activity and salivary UA were higher in S-ECC when compared to the caries-free group. This study demonstrated that decreased oxidative damage was associated with the increased activities of the enzymatic and non-enzymatic antioxidant systems in S-ECC saliva.

Keywords: dental caries, children, saliva, oxidative stress, superoxide dismutase, uric acid.

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Listas de abreviatura

S-ECC	"Severe Early Childhood Caries", cárie precoce severa da infância
$FeSO_4$	Sulfato ferroso
FRAP	"Ferric reducing antioxidant power", poder antioxidante férrico reduzido
GPx	Glutationa peroxidase
L	Litro
MDA	Malonaldeído
mL	Mililitro
mg	Miligrama
OS	"Oxidative stress", estresse oxidativo
PVS	Priscila Vieira da Silva
ROS	"Reactive oxygen species", espécies reativas de oxigênio
SEM	"Stardart error mean", erro padrão da média
SOD	Superóxido Dismutase
TAC	"Total antioxidante capacity", capacidade antioxidante total
TBARS	"Thiobarbituric acid reactive substances", substâncias reativas ao ácido tiobarbitúrico
UA	"Uric acid", ácido úrico
UE	Unidade enzimática
µL	Microlitro
µmol/L	Micromol por litro

Sumário

Sumário

The increased activity of the antioxidants systems modulates 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 children with severe early childhood caries (S-ECC).

Design: Unstimulated saliva samples were collected at the morning in 0-3 year-old children with early childhood caries (n=30) or caries-free children (n=30) for evaluation of oxidative stress (OS) and total antioxidant capacity (TAC), which were measured by FRAP method, 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 ECC children than in the caries-free group. Oxidative damage was significantly lower in saliva of S-ECC children, while salivary TAC, SOD activity and uric acid were significantly higher in saliva of S-ECC when compared to the caries-free group.

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.

Keywords: dental caries, children, saliva, oxidative stress, superoxide dismutase, uric acid.

Highlights

- High protein levels are observed in saliva of S-ECC children.
- Oxidative stress is decreased in saliva of S-ECC children.
- Total antioxidant capacity of the saliva is increased in S-ECC group.
- Enzymatic (SOD) antioxidant systems is increased in saliva of S-ECC children.
- Uric Acid, a non-enzymatic antioxidant system, is also increased in saliva of S-ECC.

Introduction

Severe early childhood caries (S-ECC) is one of the most common oral diseases in children. S-ECC is defined by the American and European Academies 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[1,-4]. High prevalence rates of S-ECC have been reported in developed and in developing countries, ranging from 46 to 96% in 3-7 year olds, reaching levels up to 17% in 0-3 years old children from low-income communities or where the access to dental services is difficult by political, economic and social factors[5-7].

Oxidative stress (OS) biomarkers are found in saliva as 8-hydroxy-desoxguanosine (8-Hodgkins), malondialdehyde (MDA), uric acid (UA), total antioxidant capacity (TAC), glutathione peroxidase (GPx), and superoxide dismutase (SOD)[8]. Oxidative 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[9,10].

Increased OS biomarkers have been observed in saliva of individuals presenting periodontal disease or dental caries[8,11]. Conversely, TAC of stimulated saliva has been shown to be significantly decreased in patients with periodontal disease[12], and in saliva of patients with peri-implant desease[13]. 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[14-18]. 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 saliva could have a significant role in modulating oxidative damages caused by dental caries in these subjects.

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). Sixty children were enrolled in the study, comprising 30 subjects with early childhood caries (ECC group) and 30 caries-free (Caries-free group) from public kindergartens in the city of Araçatuba, State of São Paulo, Brazil, at the age range of 0-3 years. Free and informed consent forms were distributed to all parents/caregivers, and those who were unsigned entered as exclusion criteria along with those with systemic diseases. Clinical examination and determination of dmfs index based on World Health Organization recommendation[19] were performed by a calibrated dentist (PVS).

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 minutes, using a Salivette® (Sarstedt, Germany). Samples were kept on ice during collection and then were centrifuged at 5500 x *g* for 10 minutes as previously described[20]. The supernatants were fractionated and kept at -80 ºC until analysis.

2.3 Determination of Total Protein Concentration

Protein was measured by the method of Lowry[21] with bovine serum albumin used as standard. The absorbance was determined at 660nm. The results were expressed in mg/mL.

2.4 Salivary Total antioxidant capacity

Salivary total antioxidant capacity was assessed according to Benzie and Strain[22], 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 $FeSO_4$.

2.5 Measurement of malondialdehyde (MDA)

MDA was determined as described by Buege and Aust[23]. Trichloroacetic acid (10% w / v) was added to the saliva samples (125µL) to precipitate proteins and to acidify the reaction solution. This mixture was then centrifuged (1000 x *g*, 3 min) and the thiobarbituric acid (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 x 105 M^{-1} cm^{-1}. The results are expressed µmol/L/mg protein.

2.6 Superoxide dismutase (SOD) activity

SOD activity was determined in saliva by the method of Maklund[24], 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 ± SEM (standard error of mean). 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.

3. Results

Sixty children aged 0-3 years participated in this study, twenty-nine boys and thirty one girls. Within the S-ECC group (*n*=30), 47% and 53% were boys and girls, respectively, while the corresponding percentages for the caries-free group (*n*=30) were 53% and 47%, respectively. Prevalence of cavitated carious lesions in the S-ECC group was 3.7 ± 0.63.

3.1. Salivary total protein

The salivary total protein was significantly higher ($p < 0.01$) in the S-ECC group (0.083 ± 0.003 mg/mL) when compared to caries-free group (0.070 ± 0.002 mg/mL) (Fig. 1).

3.2. Salivary MDA levels

Salivary MDA levels were significantly lower ($p < 0.0001$) in the S-ECC group (0.0019 ± 0.0001 µmol/L/mg protein) than in caries-free group (0.0039 ± 0.0003 µmol/L/mg protein) (Fig. 2).

3.3. TAC in saliva evaluation

TAC levels in saliva were significantly higher ($p < 0.05$) in S-ECC group (61.5 ± 3.7 µmol/L) compared to the caries-free group (49.1 ± 4.5 µmol/L) (Fig.3).

3.4. Enzymatic salivary antioxidant activity

SOD activity (36.6 ± 4.5 UE/mL) was significantly higher in S-ECC ($p < 0.05$) when compared to the caries-free group (26.8 ± 1.7 UE/mL) (Fig. 4).

3.5. Non-enzymatic salivary antioxidant activity

Salivary uric acid values were significantly increased ($p < 0.0001$) in S-ECC group (7.05 ± 0.25 mg/mL) than in the caries-free group (5.02 ± 0.29 mg/mL) (Fig. 5).

4. Discussion

In this study, increased enzymatic and non-enzymatic antioxidant systems were shown to control the oxidative damage in saliva of S-ECC children. Therefore, the study's hypothesis was accepted. Since no study had assessed oxidative stress and antioxidant system salivary profile in S-ECC children, the present findings bring novel information to the field.

The concentration of salivary total protein was higher in S-ECC children than in caries-free group. These results are in line with previous data from the literature demonstrating increased concentration of total protein in saliva of children and adults diagnosed with pneumonia[25,] cystic fibrosis[26], and in children, 3 to 5 years old, with severe caries[15].

It is possible that the higher protein concentration in saliva of S-ECC children would be a protective and/or an adaptive response of the body against dental caries, since salivary proteins have a protective role of oral tissues by forming a film of protein against enamel wear, preventing the adherence and growth of microorganisms, promoting the remineralization of enamel by attracting calcium ions, and by reducing enamel demineralization in association with salivary calcium and phosphate ions[27].

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. Reduced levels of TBARS were observed in saliva of S-ECC children when compared to caries-free children, suggesting reduced oxidative damage in the saliva of children presenting caries lesions. These results are in agreement with a previous work conducted with caries-active adults (17-50 years old)[28,29], and patients (16-46 years old) with periodontal disease and gingivitis[30,31]. However, variable levels of salivary OS of adults have been reported when associated to oral infectious diseases. No difference in salivary MDA levels[28] was observed, or higher lipid peroxidation was detected in saliva of adults with active caries than in caries-free group[32]. Moreover, the increased OS, which was observed in saliva of patients with oral lichen planus (25-75 years old), squamous cell carcinoma (34-82 years old) or periodontal disease, was associated with a reduced salivary TAC[33-35.]

Salivary TAC in S-ECC group was significantly higher than in caries-free group in the present study, in line with previous reports in children (3-5 years old) from North Iran[17]. In addition, a strong correlation between the number of decayed teeth and increased salivary TAC in children (3-5 years old) of India has been reported[16]. Together, these results corroborated the suggestion that high salivary TAC is a biomarker of early childhood caries[36] and active caries in adults (20 and 30 years old)[15], and adolescents (15- 17 years old)[14]. As recently suggested, salivary markers of oxidative stress will be used for screening and monitoring oral diseases, including dental as caries. The evaluation of these markers in saliva could be an adjuvant toll for identification of patients with poor adherence to dental visits. Furthermore, the study of such biomarkers could bring useful information for the development of novel therapies for the prevention and/or treatment of dental caries.

Based on the considerations above, we assessed whether TAC would involve the action of antioxidants systems in saliva of children presenting S-ECC, by evaluating the concentration of uric acid, which has been assessed in saliva of patients as a non-enzymatic antioxidant biomarker. It has been suggested that the increase in uric acid levels in saliva may influence TAC levels[16] since uric acid is responsible for around than 70% of salivary

antioxidant capacity[37]. While uric acid was assessed in saliva of patients with cystic fibrosis [26], chronic periodontitis[38] and in patients with cleft lip[39], to the best of our knowledge uric acid had not been previously assessed in saliva of S-ECC children. In the present study, significantly higher uric acid antioxidant response was observed in saliva of S-ECC group, which contributed to the increase in salivary TAC levels of these children.

A significant increase in SOD activity in saliva of the ECC group was observed in the present study, which might also have contributed to the lower oxidative damage in these children. The evaluation of saliva of caries active adults (25-50 years) also demonstrated increased levels of SOD and its cofactors, copper and zinc[40]. Previous studies observed higher SOD activity in patients with cleft lip and with cystic fibrosis[26]. SOD activity, which has been considered as the first line of enzymatic antioxidant defense in the organism, catalyzes the dismutation of superoxide ion (an oxygen free radical) into oxygen and hydrogen peroxide[41].

Since saliva can be used for long-term monitoring oral diseases and considering the lack of conclusive studies about oxidative stress in saliva of caries active subjects[42], our results suggest a salivary profile of S-ECC children: increased salivary proteins, reduced oxidative damage, increased TAC or FRAP and uric acid, and increased SOD activity. The reasons for the increased non-enzymatic or enzymatic antioxidant status in saliva of caries-active subjects remain unclear. Given that their function is to protect oral tissues against the deleterious effects of endogenous or exogenous oxygen or nitrogen reactive species, our results suggest that the increased UA and SOD activity might be a compensatory mechanism to reduce oxidative damage.

Based on the results of the present study, we can conclude that oxidative damage is reduced in saliva of S-ECC children as a consequence of the increased non-enzymatic and enzymatic antioxidant systems.

Acknowledgments

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References

1. Misra, S., Tahmassebi, J., & Brosman, M. (2007). Early childhood caries - a review. *Dental Update*, 34, 556-558.

2. Nunn, M. E., Dietrich, T., Singh, H. K., Henshaw, M. M., & Kressin, N. R. (2009). Prevalence of early childhood caries among very young urban Boston children compared with US children. *Journal of Public Health Dentistry*, 69, 156.

3. Losso, E. M., Tavares, M. C. R., Silva, J. Y. B., & Urban, C. A. (2009). Severe early childhood caries: an integral approach. *Jornal de Pediatria*, 295-300.

4. Vadiakas, G. (2008). Case definition, aetiology and risk assessment of early childhood caries (ECC): a revisited review. *European Archives of Paediatric Dentristry*, 9, 114-125.

5. Grund, K., Goddon, I., Schüler, I. M., Lehmann, T., & Heinrich-Weltzien, R. H. (2015). Clinical consequences of untreated dental caries in German 5- and 8-year-olds. *BioMedCentral Oral Health*, 15, 140.

6. Moimaz, S. A., Borges, H. C., Saliba, O., Garbin, C. A., & Saliba, N. A. (2015). Early Childhood Caries: Epidemiology, Severity and Sociobehavioural Determinants. *Oral Health & Preventive Dentistry*, doi 10.3290. [Epub ahead of print].

7. Warren, J. J., Blanchette, D., Dawson, D. V., Marshall, T. A., Phipps, K. R., Starr, D., & Drake, D. R. (2015). Factors associated with dental caries in a group of American Indian children at age 36 months. *Community Dentistry an Oral Epidemiology,* doi 10.1111 [Epub ahead of print].

8. Battino, M., Ferreiro, M. S., Gallardo, I., Newman, H. N., & Bullon, P. (2002). The antioxidant capacity of saliva. *Journal of Clinical Periodontology*, 29:189–194.

9. Kirschvink, N., De Moffarts, B., & Lekeux, P. (2008). The oxidant/antioxidant equilibrium in horses. *Veterinary Journal*, 177, 178–191.

10. Tunez, I., Feijoo, M., Huerta, G., Montilla, P., Munoz, E., Ruiz, A., & Collantes E. (2007). The effect of infliximab on oxidative stress in chronic inflammatory joint disease. *Current Medical Research and Opinion*, 23, 1259– 1267.

11. Tóthová, L., Celecová, V., & Celec, P. (2003). Salivary markers of oxidative stress and their relation to periodontal and dental status in children. *Disease Markers*, 34, 9–15.

12. Diab-Ladki, R., Pellat, B., & Chahine, R. (2003). Deacrease in the antioxidant activity of saliva in patients with periodontal disease. *Clinical Oral Investigations*, 7, 103-107.

13. Liskmann, S., Vihalemm, T., Salum, O., Zilmer, K., Fischer, K., & Zilmer, M. (2007). Characterization of the antioxidant profile of human saliva in peri-implant health and disease. *Clinical Oral Implants Research*, 18, 27-33.

14. Ahmadi-Motamayel, F., Goodarzi, M. T., Hendi, S. S., Kasraei, S., & Moghimbeigi, A. (2013). Total antioxidant capacity of saliva and dental caries. *Medicina Oral Patol Oral y Cirurgia Bucal*, 18, 553–556.

15. Hegde, A. M., Rai, K., & Padmanabhan, V. (2009). Total antioxidant capacity of saliva and its relation with early childhood caries and rampant caries. *Journal of Clinical Pediatric Dentistry*, 33, 231-234.

16. Kumar, D., Pandey, R. K., & Agrawal, D. (2011). Anestimation and evaluation of total antioxidant capacity of saliva in children with severe early childhood caries. *International Journal Pediatric Dentistry*, 21, 456-464.

17. Mahjoup, S., Ghasempour, M., Gharage, A., Bijani, A., & Masrourroudsari, J. (2014). Comparison of Total antioxidant Capacity in saliva of children with severe early childhood caries and caries-free children. *Caries Research*, 48, 271-275.

18. Hegde, M. N., Hegde, N. D., Ashok, A., & Shetty, S. (2013) Evaluation of total antioxidant capacity of saliva and serum in caries-free and caries-active adults: an in-vivo study. Indian Journal of Dental Research, 24, 164–167.

19. WHO: Oral Health Surveys. Basic Methods, ed 4. Geneva, World Health Organization (1997).

20. Cunha-Correia, A. S., Neto, A. H., Pereira, A. F., Aguiar, S. M. H. C. A., & Nakamune, A. C. M. S. (2014). Enteral nutrition feeding alters antioxidante activity in unstimulated whole saliva composition of patients with neurological disorders. *Research in Developmental Disabilities*, 35, 1209–1215.

21. Lowry, O. H., Rosebrough, A. L., Farr, A. L., & Randall, R. (1951). Protein measurement whit the folin phenol reagent. *The Journal Biological Chemistry*, 193, 265-75.

22. Benzie, I. F., & Strain, J. J. (1996). The ferric reducing ability of plasma as a measure of ‘antioxidant power’: the FRAP assay. *Analytical Biochemistry*, 239, 70-76.

23. Buege, J. A., & Aust, S. D. (1978). Microsomal lipid peroxidation. *Methods Enzymol*, 52, 302-310.

24. Maklund, S. (1985). Pyrogallol auto oxidation. In Greenwald RA (ed) Handbook of Methods for Oxygen Radical Research CRC. *Press Boca Raton*, 243-247.

25. Kremer, A. K., Kuzminsky, E., Bentur, L., & Nagler, R. M. (2014). Salivary and Serum Analysis in Children Diagnosed with Pneumonia. *Pediatric Pulmonology,* 49, 569-573.

26. Livnat, G., Bentur, L., Kuzmisnky, E., & Nagler, R. M. (2010). Salivary profile and oxidative stress in children and adolescents with cystic fibrosis. *Journal of Oral Pathololgy e Medicine*, 39, 16-21.

27. Amerongen, A. V. N., Bolscher, J. G. M., & Veerman, E. C. I. (2004). Salivary Proteins: Protective and Diagnostic Value in Cariology? *Caries Research*, 38, 247–253.

28. Öztürk, L. K., Furuncuoglu, H., Atala, M. H., Uluköylü O., Akyüz, S., & Yarat, A. (2008). Association between dental-oral health in young adults and salivary glutathione, lipid peroxidation and sialic acid levels and carbonic anhydrase activity. *Brazilian Journal of Medical and Biological Research*, 41, 956-959.

29. Sarode, G., Shelar, A., Sarode, S., & Bagul, N. (2012). Association between Dental Caries and Lipid Peroxidation in Saliva. *International Journal of Oral and Maxillofacial Pathology*, 3, 02-04.

30. Celec, P., Hodosya, J., Celecovac, V., Vodrazkad, J., Cervenkaa, T., Halcaka, L., Bozeke, P., Kopania, M., & Kudelab, M. (2005). Salivary thiobarbituric acid reacting substances and malondialdehyde – Their relationship to reported smoking and to parodontal status described by the papillary bleeding index. *Disease Markers*, 21, 133–137.

31. Almerich-Silla, J. M., Montiel-Company, J. M., Pastor, S., Serrano, F., Puig-Silla, M., & Dasí, F. (2015). Oxidative Stress Parameters in Saliva and Its Association with Periodontal Disease and Types of Bacteria. *Researc article*. [Epub ahead of print].

32. Rai, B. K. S., Jain, R., & Anand, S. C. (2006). Salivary LPO product malonaldeyde in varius dental diseases. *World Journal of Medical Sciences*, 1, 100-101.

33. Agha-Hosseini, F., Mirzaii-Dizgah, I., Farmanbar, N., & Abdollahi, M. (2012). Oxidative stress status and DNA damage in saliva of human subjects with oral lichen planus and oral squamous cell carcinoma. *Journal of Oral Pathology and Medicine*, 41, 736–740.

34. Sculley, D. V., & Langley-Evans, S. C. (2003). Periodontal disease is associated with lower antioxidant capacity in whole saliva and evidence of increased protein oxidation. *Clinical Science*, 105, 167–172.

35. Baltacioglu, E., Yuva, P., Aydin, G., Alver, A., Kahraman, C., Karabulut, E., & Akalin, F.A. (2014). Lipid peroxidation levels and total oxidant/antioxidant status in serum and saliva from patients with chronic and aggressive periodontitis. Oxidative stress index: a new biomarker for periodontal disease? *Journal Periodontology*, 85, 1432–1441.

36. Tulunoglu, O., Demirtas, S., & Tulunoglu, I. (2006). Total antioxidante levels of saliva in children related to caries, age, and gender. *International Journal of Paediatric Dentistry*, 16, 186–191.

37. Nagler, R.M., Klein, I., Zarzhevsk, N., Drigues, N., & Reznick, A.Z. (2002). Chacacterization of the differentiated antioxidante profile of human saliva. *Free Radical Biology and Medicine*, 32, 268-277.

38. Miricescu, D., Totan, A., Calenic, B., Mocanu, B., Didilescu, A., Mohora, M., Spinu, T., & Greabu, M. (2014). Salivary biomarkers: Relationship between stress and alveolar bone loss in chronic periodontitis. *Acta Odontologica Scandinavica*, 72, 42-47.

39. Aizenbud, D., Peri-Front, Y., & Nagler, R. M. (2008). Salivary analysis and antioxidants in cleft lip and palate children. *Achives of Oral Biology* 2008, 53, 517-522.

40. Hegde, M. N., Hegde, N. D., Ashok, A., & Shetty, S. (2014). Biochemical Indicators of Dental Caries in Saliva: An in vivo Study. *Caries Research*, 48, 170–173.

41. Varvara, G., Traini, T., Esposito, P., Caputi, S., & Perinetti, G. (2005). Copper-zinc superoxide dismutase activity in healthy and inflamed human dental pulp. *International Endodontic Journal*, 38, 195–199.

42. Tóthova, L., Kamodyová, N., Cervenka, T., & Celec, P. (2015). Salivary markers of Oxidative stress in oral diseases. *Frontiers in cellular an infection microbiology,* 5, 1-40.

Figure Captions

Figure 1. Total protein (mg/mL) in saliva of caries-free (white) and S-ECC (black) groups. Bars represent mean ± SEM. *$p < 0.05$ (Student's t test).

Figure 2. Concentration of 2-thiobarbituric acid-reactive substances (TBARS, in nmol/mg protein) in saliva of caries-free (white) and S-ECC (black) groups. Bars represent the mean ± SEM. *$p < 0.05$ (Student's t test).

Figure 3. Total Antioxidant Capacity (TAC, µmol/L $FeSO_4$) in saliva of caries-free (white) and S-ECC (black) groups. Bars represent the mean ± SEM. *$p < 0.05$ (Student's t test).

Figure 4. Superoxide dismutase (SOD) activity (UE/mL) in saliva of caries-free (white) and S-ECC (black) children. Bars represent the mean ± SEM. *$p < 0.05$ (Student's t test).

Figure 5. Uric acid (mg/mL) in saliva of caries-free (white) and S-ECC (black) groups. Bars represent the mean ± SEM. *$p < 0.05$ (Student's t test).

Graphical Abstract. In saliva of children (0-3 years) with early childhood caries (S-ECC) total protein levels are higher than in saliva of caries-free children. Oxidative damage, evaluated by TBARS, was reduced in saliva of S-ECC. This alteration was associated to increased total antioxidant capacity (TAC), evaluated by FRAP, in saliva of S-ECC children. Higher superoxide dismutase (SOD) activity and uric acid (UA), the enzymatic and non-enzymatic antioxidant systems respectively, contribute to the reduced levels of oxidative damage in saliva of S-ECC.

Figure 1

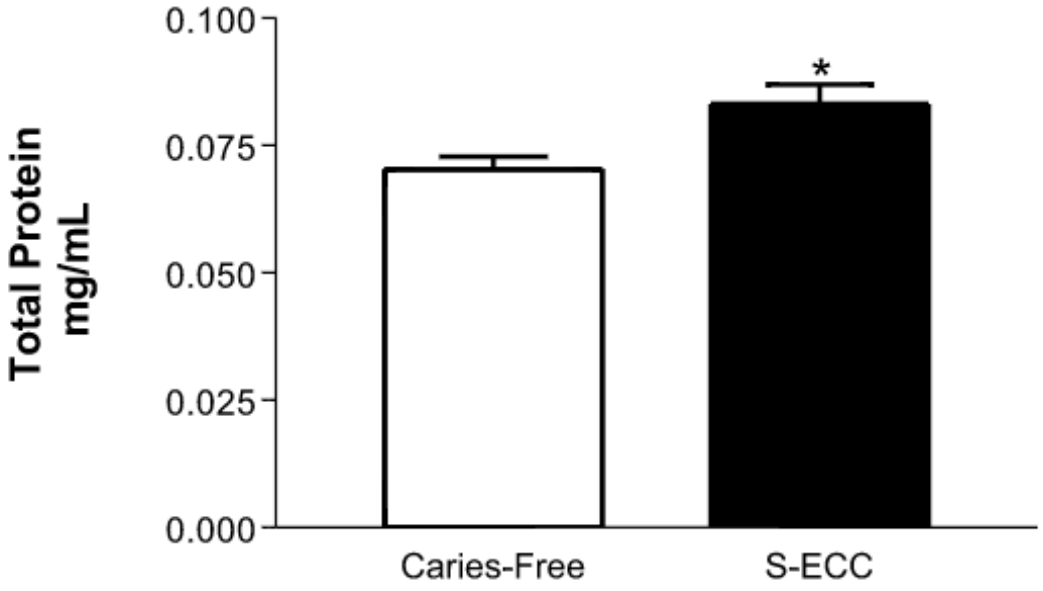


Figure 2

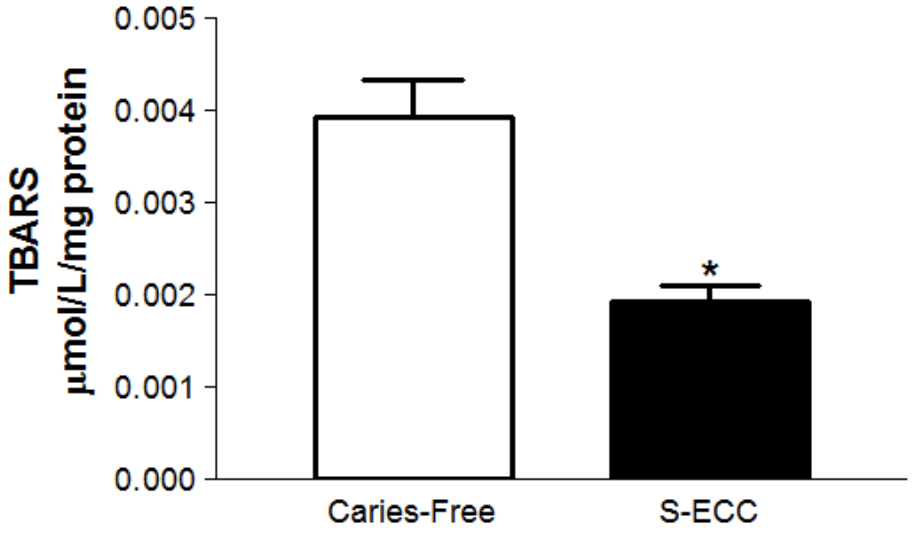


Figure 3

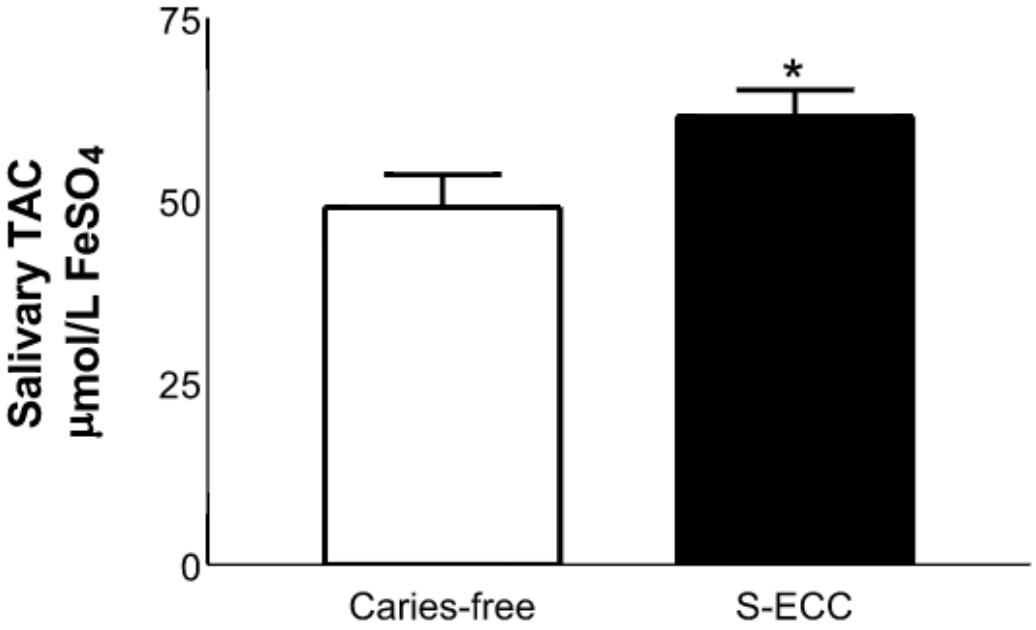


Figure 4

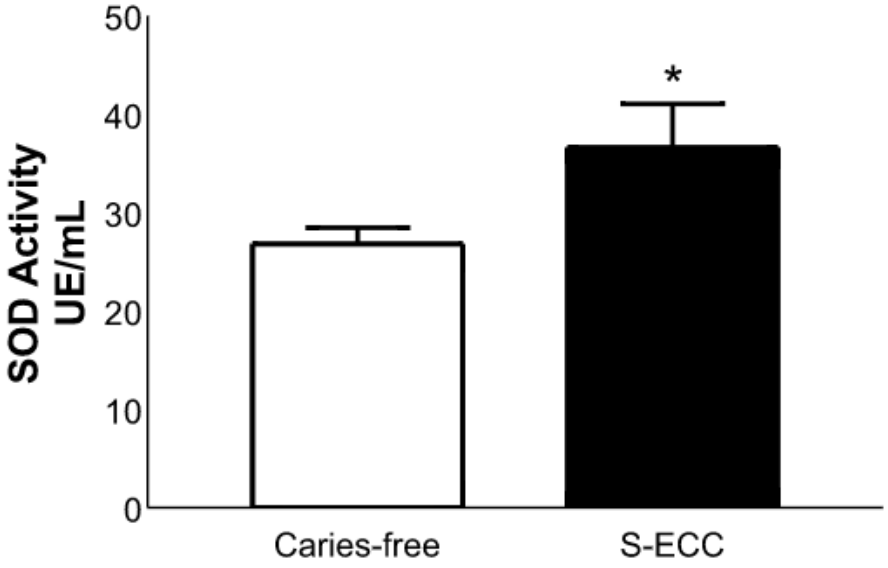
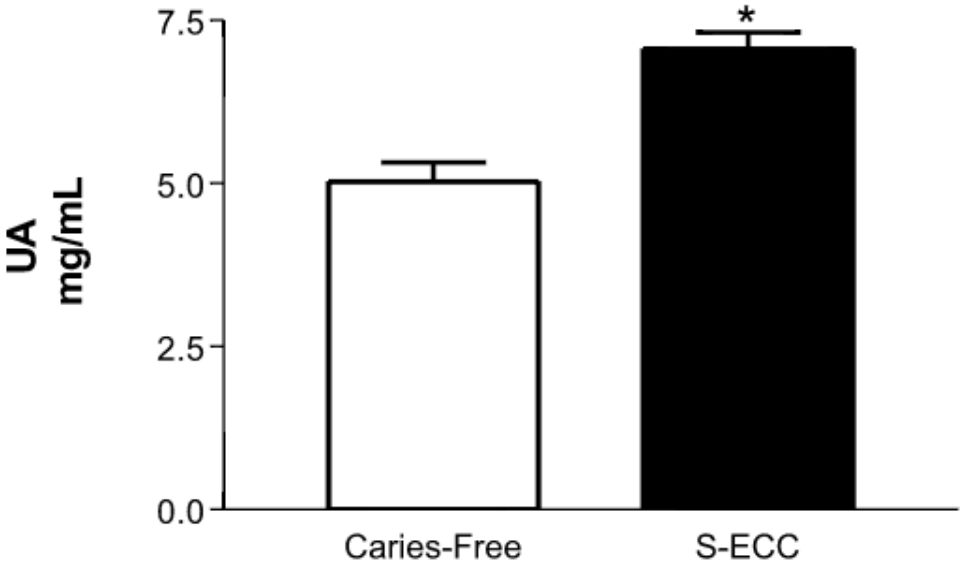
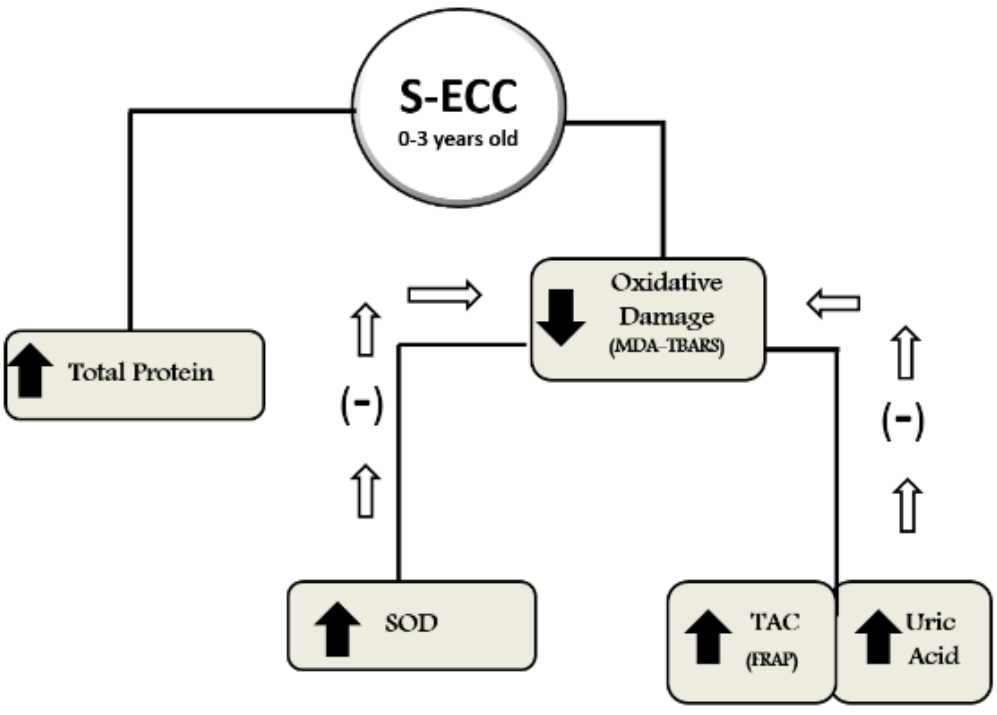


Figure 5



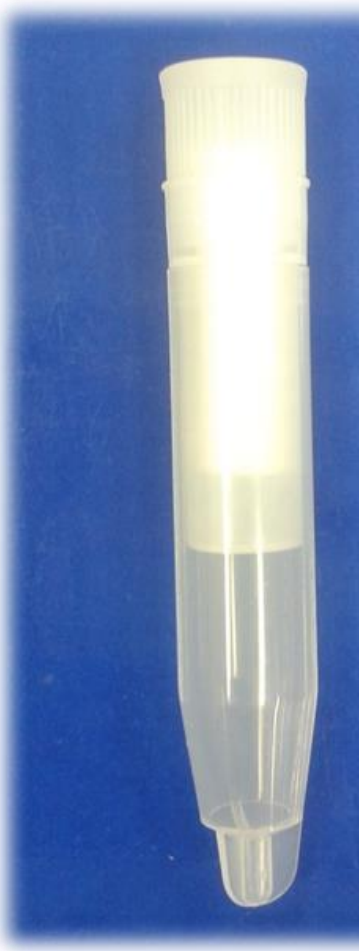
Graphical Abstract



Anexos

Anexo A

Salivette®



Anexo B

Coleta da amostra salivar