ISSN: 2230-9926

*Available online at http://www.journalijdr.com*

IJDR

*International Journal of Development Research*
*Vol. 10, Issue, 12, pp. 43022-43026, December, 2020*
https://doi.org/10.37118/ijdr.20585.12.2020

RESEARCH ARTICLE OPEN ACCESS

# ANALYSIS OF OXIDATIVE STRESS BIOMARKERS IN CARIES-FREE AND CARIES-ACTIVE CHILDREN AND ADOLESCENTS

**Guilherme Nilson Alves dos Santos[1], Amanda Maylla Ferreira Meneses[2], Urias Silva Vasconcelos[3], Regina Célia de Assis[4]†, Áurea Izabel Aguiar Fonseca e Souza[5], Gilberto Conceicao Amorim[6], Maximiliano de Souza Zierer[7] and Ayres Fran da Silva e Silva[8*]**

[1]Dental Surgeon at the Federal University of Piauí - UFPI. Master's student in Restorative Dentistry (Endodontics) at the University of São Paulo - Ribeirão Preto School of Dentistry (FORP), Ribeirão Preto, SP, Brazil; [2,3] Dental Surgeon at the Federal University of Piauí - UFPI, Teresina, Piauí, Brazil; [4]Associate Professor at the Federal University of Piauí, Department of Biochemistry and Pharmacology, Teresina, Piauí, Brazil. In memoriam; [5,6,8*]Federal Rural University of Amazonia - UFRA, Campus Parauapebas, Pará, Brazil; [7]Associate Professor at Federal University of Piauí, Department of Biochemistry and Pharmacology, Campus Ministro Petrônio Portella, Teresina, Piauí, Brazil

**ARTICLE INFO**

***Article History:***
Received 20th September, 2020
Received in revised form
07th October, 2020
Accepted 09th November, 2020
Published online 31st December, 2020

***Key Words:***
Caries; saliva; Biomarkers;
Oxidative stress; Uric acid.

***Corresponding author:***
***Ayres Fran da Silva e Silva,***

**ABSTRACT**

In the presence of dental caries, there may be an imbalance between the production of reactive oxygen species (ROS) and the ability of antioxidant enzymes to eliminate them, defined as oxidative stress, which can cause tissue damage. Several biomarkers have been used to assess oxidative stress, such as the antioxidant capacity of saliva (CAT) and salivary uric acid (SUA). The objective of this work was to evaluate the relationship between the antioxidant capacity of saliva (CAT) and salivary uric acid (SUA) with dental caries. A case-control study was carried out with 100 systemically healthy individuals, aged 12 to 15 years old. CAT and uric acid were analyzed by spectrophotometry. The data were analyzed by SPSS 18.0 using frequencies and independent t test. For groups with male and female caries, respectively, there was an increase of 72.88% and 73.01% of CAT compared to the control groups. Regarding the concentration of salivary uric acid (SUA), for groups with male and female caries, respectively, there was an increase of 44.75% and 123.33% in SUA compared to the control groups. Therefore, considering that the manifestations of CAT and SUA have a relationship with dental caries, saliva can be an important tool for the diagnosis of oxidative stress.



**Citation:** ***Guilherme Nilson Alves dos Santos, Amanda Maylla Ferreira Meneses, Urias Silva Vasconcelos, et al 2020.*** "Analysis of oxidative stress biomarkers in caries-free and caries-active children and adolescents", *International Journal of Development Research*, 10, (12), 43022-43026.

## INTRODUCTION

Saliva plays an important role in the control of dental caries, as its components, flow, viscosity and buffering capacity play an important role in the prevention, initiation and evolution of the disease. Saliva influences various aspects of the caries process and can help to produce a favorable environment to fight the disease (Erdem *et al.* 2013; Dean *et al.* 2004). Saliva can reflect the current physiological condition of the body and, therefore, is often called "the body's health mirror" because it contains proteins, hormones, antibodies and other molecules that are often measured in blood tests to monitor health and the disease. In addition, saliva is easy to collect, less painful for the patient and less infectious for the healthcare professional (Crnkovic *et al.* 2018; Pedersen *et al.* 2018; Yoshizawa *et al.* 2013; Farnaud *et al.* 2010). In the presence of dental caries, there may be an imbalance between the production of Reactive Oxygen Species (ROS) and the ability of antioxidant enzymes to eliminate them, defined as oxidative stress, and this causes tissue damage (Krifka *et al.* 2013). It was said that this imbalance plays an important role in the beginning and development of dental caries (Berra *et al.* 2006). Saliva has been used to assess oxidative stress in individuals with dental caries using biomarkers, as it is the first line of defense, as it contains antioxidant biochemical compounds such as uric acid (Araujo *et al.* 2020). Some studies have shown the relationship between caries and total antioxidant capacity (CAT) and can be used to assess disease progression (Ahmadi-Motamayel *et*

*al.* 2013; Battino *et al.* 2002; Dodwad *et al.* 2011; Preethi *et al.* 2010; Hegde *et al.* 2009). Biomarkers are able to provide information about the current physiological state of a living organism, and saliva is very reliable and can be useful in determining the presence, location and even the likelihood of developing the disease. Thus, biomarkers serve as a valuable and attractive tool in diagnosis. Understanding and validating a saliva-based biomarker can play a considerable role in interpreting the overall health reflex of the organism itself (Agatonovic-Kustrin *et al.* 2019; Bellagambi *et al.* 2017; Kaczor-Urbanowicz *et al.* 2017; Rutherfurd-Markwick *et al.* 2017). Among the biomarkers of oxidative stress are CAT and uric acid, where the first measures the total antioxidants present in saliva and the second is an important antioxidant defense molecule in saliva. Studies evaluating the relationship between caries and CAT and its components are scarce, but CAT can be considered as a sensitive and reliable marker to determine changes in oxidative stress, assessing the antioxidant levels present in the biological sample (Hamed and Abdel-Tawwab 2017; Maciejczyk *et al.* 2018; Babiuch *et al.* 2019; Jowko *et al.* 2018; Rubio *et al.* 2019; Silva *et al.* 2018; Yusuf *et al.* 2017). Thus, CAT and uric acid are valuable diagnostic biomarkers for assessing various oral health conditions. Therefore, the aim of this study was to evaluate the association of these biomarkers in young individuals with and without dental caries.

## MATERIAL AND METHODS

**Target population:** A cross-sectional case-control study carried out with 100 children and adolescents aged 12 to 15 years, diagnosed with and without caries at a municipal school in Teresina, Piauí, Brazil. The research protocol was submitted to the Research Ethics Committee of the UFPI, before starting the research, and approved with CAAE 50076115.4.0000.5214.

**Inclusion and exclusion criteria:** The inclusion criteria for the case group were: individuals diagnosed with caries according to the evaluation of a dental surgeon and those who signed the free and informed consent. Exclusion criteria were children and adolescents with systemic diseases such as diabetes, AIDS, cardiovascular diseases, rheumatoid arthritis, obesity and the presence of periodontitis, gingivitis or other oral pathologies. In addition, adolescents who drink and smoke, those who have taken vitamin supplements in the past four weeks and those who have had a fever in the past five days have also been excluded. In addition, all those who did not answer the questionnaires, due to forgetfulness or because the responsible person was not authorized to participate in the study, were also excluded.

**Clinical examination:** The clinical examination was performed by a single examiner in a chair with artificial lighting. The diagnosis of caries was performed with a flat mouth mirror (Golgran®, São Paulo, Brazil) and an exploratory probe (WHO), and the radiographic examination was not performed. The case group was selected with at least one caries and the control group with all healthy teeth.

**Collection of biological material:** Initially, children and adolescents performed oral hygiene using a small, soft bristle brush with fluoride toothpaste to avoid the influence of diet and excess bacteria. They were instructed not to drink and eat for two hours. Saliva was accumulated in the mouths of children and adolescents for two minutes and collected in a sterile graduated tube with a wide mouth, using a sputum method, collecting 4 mL. The tubes were stored in a styrofoam with ice, hermetically sealed, to be transported to the Biochemistry Laboratory at UFPI for analysis.

**Separation of saliva components:** The saliva samples were centrifuged at 15.000 rpm for 10 minutes at 4ºC. The resulting supernatants were placed in sterile plastic microtubes, duly identified and frozen immediately (-80ºC).

**Determination of total antioxidant capacity (CAT):** The total antioxidant capacity was determined by the method (Koracevic *et al.* 2001), an assay that measures the ability of the serum to inhibit the production of TBA reactive substances (TBARS) from sodium benzoate, under the influence of reactive oxygen species (ROS), derived from the Fenton reaction. The reaction was measured by a Hitachi U-3000 spectrophotometer at a wavelength of 532 nm.

**Determination of salivary uric acid (SUA):** Dosages of uric acid were made following the manufacturer's guidelines (Labtest Diadgnóstica SA, MG, Brazil) and the concentration of uric acid in each sample was expressed in mg dL-1. This method is colorimetric enzymatic from which the resulting cherry color activity is directly proportional to the uric acid concentration in the sample. The absorbance of the sample and the standard was read on the Hitachi U-3000 spectrophotometer at a wavelength of 505 nm.

**Statistical analysis:** The data were processed using the SPSS for Windows® version 18.0 program, in which the descriptive analysis (mean and standard deviation) and frequency was performed. For comparison between groups, the independent t test was applied. In the statistical analysis, the 95% confidence level, the 5% margin of error and the 50% prevalence of the disease were used.

## RESULTS

The initial population consisted of 134 children and adolescents, of which 34 participants did not bring the free and informed consent, or did not fit the exclusion criteria, and were excluded. Thus, a total of 100 children and adolescents aged 12 to 15 years participated in this study, 54 of whom were male and 46 were female. In the control group (n = 48), 56% and 44% were male and female, respectively. In the caries group (n = 52), 52% and 48% were male and female, respectively. The average age of the interviewees was 13 ± 0.96 years.

**Total Antioxidant Capacity (CAT):** The results in Table 1 show that the caries group, in both sexes, had a higher total antioxidant capacity (CAT) when compared to the control group, with a significant difference ($p < 0.05$). For groups with male and female caries, respectively, there was an increase of 72.88% and 73.01% of CAT compared to control groups (Table 1).

**Concentration of salivary uric acid (SUA):** The results in Table 2 show that the case group, in both sexes, showed a higher concentration of salivary uric acid (SUA) when compared to the control group, with a significant difference ($p < 0.05$). For groups with male and female caries, respectively, there was an increase of 44.75% and 123.33% in SUA compared to control groups (Table 2).

**Table 1. Concentration of total salivary antioxidant capacity (CAT) in children and adolescents with and without caries. Teresina - PI, Brazil, 2017**

| Gender | Caries activity | CAT level (mg/dL) |
|---|---|---|
| Male (n=27) | Without caries | 0,59±0,21 |
| Male (n=27) | With caries | 1,02±0,27 |
| | | p=0,000 |
| Female (n=21) | Without caries | 0,63±0,20 |
| Female (n=25) | With caries | 1,09±0,25 |
| | | p=0,000 |

Source: UFPI Biochemistry and Pharmacology Laboratory. p <0.05

**Table 2. Concentration of salivary uric acid in children and adolescents with and without caries. Teresina - PI, Brazil, 2017**

| Gender | Caries activity | Uric acid level (mg/dL) |
|---|---|---|
| Male (n=27) | Without caries | 2,86±1,89 |
| Male (n=27) | With caries | 4,14±1,90 |
| | | p=0,017 |
| Female (n=21) | Without caries | 1,80±1,02 |
| Female (n=25) | With caries | 4,02±1,39 |
| | | p=0,000 |

Source: UFPI Biochemistry and Pharmacology Laboratory. p <0.05

## DISCUSSION

The antioxidant defense mechanisms of saliva are very important and few studies discuss the relationship between CAT and uric acid with dental caries. Other studies have shown that the collection of saliva must be through the sputum method, that is, unstimulated saliva, since the levels of antioxidant capacity are higher in these cases (Tulunoglu *et al.* 2006; Hedge *et al.* 2009; Hedge *et al.* 2013; Erel 2004; Greabu *et al.* 2006; Pereslegina 1989; Li *et al.* 2019; Maciejczyk *et al.* 2019; Perraudin 2019; Leeuwen *et al.* 2019; Skutnik-Radziszewska *et al.* 2020). In addition, early childhood caries was associated with a higher CAT level, with a direct linear relationship between the severity of the caries and the level of antioxidant concentration (Mahjoub *et al.* 2014; Silva *et al.* 2016). The relationship between CAT and dental caries has also been reported in adults, with a linear relationship between CAT saliva and caries severity (Celecova *et al.* 2013; Tarboush *et al.* 2019; Hegde *et al.* 2009; Hegde *et al.* 2013). The level of high antioxidant concentration in children with caries can be attributed to high levels of protein. It is suggested that saliva is rich in antioxidants, especially uric acid. Uric acid is reported to be the main antioxidant in saliva, responsible for 70% -80% of the total antioxidant capacity of stimulated or unstimulated saliva (Moore *et al.* 1994). Some mechanisms have been proposed to explain why CAT could be higher in patients with dental caries. The authors reported that the highest level of CAT in children with caries is a compensatory mechanism against oxidative stress (Mahjoub *et al.* 2014). They attributed that the higher level of CAT has a direct relationship in individuals with caries and with their diet, stating that the CAT of saliva is a combination of endogenous antioxidants and food derivatives (Moore *et al.* 1994). Therefore, sugar consumption not only increases the risk of tooth decay, but also contributes to a higher level of CAT (Mahmoud *et al.* 2016).

Sugars that promote caries, such as sucrose, glucose and fructose, present in fruit juices and other sugary drinks, are easily metabolized by cariogenic bacteria to organic acids that demineralize enamel and dentin. One of the striking features of fructose is the ability to produce uric acid. Consequently, this can increase the concentration of uric acid in saliva leading to an increase in antioxidant capacity (Moore *et al.* 1994; Perheentupa & Raivio 1967; Reiser *et al.* 1989; Hegde *et al.* 2013). Thus, the habit of increasing sugar intake may be responsible for the increase in the level of uric acid and CAT, at least in part, being a confusing factor both for the development of caries and for the increase in the level of uric acid. Additional studies on the role of uric acid, its contribution to saliva CAT and its association with diet are needed to better understand this phenomenon (Kumar *et al* 2011; Hegde *et al*. 2013). When studying gender, there was a significantly smaller difference in relation to the female group (Tulunoglu *et al.* 2006; Hegde *et al.* 2009; Hegde *et al.* 2013; Erel 2004). Some studies have shown a significant relationship between CAT and gender, with the female group having a lower concentration. In the present study, the results differed, since in this group there was a higher concentration of CAT in relation to the male sex, but the difference was not statistically significant. The association between CAT and the severity of caries was confirmed later. Our study pointed to a higher uric acid concentration in individuals with caries compared to individuals free of caries. The first line of defense against tooth decay is saliva. Its composition and physiology allow a thorough investigation as it clearly influences oral health (Greabu *et al.* 2007). The result of this study was similar to that of previous studies that evaluated the relationship between the physical and chemical properties of saliva and the antioxidant status (Erel 2004; Tulunoglu *et al.* 2006; Hegde *et al.* 2009; Preethi *et al.* 2010; Ahmadi-Motamayel *et al.* 2013; Hegde *et al.* 2013; Moore *et al.* 1994; Kumar *et al.* 2011; Dodwad *et al.* 2011). Such studies concluded that the level of antioxidant concentration was higher in children with caries. They suggested that antioxidant levels could be changed in response to an infection or illness. The absence of infection in the control group may be one of the factors for levels with a lower concentration of CAT and uric acid.

## CONCLUSION

Within the limitations of this study, it can be concluded that there is an association between total antioxidant capacity and salivary uric acid and dental caries. The results reinforce the need for studies with larger samples, as well as longitudinal studies to elucidate the association between CAT levels and uric acid with the number of cavities. In addition, there may be gender differences that may be influenced by hormonal factors, which still needs to be evaluated in future studies.

## REFERENCES

Agatonovic-Kustrin, S.; Morton, D.W.; Smirnov, V.; Petukhov, A.; Gegechkori, V.; Kuzina, V.; Gorpinchenko, N.; Ramenskaya, G. (2019) Analytical Strategies in Lipidomics for Discovery of Functional Biomarkers from Human Saliva. *Disease Markers* 2019: 1-11.

Ahmadi-Motamayel, F.; Goodarzi, M.T.; Hendi, S.S.; Kasraei, S.; Moghimbeigi, A. (2013) Total antioxidant capacity of saliva and dental caries. *Medicina oral, patologia oral y cirugia bucal* 18(4): 553-556.

Araujo, H.C.; Nakamune, A.C.M.S.; Garcia, W.G.; Pessan, J.P.; Antoniali, C. (2020) Carious Lesion Severity Induces Higher Antioxidant System Activity and Consequently Reduces Oxidative Damage in Children's Saliva. *Oxidative Medicine and Cellular Longevity* 2020: 1-9.

Babiuch, K.; Bednarczyk A.; Gawlik; K.; Pawlica-Gosiewska, D.; Kesek, B.; Darczuk, D.; Stepien, P. ; Chomyszyn-Gajewska, M.; Kaczmarzyk, T. (2019) Evaluation of enzymatic and non-enzymatic antioxidant status and biomarkers of oxidative stress in saliva of patients with oral squamous cell carcinoma and oral leukoplakia: a pilot study. *Acta Odontologica Scandinavica* 77(6): 408-18.

Battino, M.; Ferreiro, M.S.; Gallardo, I.; Newman, H.N.; Bullon, P. (2002) The antioxidant capacity of saliva. *Journal of Clinical Periodontology* 29(3): 189-94.

Bellagambi, F.G.; Baraket, A.; Longo, A.; Vatteroni, M.; Zine, N.; Bausells, J.; Fuoco, R.; Di Francesco, F.; Salvo, P.; Karanasiou, G.S.; Fotiadis, D.I.; Menciassi, A.; Errachid, A. (2017) Electrochemical biosensor platform for TNF-alpha cytokines detection in both artificial and human saliva: Heart failure. *Sensors and Actuators B-Chemical* 251: 1026-33.

Berra, C.M; Menck, C.F.M.; Di Mascio, P. (2006) Estresse oxidativo, lesões no genoma e processos de sinalização no controle do ciclo celular. *Química Nova* 29(6): 1340-44.

Celecova, V.; Kamodyova, N.; Tothova, L.; Kudela, M.; Celec, P. (2013) Salivary markers of oxidative stress are related to age and oral health in adult non-smokers. *Journal of Oral Pathology & Medicine* 42(3): 263-66.

Crnkovic, D.; Peco, M.; Gelo; J. (2018) Correlation between salivary biochemical stress indicators and psychological indicators. *Acta Clinica Croatica* 57(2): 316-26.

Dean, J.A.; Avery, D.R.; McDonald, R.E. (2004) McDonald and Avery's Dentistry for the Child and Adolescent (9th ed.), Mosby/Elsevier, Maryland Heights, Mo.

Dodwad, R.; A.V. Betigeri; Preeti, B.P. (2011) Estimation of total antioxidant capacity levels in saliva of caries-free and caries-active children. *Contemporary clinical dentistry* 2(1): 17-20.

Erdem, V.; Yildiz, M.; Erdem, T. (2013) The Evaluation of Saliva Flow Rate, pH, Buffer Capacity, Microbiological Content and Indice of Decayed, Missing and Filled Teeth in Behcet's Patients. *Balkan Medical Journal* 30(2): 211-14.

Erel, O. (2004) A novel automated method to measure total antioxidant response against potent free radical reactions. *Clinical biochemistry* 37(2): 112-19.

Farnaud, S.J.C.; Kosti, O.; Getting, S.J.; Renshaw, D. (2010) Saliva: physiology and diagnostic potential in health and disease. *The Scientific World Journal* 10: 434-56.

Greabu, M.; Battino, M.; Mohora, M.; Totan, A.; Spinu, T.; Totan, C.; Didilescu, A .; Duţa, C. (2006) Could constitute saliva the first line of defence against oxidative stress? *Romanian journal of internal medicine* 45(2): 209-13.

Hamed, H.S.; Abdel-Tawwab, M. (2017) Ameliorative effect of propolis supplementation on alleviating bisphenol-A toxicity: Growth performance, biochemical variables, and oxidative stress biomarkers of Nile tilapia, Oreochromis niloticus (L.) fingerlings. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology* 202: 63-69.

Hegde, A.; 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(3): 231-34.

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(2): 164-167.

Jowko, E.; Rozanski, P.; Tomczak, A. (2018) Effects of a 36-h Survival Training with Sleep Deprivation on Oxidative Stress and Muscle Damage Biomarkers in Young Healthy Men. *International Journal of Environmental Research and Public Health* 15(10): 2066-2077.

Kaczor-Urbanowicz, K.E.; Carreras-Presas, C.M.; Aro, K.; Tu, M.; Garcia-Godoy, F.; Wong D.T.W. (2017) Saliva diagnostics - Current views and directions. *Experimental Biology and Medicine* 242(5): 459-72.

Koracevic, D.; Koracevic, G.; Djordjevic, V.; Andrejevic, S.; Cosic, V. (2001) Method for the measurement of antioxidant activity in human fluids. *Journal of clinical pathology* 54(5): 356-61.

Krifka, S.; Spagnuolo, G.; Schmalz, G.; Schweikl, H. (2013) A review of adaptive mechanisms in cell responses towards oxidative stress caused by dental resin monomers. *Biomaterials* 34(19): 4555-4563.

Kumar, D.; Pandey, R.K.; Agrawal, D.; Agrawal, D. (2011) An estimation and evaluation of total antioxidant capacity of saliva in children with severe early childhood caries. *International journal of paediatric dentistry* 21: 459-64.

Leeuwen, S.J.M.; Potting, C.M.J.; Huysmans, M.C.D.N.J.M.; Blijlevens; N.M.A. (2019) Salivary Changes before and after Hematopoietic Stem Cell Transplantation: A Systematic Review. *Biology of Blood and Marrow Transplantation* 25(6): 1055-61.

Li, Y.; Wang, W.; Wang, T.; Wouters, M.A.; Yin, Y.; Jiao, Z.; Ma, L.; Zhang, F. (2019) Regulation through microRNAs in Response to Low-Energy N+ Ion Irradiation in Oryza sativa. *Radiation Research* 191(2): 189-200.

Maciejczyk, M.; Szulimowska, J.; Skutnik, A.; Taranta-Janusz, K.; Wasilewska, A.; Wisniewska, N.; Zalewska, A. (2018) Salivary Biomarkers of Oxidative Stress in Children with Chronic Kidney Disease. *Journal of Clinical Medicine* 7(8): 209-27.

Maciejczyk, M.; Zalewska, A.; Ladny J.R. (2019) Salivary Antioxidant Barrier, Redox Status, and Oxidative Damage to Proteins and Lipids in Healthy Children, Adults, and the Elderly. *Oxidative Medicine and Cellular Longevity* 2019: 1-12.

Mahjoub, 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: 271-75.

Mahmoud, R.; Ghoorchi, V.; Rezaei; F.; Raygani, A.V. (2016) Evaluation of total antioxidant capacity of saliva in high school students. *Global journal of health science* 8(9): 89-94.

Moore, S.; Calder, K.A.C.; Miller, N.J.; Rice-Evans, C.A (1994) Antioxidant activity of saliva and periodontal disease. *Free radical research* 21(6): 417-25.

Pedersen, A.M.L.; Sorensen, C.E.; Proctor, G.B.; Carpenter, G.H.; Ekstrom, J. (2018) Salivary secretion in health and disease. *Journal of Oral Rehabilitation*, 45(9): 730-46.

Pereslegina; I.A. (1989) The activity of antioxidant enzymes in the saliva of normal children. *Laboratornoe delo* 11: 20-23.

Perheentupa, J.; Raivio, K. (1967) Fructose-induced hyperuricaemia. *The lancet* 290(7515): 528-31.

Perraudin, J.P. (2019) The biochemical aspects of diabetes in oral health. *British Journal of Diabetes* 19(2): 93-98.

Preethi, B.P.; Reshma, D.; Anand, P. (2010) Evaluation of flow rate, pH, buffering capacity, calcium, total proteins and total antioxidant capacity levels of saliva in caries free

and caries active children: an in vivo study. *Indian Journal of Clinical Biochemistry* 25: 425-28.

Reiser, S.; Powell, A.S.; Scholfield, D.J.; Panda, P.; Ellwood, K.C.; and Canary, J.J. (1989) Blood lipids, lipoproteins, apoproteins, and uric acid in men fed diets containing fructose or high-amylose cornstarch. *The American journal of clinical nutrition* 49(5): 832-39.

Rubio, C.P.; Mainau, E.; Ceron, J.J.; Contreras-Aguilar, M.D.; Martinez-Subiela, S.; Navarro, E.; Tecles, F.; Manteca, X.; Escribano, D. (2019) Biomarkers of oxidative stress in saliva in pigs: analytical validation and changes in lactation. *Bmc Veterinary Research* 15:144.

Rutherfurd-Markwick, K.; Starck, C.; Dulson, D.K.; Ali, A. (2017) Salivary diagnostic markers in males and females during rest and exercise. *Journal of the International Society of Sports Nutrition* 14: 27.

Silva, M.R.; Matono, D.; Bosco, A.M.; Baptistiolli, L.; Torrecilha, R.B.P.; Ciarlini, P.C. (2018) Oxidative stress in dogs with periodontal disease: comparison of plasmatic and salivary biomarkers. *Arquivo Brasileiro de Medicina Veterinaria e Zootecnia* 70(5): 1369-77.

Silva, P.V.; Troiano, J.A.; Nakamune, A.C.M.S.; Pessan, J.P.; Antoniali, C. (2016) Increased activity of the antioxidants systems modulate the oxidative stress in saliva of toddlers with early childhood caries. *Archives of Oral Biology* 70: 62-66.

Skutnik-Radziszewska, A.; Maciejczyk, M.; Fejfer, K.; Krahel, J.; Flisiak, I.; Kolodziej, U.; Zalewska, A. (2020) Salivary Antioxidants and Oxidative Stress in Psoriatic Patients: Can Salivary Total Oxidant Status and Oxidative Status Index Be a Plaque Psoriasis Biomarker? *Oxidative Medicine and Cellular Longevity* 2020: 1-12.

Tarboush, N.A.; Masoodi O.A.; Bdour, S.A.; Sawair, F.; Hassona, Y. (2019) Antioxidant capacity and biomarkers of oxidative stress in saliva of khat-chewing patients: a case-control study. *Oral Surgery Oral Medicine Oral Pathology and Oral Radiology* 127: 49-54.

Tulunoglu, Ö.; Demirtas, S.; Tulunoglu, I. (2006) Total antioxidant levels of saliva in children related to caries, age, and gender. *International Journal of Paediatric Dentistry* 16: 186-91.

Yoshizawa, J.M.; Schafer, C.A.; Schafer, J.J.; Farrell, J.J.; Paster, B.J.; Wong, D.T.W. (2013) Salivary biomarkers: toward future clinical and diagnostic utilities. *Clinical microbiology reviews* 26: 781-91.

Yusuf, A.O.; Mlambo, V.; Sowande, O.S.; Solomon, R. (2017) Oxidative stress biomarkers in West African Dwarf goats reared under intensive and semi-intensive production systems. *South African Journal of Animal Science* 47(3): 281-89.

*******