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Evaluation of Some Caries-Related Factors in the Saliva of 3-5 Year Old Children in Sari, Northern Iran

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Abstract

Background: Dental caries is one of the most common oral diseases in pre-school children. Several factors can affect caries process. Aim of this study was comparison of some of the chemical properties of saliva such as total antioxidant capacity, total protein, pH, nitric oxide level in caries free (CF), and caries active (CA) children.

Materials and Methods

This cross-sectional based study was designed with random selection of 80 healthy population including 40 CF and 40 CA children (3-5 years old) from several public kindergartens in Sari, Iran in 2019. Caries status was assessed using DMFT (Decayed/Missing/Filled Teeth) index according to WHO criteria .Un-stimulated saliva samples were collected from children in the morning. Then, several caries-related factors including total antioxidant capacity, nitric oxide, total protein concentration and pH were assessed in saliva samples. Data were analyzed using SPSS software version 16.0.

Results: Significant higher total antioxidant capacity and total protein concentration were observed in the saliva of CA than in the CF children. On the other hand, nitric oxide level was markedly lower in CA samples. In addition, a decrease in pH of saliva was observed in CA children.

Conclusion

Based on the results, increase in the total antioxidant capacity and total protein as well as decrease in nitric oxide levels in the saliva of CA children can be considered as valuable evidence of dental caries occurrence among children.

Key Words: Dental Caries, Nitric Oxide, Total Antioxidant Capacity, pH, Total Protein.

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1- INTRODUCTION

Dental caries is a chronic and multifactorial local disease which is one of the major concerns of world health organization (WHO) about oral health (1). Several factors contribute to the incidence of dental caries, among which, inattention to dental and oral hygiene, presence of more bacteria in oral cavity, low salivary flow rate, family history of caries and also low level of fluoride in drinking water are the most important (2). Saliva as a biological fluid, is a mixture of different enzymes, antibacterial constitutes, hormones and other materials. Because of some unique properties of saliva such as its antimicrobial activity, flow rate, pH buffering capacity, immunological defense system, natural antioxidant capacity, it has a protective role in various pathologic conditions in oral cavity (3, 4).

Recently, the role of oxidative stress in the development of dental caries has been considered. Oxidative stress is defined as an imbalance between free radical production and antioxidant defense system in the body (5). Increased oxidative damage markers have been reported in the saliva samples of individuals with dental caries, for example, Subramanyam et al. showed that malondialdehyde level rose in the saliva of caries affected children (6).

Therefore, increased level of oxidative markers can promote dental caries. On the other hand, it has been shown that salivary proteins can have inhibitory effects against development of caries process, especially due to their free radical scavenging activity. So, evaluation of total salivary protein can be representative of salivary defense against oral diseases (7). Nowadays, the role of nitric oxide (NO) in dental caries has gained more attention. Although NO has been known to be historically responsible for deleterious adverse effects in several organs due to its free radical activity, it is reported that it has beneficial effects in amelioration of oral diseases, especially dental caries (8). With respect to the role of oxidative stress in pathogenesis of dental caries, there is a lack of comprehensive study about oxidative stress and caries process in 3-5 year old children. Therefore, aim of this study was to evaluate several factors, which can affect caries process including total protein, total antioxidant capacity (TAC), pH and NO in the saliva samples of two populations of caries active and caries free children at the age of 3-5 years old in Sari city (Northern Iran).

2- MATERIALS AND METHODS

2-1. Patient Selection

A cross-sectional based study was designed with random selection of eighty healthy population including 40 caries free (CF) and 40 caries active (CA) children with the age range of 3 to 5 years old. The study population was carried out from several public kindergartens in the city of Sari, Iran. Inclusion criteria were: being generally healthy, without periodontal disease, having no dental caries in CF group, having at least five decayed tooth surfaces in CA group. Also, exclusion criteria were: having systemic or local disease, using medication in the last three months, poor oral hygiene, pathological lesions in oral cavity and subjects with dental fluorosis. The study protocol was approved by the Ethics Committee of Mazandaran University of Medical Sciences, Sari, Iran (Ethics number: 96.2916).

2-2. Clinical Examination

Before the beginning of examination, the nature of the study was completely explained for children's parents and written consent was obtained from them. A single examiner did all clinical examinations. Caries evaluation was based on clinical observation with dental mirror and explorer. The level of Caries status was assessed using DMFT

(Decayed/Missing/Filled Teeth) index according to WHO criteria.CA group were selected within the children which had at least five clinical caries surfaces and CF group considered children that did not have any caries and filling and sign and symptom of sensitivity of teeth (DMFT=0) (9, 10).

2-3. Saliva Collection and Sample Preparation

Children were in sitting position while anterior head protrusion position. Unstimulated saliva samples (about 2 ml) were collected for the study by spitting method. Saliva specimens were collected in the morning, and all selected students were asked not to consume any oral stimulant such as eating and drinking for 120 min prior to collection (11). Samples were kept on ice during collection and immediately transferred to the laboratory, then centrifuged at 10,000*g* for 5 min by using a Refrigerated *Centrifuge* to remove bacterial and cellular debris and finally stored at -80 $^{\circ}$C until the analysis time (12).

2-4. Estimation of pH

pH of saliva was measured by using commercial pH meter (paper strip manufactured by MERCK, Germany).

2-5. Determination of Total Protein Concentration

Protein content was determined in saliva samples with Bradford method. Bovine serum albumin was used as standard. 100 µl of prepared samples added to 5 ml coomassie blue reagent and after vortexing, kept in a dark place. After 10 min, absorbance was determined at 595 nm by spectrophotometer (13).

2-6. Measurement of Total Antioxidant Capacity

The total antioxidant capacity of saliva was determined by measuring the ability of plasma to reduce Fe^{3+} to Fe^{2+} using the Ferric reducing antioxidant power (FRAP) test. Briefly, the prepared samples exposed to Fe^{3+} and the antioxidants present in the samples produce Fe^{2+} as a result of antioxidant activity. The reagents required for this experiment were buffer solution, 10 mM, TPTZ (2, 4, 6-tripyridyl-s-triazine) in 40 mM HCL, 20 mM $FeCl_3.6H_2O$. The working FRAP reagent was prepared by mixing 25 ml acetate buffer, 2.5 ml TPTZ solution, and 2.5 ml $FeCl_3.6H_2O$ solution. Then, 10 microliters of H_2O-diluted sample was added to 300 µl freshly reagent warmed at 37°C. The complex between $Fe_2^{+}$and TPTZ led to formation of a blue color with absorbance at 593 nm (14).

2-7. Measurement of Nitric Oxide

Nitric oxide (NO) was evaluated by using the commercial kits based on the Griess reagent. In this method, sulfanilic acid is converted to a diazonium salt by reaction with nitrite in acid solution. Then the diazonium salt is coupled to N-(1-Naphthyl) ethylenediamine, forming an azo dye which can be spectrophotometrically quantitated on the 548 nm (15).

2-8. Statistical Analysis

Data are expressed as Mean ± Standard error of mean (SEM). All Statistical analyses were performed using independent Student t-test (SPSS software, version 14.0). Values of $p < 0.05$ were considered as statistically significant.

3- RESULTS

3-1. Salivary pH

According to **Figure.1**, mean salivary pH in CA group was lower in comparison with CF group.

3-2. Salivary Total Protein

Salivary total protein concentration was significantly higher ($p < 0.01$) in the CA group when compared to CF group (**Figure.2**).

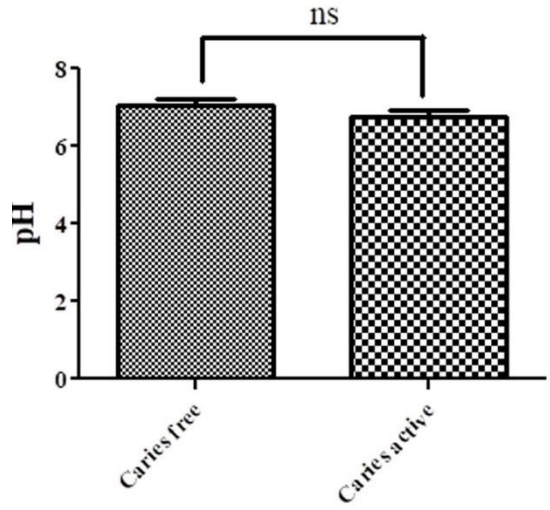

Fig 1. Comparison of salivary pH between Caries active (CA) and Caries free (CF) children. Values are represented as Mean ± SEM. NS: non-significant.

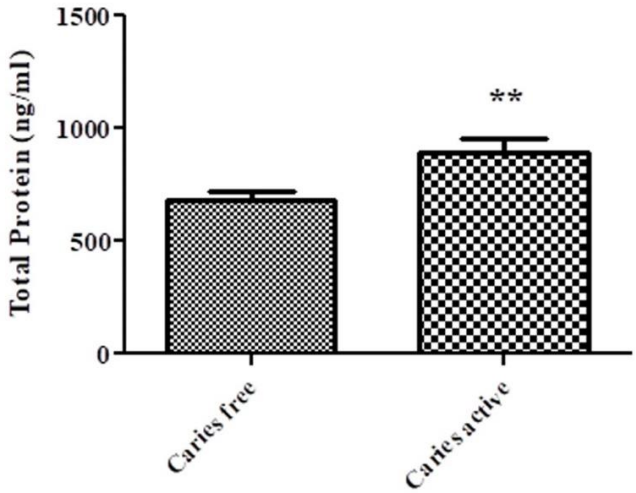

Fig 2. Comparison of salivary total protein concentration between Caries active (CA) and Caries free (CF) children. Values are represented as Mean ± SEM.* *significantly different compared to the CF group (P<0.01).

3-3. Salivary Total Antioxidant Capacity

Salivary total antioxidant capacity is the cumulative effect of all antioxidants present in saliva and is an important factor against oral oxidative damages. As shown in **Figure.3**, it was markedly ($p < 0.05$) higher in CA children rather than CF group.

3-4. Salivary Nitric Oxide Level

Salivary nitric oxide levels were significantly lower ($p < 0.001$) in the group of children with caries (CA) compared to CF group (**Figure.4**).

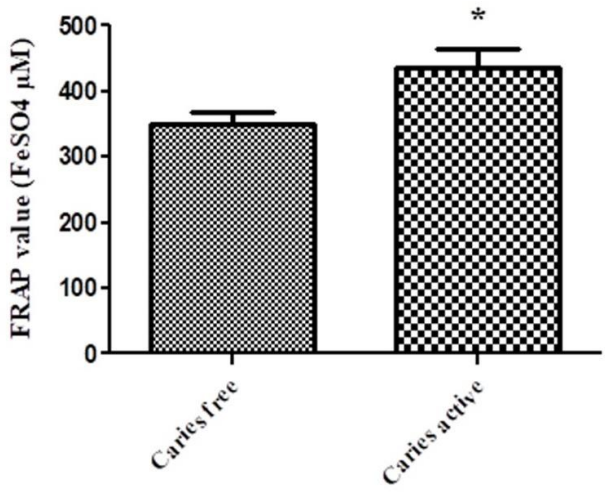

Fig 3. Comparison of salivary total antioxidant capacity between Caries active (CA) and Caries free (CF) children by ferric reducing antioxidant power (FRAP) test. Values are represented as Mean ± SEM.*significantly different compared to the CF group (P<0.05).

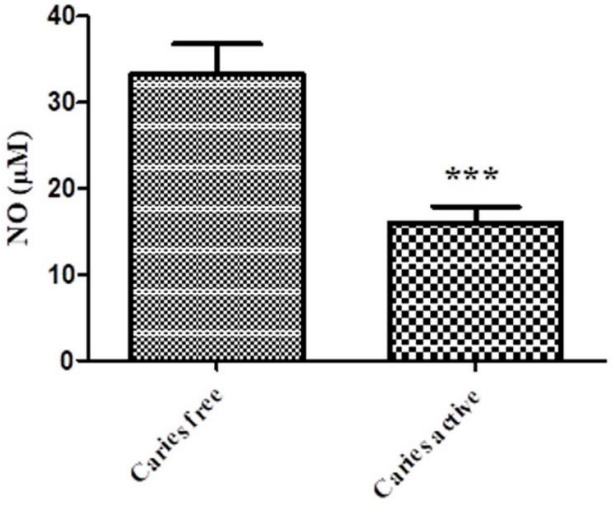

Fig 4. Comparison of salivary nitric oxide levels between Caries active (CA) and Caries free (CF) children. Values are represented as Mean ± SEM.***significantly different compared to the CF group (P<0.001).

4- DISCUSSION

In this study, we assessed the most important salivary factors, which can affect dental caries process in 3- 5-year-old children. We found that TAC and total protein were markedly higher in CA children in comparison to CF group. While NO level was significantly, lower in CA children. Despite the advances in oral disease sciences, dental caries continues to grow in different countries and affects individuals of all ages, especially children. According to the reports, 46-96 percent of 3-7 year old children are involved in early childhood caries in developing countries (16). This oral health problem is the result of interaction between internal defense factors including saliva, and hormonal status, tooth surface and impact of external

factors such as oral hygiene, diet, microbial flora, and fluoride access (17). Previous studies reported that oxidative stress has been implicated as one of the important mechanism of oral integrity pathologies, like dental caries (18). A free radical is defined as an atomic or molecular species with un-paired electron (s) in its structure. The main type of free radical is called reactive-oxygen- species (ROS) which has strong oxidizing capability and can cause oxidative damage to biological systems (19). Antioxidant are beneficial endogenous or exogenous substances that prevent or delay oxidative damages. They are found in all biological fluids as well as tissues and protect the body against potentially adverse effects of ROS (9). Antioxidant defense system can be classified as two different categories: non-enzymatic antioxidants including vitamin E and C, uric acid and enzymatic antioxidants such as superoxide dismutase, catalase, and glutathione peroxidase (20).

On the other hand, there are different compounds in saliva which are known to act as an antioxidant including: uric acid, albumin, glutathione, ascorbic acid (21). Although the concentration of salivary antioxidants can be determined individually, the more accepted approach is the measurement of TAC to evaluate cumulative effect of all antioxidant components present in the saliva (20). In our study, the level of TAC in saliva sample of caries active group was significantly higher than caries free group. Previous studies also confirmed our data. For example Hegde et al. observed that TAC levels were higher in the saliva of caries active children when compared to those who do not have caries (22). Furthermore, we should also mention the impact of salivary peroxidase system in the prevention of dental caries. As declared in many scientific papers, saliva contains several antibacterial proteins such as lactoferin, lysozyme and peroxidase (23).

One of the main functions of salivary peroxidase system is the control of oral bacteria such as *Lactobacillus casei*, *Streptococcus mutans*, *Streptococcus Sobrinus*, which is involved in the of dental plaque formation and progression of dental caries process by catalyzing the peroxidation of thiocyanate (SCN) to generate a more stable oxidation product such as hypothiocyanate (OSCN) that inhibits the growth of microorganisms (24). This enzymatic system has another role in oral cavity, which is the same function of catalase. Actually it serves as a protective defense system against H_2O_2 that is a highly reactive radical and converts it to non-harmful molecular oxygen and water (25). Therefore, we can express that increased TAC level in the saliva of caries active children can be attributed to alteration of antioxidants levels in response to caries incidence. TAC level elevation in carries active children may also be due to the activation of salivary peroxidase system to counteract with caries (26).

Total protein concentration of saliva was also evaluated and we observed that it was more in the caries status. Several researchers also reported that salivary total protein was increased in individuals with caries, that confirmed our data (10, 23). Since saliva has many antioxidant compounds such as uric acid, albumin, glutathione and peroxidases, and all of them are protein or have proteins in their structures, so, the higher level of TAC in caries active children can also be attributed to elevated proteins. Saliva also contains many other important proteins including immunoglobulins and antibacterial proteins such as lactoferin, lysozyme and lactoperoxidase and also several peptides such as histatins, defensins which have antibacterial activity (27). It is reported that the higher concentration of proteins in caries active children is a protective and/or adaptive response against dental caries

(28). It is the result of a protein film formation against enamel wear, restriction of microorganism's adherence and growth, induction of re-mineralization of teeth enamel by attracting calcium ions (27). pH of the saliva is an important factor to maintain the integrity of oral cavity (1). Low pH of saliva induces the growth of salivary aciduric bacteria and allows formation of inhospitable environment for the protective oral bacteria present in the saliva (29). Furthermore, it has been documented that acidic pH of saliva promotes dissolution of teeth enamel (30). Our result indicated that pH value of caries active children's saliva samples was lower than caries free group, although it was not statistically significant. Preethi et al. also observed that salivary pH was markedly lower in caries active group (23).

In another study, Muchandi et al., also reported the same results as ours, but their data was significant between two groups (4). Recently, there is a lot of interest in the role of nitric oxide in dental caries prevention. It seems that nitric oxide acts as a double-edged sword. Nitric oxide is primarily considered as a free radical, which can promote oxidative stress reactions in biological systems. On the other hand, it has a protective role in oral cavity against caries development. It is believed that NO expresses its protective effects by bacterial growth inhibition (antibacterial features), and also by induction of macrophage-mediated cytotoxicity (15). Syed et al. showed that elevation of NO levels was contributed to lower occurrence of caries in children (15). Interestingly, our data showed increased level of NO in caries free children, which confirmed the result of previous studies.

4-1. Study Limitations

Our study has the following main limitations: Detection of dental caries was done by using dental mirror and explorer while radiographic examination was not performed because of lack of instruments and impossibility of using them in the place. Therefore, dental caries was identified only with clinical diagnosis. In addition, it should be mentioned that because of low age and anxiety of some of the children they did not cooperate in sampling process. As suggestions, further studies can be focused on evaluation of the relationship between salivary and serum total antioxidant capacity and nitric oxide levels. We also suggest comparison of these markers before and after treatment of dental caries. Also, the impact of different foods and supplements consumption on the occurrence of dental caries can be studied.

5- CONCLUSION

The present study showed that salivary total protein and total antioxidant capacity increased significantly in response to dental caries incidence. Furthermore, nitric oxide level was higher in caries free children which can be a protective mechanism for prevention of caries development.

6- ACKNOWLEDGMENT

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7- CONFLICT OF INTEREST: None.

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