http:// jpp.mums.ac.ir

Original Article (Pages: 19475-19482)

Investigating the Relationship between Salivary Nitric Oxide Concentration and Tooth Decay Index in Different Age Groups of Children

Mahsa Shabouei Jam [1], Yasaman Karbalaei [1], Seyede Solmaz Taheri [2], * Bahareh Nazemi Salman [1]

[1] School of Dentistry, Zanjan University of Medical Sciences, Zanjan, Iran.
[2] Department of Biostatistics and Epidemiology, Faculty of Medicine, Zanjan University of Medical Sciences, Zanjan, Iran.

Abstract

Background: Nitric oxide (NO) is a highly active radical that prevents the growth of oral bacteria by inhibiting their growth and increasing the cytotoxicity of salivary macrophages as a non-specific defense mechanism. The aim of this study was to investigate the salivary NO concentration and its relationship with the DFS caries index and salivary pH in children.

Materials and Methods: In a cross-sectional study, 177 children aged 4-12 years with different levels of DFS were selected. 2 ml of unstimulated saliva was collected from each child, and the pH and NO concentration of the collected saliva were measured using a pH meter and the enzyme-linked immunosorbent assay (ELISA) method, respectively. Statistical analyzes were done with the help of one-way analysis of variance and pairwise comparisons.

Results: The mean concentration of salivary NO in children with severe caries (dfs>8) was significantly lower than in children with mild caries (dfs≤2) (p=0.008). The pH of saliva in children with severe caries (dfs>8) was significantly higher than in children with moderate caries (2 ≥dfs>8) (p=0.027), and in children aged 4-6, it was higher than in children aged 6-9(p=0.039).

Conclusion: Increasing the amount of NO in saliva can decrease decay, as the production of nitrous oxide is actually the body's defense mechanism to neutralize the acids produced by decay-causing bacteria. Salivary pH decreases first and then increases with increasing caries.

Key Words: Children, Dental caries, Salivary nitric oxide, Salivary pH.

* Please cite this article as: Shabouei Jam M, Karbalaei Y, Taheri S.S, Nazemi Salman B. Investigating the Relationship between Salivary Nitric Oxide Concentration and Tooth Decay Index in Different Age Groups of Children. J Ped Perspect 2025; 13 (5):19475-19482. **DOI: 10.22038/jpp.2025.88218.5551**

Corresponding Author:

Bahareh Nazemi Salman, Associate Professor of Pediatric Department, School of Dentistry, Zanjan University of Medical Sciences, Zanjan, Iran. Tel: 09123777185; E-mail: dr.b.nazemi@gmail.com

1- INTRODUCTION

Caries of milk teeth is a common issue in pediatric dentistry, that causes destruction of tooth structure and pain. It can lead to infection, an increased risk of new caries in both milk and permanent teeth, and adverse effects on the growth of permanent teeth (1,2). These caries progress rapidly and considering that they start at a very young age, they are considered a problem for the patient and the dentist (3). Therefore, caries prevention methods, relying on the role of microorganisms, emphasize increasing the defensive power of teeth and improving their repair ability. Saliva is effective in the caries process through its antibacterial activities and buffering effect (neutralization of acid produced by caries-causing bacteria) (4). The high level of stimulated saliva secretion and the high concentration of free bicarbonate cause the pH to rise and increase the buffering power of saliva. On the other hand, hydrogen that is secreted by microorganisms and food or by salivary glands in organic and inorganic form causes a decrease in pH (5). Individuals with higher salivary nitrate and nitrite have been reported to experience less caries compared to controls (6). On the other hand, nitrite conversion takes place in the acidic (pH less than 7) environment of the plaque (7). In the oral cavity, saliva nitrate comes into contact with bacteria that are able to convert nitrate to nitrite during the breathing process. The acid produced by Lactobacillus, Streptococcus mutans, and Actinomyces present in dental plaque causes nitrite to become acidic. This unstable acid decomposes and produces a mixture of nitrogen oxides, especially nitric oxide (NO) (8). NO, in another way, is made by a group of enzymes called nitric oxide synthase. These enzymes convert arginine into citrulline, in which NO is also synthesize (9). NO as an unstable gas enables chemical reactions. The strong reactive radical NO in the non-specific antibacterial defense mechanisms of the oral cavity, by inhibiting the growth of bacteria (inducing deamination and breakage of DNA by direct binding to it and inhibiting the replication of bacteria by disrupting the inhibition of metalloproteins involved in DNA synthesis) and increases the cytotoxicity of macrophages (production of NO by some cells involved in the immune response, especially cytokine-activating macrophages that produce a high concentration of NO to kill target cells such as bacteria or tumor cells) (10,11). NO production is the main key to many capabilities and characteristics of immune cells, including dendritic cells, NK cells, mast cells, macrophages and other phagocytes, and both inducible nitric oxide synthase (iNOS) and eNOS genes are found in all these cells (12). In some studies, it has been acknowledged that reducing the concentration NO, increases the prevalence of caries in children (13–14). It seems that saliva nitrite has an inhibitory effect on the growth and survival of caries-causing bacteria in acidic conditions (15). A significant increase in NO, as a vasodilator and with antimicrobial properties, is associated with a decrease in intraoral pH (16). On the other hand, the results of some studies also indicate that there is no correlation between NO levels in saliva with caries experience (17) and the lack of effect of high concentrations of nitrite and nitrate to prevent caries (18). Clinically, very few studies have evaluated the relationship between salivary NO with caries severity and salivary pH in children and have obtained mixed results. Therefore, this study was designed and carried out with the aim of investigating the relationship between salivary NO concentration and tooth decay index and salivary pH in different age groups of children.

2- MATERIALS AND METHODS

A total of 177 healthy children between the ages of 4 and 12 years were included in this case-control study. Children suffering from any acute or chronic disease, with a history of allergies, stress, anxiety, respiratory infections, fever, and children who had a history of taking antibiotics in the last 3 weeks were excluded from the study. The children were then divided into three age groups: 4-6, 6-9, and 9-12 years. The smooth and occlusal surfaces of the teeth were cleaned and dried with a soft-bristled brush, and then examined to determine the dfs index (decayed and filled surfaces).

Based on the dfs index, children were placed in 3 spectrums: no caries (control group) (dfs ≤2), moderate caries (2< dfs ≤8) and severe caries (dfs >8). Children were assigned to three dfs groups based on their caries status. The groups were matched for age and gender distribution to ensure homogeneity. A low DMFS score (like below 2) indicates that the individual has likely maintained good oral hygiene practices, has had limited exposure to sugary foods and drinks, and has received regular dental care, leading to fewer instances of tooth decay.

Age and gender distribution in three groups was checked through interim analysis. The participants in the study were prohibited from eating and drinking for 2 hours before sampling, and before collecting the saliva sample, they brushed their teeth without using toothpaste.

Then unstimulated saliva was collected by spitting in sterile tubes in the amount of 5 cc.

The collected saliva samples were centrifuged at 3000 rpm for 20 minutes at 15°C using a refrigerated centrifuge and then stored at -70°C until analysis. Salivary pH was obtained using a pH meter and NO concentration, with the help of a nitrous oxide standard measurement kit (Cat. No: E1150Hu saliva (using ELISA). An enzyme-linked immunosorbent assay (ELISA) test is used to measure nitrous oxide levels in a lab report by detecting and quantifying a specific protein or molecule related to nitrous oxide production or its effects. In this context, the ELISA would likely be used to measure the levels of NOS, particularly iNOS, an enzyme responsible for producing NO. The steps of ELISA for the quantitative measurement of nitrous oxide were carried out according to the protocol of the BIOTECH DAY CRYSTAL commercial-research kit made in China.

3- RESULTS

The results of the one-way analysis of variance test showed that the average concentration of NO (p=0.009) and pH in saliva (p=0.014) were significantly different in various study groups (Table 1). The concentration of NO in saliva in children with dfs > 8 is significantly lower than in children with dfs ≤ 2 (p=0.008). (Table 2).

Additionally, the pH level in children with dfs>8 is significantly higher than in children with 2 ≥dfs>8 (p=0.027) (Table 2).

The results of the one-way analysis of variance did not show any difference in the average concentration of salivary nitroxide among the studied age groups. However, the average pH levels in different age groups had a statistically significant difference (p=0.047) (Table 1). It can be concluded that salivary NO concentration is related to the dfs index regardless of age. The pH levels in children aged 6-9 years were significantly lower than in children aged 4-6 years (p=0.039) (Table 3).

Table-1. Comparison of average of pH and NO in three dfs groups and age groups of study.

Variables	Categories	Mean ± SD	
		NO	pH
dfs	dfs≤ 2(no decay) (control)	161.6 ± 62.21	6.37 ± 0.41
	2≤ dfs<8(moderate decay)	132.8 ± 42.53	6.17 ± 0.53
	dfs>8(high decay)	130.4 ± 45.54	6.51 ± 0.54
	p-value	0.009	0.014
Age	4-6 years	130.6 ± 54.51	6.37 ± 0.51
	6-9 years	134.9 ± 43.58	6.12 ± 0.58
	9-12 years	146.9± 44. 46	6.27 ± 0.46
	p-value	0.152	0.047

Table-2. Tukey's post hoc test for pairwise comparisons of NO and pH averages in differe groups.

Variables		dfs≤ 2	2≤ dfs<8
NO	2≤ dfs<8	0.063	-
	dft<8	0.008	0.977
pH	8≤ dft<2	0.176	-
	dft<8	0.623	0.027

Table-3. Tukey's post hoc test for pairwise comparisons of pH averages in different age groups

Variables		4-6 years	6-9 years
pH	6-9 years	0.039	-
	9-12 years	0.583	0.291

4- DISCUSSION

The antimicrobial role of NO in protecting against digestive, skin and oral diseases has raised the possibility of this substance being associated with dental caries (19). This study was designed and implemented to investigate the relationship between salivary NO concentration, tooth decay index, and salivary pH in different age groups of children. The results of the study showed that the concentration of salivary NO in children with high caries is lower than in children without caries. Additionally, the pH of saliva was higher in children with severe caries compared to children with moderate caries.

The pH of saliva in children aged 6-9 years was lower than in children aged 4-6 years. The results of the present study are in line with the study of Doel et al., which found that people who had more nitrates and nitrites in their saliva had fewer caries than the control group (6). The study by Radcliffe et al. also showed that saliva nitrite has an inhibitory effect on the growth and survival of caries-causing bacteria in acidic conditions (15). Other studies have reported a relationship between the decrease in the concentration of nitric oxide and the increase in the prevalence of caries in children, attributed to the antimicrobial effect of NO (13,14). It seems that the reason for the lower concentration of NO in children with more caries compared to other children is that with the increase in caries, instead of increasing the production of NO, negative feedback occurs in a self-controlled manner and the amount of NO production decreases in this way. Although the reduction of dfs is related to the increase of nitrite and nitrate concentration in saliva, a high concentration of nitrite and nitrate in saliva is not enough to prevent caries (18). In another study, the concentration of NO in the saliva and gingival crevice fluid of people with periodontitis was higher

compared to healthy people (20). In other studies, the amount of nitrate, nitrite, and NO in the saliva of people with gingivitis was higher than that of healthy people, and they concluded that by measuring the concentration of NO as an inflammatory mediator in the vascular tissues of the gingival grooves and saliva, it can be used to investigate the process of caries and prevention (21–23). In addition to gum grooves, dental plaque is also very important. An increase in the amount of decay with an increase in NO concentration may be considered as a host defense mechanism. It is difficult to evaluate any type of anti-caries factor alone, because saliva has the ability to change all compounds in different ways. Saliva antimicrobial proteins oppose each other by weakening or strengthening their effect, so according to these findings, there is a need for more clinical studies to determine the relationship between NO and caries (7). Byinder et al found that the concentration of NO in plaque is higher than in saliva. When dental caries increases or the person lacks adequate oral and dental hygiene, NO production may be a kind of host defense mechanism to prevent the progress of caries (24). The association of high levels of NO with more caries suggests the theory that high levels of NO alone cannot prevent dental plaque caries and perhaps the increase in plaque thickness and the level of caries activity in a person causes the concentration of NO in plaque to be higher than in saliva (25). In this study, the saliva of children with more caries and children with younger ages (4-6 years) was more alkaline than others. It seems that the cause of the increase in pH of saliva with the increase in caries is the dominance of Lactobacillus bacteria at the same time as the caries levels increase and the caries lesions deepen. The high concentration of free hydrogen inside the mouth causes a decrease in pH or oral acidity and ultimately damages the dental tissues (26). At younger ages, the number of decayed teeth in children is less, and as a result of food consumption, increased production of acid and hydrogen ions, NO production by nitrate reductase increases, and by creating a buffering effect, the pH of the mouth increases. The buffering property of NO production is related to oral pH, and the initial decrease in oral pH stimulates NO production, which ultimately makes oral pH more alkaline with NO production. In another study, high concentrations of nitrate, nitrite, nitrate reductase, and NO were associated with a decrease in lactobacilli in saliva, but were not related to the number of Streptococcus mutans. It seems that the increase in acid produced by lactobacillus causes the reduction of nitrate to nitrite by nitrate reductase, and as a result, increases the concentration of NO and inhibits the growth of bacteria. On the other hand, the pH of the mouth becomes alkaline due to the consumption of hydrogen ions during the synthesis of NO by nitrate reductase (17). As an electron acceptor, nitrite prevents acid fermentation in anaerobic conditions and reduces the amount of hydrogen ions in saliva. In the process of converting saliva nitrate to NO, the acids produced by caries-causing bacteria are reduced, and as a result, the pH of the mouth becomes more alkaline (26). The relationship between pH and NO in the first stages of caries causes the remineralization of the initial caries lesions, but if the lack of hygiene control and the frequent consumption of sweet foods continue, there is no possibility of compensating and neutralizing the acid in the mouth and the mouth will remain acidic. In this regard, Vanhatalo et al observed that when sucrose mouthwash was given to people who did not have caries and periodontal disease, NO production significantly increased with a decrease in pH after 5 minutes. In their study, they concluded that the intraoral production of NO is dependent on many factors such as nitrate consumption, saliva

flow, the amount of nitrate reductase activity of the microbial flora, and a significant increase in NO, as a vasodilator and an antimicrobial agent, with a decrease in pH intraoral is related (16). One of the limitations of the study is the impossibility of measuring children's saliva at several different times during the day and night. It was also not possible to measure NO in dental plaque. The time taken to transfer the samples from schools to the freezer, and the non-cooperation of some children, as well as the uncertainty of whether the children were fasting, were other limitations of this study.

5- CONCLUSION

Caries plays an important role in the increase of NO in saliva. However, there is no reliable evidence of a direct effect of NO and age on caries. More long-term research with larger sample sizes in all three age groups is needed to better understand the role of NO in caries and its relationship with age.

6- SUGGESTIONS

In order to conduct more detailed investigations regarding the concentration of NO with decay, it is suggested to also evaluate the concentration of bacteria. Additionally, due to changes in salivary conditions, it is recommended to measure the amount of NO and other influencing factors for each of the sampled people at different times of the day and night. To examine the effect of age more precisely, it is suggested to consider a wider range of ages, including both young and middle-aged individuals.

7- ETHICAL CONSIDERATIONS

This study was conducted after receiving approval from the research council committee of Zanjan University of Medical Sciences Faculty of Dentistry and obtaining the code of ethics (IR.ZUMS.REC.1396.263). All ethical principles were observed, including the preservation of participants' information.

8- AVAILABILITY OF DATA AND MATERIALS

The dataset used in the study is available upon request from the corresponding author, both during submission and after publication. The data is not publicly accessible due to privacy concerns.

9- REFERENCES

1. Kumarihamy SL, Subasinghe LD, Jayasekara P, Kularatna SM, Palipana PD. The prevalence of Early Childhood Caries in 1-2 yrs olds in a semi-urban area of Sri Lanka. BMC research notes. 2011 Dec;4:1-6.

2. Broumand S, Sharififar S, Alikhani SH. The Study Of Caries Free Indicator Of Milk Teeth In Children Age 3-6 At Dare Care Center Affiliated To Health Centers Of Army. Vol. 4. 2006. p. 828–35.

3. Rosenblatt A, Zarzar P. Breast-feeding and early childhood caries: an assessment among Brazilian infants. International Journal of Paediatric Dentistry. 2004 Nov;14(6):439-45.

4. MC LP. The role of saliva in maintaining oral health and as an aid to diagnosis. Med oral, Patol oral y cirugía bucal Ed inglesa. 2006;11(5):14.

5. Tabak LA. A revolution in biomedical assessment: the development of salivary diagnostics. Journal of Dental Education. 2001 Dec;65(12):1335-9.

6. Doel JJ, Hector MP, Amirtham CV, Al-Anzan LA, Benjamin N, Allaker RP. Protective effect of salivary nitrate and microbial nitrate reductase activity against caries. European journal of oral sciences. 2004 Oct;112(5):424-8.

7. Javadinejad SH, Talebi MO AG. Nitric oxide concentrations in saliva in relation to caries; experience in 6-12 years old children. J Isfahan Dent Sch. 2008;3(2):71–5. (In Persian)

8. Zhao Y, Vanhoutte PM, Leung SW. Vascular nitric oxide: Beyond eNOS. Journal of pharmacological sciences. 2015 Oct 1;129(2):83-94.

9. Yu B, Ichinose F, Bloch DB, Zapol WM. Inhaled nitric oxide. British journal of pharmacology. 2019 Jan;176(2):246-55.

10. Pašić E, Dedić A, Huskić J, Hadžić S. Concentration of nitric oxide in patients' saliva from various metal restorative activities in the oral cavity. Acta Medica Academica. 2011 Nov 11;40(2):159-65.

11. Šurdilović D, Stojanović I, Apostolović M, Igić M, Kostadinović L. The role of nitric oxide in saliva in reduction of caries. Acta Facultatis Medicae Naissensis. 2008;25(2):93-5.

12. Bogdan C. Nitric oxide and the immune response. Nature immunology. 2001 Oct 1;2(10):907-16.

13. Eagappan AS, Rao VA, Sujatha S, Senthil D, Sathiyajeeva J, Rajaraman G. Evaluation of salivary nitric oxide level in children with early childhood caries. Dental research journal. 2016 Jul 1;13(4):338-41.

14. Assudani HG, Hugar SM, Patil A, Sogi SH, Dhariwal NS, Thakkar P. Evaluation of salivary nitric oxide levels in caries-free children and children with early childhood caries: An: in vivo: study. Indian Journal of Health Sciences and Biomedical Research kleu. 2016 May 1;9(2):190-5.

15. Radcliffe CE, Akram NC, Hurrell F, Drucker DB. Effects of nitrite and nitrate on the growth and acidogenicity of Streptococcus mutans. Journal of dentistry. 2002 Sep 1;30(7-8):325-31.

16. Vanhatalo A, Blackwell JR, L'Heureux JE, Williams DW, Smith A, van der Giezen M, et al. Nitrate-responsive oral microbiome modulates nitric oxide homeostasis and blood pressure in humans. Free Radical Biology and Medicine. 2018 Aug 20;124:21-30.

17. Han DH, Kim MJ, Jun EJ, Kim JB. Nitric oxide modulates levels of salivary Lactobacilli. Acta Odontologica Scandinavica. 2013 Sep 1;71(5):1156-61.

18. Ghasempour M, Qujeq D, Rabiee M, Hamzeh M. Measurement of nitrite and nitrate in saliva of children with different caries activity. The Journal of Contemporary Dental Practice. 2014 Sep 1;15(5):623-5.

19. Cuzzocrea S, Mazzon E, Calabro G, Dugo L, De Sarro A, van de Loo FA, et al. Inducible nitric oxide synthase—knockout mice exhibit resistance to pleurisy and lung injury caused by carrageenan. American journal of respiratory and critical care medicine. 2000 Nov 1;162(5):1859-66.

20. Bejeh-Mir AP, Parsian H, Khoram MA, Ghasemi N, Bijani A, Khosravi-Samani M. Diagnostic role of salivary and GCF nitrite, nitrate and nitric oxide to distinguish healthy periodontium from gingivitis and periodontitis. International Journal of Molecular and Cellular Medicine. 2014;3(3):138.

21. Reher VG, Zenóbio EG, Costa FO, Reher P, Soares RV. Nitric oxide levels in saliva increase with severity of chronic periodontitis. Journal of oral science. 2007;49(4):271-6.

22. Parwani SR, Chitnis PJ, Parwani RN. Salivary nitric oxide levels in inflammatory periodontal disease–A case-control and interventional study. International journal of dental hygiene. 2012 Feb;10(1):67-73.

23. Menaka KB, Ramesh A, Thomas B, Kumari NS. Estimation of nitric oxide as an inflammatory marker in periodontitis. Journal of Indian Society of Periodontology. 2009 May 1;13(2):75-8.

24. Bayindir YZ, Polat MF, Seven N. Nitric oxide concentrations in saliva and dental plaque in relation to caries

experience and oral hygiene. Caries research. 2005 Feb 22;39(2):130-3.

25. Carossa S, Pera P, Doglio P, Lombardo S, Colagrande P, Brussino L, et al. Oral nitric oxide during plaque deposition. European Journal of Clinical Investigation. 2001 Oct;31(10):876-9.

26. Li H, Thompson I, Carter P, Whiteley A, Bailey M, Leifert C, et al. Salivary nitrate–an ecological factor in reducing oral acidity. Oral microbiology and immunology. 2007 Feb;22(1):67-71.