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ORIGINAL RESEARCH

Measurement of Nitrite and Nitrate in Saliva of Children with Different Caries Activity

[1]Maryam Ghasempour, [2]Durdi Qujeq, [3]Maryam Rabiee, [4]Mahtab Hamzeh

ABSTRACT

Objective of the study: Recently, there has been growing interest in the role of salivary nitrate and nitrite in caries protection. Nitrate is a natural compound found in fruits and vegetables and when secreted in saliva, is reduced to nitrite through bacterial respiration and subsequently reduced to nitric oxide in acidic condition. Nitric oxide takes part in oral non-specific immune system and prevents bacterial growth. The aim of present study was to determine the concentration of nitrite and nitrate in saliva of children with different caries activity.

Materials and methods: Ninety three children, 4 to 6 years old, enrolled in this case-control study and were divided into 3 groups; 31 caries free children, 31 with 5 <DFS ≤ 10 and 31 with DFS >10. Unstimulated saliva was collected and stored in 4ºC. Measurement of nitrate and nitrite concentration was performed using Griess reaction. Data were analyzed by T-test, Chi-square, ANOVA and multiple comparisons using SPSS 18. $p < 0.05$ was considered significant.

Results: Mean value of DFS in the first, second and third were 0, 7.12 and 12.61 respectively. Mean value of nitrite and nitrate in the third group was significantly higher than two others ($p < 0.05$), but the difference between first and second group was not significant.

Conclusion: Increase in DFS was associated by increase in salivary nitrite and nitrate concentration.

Clinical significance: High concentration of nitrate and nitrite is not enough for caries prevention.

Keywords: Case-control study, Nitrate, Nitrite, Saliva, DFS.

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[1]Associate Professor, [2]Professor, [3]Private Dentist, [4]Assistant Professor

[1,3,4]Department of Pediatric Dentistry, Faculty of Dentistry Babol University of Medical Sciences, Babol, Iran

[2]Department of Biochemistry and Biophysics, Babol University of Medical Sciences, Babol, Iran

Corresponding Author: Mahtab Hamzeh, Assistant Professor Department of Pediatric Dentistry, Faculty of Dentistry Babol University of Medical Sciences, Babol, Iran, Phone: 00981113232456, e-mail: dr.mahtabhamzeh@yahoo.com

INTRODUCTION

Dental caries is a carbohydrate-mediated infectious disease that is specially regulated by saliva.[1] Caries procedure results from interactions between several risk factors such as microbial flora, diet, host and time.[1] Saliva is known as a part of host factor and a principal part of caries procedure, that prevents caries incidence and development via four different mechanisms:

1. Cleansing effect.
2. Buffering effect.
3. Antibacterial effect.
4. Aupersaturity of calcium and phosphate.[2-4]

On the other hand, saliva contains nitrite and nitrate that may be related to carious defects in the oral cavity.[5] Nitrate is a natural compound, which is found in several types of fruits and vegetables such as lettuce, celery and beetroot and in drinking water. More than 80% of dietary nitrate originates from fruits and vegetables.[6] Exogenous sources of nitrite are less than nitrate and it is found in some kinds of food as an additive ingredient.[7] About 90% of salivary nitrite is the reduced form of salivary nitrate and less than 10% comes from foods.[8] About 25% of the nitrate in plasma is taken up and actively concentrated by the salivary glands, so that salivary concentrations are approximately 10-fold higher than those found in plasma.[5,9-11] About one-fifth of salivary nitrate (approximately 5% of the whole ingested nitrate) is converted to nitrite by the action of nitrate reductase enzymes expressed by microorganisms, in particular those on the surface of the tongue.[12,13] Radcliffe et al proved the inhibitory effect of salivary nitrate [possibly by generating nitric oxide (NO)] on growth and survival of cariogenic bacteria in acid environment.[10] Xia et al have shown that nitrite has cytostatic and cytocidal effect on *Streptococcus mutans, Lactobacillus casei, Salmonella enteritidis, Candida albicans and Streptococcus pyogenes.*[14] Preliminary studies revealed that NO expresses its antibacterial effect in two ways- by inhibition of bacterial growth and/or by increase of macrophage-mediated cytotoxicity.[15] Recently, gross attention is paid to the role of salivary nitrate and nitrite in protection against oral diseases so the aim of this study was to evaluate the amount of salivary nitrate and nitrite in children with different caries activity.

MATERIALS AND METHODS

Study cases were collected from 8 randomly selected kindergartens in Babol, Iran. Oral informed consent was obtained from all parents before sample collection and the study protocol was approved by the Ethics Committee of Babol University of Medical Sciences, Iran. About 93 cases, 4 to 6 years old, were examined. Children had no history of systemic disease, metabolic disorders and did not use iron supplements. Using antibiotic drugs and antibacterial mouthrinse and topical fluoride application during a month prior to the study were considered as exclusion parameters. Index of diseased and filled surfaces (DFS) was determined by one examiner using dental mirror under room light and the children were divided into three groups (31 objects in each group) according to caries experience. Group 1 presented DFS < 1 and considered as control group. Group 2 and 3 presented 5 < DFS ≤ 10 and DFS > 10 respectively and considered as case groups. These three groups were matched by sex and age.

Saliva sampling was performed from 9 to 11 AM. Children had to eat nothing 1 hour prior to sampling. About 0.5 cc of nonstimulated saliva was obtained from each child and collected in sterile plates. Samples were kept in 4ºC refrigerator before laboratory tests. Samples were removed from refrigerator just before examination and transferred to test tubes after reaching the room temperature. Amounts of nitrite and nitrate were assayed spectrophotometrically at 540 nm using Griess reagent (1,000 µl of 1% 1-naphthylethylendiamine, 200 µl of 1% sulfanilamide and 200 µl of 5% phosphoric acid) according to method of Hortelano et al.[16] Data were analyzed using T-test, Chi-square, ANOVA and multivariate comparisons and $p < 0.05$ considered statistically significant.

RESULTS

This study was performed on 93 children, 4 to 6 years old (48 boys, 45 girls). Mean DFS was 0 in group 1 (DFS < 1), 7.12 ± 1.38 in group 2 (5 < DFS ≤ 10) and 12.61 ± 1.68 in group 3 (DFS > 10).

Table 1 shows mean amounts of nitrite and nitrate in saliva of the aforementioned groups.

Amount of nitrite and nitrate in the third group was significantly more than control and second groups ($p < 0.05$). Similarly, amount of nitrite and nitrate in second group was more than control, but the difference was not statistically significant ($p > 0.05$). The results showed that increase in DFS was accompanied by increase in amount of nitrite and nitrate in saliva of the studied children.

DISCUSSION

Based on the results of present study, a positive relationship was found between score of DFS and nitrite and nitrate level in saliva of children with different caries activity means that increase in caries activity was associated by significant increase in nitrite and nitrate level in saliva. Surdilovic found similar outcomes, while evaluating NO and its metabolites concentration in saliva of children with mean age of 13 years.[9] Javadinejad et al performed a study on 6 to 12 years old children and found a positive relationship between degree of dental caries and NO concentration.[17] Zetterquist showed that amount of NO increases along with increase in degree of dental caries.[18] Bayindir found higher NO concentration in plaque of adults with higher diseased missed and filled teeth (DMFT) score.[19] In a study performed by Carossa, it was found that increase in dental plaque is accompanied by increase in NO concentration.[20] As the aforementioned studies showed, amount of NO increases in subjects with high-caries activity. Poor oral hygiene results in high amount of NO, but caries development shows that high concentrations of NO is not sufficient for caries prevention.[9,21] Increase in amount of NO in presence of dental caries, indicates host response to bacterial growth. NO is produced by induction of inducible nitric oxide synthase (iNOS) by microorganisms. Once NO is made, it is rapidly transformed to nitrite and nitrate.[13,19,20] On the other hand, some authors reported contradicted results. Hedge studied children of two different age groups, 6 to 12 years and 71 months and less. Results of their study indicated that amount of NO and nitrite in children with early childhood caries (ECC) and rampant caries is lower than control group with no dental caries.[22] Doel performed a study on children at the mean age of 7 years and Li studied 20 to 48 years objects and both resulted that higher amount of nitrite and nitrate led to less degree of dental caries.[5,6] Li suggested that nitrate is an electron receptor that's presence in anaerobic condition can arrest acidic fermentation and acidity of saliva may be reduced due to its action.[6] It should be mentioned that evaluation of each caries-related factor alone, is difficult and no antibacterial salivary compound solely can be determinant in evaluating the risk of dental caries.[23] Regarding to these results, more clinical studies about the relationship between nitrate and nitrite and dental caries seems to be necessary.

Table 1: Amount of nitrite and nitrate in saliva of studied groups

	Dfs (mean ± SD)	*Amount of nitrite (µM/L) (mean ± SD)*	*Amount of nitrate (µM/L) (mean ± SD)*
Group 1	0	9.30 ± 5.96	60.77 ± 47.70
Group 2	7.12 ± 1.38	11.09 ± 5.96	76.24 ± 52.81
Group 3	12.61 ± 1.68	17.21 ± 11.31	124.78 ± 92.55
p-value	—	0.001	0.001

CONCLUSION

Collectively, there was a significant positive relationship between degree of dental caries and amount of nitrate and nitrite in saliva, but the difference between the amount of these metabolites in children with moderate caries and children with no caries was less accentuated.

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REFERENCES

1. Pinkham JR, Casamassimo Ps, Fields HW, Mcatigue DJ, Nowak AJ. Pediatric Dentistry Infancy Through Adolescence. 4th ed. Elsevier Inc. St. Louis, Missouri 2005;199-204.
2. Mc Donald R, Avery D, Dean J. Dentistry of child and adolescent. 8th ed. Blackwell Munksgaard 2004;215-232.
3. Zehetbauer S, wojahn T, Hiller KA, schmalz G, Ruhl S. Resemblance of salivary protein profiles between children whit early childhood caries and carie-free controls. Eur J Oral Sci 2009 Aug;117(4):369-373.
4. Dowd FJ. Saliva and dental caries. J Dent clin of North Am 1999 Oct;43(4):579-597.
5. Doel JJ, Hector MP, Amirtham CV, AI-Anzan LA, Benjamin N, Allaker RP. Protective effect of salivary nitrate and microbial nitrate reductase activity against caries. Eur J Oral Sci 2004 Oct;112(5):424-428.
6. Li H, Thompson I, Carter P, Whiteley A, Bailey M, Leifert C, Killham K. Salivary nitrate–an ecological factor in reducing oral acidity. Oral Mic Robiol Immunol 2007 Feb;22(1):67-71.
7. Pegg RB, Shahidi F. Unraveling the chemical identity of meat pigments. Crit Rev food Sci Nutr 1997 Oct;37(6):561-589.
8. Archer DL. Evidence that ingested nitrate is beneficial to health. J Food Port 2002 May;65(5):872-875.
9. Surdilovic D, Stojanovic I, Apostolovic M, Igic M, kostadinovic L. The role of nitric oxide in saliva in reduction of caries. ACTA FAC MED NAISS 2008;25(2):93-95.
10. Radcliffe CE, Akram NC, Hurrell F, Drucker DB. Effect of nitrae on the growth and acidogenicity of Streptococcus mutans. J Dent 2002 Sep-Nov;30(7-8):325-331.
11. Sato EF, Choudhury T, Nishikawa T, Inoue M. Dynamic aspect of reactive oxygen and nitric oxide in oral cavity. J Chin Biochem Nutr 2008 Jan;42:8-13.
12. Carlsson S. Antibacterial effects of nitrite in urine. Thesis, Karolinska Institute, Stockholm, Sweden. 2005.
13. Surdilovic D, Stojanovic I, Apostolovic M. Salivary nitric oxide as biomarker of caries risk in children. Acta stomatol croat 2009;43(1):39-44.
14. Xia DS, Liu Y, Zhang CM, Yang SH, Wang SL. Antimicrobial effect of acidified nitrate and nitrite on six common oral pathogens in vitro. Chin Med J 2006 Nov 20;119(22):1904-1909.
15. MacMicking J, Xie QW, Nathan C. Nitric oxid and macrophage function. Annu Rev Immunol 1997;15:323-350.
16. Hortelano S, Dewez B, Genaro AM, Díaz-Guerra MJ, Boscá L. Nitric oxide is released resulting in regenerating liver after partial hepatectomy. Hepatology 1995 Mar;21(3):776-786.
17. Javadinejad SH, Talebi M, Aslani G. Nitric oxide concentration in saliva in relation to caries. J Isfahan Dent School 2007;3(2):71-75.
18. Zetterquist W, Pedroletti C, Lundberg JO, Alving K. Salivary contribution to exhaled nitric oxide. Eur Respir J 1999 Feb; 13(2):327-333.
19. Bayindir YZ, Polat MF, Seven N. Nitric oxide concentration in saliva and dental plaque in relation to caries experience and oral hygiene. Caries Res 2005 Mar-Apr;39(2):130-133.
20. Carossa S, Pera P, Doglio P, Lombardo S, Colagrande P, Brussino L, Rolla G, Bucca C. Oral nitric oxide during plaque deposition. Eur J Clin Invest 2001 Oct;31(10):876-879.
21. Choudhury T, Sato EF, Inoue M. Nitrite reductase in Streptococcus mutans plays a critical role in the survival of this pathogen in oral cavity. Oral Microbial Immunol 2007 Dec;22(6):384-389.
22. Hedge AM, Neekhra V, Shetty S. Evaluation of nitric oxide in saliva of children with rampant caries and early childhood caries: a comparative study. J Clin Pediater Dent 2008 Summer; 32(4):283-286.
23. Kirstilä V, Häkkinen P, Jensch H, Vilija P, Tenovuo J. Longitudinal analysis of the association of human salivary antimicrobial agent with caries increment and cariogenic microorganisms: a 2-year cohort study. J Dent Res 1998 Jan; 77(1):73-80.