Evaluation of Levels of Nitric Oxide in Saliva of Children with Rampant Caries and Early Childhood Caries : A Comparative Study

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It is considered that caries incidence might be low in subjects with high salivary Nitric Oxide (NO) levels. Thus the objective of the present study was to determine the levels of nitric oxide in saliva of children with Rampant Caries (RC) and Early Childhood Caries (ECC). A total of 120 children were divided into 4 groups of 30 each belonging to two age groups of 6-12 yrs and 71 months or less respectively. Children between the age of 6-12 yrs were either with RC or their control and children between the age of 71months or less were either with ECC or their control respectively. The study and control subjects were divided equally. Oral health status was recorded followed by unstimulated salivary flow rate estimation. Estimation of salivary nitric oxide was measured by the concentration of its stable metabolite nitrite using Classical Griess Reaction. The mean nitrite levels of both the control groups were much higher when compared with the study groups, which was statistically very highly significant.

Keywords: *Nitrate; Nitrite; Nitric Oxide; Rampant Caries; Early Childhood Caries; Salivary Flow Rate.*
J Clin Pediatr Dent 32(4): 283–286, 2008

INTRODUCTION

Dental caries is a multifactorial local disease which involves destruction of the hard tissues of the teeth by metabolites produced by oral microorganisms. The uniqueness of dental caries makes it a fascinating study from a scientific standpoint.[1]

Since the caries process involves local, exogenous causal factors it is not surprising that components of salivary secretions can dramatically affect the rate of caries development. Significant factors appear to be elements of the salivary defense system. Saliva and its organic and inorganic components have therefore been extensively studied.[1] Recently, there has been growing interest in the role of nitrate and nitrite in protecting against oral disease.

In humans, ingested nitrate is absorbed from the duodenum and upper ileum into the blood stream and concentrated in the salivary glands by an active transport system, where it increases on concentration up to 10 times that found in plasma.[2] The nitrate is then rapidly reduced to nitrite in the mouth by action of nitrate reductase enzymes expressed by microorganisms. Nitrite may then be acidified through encounter with the acid environment around the dental tissue provided by dental plaque microflora including *Lactobacillus*, *Streptococcus mutans* and *Actinomyces*, which are implicated in dental caries.[3] The acidification of nitrite produces a complex mixture of nitrogen oxides as well as nitrous acid. Nitrous acid is unstable and will spontaneously decompose to produce nitric oxide (NO).[4] NO, being a highly reactive radical, participates in the non-immune system-mediated mechanism of defense of the oral cavity to prevent bacteria from overgrowing.[2]

In vitro studies demonstrated that at acidity levels below pH 7, low concentrations of nitrite caused effective complete killing of *S. mutans*.[5,6] *S. mutans* has been found to be significantly increased in the saliva of patients with rampant caries.[7] In addition saliva from infants with nursing bottle caries contains unusually high levels of *S. mutans*.[8]

The etiology behind the sudden appearance of Rampant Caries (RC) is still unclear and suggests that an overwhelming imbalance of the oral environment seems to accelerate it so that it becomes uncontrollable, when compared to the normal caries pattern.[9]

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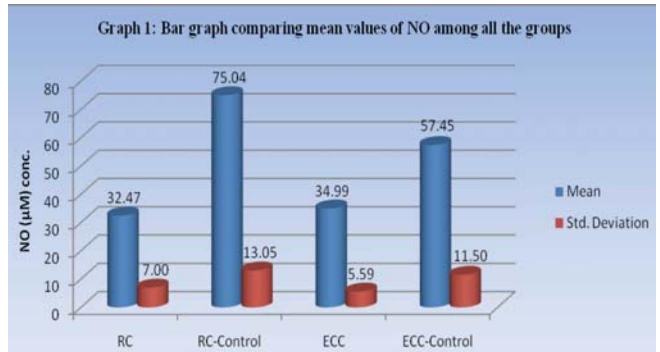
Table 1. Comparison of mean scores of nitrite levels among study and control group

	Group	N	Mean	Std. Deviation	Sig. p value
NO (μM) conc.	RC	30	32.4700	±7.00114	.000 (vhs)
	RC-Control	30	75.0400	±13.04812	
NO (μM) conc.	ECC	30	34.9900	±5.58615	.000 (vhs)
	ECC-Control	30	57.4533	±11.50106	

vhs = very highly significant



Because of the paucity of information available regarding the role of nitrate and nitrite against dental caries, the present study was planned to compare the salivary nitric oxide levels between healthy controls and children with Rampant Caries and Early Childhood Caries (ECC).

MATERIAL AND METHODS

Subjects

Patients reported to the Department of Pedodontics and Preventive Children Dentistry at A.B. Shetty Memorial Institute of Dental Sciences, were included in the study after getting approval by the local ethical committee. The study included 4 groups of 30 children each from two age groups; children of 6-12 years of age for RC[10] and children of 71 months of age or younger for ECC[9] respectively. Children with RC are in group I and their healthy controls are in group II. Similarly, children with ECC are in group III and their healthy controls in group IV respectively.

Clinical Procedure

Oral health status was recorded using a modified WHO format[11] by the same examiner in all the groups. Caries assessment was done using 'DMFT' and 'dft' index. Oral hygiene assessment was done using 'OHI-S' index for mixed dentition and ('OHIS-M')[12] index for primary dentition.

All the subjects were instructed to refrain from eating or drinking for a minimum of 2 hours before the saliva sample were collected. Before treatment unstimulated saliva (1 – 1.5ml) was collected by allowing the patient sitting in the coachman position, the patient was asked to passively drool into a funnel inserted into a graduated cylinder for 5 min. The volume of saliva collected in the cylinder after 5 minutes was divided by 5 to determine the unstimulated salivary flow rate.[9] The collected saliva was stored in glass or plastic vials, in the chiller at 4°C temperature and the NO evaluation was done within 24 hours.

Estimation of salivary nitric oxide was measured by the concentration of its stable metabolite nitrite using Classical Griess Reaction.[13] The results were statistically evaluated using Student's-t test and Pearson's coefficient of correlation with SPSS data processing software version 11.0.

RESULTS

Among the 120 children, the mean nitrite levels of both the control groups were much higher when compared with the study groups, which was statistically very highly significant (Table 1, Graph 1).

The mean nitrite level among the children of 6-12 yr of age was higher when compared with the children of 71 months or younger, which was statistically very highly significant (Table1). There was a positive correlation between the mean nitrite level and age, suggesting that as the average age increases, the salivary nitrite level also increases, which was statistically very highly significant (Table2).

Table 2. Correlation between the age & mean nitrite levels among healthy controls

Age (yr) NO (μM) conc.	Mean
3.00	50.0444
4.00	63.6000
5.00	71.0500
6.00	62.0900
7.00	71.8429
8.00	83.9000
9.00	91.0333
10.00	85.7500
11.00	84.3400
12.00	93.3000

p value = .000

The mean salivary flow rates of both the control groups were higher when compared with the study groups but the difference was not statistically significant. However, a positive correlation was observed between the total nitrite level and the mean salivary flow rate, suggesting that as the average flow rate increases, the mean nitrite level also increases, which was statistically highly significant (Table 4).

A minimal increase in salivary flow rate was observed among all the groups with age but was not statistically significant.

The mean OHI-S score of RC and ECC group was interpreted as 'poor' and 'fair' respectively, whereas of both the control groups was 'good' (Table 5).

DISCUSSION

The role of nitric oxide in saliva and the factors affecting its concentration is still unclear. We attempted to ascertain the correlation between the salivary nitric oxide level and the occurrence of dental caries.

In our study, the mean salivary nitrite levels in control

Table 3. Comparison of mean scores of dft among study group and its correlation with nitrite levels

Group	N	Mean NO (μM) conc.	Mean dft	Correlation between total NO and dft
RC	30	32.4700	4.4667 SD = ±1.59164	
ECC	30	34.9900	5.7667 SD = ±1.22287	
Total	60	33.7300	5.1167 SD = ±1.55238	r = -.566, p = .000 (vhs)

Table 4. Comparison of mean scores of flow rate among study and control group & its correlation with nitrite levels

Group	N	Mean NO (μM) conc.	Mean Flow rate (ml/min.)	Correlation between total NO and Flow rate
RC	30	32.4700	.2660 SD = ±.03539	
RC-Control	30	75.0400	.2847 SD = ±.03329	
ECC	30	34.9900	.2653 SD = ±.04416	
ECC-Control	30	57.4533	.2707 SD = ±.03532	
Total	120	49.9883 SD = ±19.99041	.2717 SD = ±.03763	r = .255, p = .005 (hs)

hs = highly significant

Table 5. Comparison of mean scores of OHI-S & OHIS-M among study and control group & their interpretation

Group	N	Mean NO (μM) conc.	Mean OHI-S & OHIS-M	Interpretation*
RC	30	32.4700	3.2467 SD = ±.64044	Poor
RC-Control	30	75.0400	1.1333 SD = ±.46708	Good
ECC	30	34.9900	2.2967 SD = ±.75589	Fair
ECC-Control	30	57.4533	1.0733 SD = ±.65545	Good

*Good = 0.0 – 1.2; Fair = 1.3 – 3.0; Poor = 3.1 – 6.0

subjects of both the age groups were significantly higher than the study subjects, probably due to the presence of an oral flora with a high capacity to reduce nitrate to nitrite.[14] However, the mean salivary nitrite levels in study subjects of both age groups were not significantly different, but there was a significant difference between the mean salivary nitrite levels in control subjects of different ages, indicating that there could be changes in the salivary nitrite levels among healthy individuals as the age advances.

Since in healthy mammals, nitrate is considered to be largely an inert material, with no enzymes capable of its reduction, the production of the nitrite in the oral cavity is largely depend upon the number of microorganisms capable of reduction of nitrate to nitrite,[15] the succession in the oral microbial ecology with the advancing age could be one of the reason for the alteration in the salivary nitrite levels with age. As shown in Table 2 there is a positive correlation between salivary nitrite concentration and age among the healthy children. Also Mirvish *et al.* showed that salivary nitrite levels increased at night and were higher in older people, especially older men, and salivary nitrate and nitrite levels varied little from day to day, but varied more after 4-6 years.[16]

Human salivary nitrate and nitrite concentrations are greatly influenced by the amount of nitrate in the diet. It is estimated that some 25% of dietary nitrate is secreted into saliva, and most of this nitrate is converted to nitrite by nitrate reductase. The reduction of nitrate to nitrite is enhanced by chewing, which increases salivary contact with the tongue. After intake of a nitrate-rich meal, up to 1.5 mmol of nitrite could enter the stomach. Therefore, it appears that the concentration of nitrite in saliva varies according to dietary nitrate intake, activity of bacterial nitrate reductase, salivary flow rate, and endogenous production of nitrate.[17] Even in the present study we had found a positive correlation between salivary nitrite concentration and the salivary flow rate, indicating that increase in the salivary flow rate probably would result in increase in concentration of salivary nitrite levels.

The etiology behind the sudden appearance of Rampant Caries (RC) is still unclear and suggests that an overwhelming imbalance of the oral environment seems to accelerate it so that it becomes uncontrollable, when compared to the normal caries pattern.[9] *S. mutans* has been found to be significantly increased in the saliva of patients with rampant caries.[7] In addition saliva from infants with nursing bottle caries contains unusually high levels of *S. mutans*.[8] *In vitro* studies demonstrated that at acidity levels below pH 7, low concentrations of nitrite caused effective complete killing of *S. mutans*.[5,6] If this is the case, the presence of less amount of salivary nitrite in children with RC and ECC as found in our study, could be set as one of the salivary factor responsible for the sudden acceleration of caries in the oral cavity of children.

Oral NO production is said to increases during de novo deposition of dental plaque and thus, NO might be an early host defense mechanism against bacterial proliferation in the plaque.[18] in addition the thickness of mature plaque is said to limit the diffusion of oxygen to deeper layers, which would favors the facultative anaerobes that survive in the deeper parts of the plaque for the nitric oxide production[19] which explains that NO level might be higher in individuals with more plaque and ‘poor’ OHI-S score. One interesting finding we found in our study which was contradictory to the previous that, in spite of ‘poor’ OHI-S score in study group, the mean nitric oxide level was less when compared with control group having ‘good’ OHI-S score. This could be because the salivary nitric oxide level in plaque will not only be influenced by the plaque thickness, but may also be influenced by its composition, the plaque pH, the cariogenecity of plaque and the oral microflora.

To date little has been published about NO on its role in oral diseases, but it almost certainly has important and damaging actions against acidogenic bacteria. As nitrate is found in high concentration in green leafy vegetables, some authors hypothesized that increase intake of these vegetables may be especially important in suppressing the growth of acid forming bacteria in children with high caries risk.[5] Although the findings of our study may not finally conclude whether salivary NO concentrations have prognostic significance or not but definitely open some intriguing questions regarding the potential activity of NO in many oral diseases including dental caries and invites for more research in this direction.

CONCLUSION

The salivary nitrite levels are higher in children without caries when compared to children with caries.

As the age advances there is an increase in the salivary nitrite level.

There is a linear relationship between the salivary nitrite level and the salivary flow rate.

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