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Research Article

SALIVARY NITRIC OXIDE (NO_2+NO_3) AS BIOMARKER OF DENTAL CARIES IN ADULTS: AN INVIVO STUDY

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ABSTRACT

The purpose of the study was to evaluate salivary nitric oxide (NO_2+NO_3) as a biomarker of dental caries in adults and to determine the correlation between salivary nitric oxide levels and DMFT index. Eighty healthy adults were divided into two groups: as caries free consisting of 20 individuals and caries active group consisting of 60 individuals. The caries active group was further divided into three subgroups based on the DMFT score (D=decayed, M=missing, F=filled, T=teeth) as follows: Group I (DMFT<3), Group II (DMFT<10) and Group III (DMFT>10), each group consisting of 20 individuals. Saliva collected was estimated for nitric oxide (NO_2+NO_3) level by Griess reaction method. One-way ANOVA was used to correlate between concentration of nitric oxide (NO_2+NO_3) in saliva and DMFT index in caries free and caries active groups. Results are presented as mean ± standard deviation value. The mean level of nitric oxide in saliva of control group was 49.91±15.90 and that of group I, II and III were 32.10±5.91, 32.09±7.61 and 30.14±3.06 respectively. The mean level of nitric oxide (NO_2+NO_3) was higher in caries free group when compared to caries active group and was statistically significant and suggests that increased NO production might contribute to lower caries incidence in adults.

Key words: Nitric oxide, Saliva, Dental Caries

INTRODUCTION

A number of studies are carried out on the onset and prevalence of dental caries, but the etiology of caries is not fully understood and it still remains as the most common disease of modern times[1]. Caries is said to be a multifactorial disease. Different individuals of the same age, sex, race and geographic area sustaining on the similar diets under the same living conditions accentuate the complexity of the caries problem [2].

The multiple functions of saliva play a significant role in the prevention of dental caries. Saliva is often referred to as the "mirror of the body" as it is the indicator of health not just in the oral cavity but also throughout the body [3]. Saliva has an old history of study but its physiological importance has only been recognized recently[4]. Saliva consists of therapeutic, hormonal, immunologic and toxicological molecules, which can provide vital clues to systemic health [5].

Theoretically saliva can affect prevalence of dental caries in four general ways, firstly as mechanical cleansing which result in less accumulation of plaque, secondly by reducing enamel solubility by means of calcium, phosphate, and fluoride, thirdly by buffering and neutralizing the acids produced by cariogenic organisms or introduced directly through diet and finally by anti-bacterial activity [6]. The elements of salivary defense system, i.e., organic and inorganic compounds are the significant factors in caries. A large number of salivary substances have direct or indirect role in caries onset [7-9].

Human oral cavity represents the environment with a constant supply of concentrated nitrates, the metabolic products of nitric oxide (NO). There is a large body of evidence that NO is involved in several inflammatory disorders. Indeed, virtually every cell and many immunological parameters are modulated by NO[10]. The origin of nitric oxide (NO) in oral cavity appears in two ways. It can occur chemically, by physiological reduction of nitrates from food and enzymatically from L-arginine by inducible nitric oxide synthase (iNOS), an enzyme expressed in salivary- glands[11,12]. NO is known to have antimicrobial activity in oral cavity [12]. In humans, dietary nitrates are absorbed in duodenum and upper parts of illeum in blood circulation, and then concentrated in salivary glands by the mechanisms of active transport, reaching the concentration up to ten times higher than the concentration in plasma [13]. In the oral cavity, nitrate reduces into nitrites by the activities of nitrate-reducing microorganisms, present on tongue surface, in fact their enzyme – nitrate reductase. Acid surrounding obtained by existing microflora including *Lactobacillus*, *Streptococcus mutans, Actimomyces* micro organisms implied in dental caries [14], as well as *Staphylococcus Aureus* and *Staphylococcus Epidermidis*[15] brings about the acidification of nitrite in teeth tissues. Nitrite acidification leads to the formation of nitrous oxide and nitrous acid mixture. Nitrous acid is unstable and spontaneously converted into nitric oxide (NO) and nitric dioxide (NO_2).

Low pH value, inevitable for previously mentioned reactions, is obtained in caries lesion, where pH value can decrease even to 3.6. These local sites of extreme pH depression make nitrite conversion to antimicrobial components possible, resulting in autoinhibition of acidogenic bacteria, such as S. Mutans [15].

The aim of the present study was to determine the relationship between salivary nitric oxide (NO_2+NO_3) concentration in saliva of adults in correspondence to DMFT index.

MATERIALS AND METHODS

This study was approved by the Committee for Ethics in Research, Dental College, Nitte University, Karnataka, India. 12,500 healthy adult patients coming to the OPD of Department of Conservative Dentistry and Endodontics, A.B.Shetty Memorial Institute of Dental Sciences under the age group of 25-50 years between December 2010- June 2011 were randomly selected. The patients fulfilling the

inclusion criteria were free from systemic or local disease which affects salivary secretions and their caries status was assessed according to World Health Organization "W.H.O. recommendations 1997" to calculate dental caries index [16]. Patients with periodontal disease, hypertension, diabetes, radiotherapy, chemotherapy, systemic disease of the vital organs and history of long term medications were excluded from the study.

Out of these, 80 healthy adults were selected for the study and divided into groups as caries free consisting of 20 individuals and caries active group consisting of 60 individuals. The caries active group was further divided into three subgroups based on the DMFT score as follows, Group I (DMFT<3), Group II (DMFT<10) and Group III (DMFT>10), each group consisting of 20 individuals.

A detailed case history of the patient was recorded, informed consent read and duly signed by each patient.

The smooth and occlusal surfaces of teeth were cleaned with soft bristle brush, dried and examined. DMFT score calculated.

Collection of saliva was done in the noon time before food to maintain the uniformity of the composition.

Unstimulated saliva was collected from a patient who is not involved in any masticatory function in the last two hours prior to saliva collection and is seated in an ordinary chair and not on any dental / operatory chair to avoid anxiety.

5ml of saliva was collected from the patient, centrifuged and the supernatant obtained was stored at 4°C for subsequent analysis.

Estimation Of Nitric Oxide (NO_2+NO_3) Concentration By Griess Reaction Method

Nitric oxide concentration was measured as total nitrates and nitrites (NO_2+NO_3) by the Griess reaction method. The nitrate present in the sample is reduced to nitrite by reduced nicotinamide adenine dinucleotide phosphate (NADPH) in the presence of the enzyme nitrate reductase (NR).

Nitrate+ NADPH+ H^+→Nitrite+ NADP+ H_2O.

The nitrite formed reacts with Sulphanilamide and N-(1-naphthyl)-ethylene diamine dihydrochloride to give a red violet diazodye.

Nitrite+ Sulphanilamide+ N-(1-naphthyl)-ethylene→ Diazodye.

The diazodye is measured on the basis of its absorbance in the visible reagent at 550nm.

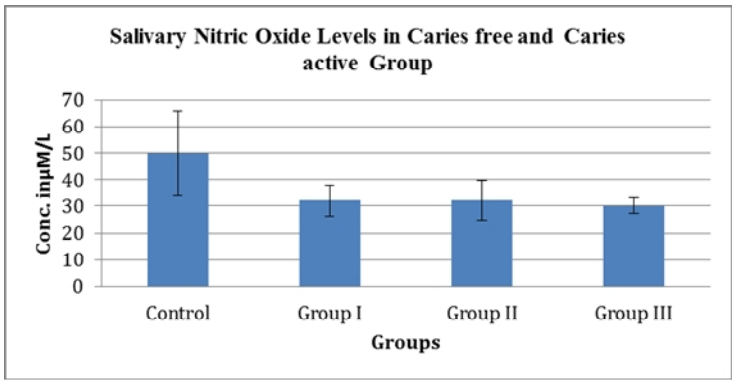
Statistical Analysis

One-way ANOVA was used to correlate nitric oxide levels (NO_2+NO_3) in saliva and DMFT index. Results are presented as mean ± standard deviation value. 'p' value of 0.05 or less was considered significant.

Table 1: Correlation between Salivary Nitric Oxide (NO_2+NO_3) Levels In Caries Free And Caries Active Adults.

Parameters	Caries Free Group Mean±SD (µM/L)	Caries Active Group			'p' value
		Group I Mean±SD (µM/L)	Group II Mean±SD (µM/L)	Group III Mean±SD (µM/L)	
Nitric Oxide (NO_2+NO_3)	49.91±15.90	32.10±5.91	32.09± 7.61	30.14± 3.06	P<0.0001

*p<0.05 is statistically significant. Statistical comparison were performed by One-way ANOVA. Data expressed as Mean±SD



Graph 1: Comparison Of Salivary Nitric Oxide Levels In Caries Free And Caries Active Adults

RESULTS

The mean level of nitric oxide in saliva of control group was 49.91±15.90 and that of group I, II and III were 32.10±5.91, 32.09±7.61 and 30.14±3.06 respectively. 'p' value was statistically significant (P<0.05).

DISCUSSION

Saliva plays an important role in maintaining the equilibrium of the oral ecosystems. This is essential for dental caries control. Whole saliva is a complex mixture of proteins and other molecules which originate from several sources. Saliva promotes bacteria that do not produce acids, and it helps kill undesirable and excess bacteria with the use of nitrate. Dietary nitrate floats unused in our blood until we excrete it via urine as cells do not have much use of nitrate. Some bacteria, however, can use nitrate (NO_3^-) instead of oxygen for respiration, turning it into nitrite (NO_2^-). When nitrite gets in contact with acid it becomes a strong poisonous agent that can kill bacteria in close vicinity. Salivary glands actively accumulate nitrate from the blood and secrete it with the saliva into the mouth [17, 18]. To test the hypothesis that nitric oxide has antimicrobial activity in the oral cavity and could be protective against dental caries, we examined the levels of salivary nitric oxide (NO_2+NO_3), and caries experience in adults.

The results of this study showed that the presence of NO and its metabolites in saliva of adults with natural healthy teeth is significantly higher compared to caries active group, suggesting the protective role of NO in relation to caries and is statistically significant. Similar results are obtained in our previous studies [19].

Nitric oxide is a highly reactive radical, taking part in nonspecific natural defense mechanisms of oral cavity, aiming to prevent bacterial growth and development. Numerous authors [15, 20] prove the ability of salivary nitrites to have an inhibitory effect on growth and survival of cariogenic

bacteria in acid environment. It is believed that NO expresses its antibacterial effect in two ways – by inhibition of bacterial growth and/or by increase of macrophages-mediated cytotoxicity from saliva. Nitric oxide easily passes through cell membranes and can provoke damage of microorganisms by different mechanisms, such as impairment of biological oxidation in mitochondria [21], DNA damage [22] and formation of highly toxic peroxinitrite [23]. Some researches indicate that caries incidence is lower with high level of NO in saliva [20, 24]. Continuous plaque deposition (plaque maturity) makes nitrite conversion with pH level below 7 and iNOS induction possible, leading to the conversion of -arginine into NO [20, 24]. The obtained results suggest that increased NO production might contribute to lower caries incidence in adults. This is in accordance with the results of Carossa et al., who suggested the role of NO in the defense against bacterial proliferation in dental plaque.

CONCLUSION

Dental caries is one of the common diseases in children as well as in adults. Saliva is one of the important factors that influence the development of caries. From the present study, it can be concluded that nitric oxide (NO_2+NO_3) serves as a potential biomarker of caries risk in adults. The results of the study suggest the antimicrobial activity of nitric oxide.

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