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Original Article

EVALUATION OF THE STATUS OF SALIVARY NITRIC OXIDE IN PATIENTS WITH DENTAL CARIES

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Abstract :
The aim of the present study is to evaluate the status of salivary nitric oxide in patients with dental caries. The study consisted of 80 individuals. According to DFMT index, the subjects were divided into control group (DMFT=0) and study group (DMFT>5).Saliva sample was collected from each subject. Nitric oxide concentration was measured as total nitrates and nitrites by the Griess reaction method. The results of this study show that the presence of NO and its metabolites in saliva of adults with natural healthy teeth is significantly higher compared to high risk group, suggesting the protective role of NO in relation to caries. From the study it can be concluded that highly significant increase of nitrates and nitrites in stimulated saliva of high caries risk group could be the host defense response opposing bacterial growth. The obtained results support the role of NO as modulator of bacterial proliferation and suggest that increased NO production might contribute to lower caries incidence in adults.

Keywords: Nitric Oxide, Dental Caries, Saliva, DMFT

Introduction :

Dental caries is the most prevalent dental disease affecting human race although the prevalence of dental caries has significantly reduced; it is still a major problem. The etiology and pathogenesis of dental caries are known to be multifactorial. Saliva is undoubtedly the most important component of oral environment and an integral component of oral health. Saliva helps antimicrobial function by the effects of immunoglobulins and proteins present in fluid. The secretion rate and quality of saliva are important not only in caries development but also for remineralization. The significant factors in caries could be the elements of salivary defense system, i.e. organic and inorganic compounds present in saliva. The most possible reasons for change in the oral balance that is favoring demineralization may be answered by measuring important salivary parameters. Evaluating the causative factors in saliva of individual's at risk to dental caries can pay the way to make recommendations that will cater specifically to individual's needs [1,2].

Human oral cavity represents the environment with a constant supply of concentrated nitrates, the metabolic products of nitric oxide (NO). In oral cavity NO and nitrite, the stable NO metabolite, originate either from physiological reduction of dietary nitrates or from L-arginine undergoing the reaction catalyzed by inducible nitric oxide synthase (iNOS), the enzyme expressed in salivary glands and duct epithelial cells which may be induced by proinflammatory

stimuli. Nitric oxide is a highly reactive radical, taking part in nonspecific natural defense mechanisms of oral cavity, aiming to prevent bacteria growth and development. Numerous authors have proved the ability of salivary nitrites to have an inhibitory effect on growth and survival of cariogenic bacteria in acid environment [3-6].

Nitrites acidification occurs in acid environment of teeth tissues. Acid surrounding is obtained by existing microflora

including Lactobacillus, Streptococcus mutans, Actimomyces, microorganisms implied in dental caries, as well as Staphylococcus aureus and Staphylococcus epidermidis[7]. Nitrite acidification leads to the formation of nitrous oxide and nitrous acid mixture:

NO + H <=> HNO

Nitrous acid is unstable and spontaneously converted into nitric oxide (NO) and nitric dioxide

2HNO <=> H O + N O

N O <=> NO + NO

Low pH value, inevitable for above mentioned reactions, is obtained in caries lesion, where pH value can decrease even to 3. These local sites of extreme pH depression make nitrite conversion to antimicrobial components possible, resulting in auto inhibition of acidogenic bacteria, such as S. mutans .It is well known that NO has strong anti bacterial effect. Nitric oxide easily passes cell membranes and can provoke damage of microorganisms by different mechanisms, such as impairment of biological oxidation in mitochondria, DNA damage and formation of highly toxic peroxinitrite[8,9].

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

Materials And Methods :

This study was conducted in the Central Research Laboratory of Nitte University after the approval from institutional ethical committee.

Subjects :

Study group:

60 adult patients coming to the OPD of Department of Conservative Dentistry and Endodontics, A.B.Shetty Memorial Institute of Dental Sciences, Mangalore with DMFT>5 under the age group of 25-50 years were included in the study.

Control group:

20 healthy adults without caries, in the same age group were taken.

Patients fulfilling the inclusion and exclusion criteria were selected for the study. The inclusion and exclusion criteria used are as follows:

Inclusion criteria :

- Free from systemic or local disease which affect salivary secretions.
- Caries status was assessed according to WHO criteria. Caries active adult having atleast 5 decayed tooth surfaces.

Exclusion Criteria :

Patients with hypertension, diabetes, radiotherapy, chemotherapy, systemic disease of the vital organs and history of long term medications.

A detailed case history of the patient was taken. A case history format was filled, with an informed consent which was duly signed by each patient.

Calculation of DMFT :

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

Collection of saliva :

Saliva was collected from the patients. Centrifuged and only the supernatant was used for the study.

Estimation of Nitric oxide concentration by Griess reaction method

Nitric oxide concentration was measured as total nitrates and nitrites (NO_2+NO_3) by the Griess reaction method. Absorbance is read at 550nm.Concentration is determined using standard graph.

Statistical analysis :

Results are presented as mean $\pm$ standard deviation value. Student 't' test was used to correlate between total antioxidant level and dental caries in study and control groups. A 'p' value of 0.05 or less was considered significant.

Results :

The nitric oxide (NO_2+NO_3) concentration of saliva was higher in control group when compared to that of the study

group. It was found that nitric oxide levels in control group was 6.55±1.57 and that of study group was 1.66±0.49 and it was statistically significant ($p<0.05$) (Table-1,fig-1).

Discussion :

Nitrate arises from oxidation of nitric oxide, one of the most powerful antibacterial compounds, acting through inhibition of bacterial growth or through enhancement of macrophage-induced cytotoxicity. Salivary nitrate is reduced to nitrite and nitrous oxide by oral microorganisms in humans and animals, particularly against highly cariogenic species [10-12].

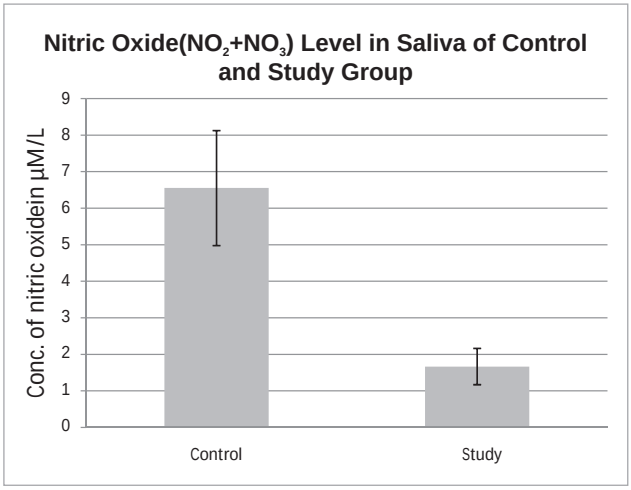
NO can easily penetrate the cell membrane and induce microbial damage through several mechanisms, such as inhibition of iron-containing DNA synthases, reaction with iron-sulphur center of mitochondrial respiratory chain enzymes and combination with superoxide to highly reactive hydroxyl radical [13-14]. Consequently, it could be considered that incidence might be low in subjects with high NO levels.

The results of this study show that the presence of NO and its metabolites in saliva of adults with natural healthy teeth is significantly higher compared to high risk group, suggesting the protective role of NO in relation to caries. The obtained results support the role of NO as modulator of bacterial proliferation and 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[15]. Previously researches have demonstrated the possibility that nitrite in saliva has a dramatic effect on cariogenic bacteria growth and survival, and it is a well known fact that the impairment of saliva excretion promotes dental caries, we believe that the amount of NO in saliva could be an important factor of host defense mechanisms against caries-producing microorganisms[16].

Table 1: Nitric oxide (NO_2+NO_3) concentration in the control group and the study group

Group	N	Mean	Std. Deviation	Std. Error Mean	p value
Control	20	6.55	1.574222	0.352	p<0.0001 (significant)
Case	20	1.66	0.496538	0.111	

Figure1: Graphical Representation of Nitric oxide (NO_2+NO_3) concentration in study group with respect to the control group



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 results obtained it can be concluded that nitric oxide levels in saliva can act as a potential biomarker of caries risk in adults. The results of the study suggest the antimicrobial activity of nitric oxide. The obtained results support the role of NO as modulator of bacterial proliferation and suggest that increased NO production might contribute to lower caries incidence in adults.

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