INTRODUCTION

Theoretical 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. 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. 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. 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. NO is known to have antimicrobial activity in oral cavity. 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. 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, Actinomyces micro organisms implied in dental caries, as well as Staphylococcus Aureus and Staphylococcus Epidermidis brings about the acidification of nitrite in teeth tissues. Nitrite acidification leads to the formation of nitrous oxide and nitrous acid mixture. Nitrosyl acid is unstable and spontaneously converted into nitric oxide (NO) and nitric dioxide (NO2).

ABSTRACT

The purpose of the study was to estimate salivary nitric oxide (NO2+NO3) 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 (NO2+NO3) level by Griess reaction method. One-way ANOVA was used to correlate between concentration of nitric oxide (NO2+NO3) 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 49.9±15.90 and that of group I, II and III were 32.10±5.91, 32.09±7.46 and 30.14±3.06 respectively. The mean level of nitric oxide (NO2+NO3) 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
Dietary nitrate floats unused in our blood until we excrete it.

**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 (NO₃⁻) instead of oxygen for respiration, turning it into nitrite (NO₂⁻). 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 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).

Statistical Analysis

One-way ANOVA was used to correlate nitric oxide levels (NO₂⁻+NO₃⁻) in saliva and DMFT index. Results are presented as mean ± standard deviation value. 'p' value of 0.05 or less was considered significant.

**Estimation Of Nitric Oxide (NO₂⁻+NO₃⁻) Concentration By Griess Reaction Method**

Nitric oxide concentration was measured as total nitrates and nitrites (NO₂⁻+NO₃⁻) 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+ H₂O.

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.

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

**Table 1: Correlation between Salivary Nitric Oxide (NO₂⁻+NO₃⁻) Levels In Caries Free And Caries Active Adults.**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Caries Free Group Mean±SD (μM/L)</th>
<th>Caries Active Group Mean±SD (μM/L)</th>
<th>'p' value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitric Oxide (NO₂⁻+NO₃⁻)</td>
<td>49.91±15.90</td>
<td>32.10±5.91</td>
<td>32.09±7.61</td>
</tr>
</tbody>
</table>

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

**REFERENCES**


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