

**EVALUATION OF SALIVARY NITRIC OXIDE WITH RESPECT TO DENTAL CARIES
AND PERIODONTAL STATUS IN CHILDREN WITH DOWN'S SYNDROME.**

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ABSTRACT

Aim – To determine correlation of salivary nitric oxide (NO) levels with dental caries and periodontal status in children with Down's syndrome. **Methodology**- Fifty children in the age group of 4-14 years included in the study were divided into control group consisting of 25 healthy children, and study group consisting of 25 children with Down's syndrome. Caries score of each child was calculated using DMFT/dmft index, oral hygiene status was recorded using simplified oral hygiene index (OHI-S) and periodontal status was recorded using WHO oral health assessment form for children (2013). Unstimulated saliva was collected from each child and NO was estimated using modified Greiss reaction method. **Results**- Mean DMFT/dmft score of normal children was significantly higher ($p < 0.001$) than that of children with Down's syndrome. Higher gingival bleeding scores on probing and salivary NO levels were obtained in children with Down's syndrome, than in normal children. Negative correlation was found between salivary NO levels and caries experience in both groups. **Conclusion** – Salivary NO plays a protective role against caries pathogens, but does not show a protective role against periodontal pathogens. Increased dietary nitrate may help in increasing salivary NO levels further decreasing dental caries experience among children.

KEYWORDS: Salivary nitric oxide, dental caries and Down's syndrome.

INTRODUCTION

Down's syndrome, a genetic disorder resulting from an extra copy of chromosome 21, is known to be the most common genetic cause of mental retardation.^[1] The incidence of dental caries in individuals with Down's syndrome has been reported to be extremely low, which still remains controversial.^[1,2] However, authors have found periodontal diseases to be more prevalent in these individuals.^[1,3,4] Saliva is one of the primary needs for the protection of dentition against dental caries. It shows multiple anticariogenic functions which are related to its fluid characteristics especially the dilution and washing effects.^[5] Salivary nitrite and nitrate obtained from both metabolic and dietary sources have been seen to play a role in protection against various oral diseases through production of nitric oxide (NO), which is a free radical gas present in atmosphere and also in saliva in trace

amounts.^[6] It has an ability to penetrate bacterial cell membrane easily and shows bacteriostatic effect by inhibiting bacterial DNA synthetase and combining with mitochondrial enzymes required for bacterial respiration.^[7] This suggests that it is a strong antibacterial compound which may show an antibacterial effect against caries pathogens as well. Therefore, salivary NO levels can be of value for future caries prediction and diagnosis. Hence, the aim of the present study was to determine the correlation of salivary NO levels with dental caries and periodontal status in children with Down's syndrome.

MATERIALS AND METHODS

The control group consisted of 25 healthy children in the age group of 4-14 years, who attended as outpatients of the department. The study group consisted of 25 children

in the age group of 4-14 years, diagnosed with Down's syndrome. Following the institutional ethical committee approval, saliva samples were collected with the parent's consent. Caries status was recorded with DMFT / dmft index, oral hygiene status was recorded using OHI-S index and periodontal status was recorded using WHO oral health assessment form for children (2013).^[8]

Saliva sample collection

Unstimulated saliva was collected in the morning hours and all the participants were asked to refrain from eating, drinking and tooth brushing 90 min before saliva collection. Instructions were given to the caretakers of the children with Down's syndrome. In order to eliminate the effect of the sympathetic tone, the participant in an upright position was asked to sit in a relaxed position with no movements and talking for few minutes. 1 ml of saliva sample was collected in wide mouthed sterile containers and refrigerated.

Nitric oxide estimation

NO estimation was done by modified Greiss reaction as suggested by Miranda. *et.al.*^[9] This method determines the total nitrite/nitrate level based on the reduction reaction of any nitrate to nitrite by vanadium, followed by the detection of total nitrite (intrinsic + nitrite obtained from reduction of nitrate) by Griess reagent. The Griess reaction involves the formation of a pink colored chromophore from the diazotization of sulfanilamide by acidic nitrite, followed by coupling with bicyclic amines such as N-(1-naphthyl) ethylenediamine, which can be measured calorimetrically at 540 nm.

Procedure

An Eppendorf tube was taken and 0.75 ml cold absolute ethanol was added to 0.75 ml saliva, which was left for 48 hours in the refrigerator to attain complete protein

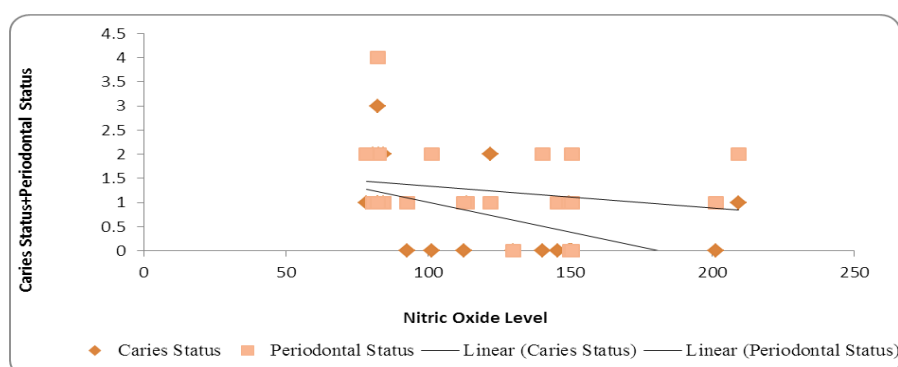
precipitation. The mix was then centrifuged at 4000 rpm at 12°C for 30 min using cooling centrifuge. 250 µl of the obtained supernatant was used, to which 250 µl vanadymyrichloride was added followed by rapid addition of 125 µl sulfanilamide (2% (w/v) in 5% HCl) and 125 µl of N-(1-Naphthyl) ethylenediamine dihydrochloride (0.1% (w/v) in distilled water). The mixture was then left at room temperature for 30 min, after which the absorbance of the obtained pink coloured chromophore was measured at 540 nm using a double beam spectrophotometer against a blank treated in the same manner to the test but using 250 µl distilled water instead of the sample. The standard was treated exactly as the supernatant and measured against a blank reagent containing 250 µl distilled water.

RESULTS

On comparison of caries status in both the groups, mean DMFT/dmft score for healthy children was 3.16 which was significantly higher ($p < 0.001$) than a mean score obtained for children with Down's syndrome which was 0.72, which is suggestive of lower incidence of caries in children with Down's syndrome. The OHI-S scores for children with Down's syndrome were higher with increased gingival bleeding on probing scores as compared to normal children. On comparing the NO levels in both the groups, significantly higher ($p < 0.001$) level was found in children with Down's syndrome which was 123.16 µm/l, than the level obtained in normal children which was about 82.79 µm/l [Table 1]. On correlating the mean DMFT/dmft score with the mean NO levels in both the groups, a decrease in caries experience was noted with increasing levels of salivary NO, which is suggestive of the negative correlation between the two. Also, a negative correlation was found between the oral hygiene and periodontal status and NO levels [Graph 1].

Table 1- Caries status and nitric oxide levels in study subjects

Parameters	Children with Down's syndrome	Normal Healthy Children	t-value	p-value
Nitric Oxide Level	123.16±37.94	82.79±22.16	4.59	S, $p < 0.001$
Caries Status	0.72±0.89	3.16±1.28	7.82	S, $p < 0.001$
Oral hygiene Status	1.24±0.83	0.36±0.48	4.56	S, $p < 0.001$



Graph 1- Correlation of caries status, oral hygiene status and salivary Nitric oxide levels

DISCUSSION

Dental caries is the most prevalent infectious disease which is caused by acid-producing pathogens inhabiting the mouth that dissolve the tooth structures in the presence of fermentable carbohydrates.^[10] Any infectious disease occurs only when the pathogenic organisms are sufficient in number to surmount the intra-oral defence mechanisms. Hence, innate host defence mechanism against bacteria causing dental caries plays an essential role.^[5] A number of salivary factors play a role in protection of dentition against dental caries. Evidences have suggested a beneficial, antimicrobial role of inorganic nitrate in several systems in humans, including the gastrointestinal tract, oral cavity and skin.^[11,12,13] In the oral cavity, nitrate reduces into nitrites by the activities of nitrate-reducing microorganisms, 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 (NO₂).^[14] In 2004, J.J Doel *et.al.*^[15] stated that salivary NO may limit the growth of cariogenic bacteria. NO produced in large amounts shows antibacterial properties by causing modifications in bacterial DNA, respiratory complexes and interaction with other reactive species.^[16] It is the part of the mechanism for killing used by macrophages through action of inducible NO synthetase enzyme.^[17] Accordingly, when nitrite comes in contact with acids that are produced by acidogenic bacteria such as *S.mutans*, a bolus of NO gets formed resulting in its antimicrobial effect.^[18] Hence, NO through its antibacterial property might act as innate host defence mechanism against dental caries.

Dietary nitrates which are present in large quantities in foods such as green leafy vegetables are absorbed from the small intestine into the bloodstream, which are then actively concentrated by the salivary glands. These glands contain NO synthetase, immunoreactive neurons and periacinar, periductal and perivascular nerve fibres, which help in the synthesis of NO in acinar cells of salivary glands. It has seen to be important in salivary vasoregulation and secretion.^[19] The salivary nitrate concentration is approximately 10-fold higher than that found in plasma.^[20] It is found to be about 1500 μ M, depending largely upon dietary nitrate intake. However, nitrate concentration varies widely according to the quality and preparation of the food, for example, boiling results in a loss of nitrate from most vegetables.^[15] Saliva collection being easy and non-invasive can be used as a biomarker for caries risk. NO in saliva can be measured using various direct and indirect methods.^[5] In earlier studies Greiss reaction method was used in which Cadmium was used as a reducing agent. Use of modified Greiss reaction method was suggested by Miranda *et.al.*^[9] which is seen to measure the levels of unstable NO more accurately. Hence, Modified Griess reaction method was used in the present study.

Salivary NO levels showed a negative correlation with the caries status in children with Down's syndrome,

which may be suggestive of protective role of NO in saliva. Similar results were seen in the previous studies by T. Choudhury *et.al.*^[21] and Dusans *et.al.*^[22] where higher NO levels were seen in caries free group when compared with caries active group. However, results were contradictory to results obtained by Bayindir. Y.Z *et.al.*^[6] where it was difficult to consider NO as a host defence mechanism in presence or absence of dental caries. In the present study, higher gingival bleeding scores and higher NO levels in children with Down's syndrome were obtained. However, in a study carried out by Andrej Aureur *et.al.*^[19], it was found that NO levels were decreased in subjects with periodontal conditions as compared to normal subjects. The reason for increased gingival inflammation in children with Down's syndrome inspite of higher levels of salivary NO as compared to normal children may be due to genetically influenced decreased immune response in this condition. In 1971, Dr. Cutress^[23] found that prevalence of periodontal disease is due to the immunological factor inherent to Down's syndrome as well as to environmental factors. Several studies have revealed an important change in the host's immune response, where many factors are altered such as chemotaxis, phagocytosis, oxidative response as well as an abnormal bactericidal activity of polymorphonuclear leucocytes.^[24, 25,26] Dr. Armano *et al*^[27] incorporated other factors to the cause, such as weak periodontal tissue, microrizosis, unfavorable root-crown relationship and poor masticatory function. It has been suggested that certain local factors exert influence on periodontal disease: these could be macroglossia, malocclusion and bruxism, all of which are frequently exhibited by Down's syndrome patients.^[23] 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.^[28] It was observed that in addition to the dietary sources, other reasons for the differences include differences in local parasympathetic, hormonal, cytokine, inflammatory or endothelial cell activity. Various genetic and environmental factors influence the oral defense mechanism in an individual, so do they affect the production of NO in saliva.^[19] In the present study, higher levels of salivary NO levels were found in children with Down's syndrome as compared to normal children. This finding may confirm a protective role of NO against dental caries in children with Down's syndrome. Consumption of diet rich in green leafy vegetables providing adequate dietary nitrate may improve the oral defense mechanism and help in decreasing caries experience among children.

CONCLUSION

Salivary NO plays a protective role against caries pathogens. However, it does not show a protective role against periodontal pathogens. Increased dietary nitrate may help in increasing NO levels in saliva further decreasing dental caries experience among children.

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