



QUALITATIVE ANALYSIS OF SALIVARY PH IN DENTAL CARIES USING SNYDER'S TEST

Dental Science

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ABSTRACT

INTRODUCTION: Dental caries is a disease with multifactorial etiology. Early detection along with preventive measures is essential for the control of caries. Caries activity tests are the methods of early detection of caries. **AIM:** The purpose of this study was to test the salivary pH in dental caries patients using Snyder test. **MATERIALS AND METHODS:** A total of 100 saliva samples were collected. Snyder's solution was prepared and 0.2ml of collected saliva was incorporated in each test tube containing 10 ml of solution and mixed by gentle shaking of test tubes. pH and colour change of the solutions were recorded. Caries activity was assessed. **RESULTS:** Statistically highly significant difference seen for the values between the groups for DMFT index, pH values. Conclusion: Our study showed Snyder test to be a simple and an inexpensive method for assessment of caries activity. We also found a close relationship between DMFT index and salivary pH value which could be suggestive of increased risk of dental caries within an acidic environment. We can conclude by stating that salivary flow, pH and buffering capacity are contributory factors in maintaining the integrity of oral cavity. The increase in these factors can result in reduction of caries activities. **CLINICAL SIGNIFICANCE:** As there is marked increase in the prevalence of dental caries, caries activity tests are a valuable adjunct for patient motivation in plaque control program.

KEYWORDS

Dental Caries, Caries Activity, Snyder Test

INTRODUCTION

Dental caries is considered to be the most prevalent oral disease worldwide and main cause of tooth loss among the population. The word caries is derived from Latin word means 'rot' or rotten.^[1] At the 1948 consensus conference, dental caries was defined as a disease of the calcified tissues of the teeth. It is caused by acids resulting from the action of microorganisms on carbohydrates, is characterized by a decalcification of the inorganic portion and is accompanied or followed by a disintegration of the organic substance of the tooth.^[2] Worldwide, approximately 36% of the population have dental caries in their permanent teeth. In deciduous teeth it affects 9% of the population. It is the primary pathological cause of tooth loss in children.^[3]

The DMFT index that measures the number of permanent teeth decayed, missing and filled teeth. Distribution of caries in the world was analyzed by DMFT index in which the average was 2.11 (\pm 1.32). The Southeast Asian region alone showed an average of 1.95 (\pm 1.24).^[4]

The destructive process of the tooth by the demineralization were proposed by several modern theories: Acidogenic (Chemico-parasitic) theory by D. Miller 1890, Proteolytic theory by Gottlieb, Proteolytic chelation theory by Schwartz, Martin and coworker, Sucrose chelation theory by Egger-Lura, Phosphate Sequestra theory by Kreitzman et al, Autoimmunity theory by Jackson and Burch 1972 and Genetic theory. One such theory that has been accepted almost universally is the 'chemo-parasitic theory.' This theory proposed by W. D. Miller in 1881. According to this theory, 'all and any of the salivary microorganisms' that were acidogenic were responsible for the decalcification of the tooth structure. In 1898 G.V Black and J.L. Williamin explained the entity of 'dental plaque,' a colonization of endogenous microorganisms on the tooth surface that caused tooth dissolution. In 1950s to 60s, the landmark studies of Orland et al. and the "Keyes and Fitzgerald" had proven the strong causal relationship of certain specific microorganisms such as Streptococci, Lactobacilli, and Actinomyces present in the dental plaque with the incidence of caries.^[1] These microorganisms along with accordance with the low pH of saliva and lack of fluoride can disrupt the equilibrium between the microbiota and

tissue resulting in demineralization of enamel, dentin and cementum, due to fall in pH below a critical value.^[5]

Worldwide, the main species associated with caries in humans is *Streptococcus mutans*, which is part of the "mutans streptococci group", including species with different natural hosts, such as *S. mutans* and *S. sobrinus*, *S. cricetus*, *S. rattus*, *S. ferus*, *S. macacae* and *S. downei*.^[6] Host related factors are important contributors to a person's dental caries susceptibility, resistance or both. It is well established that saliva plays an important role in the health of soft and hard tissues in the oral cavity. Chronically low salivary flow rate is one of the strongest indicators of increased caries risk.^[7]

Caries activity can generally be defined as the occurrence and the rate at which, teeth are destroyed by acid production by the plaque bacteria. It is very difficult to measure all the factors responsible for caries using one caries activity test. Hence, until date the ideal method to evaluate caries activity in terms of sensitivity, specificity and reliability has not been found.^[8,9]

Snyder Test Agar, also known as BCG Dextrose Agar, is used for estimating the relative number of lactobacilli in saliva based on production.^[3] It measures the rapidity of acid formation when a sample of stimulated saliva is inoculated into glucose agar adjusted to pH 4.7 to 5 and with bromocresol green as color indicator. Indirectly the test also measures the acidogenic and aciduric bacteria.^[3] Hence the purpose of this study was to test the salivary pH in dental caries patients using Snyder test, to assess and analyze the caries susceptibility in respect to gender versus time and age versus time.

MATERIALS AND METHOD

The present study was carried out in the Department of Oral pathology and Microbiology, BIDS, Patna, Bihar. A total of 100 saliva samples were collected.

Procedure

Patients were asked to rinse the mouth with water; then given to chew paraffin and had requested to spit saliva that had collected in a sterile

vial. Snyder's solution was prepared and 0.2ml of collected saliva was incorporated in each test tube containing 10 ml of solution and mixed by gentle shaking of test tubes. The test tubes were kept in rack in vertical direction and placed in incubator. Temperature of the incubator was maintained at 37°. The medium was melted and then allowed to cool to 45°. pH and colour change of the solutions were recorded. Data was obtained on MS office Excel sheet and then subjected to statistical analysis using statistical package SPSS V210 IBM. For all the statistical tests, $P < 0.05$ was considered to be statistically significant, keeping α error at 5% and β error at 20%, thus giving a power to the study as 80%.

RESULTS

The mean age was 34.47 ± 12.00 . [Table no: 1] 41% were females and 59% were males. [Table no: 2] The mean DMFT index of the sample was 6.30 ± 1.850 . [Table no: 3]

Statistically high significant difference between groups for DMFT index, pH values at 24 hrs and 48 hrs and 72 hrs ($P < 0.01$). [Table no: 4]

Statistically highly significant difference seen for the values between the groups for DMFT index, pH values at 24 hrs, 48 hrs and 72 hrs ($p < 0.01$) [Table no: 5]

A comparison of numerical value between the genders was done. There was a statistically non-significant difference seen for the value between the gender ($P > 0.05$) thus indicating that gender did not have an influence on the outcome of variable. [Table no: 6]

When Bivariate correlation of age versus DMFT & pH at various time intervals was carried out, statistically non significant correlations were seen with age, indicating that age did not have an influence on the outcome variables. [Table no: 7]

DISCUSSION

Dental caries is a multifactorial disease influenced by many factors including age, gender, micro-organisms, salivary flow rate, diet and oral hygiene.^[10] Dental caries is established in the mouth long before it becomes clinically manifested as a viable lesion.^[11] Several caries activity tests have been developed to detect the presence of oral conditions associated with increased risk of caries. Currently no single caries activity test can predict caries with a high degree of accuracy. Because many of these tests rely on samples of salivary bacteria, the reliability of such tests is limited; bacteria that are free floating in the saliva may not be representative of the bacteria in plaque. Other tests measure the plaque index (amount of plaque present), but also are insufficient for complete assessment of caries risk.

Individual caries activity tests, despite their limitations, can be a useful adjunct and can be used to motivate patients and to determine patient compliance with treatment regimens.^[12]

A variety of microbial factors have been associated with caries activity. Various observations indicate an important relationship between *Streptococcus mutans* and the development of the early carious lesion of enamel. Lactobacilli, however seem to be associated with dentinal caries and *Actinomyces* strains with root surface caries. The fact that *S. mutans* is closely associated with caries does not mean that it is the only microorganism causing caries, nor does it imply that *S. mutans* is always cariogenic. The ability to produce acid is a prerequisite for caries induction, but not all acidogenic organisms are cariogenic.^[13]

Borgstrom and colleagues reported that the most common method used to identify caries-susceptible people is estimating the number of cariogenic bacteria such as lactobacilli and mutants streptococci mutans in saliva or plaque samples taken from the patient.^[14]

Saliva serves as a major component in most caries activity tests, and aids in the categorization of patients into high, medium and low caries activity. Although a multiplicity of tests have been described in the literature, none of them are currently available methods with satisfactory results. A study carried out by Ferraro M (2010) revealed female were more prone to caries when compared to males. The possible reason for this disparity was postulated to be multifactorial. He said that males may be expressing a greater amount of amelogenin contributing to the strength of the tooth hence less caries susceptible.

Salivary IgA is an immunoglobulin found in the oral cavity which acts as a protective mechanism against caries, was detected in low concentration in women saliva. Pregnancy also has negative effects on salivary flow, impairing the protective washing and buffering mechanisms of saliva against caries development.^[15]

Hormonal fluctuation of estrogen also occurs in females during pregnancy, menstruation & puberty. The elevated estrogen level can lead to significant changes in the environment of the oral cavity. The earlier exposure of female teeth to the oral cavity provides long exposure to oral environment, bacteria, when compared to males of same age providing more opportunity for caries process to develop.^[15]

The Gender distribution of samples in our study showed increased incidence of males (59%) as compared to females (41%) while the studies conducted by Ellaisson et al (2006)^[16] showed decrease in the concentration of salivary IgA in females, hence increase in the incidence of dental caries in females.

In our study mean DMFT index of the sample was found to be 6.30 ± 1.850 . The mean DMFT of the sample subject was 6.30 ± 1.850 . Based on the DMFT index an intergroup comparison of variables was carried out. A statistically highly significant p value of 0.000 was found between the DMFT index and pH of the solution. [Table 4] A study carried out by Kunte et al^[8] showed similar result, where the DMFT index co-related well with the existing caries status of the individuals.

Comparison was made between the DMFT index of the patient with the pH values of solution found at the time of inoculation of saliva, of the 24, 48, and 72 hours. A statistically highly significant difference was seen. [TABLE 5]

In our study all the sample were categorized as subgroup A having DMFT index between 1 to 5 and Subgroup B having DMFT index between 6 to 10. Hence it was found that with increase in the DMFT index the pH value of saliva was found to be decreasing this finding was in accordance with Chitharanjan shetty et al. They found out that the salivary parameters in the saliva of young healthy adults of the age group of 20 to 30 years studies as pH, flow rate, buffering capacity which showed a study with an increase in the DMFT value and was statistically significant.^[17]

In our study changes in the color of the Snyder solution was recorded and subjected to static analysis. The change in the color of the solution was recorded at the time of inoculation of saliva as well as at 24, 48, and 72 hours. There was statistically high significant difference seen for the frequencies of various types of color at 48 and 72 hours between the DMFT sub groups 1 and 2 with lower frequency for yellowish green in subgroup 1 while higher of yellowish green in subgroup 2 at 48 hours. The change in the color of the solution was recorded in relation to the change in the pH of the solution. In a study carried out by Ramesh et al. sensitivity of Snyder's test was found to be 87.5% along with positive predictive value of 18.9%. Specificity of 18.9% was obtained with negative predictive value of 87.5% in caries free individuals.^[11]

In our study DMFT index was co-related with gender and numerical value of pH, but a statistically non-significant difference seen for the values between the genders indicating that gender did not have an influence on the pH changes. [Table: 6] Similar study was carried out by Lorne et al^[9] and they showed similar finding. They stated that there was no significant difference found between females and males with respect to the caries indices (DMFT).

A Bivariate correlation of age versus DMFT and pH at various time intervals was carried out, statistically non significant correlations were seen with age, indicating that age did not have an influence on the outcome variables. [Table: 7] This finding was in contrary to the findings of Lorne et al. where they stated that caries was significantly age related.^[9]

Thus this present study shows that there exists close correlation between the salivary pH and the DMFT Index.

CONCLUSION

Dental caries is a disease with multifactorial etiology which begins with the loss of ions from the apatite crystals that leads to cavitation. An objective evaluation of caries activity requires clinical examination

and quantification of factors associated with the pathogenesis of caries like host, microorganisms and diet. Caries activity tests are valuable adjunct for patient motivation in plaque control program. In our study Snyder test was performed and it was found to be a simple and an inexpensive method for assessment of caries activity. We observed a close relationship between DMFT index and salivary pH value which could be suggestive of increased risk of dental caries within an acidic environment. All caries activity test developed so far are based on microbiological aspects of dental caries. There is no ideal test at present time for accurate establishment of caries activity. Further detailed studies are required for other factors associated with dental caries prevalence to know prognosis as well as prevention of dental caries. We can conclude by stating that salivary flow, pH and buffering capacity are contributory factors in maintaining the integrity of oral cavity. The increase in these factors can result in reduction of caries activities.

Clinical significance

The ability to utilize saliva to monitor the health and disease state of individuals is a highly desirable goal for health promotion and health-care research.

REFERENCES

1. Usha C, Ramarao S. Dental caries - A complete changeover Part I. *J Conserv Dent*. 2009;12(2):46-54.
2. Ismail A, I, Hasson H, Sohn. W. Dental Caries in the Second Millennium. *Journal of Dental Education* 2001; 65(10): 953-59.
3. Yadav K, parkas S. Dental Caries: A Review. *Asian Journal of Biomedical and Pharmaceutical Sciences*. 2016; 6(53)01-07.
4. Moreira R. Epidemiology of Dental Caries in the World, *Oral Health Care - Pediatric Research, Epidemiology and Clinical Practices*, Prof. Mandeep Viridi (Ed.), 2012; ISBN: 978-953-51-0133-8, Intech.
5. Veiga N et al. Dental Caries: A Review. *Journal of Dental and Oral Health*. 2016; 2 (5) 43.
6. Carletto-Korber F P M et al. Serotype diversity of Streptococcus mutans and caries activity in children in Argentina. *European Journal of paediatric dentistry*. 2015; 16(3) 177-80.
7. Zero D T et al. The biology, prevention, diagnosis and treatment of dental caries. *American Dental Association*. 2009; 140 (9) 25-34.
8. Kunte S Sanket, Chaudhary S, Singh A, Chaudhary. Evaluation and co-relation of the oratest, colorimetric Snyder test and Salivary Streptococcus mutans count in children of age group of 6-8 years. *Journal of International society of preventive and community dentistry*. 2013; 3 (2) 59-66.
9. Koroluk L, Hoover N J, Komiya K. The sensitivity and specificity of a colorimetric microbiological caries activity test (Cariostat) in preschool children. *Journal of pediatric dentistry*. 1994;16(4) 276-281.
10. Deshpande S N, Kadam D G, Ingle S B. Studies on determination Pharma Bioallied of susceptibility to dental caries among school children. *International Journal of pharma and Bio Sciences*. 2012;3(2):809-13.
11. Ramesh K, Kunjappan S, Ramesh M, Shankar S, Reddy S. Comparative evaluation of predictive value of three caries activity test-snyder, lactobacillus count and cariostat in mixed dentition children with and without caries. *J Pharma Bioall Sci*. 2013;5:63-8.
12. Robertson TM, Heymen HO, Swift EJ, Sturdevant's Art and science of operative dentistry, 5th Edition, 2006, Elsevier India private Limited.
13. Peter. S Essentials of Preventive and Community Dentistry, 4th edition, 2009, Arya Medi Publishing House Pvt. Ltd.
14. Namarugommula. N, Arun A, Mythri H. Caries activity tests. *Research and Reviews: Journal of dental Sciences*. 2013;1(3) 50-58.
15. Ferraro M, Vieira A R. Explaining gender differences in caries: A multifactorial approach to a multifactorial disease. *Hindawi Publishing Corporation International Journal of Dentistry* Volume 2010; Article ID 649643, doi:10.1155/2010/649643:1-5.
16. L. Eliasson, D. Birkhed, T. Osterberg, A. Carlen. Minor salivary gland secretion rates and immunoglobulin A in adults and the elderly. *European Journal of Oral Sciences* vol. 114, 6 pp. 494-499, 2006
17. Chitharanjan Shetty et al. Correlation between dental caries with salivary flow, pH, and buffering capacity in adult south indian population: an in- vivo study. *Int. j. Res. Ayurveda Pharm*. 2013; 4(2):219-23.