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ASSESSMENT OF PHYSIOCHEMICAL PROPERTIES AND BACTERIOLOGY OF SALIVA IN INDIVIDUALS WITH DIFFERENT CARIES RISK SEVERITY: A RETROSPECTIVE RANDOMIZED STUDY

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ABSTRACT

Introduction: This study assesses the salivary parameters in individuals using the modified caries risk assessment form. **Methods:** Fifty male (n=29) and female (n=21) patients in the OPD of Conservative Dentistry and Endodontics of BVDUDCH, Navi Mumbai, were enrolled after obtaining written consent. All participants underwent modified caries risk assessment form, salivary parameters (stimulated and unstimulated flow, pH, buffering capacity, consistency), salivary CFU count for Str. mutans and Lactobacilus using the CRT kit, and presence of Str. mutans using the Saliva-Check mutans rapid detection kit. **Results:** Eight patients were in moderate-risk, 32 in high-risk and 10 were in extremely high-risk category as per the modified caries risk assessment form. The mean unstimulated salivary flow rates were lower (p, 0.001) in the higher risk categories. The buffering capacity was 'very low' in 70.0% patients with extremely high-risk, 43.7% patients with high-risk and all moderate-risk patients had normal buffering capacity. None of the patients had highly acidic salivary pH, whereas more patients in extremely high-risk group had higher pH than those with high-risk and moderate-risk groups (p, 0.326). Saliva viscosity was normal in all moderate-risk patients, and increased viscosity was observed in 59.4% patients with high-risk and 80.0% patients with extremely high-risk (p, 0.0019). Higher CFU/ml was observed for Str. mutans (p, 0.0196) and Lactobacillus (p, 0.0031) in patients with extremely high-risk and high-risk compared to moderate-risk patients. **Conclusion:** Caries risk assessment form could be a quick and convenient method for identifying potential caries susceptibility and should be adopted in regular clinical practice

KEYWORDS: Caries risk assessment, Saliva, Bacteriology, Streptococcus mutans, Lactobacillus.

INTRODUCTION

Dental caries, one of the most prevalent chronic disease, affects 97% of the adults and children, with continuous susceptibility and risk throughout life. Dental caries is a demineralisation process which invariably leads to the formation of a cavity if left untreated. The process is multifactorial, and the aetiology could include poor oral hygiene, reduced salivary flow, increased frequency of fermentable carbohydrate intake, acidogenic bacteria in dental plaque especially Streptococcus Mutans and Lactobacillus sp., etc. To bring this process to a halt, there must be a balance between the protective and pathogenic factors.[1]

Caries risk is the probability that the individual will develop a carious lesion over a specified period of time. Complete oral health care entails treatment at two levels: 1) Therapeutic 2) Risk and preventive. Conventional therapy for dental caries usually involves a surgical approach at the tooth level. Rather than sole focus on restoration, 'Caries Risk Assessment' should be the first step of treatment protocol which categorizes risk levels based on its assessment of risk factors and protective factors that helps develop individualised intervention strategies for the same. It is an evidence-based approach that ensures thorough management of dental caries.[2,3] The consequential sequel of dental caries includes but is not limited to pain, aesthetic compromise, multiple

sitting treatment procedures and treatment-based expense. Hence, it is prudent to prevent this disease by incorporating certain diagnostic tests to the CRA protocol especially those that diagnose important causative factors like Streptococcus Mutans, and Lactobacilli counts, salivary pH, buffering capacity flow rate and salivary viscosity, and past caries experience, apart from visual and radiographs.

Many studies have been carried out to describe and demonstrate the validity of the Caries Risk Assessment approach along with the use of bacterial culture and salivary tests. However very few have been carried out in India, as a result of which its adoption into regular practice seems to be insignificant. This study aimed to assess the caries risk severity using a modified caries risk assessment form, the physicochemical properties such as salivary flow rate, pH, buffering capacity and viscosity, and bacteriology of saliva in terms of Streptococcus mutans and Lactobacillus strain in individuals with high and moderate caries risk severity.

SUBJECTS AND METHODS

Study design

This retrospective randomized study was approved by the Institutional Ethical Committee. The study was carried out following the principles of the Declaration of Helsinki (2013). All subjects integrated in the study accepted and signed an informed consent form.

Selection of patients

50 patients, aged 18-60 years, were included from the OPD of Department of Conservative Dentistry and Endodontics of Bharati Vidyapeeth Dental College and Hospital, Navi Mumbai.

The sample size was empirical as it was an exploratory study. The inclusion criteria were individuals with moderate and high caries risk severity as assessed using the modified CRA form (Figure 1) and individuals who had discontinued antibiotic use in the last 2 weeks and use of antibacterial mouth rinse in the last 12 hours prior to enrolment. The exclusion criteria were individuals below 18 years and over 60 years of age, Individuals with mild caries risk severity as assessed using the modified CRA form, individuals who were not willing to sign the written informed consent form, individuals on antibiotic therapy within 2-week time frame from time of salivary test and on antibacterial mouth rinse within 12-hour time frame from time of salivary test.

Study procedure

50 patients [male (n=29) and female (n=21)] were randomized using computer generated random number tables after assessing for eligibility and were categorised into low, moderate, and high-risk levels using the modified caries risk assessment form. Patients with moderate and high-risk levels were selected for further analysis. This was an open study as blinding could not be done due to the nature of the tests.

Salivary tests

Resting saliva was tested for hydration which was visible as droplets at the orifices of labial minor glands on the lower lip within 60 seconds. Alongside, consistency (frothiness) was also visually assessed. Saliva expectoration (pooled in the mouth for 60 secs) was used for pH measurement using a pH strip (Figure 2. A.) The colour of the strip changed in 10 seconds and indicated the pH quantitatively. Later stimulated saliva was tested using chewable paraffin wax. Stimulated saliva was expectorated in a collecting jar for 5 minutes to measure the quantity of saliva. The buffering capacity of saliva was checked using Saliva-Check Buffer test strips over which saliva was dispensed onto 3 test pads using a pipette. A change in colour over 2 minutes indicated the buffering capacity range.

Bacterial culture tests

CRT-Bacteria were used for qualitative assessment. (Figure 2. D. and 2. E.) Patient's saliva was stimulated using a paraffin pellet and collected in a container. The agar carrier was removed from the test vial, sodium bicarbonate tablet was placed at the bottom of the container and after unwrapping the vial off the foil, the agar surfaces were wet by the saliva using a pipette. Agar was then placed back into vial and incubated at 37C for 48 hours followed by quantitative comparison of mutans streptococci and lactobacilli. Saliva Check-Mutans (Figure 2. C.) was then used to confirm the presence of Streptococcus. This rapid procedure took 15 minutes and involved stimulated saliva collection in a container. 1 drop of reagent 1 and 4 drops of reagent 2 was added with intermittent shaking and tapping of the container. Saliva sample turned green. Saliva sample using the graduated pipette 3 scales was dispensed into the sample window of the test device. A bench rest of 15 mins was allowed. A positive result was indicated by a red line on the T window and a valid test was indicated by a red line on the C window. A display of a thin red line indicated high MS level. (Figure 2. B.)

Statistical analysis

The statistical analysis was done using MedCalc Statistical Software version 16.8.4 (MedCalc Software bvba, Ostend, Belgium; http://www.medcalc.org; 2016). One way analysis of variance for salivary flow rates was calculated. Chi square tests were used for analysis of buffering capacity, pH, and consistency of saliva. Correlation between the various parameters was established.

RESULTS

Amongst the 50 subjects enrolled, 8 subjects were moderate risk, 32 subjects were high risk and 10 subjects were in extremely high risk category upon assessing through the modified CRA form.

Salivary flow rate

One way analysis of variance was used, and it was seen that the mean unstimulated salivary flow rates were

significantly lower in the higher risk categories (P = 0.001).

Buffering capacity
Chi square test showed significant differences in the buffering capacity in extremely high-risk categories than the high-risk category. (P=0.0039).

Salivary pH
Chi square test showed no significant differences in the salivary pH in the moderate, high risk and extremely high-risk categories. (P=0.0506)

Consistency
Chi square test showed significant differences in the viscosity of saliva in the extremely high-risk category and high-risk category. (p= 0.0019)

Significant correlations were found between pH and buffering capacity (p=0.0001), buffering capacity and consistency (p-0.0004), salivary flow and consistency (p= 0.0152), pH and streptococcus mutans (p =0.107). No significant correlations were seen between pH and consistency, buffering capacity and Streptococcus mutans, buffering capacity and Lactobacillus, pH, and Lactobacillus.

Higher CFU/ml was observed for Str. mutans (p, 0.0196) and Lactobacillus (p, 0.0031) in patients with extremely high-risk and high-risk compared to moderate-risk patients.

DISCUSSION
Caries risk evaluation can be compared to a weather forecast i.e., one needs information on several factors, hence this study was carried out to see the correlation between the CRA form and salivary tests, as well as the interrelation between different properties of saliva, as it may not be possible to do these tests regularly and often in the clinical scenario.

Dental caries, which is a multifactorial disease, is highly prevalent in the world. The properties and constituents of saliva play an important role in occurrence and progression of caries as the teeth are constantly surrounded by this oral fluid. Various components of saliva may protect the teeth via several functions such as food debris clearance, elimination of microorganisms, neutralizing acid by buffering actions, etc.

There is evidence associating dental caries and salivary parameters such as flow rate, buffering capacity and mutans streptococci abundance. It is seen that caries prevalence may be elevated in persons with pathological low salivary flow rates, in persons where the buffering capacity is compromised and where there are high titres of mutans streptococci in saliva.[4]

In the present study, patients in the descending order of risk had a salivary flow rate of 0.47 ml/min, 0.7 ml/min and 1.7 ml/min respectively thereby, endorsing previous studies of the fact that it acts as a cleansing solution and the normal salivary rate imparts a strong protective factor against dental caries.[5,6,7] A rate > 1.0ml/min is considered normal.

Leone & Oppenheim have stated that buffering capacity prevents the sudden drop in pH by neutralizing acid in oral cavity after sugar intake.[6] Other studies have also shown that higher the buffering capacity lesser is the microbial attacks. The results of this study too showed the same as patients in extremely high & high-risk groups had 70%, 43.7% buffering capacity, and all the subjects in the moderate risk group had normal buffering capacity. Salivary flow rate and consistency had a correlation with Buffering capacity.[7]

The pH had a correlation with buffering capacity while pH & consistency had no correlation. The results are in accordance with studies performed by Prabhakar et al in 2009.[8]

In extremely high risk & high-risk groups, >60% had frothy & bubbly saliva, similar results have been seen in previous studies.[8] It also showed a significant relationship with buffering capacity, & salivary flow (rest).

Higher CFU/ml was observed for Mutans Streptococcus and Lactobacillus in patients with EH-risk and H-risk, when using the CRT bacteria kit. The Rapid MS matched the MS CRT test 82% of the times. As the Rapid test gave 18 false positive results, hence it was concluded that the test was sensitive (95.833%) but not specific (22.222%). Alaluusua et al (1990) have also reported higher sensitivity & lower specificity for Lactobacillus tests. Mutans Streptococcus & Lactobacillus culturing appears to be a valid and reliable caries risk predictive approach.[9,10]

However, a more extensive research is required as the sample size of 50 may have a limitation of not being a true representative of the entire population.

CARIES RISK ASSESSMENT
Dept. of Conservative Dentistry Endodontics

Adapted caries risk assessment form			
ID:		Sr.	
Name:			Date: .....
	Male Female	Contact:	Age: .....

Risk category				
	High Risk Factors	Yes	No	Notes
A	Visible Cavitations (carious) or caries into dentin by radiograph			
B	Caries restores in last 3 years			
C	Readily visible heavy plaque on teeth			
D	Frequent (>3 times daily) between meals snacks of sugar coated starch			
E	Saliva reducing factors			
i.	Hyposalivatory medications			
ii	Radiation to head and neck			
iii	Systemic reasons e.g. Sjogren syndrome			
F	Visually inadequate saliva flow			
G	Appliances present fixed or removable e.g orthodontic brackets/bands/retainer or removable partial dentures or FPD or adhesively retained FPD			

If YES to A and hyposaivation confirmed then classify patient as Extreme High Risk & perform bacterial & salivary tests
If YES to A or any two of B-G define patient as High Risk & perform bacterial & salivary tests
If YES to F measure saliva flow <0.7 ml/min by test = hyposaivation - define patient as High Risk
If YES to G only define patient as Moderate Risk

	Moderate Risk Factors	Yes	No	Notes
A	Exposed Roots			
B	Deep pits and fissures /develomental defects			
C	Interproximal enamel lesions /radiolucencies			
D	Enamel white spots lesions or occlusal discoloration			
E	Use of recreational drugs			

	Protective Factors	Yes	No	Notes
A	Salivary flow visually adequate or > 1ml/mm by test			
B	lives in flouridated community			
C	uses flouride toothpaste daily			
D	Uses flouride mouthwash rinse /gel daily			
E	Uses xylitol gum or mints 4x/day			
F	Uses chlorhexidine rinse			

Figure 1: Modified caries risk assessment form.

Figure 2: A. pH is measured by pH strips using expectorated saliva pooled in the mouth for 60 sec, B. A display of a thin red line on the test device indicates high MS level, C. Saliva check mutans test kit for quantitative assessment of saliva, D. Qualitative assessment- CRT bacteria for Mutans Streptococci, E. Qualitative assessment- CRT bacteria for Lactobacilli.

CONCLUSION

Within limitations of this study, we can conclude that alterations in physiochemical properties of saliva play a major role in development and prediction of caries risk. Evaluation of these factors specific to the individual should be done to cater to the needs of the patient, because if only conventional dental treatment is done the dentist is sure to see recurrence of dental caries in a relatively short time. CRA form could be a quick and convenient method for identifying potential caries susceptibility and should be adopted in regular clinical practice.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest in the present study.

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Nil

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