Original Article

A clinical evaluation of buffering capacity comparing four tooth pastes and mouthwashes

Fazeela Ayub, Jonathan Emil Sam, Bejoy John Thomas, Paulaian Benin, Jacob Raja[1], Gnana Seelan

Departments of Conservative Dentistry and Endodontics and [1]Periodontics, Rajas Dental College, Tirunelveli, Tamil Nadu, India

ABSTRACT

Introduction: To evaluate the efficacy of fluoride, chlorhexidine (CHX), herbal, and xylitol containing toothpastes and mouthwashes against in maintaining the buffering capacity (BC) in subjects within the age group of 18-22 years at time intervals of 1, 3, and 6 months. **Materials and Methods:** One-hundred subjects were randomly divided into four groups with 25 subjects in each group. Group I: Fluoride, Group II: CHX, Group III: Herbal, Group IV: Xylitol. Salivary samples were collected from the subjects for BC evaluation. In each group, subjects were advised to use toothpaste in the morning and mouthwash at night. BC of the saliva was evaluated with CRT BUFFER strips. Then it was visualized and correlated with the standard colors provided by the manufacturer. **Statistical Analysis:** Data were analyzed using Statistical Package for Social Sciences (SPSS) version 20.0. To compare the mean difference between the four groups at four different time interval analysis of variance test was used. To find out the individual significance *post-hoc* test with a Bonferroni test of multiple correction was used. For the entire analysis $P < 0.05$ was considered as significant. **Results:** During intra group comparison of BC, Group I showed a statistically significant difference among 4 times intervals. The mean value was 2.72 and *P* value was < 0.001. The mean difference on comparing the baseline values with 6 months was 0.8 in Group I which was statistically significant. After 6 months, the mean values in Group I was found to be significant when compared with Group II, Group IV, and Group III. **Conclusions:** Within limitation of the present study, it can be concluded that while using fluoride BC was elevated during 1, 3, and 6 months evaluations. In CHX BC was elevated during 6 months evaluations but not after 1 and 3 months. While using xylitol an elevation in BC was observed during 6 months evaluation but not after 1 and 3 months.

Key words: Buffering capacity, chlorhexidine, fluoride, herbal, xylitol

INTRODUCTION

Carious process is recognized as a balance between protective factors (fluoride, calcium, phosphate, saliva, and antibacterial agents) and pathological factors (cariogenic

Address for correspondence:
Dr. Benin Paulaian, Department of Conservative Dentistry and Endodontics, Rajas Dental College, Kavalkinaru, Tirunelveli, Tamil Nadu, India.
E-mail: drbenin@yahoo.com

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bacteria, dietary habits-especially frequent intake offer fermentable carbohydrates, and lack of saliva). Among 25-40% of adult population are now at caries risk.[1,2] Factors such as buffering capacity (BC) of saliva have been shown to be strong risk factors associated with the initiation and progression of dental caries. To prevent dental caries, the reduction of high counts of bacteria, an improvement in oral hygiene and a high BC of saliva is necessary.[3]

Studies have also shown that the BC of saliva influences the prevalence of dental caries. Subjects who have high BC are more likely to neutralize the pH, and hence, have a lower prevalence of dental caries. Therefore, assessing caries risk will lead to a therapeutic management of dental caries.[4] With accurate risk assessment caries preventive modalities including fluoride, chlorhexidine (CHX), xylitol, and herbal products can be used with confidence and invasive restorative procedures can be more conservative, preserving tooth structure, and better benefiting patient oral health.

Fluoride toothpaste and mouthwash are promising agents in maintaining the BC.[5] CHX is an antimicrobial ingredient in oral health care. Its mechanism of action is that the cationic molecule binds to the negatively charged cell walls of the microbes, decreasing their osmotic balance.[6] Herbal extracts have been successfully used in dentistry for tooth cleaning and as antimicrobial plaque agents. These natural phytochemicals could offer an effective alternative to antibiotics and represent a promising approach in the maintenance of BC.[7] Xylitol causes salivary stimulation that leads to an increase in the BC of saliva.[8]

The aim of this study was to evaluate the BC of fluoride, CHX, herbal, and xylitol containing toothpastes and mouthwashes in subjects within the age group of 18-22 years at time intervals of 1, 3, and 6 months.

MATERIALS AND METHODS

Subject selection

This randomized clinical trial was conducted among 100 college going students in the age group of 18-22 years. The procedures were explained to them verbally and a written informed consent was obtained from each student. The study protocol was submitted to institutional research committee and ethical board and approval was obtained.

The subjects were randomly divided into four groups with 25 subjects ($n = 25$) in each group. Salivary samples were collected from the subjects for BC evaluation. A simple randomization technique was followed for assigning subjects into their designed groups. The processes of allocation, concealment, and actual implementation of assignments follow the sequence generation.

Agents	Toothpaste	Mouthwash	Usage
Fluoride	Colgate	Senquel AD	Morning and night
CHX	Elgydium	OL sept	Morning and night
Herbal	Himalaya complete care	Hi Ora	Morning and night
Xylitol	Spry	Spry	Morning and night

CHX: Chlorhexidine

- Group I: Fluoride ($n = 25$)

Subjects were asked to use Colgate toothpaste in the morning with a soft toothbrush using Stillman's technique for tooth brushing and Senquel AD mouthwash was used at night.

- Group II: CHX ($n = 25$)

Subjects were asked to use Elgydium toothpaste in the morning and OL sept mouthwash at night which contains CHX as the main ingredient.

- Group III: Herbal ($n = 25$)

Subjects were asked to use Himalaya Complete Care toothpaste in the morning and Hi Ora mouthwash was also used in the night.

- Group IV: Xylitol ($n = 25$)

Subjects were instructed to use spry toothpaste in the morning and spry mouthwash at night which contains xylitol as the main ingredient.

Evaluation criteria

The evaluation was performed before using the study materials, after 1, 3, and 6 months.

Collection of saliva

The subjects were given paraffin wax and asked to chew for a period of 30 s and then to swallow saliva but not the paraffin. Thereafter, the subjects continued to chew the wax and saliva was collected at 2 min interval for a total period of 6 min in a calibrated beaker. The accumulated saliva was used for buffer evaluation.

Saliva check buffer

BC of the saliva was evaluated with CRT BUFFER (Ivoclar Vivadent, FL 9494, Liechtenstein) strips. In this technique, the strip was placed in a stable, horizontal position, and a drop of saliva was pipetted onto the strip and left undisturbed for 5 min. Then it was visualized and correlated with the standard colors provided by the manufacturer.

Green	High buffering capacity
Yellow	Medium buffering capacity
Blue	Low buffering capacity

The data obtained at periodical time duration were entered in the evaluation sheet corresponding to the

various time intervals. These data were used for the statistical analysis.

RESULTS

Data were analyzed using Statistical Package for Social Sciences (SPSS) version 20.0. To compare the mean difference between the four groups at four different time intervals analysis of variance [Annexure 4] was used. To find out the individual significance *post-hoc* test with a Bonferroni test of multiple corrections [Annexure 5] was used. For the entire analysis, $P < 0.05$ was considered as significant.

Table 1 shows the comparison of levels of BC within the different study groups at various time periods. BC values in Group I showed the statistically significant difference among 4 times intervals. The mean value was 2.72 and *P* value was <0.001, and hence, it was very highly significant. In Group II also the mean value was 2.56 and *P* value was <0.016. Hence, it was considered to be highly significant. On comparing Group IV, statistically significant difference was found at all time intervals. The mean value was 2.4 and *P* value was <0.047. In Group III, no statistically significant difference was found at any time intervals. The mean and *P* values were 2.32 and 0.436, respectively. The intra group comparison of BC levels among different agents. The mean difference on comparing the baseline values with 3 and 6 months and 1-month with 6 months were 0.8 and 0.72 in Group I which showed a statistically significant difference. For the remaining time periods, no statistically significant difference was found. In Group II, while comparing the baseline with 6 months mean difference was 0.6 which showed a statistically significant difference. For all the remaining time intervals in Group II, no statistically significant difference was found. The mean difference on comparing the baseline values with 6 months was 0.52 in Group IV which was considered as statistically significant. For the remaining comparisons, there was no statistically significant difference at any time intervals. On comparing Group III, no statistically significant difference was found at any time interval.

Table 2 shows the inter group comparison of BC at different time periods. During inter group comparison calculated mean values after 1-month for Group II, Group I, Group III, and Group IV were 2.08, 2, 2.04, and 1.96, respectively. Group II was more significant as the mean value was high followed by Group I, Group III, and Group IV. For 3rd month, Group I was found to be more significant followed by Group II, Group IV, and Group III was found to be significant by comparing the mean value of 2.36, 2.28, 2.16, and 2.12, respectively. Calculated mean values after 6 months Group I, Group II, Group IV, and Group III were 2.72, 2.56, 2.40, and 2.32, respectively. Group I was found to be more significant as the mean value was high followed by Group II, Group IV, and Group III.

Table 1: Intra group comparison of BC at different time periods

Groups	Parameter	Mean	SD	*F*	Significant
Fluoride	Baseline	1.92	0.702	7.587	0
	1-month	2.00	0.707		
	3 months	2.36	0.700		
	6 months	2.72	0.542		
CHX	Baseline	1.96	0.735	3.59	0.016
	1-month	2.08	0.702		
	3 months	2.28	0.678		
	6 months	2.56	0.651		
Herbal	Baseline	2.00	0.764	0.917	0.436
	1-month	2.04	0.79		
	3 months	2.12	0.726		
	6 months	2.32	0.69		
Xylitol	Baseline	1.88	0.726	2.753	0.047
	1-month	1.96	0.735		
	3 months	2.16	0.688		
	6 months	2.40	0.645		

SD: Standard deviation; BC: Buffering capacity; CHX: Chlorhexidine

Table 2: Inter-group comparison of BC at different time periods

Groups	Parameter	Mean	SD	*F*	Significant
Baseline	Fluoride	1.92	0.702	0.124	0.945
	CHX	1.96	0.735		
	Herbal	2.00	0.764		
	Xylitol	1.88	0.726		
1-month	Fluoride	2.00	0.707	0.124	0.946
	CHX	2.08	0.702		
	Herbal	2.04	0.790		
	Xylitol	1.96	0.735		
3 months	Fluoride	2.36	0.700	0.622	0.602
	CHX	2.28	0.678		
	Herbal	2.12	0.726		
	Xylitol	2.16	0.688		
6 months	Fluoride	2.72	0.542	1.954	0.126
	CHX	2.56	0.651		
	Herbal	2.32	0.690		
	Xylitol	2.40	0.645		

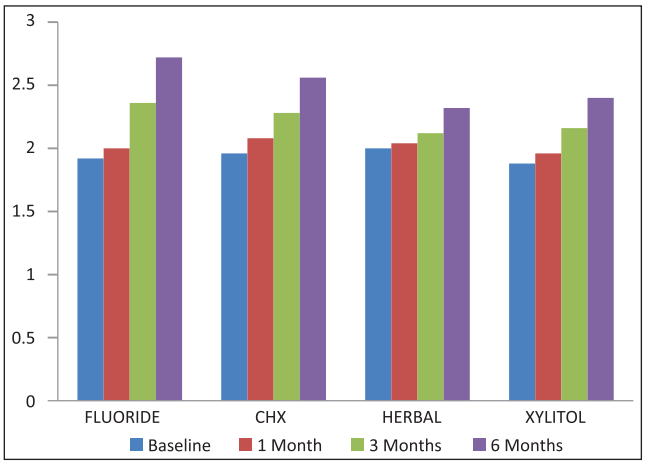
SD: Standard deviation; BC: Buffering capacity; CHX: Chlorhexidine

Graph 1 shows the intra group comparison of BC at different time periods. The different antimicrobial agents were plotted along the X-axis and the mean value along the Y-axis. The order of antimicrobial efficacy among the group tested was Group I > Group II > Group IV > Group III.

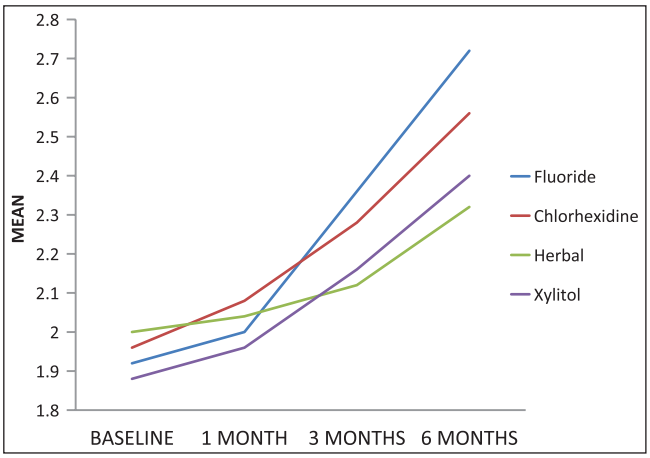
Graph 2 shows the inter group comparison of BC at different time periods. The different antimicrobial agents were plotted along the X-axis and the mean value along the Y-axis. The order of antimicrobial efficacy among the group tested was Group I > Group II > Group IV > Group III.

DISCUSSION

Dental caries occurs through a complex interaction over time between acid producing bacteria and fermentable



Graph 1: Intra group comparison of buffering capacity at different time periods



Graph 2: Inter group comparison of buffering capacity at different time periods

carbohydrate, and many host factors including teeth and saliva. Tooth brushing plays a pivotal role in the prevention and control of dental caries.[6] Professional help may not be essential to apply this technique and is commonly practiced by patients. This also enhances the patient motivation.[9] There is a scarcity of researches in the literature comparing BC after sequential time periods of 1, 3, and 6 months by using caries preventive agents.

In this study, the decision to select saliva as a parameter for caries risk assessment was made because saliva samples can provide useful information on the component cause for the caries process.[10] No special equipment is needed for collection of the fluid. Buffering action of saliva is an important factor which can influence dental caries. An important buffering system is a bicarbonate which diffuses into the plaque and acts as a buffer by neutralizing the acid. BC is important for maintaining a neutral pH range in the oral biofilm. Those having high BC are more likely to neutralize the pH and have a lower prevalence of dental caries.[11,12]

The four antimicrobial agents used in this study are fluoride, CHX, herbal, and xylitol containing toothpastes and mouthwashes. The mechanism by which fluoride inhibits demineralization is by facilitating reprecipitation of dissolved calcium and phosphate ions on the remaining crystals. This mechanism prevents these tissue ions from being leached out into the saliva. Precipitated ions at the tooth surface decreases the pores in the enamel, obstruct the diffusion pathways of plaque acids and hamper acid penetration into the enamel.[13] CHX interferes with cell wall transportation and metabolic pathways, whereas in higher concentrations it can cause precipitation of intracellular cytoplasm. CHX formulations are considered to be the "gold standard among mouthrinses"[14] due to their prolonged broad spectrum antimicrobial activity and plaque inhibitory potential. Herbal extracts have been successfully used in dentistry as antimicrobial agents. The natural phytochemicals offer an effective alternative to antibiotics and represent a promising approach in prevention and therapeutic strategies for dental caries management.[15] Various studies indicate that many Asian botanical formulae including their individual herbal compounds and chemical constituents, exhibit antibacterial properties, which may significantly delay the development of dental caries.

Xylitol is a naturally occurring five carbon sugar alcohol that hampers bacterial metabolism and controls the pH decrease. The polyol is incorporated by oral bacteria with the fructose specific phosphotransferase system and phosphorylated to xylitol 5 phosphate. These properties suggest that xylitol has a dual effect on the caries balance by decreasing the acid challenge on the pathologic side and by promoting remineralization through saliva stimulation on the protective side.[16,17]

In this study, the patients chewed the paraffin wax for total of 6 min to stimulate the secretion of saliva. There are various studies which states that "quantitative assessment of *Streptococcus mutans* should be based on stimulated rather than unstimulated saliva samples."[18] Stimulated saliva helps to transfer more microorganisms from teeth to oral cavity. CRT buffer strips were used for the determination of BC of saliva. It forms the basis of targeted treatment and this can be used as a comprehensive treatment to determine the caries risk status.[19] This test enables individualized recall intervals for the long-term maintenance of teeth. This technique is quick, easy to use, and results can be obtained within 5 min. Though BC was not strongly associated with dental caries on its own, saliva as part of bacterial counts could be a useful predictor of dental caries.[20]

Comparison of buffering capacity

During intra group comparison of BC at different time periods (base line, 1-month, 3 months, and 6 months) it was seen that Group I showed a significant increase in BC with the mean value of 2.72 and $P < 0.001$ followed by Group II

(mean value was 2.56 and $P < 0.016$). Group IV also showed increase in BC of saliva.

The presence of fluorides in the ambient solution effectively protects enamel during acid challenges. Therefore, a frequent availability of fluoride ions in the oral fluids is important. The mechanism by which fluoride maintains BC is by facilitating the reprecipitation of dissolved calcium and phosphate ions on the remaining crystals.[21] This mechanism prevents the tissue ions from being leached out to the environment into the saliva. Precipitated ions at the tooth surface, obstruct the diffusion pathways for plaque acids, and hamper acid penetration into the enamel.[22]

CHX was effective in increasing the BC as fluoride. CHX indirectly affects the enzymatic function of dehydrogenase and adenosine triphosphatase present in the cell wall of bacteria resulting in the disruption of cell membrane. It is evident in this study that the CHX showed a definite increase in BC which resulted in marked anti cariogenic effect.[23] However, there is a lack of convincing clinical data and long-term clinical evidence for maintaining BC with CHX.

Xylitol was also found to elevate the BC. A study done by Ly *et al*. verified that xylitol promotes remineralization by increasing salivary flow. Apart from that, xylitol exerts a stimulating effect on saliva secretion. The herbal group did not show any significant increase in the BC as per results of our study.[24]

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Conflicts of interest
There are no conflicts of interest.

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