EGYPTIAN DENTAL JOURNAL
www.eda-egypt.org
VOL. 70, 3859:3868, OCTOBER, 2024
PRINT ISSN 0070-9484 • ONLINE ISSN 2090-2360
CONSERVATIVE DENTISTRY AND ENDODONTICS
Submit Date : 07-07-2024 • Accept Date : 11-08-2024 • Available online: 05-10-2024 • DOI : 10.21608/edj.2024.302439.3108

EFFECT OF XYLITOL AND CALCIUM COMBINATION TO FLUORIDE GEL VERSUS CONVENTIONAL FLUORIDE GEL ON PLAQUE BACTERIAL COUNT AND SALIVARY PH IN HIGH CARIES RISK ADULT PATIENT OVER THREE MONTHS FOLLOW UP: A RANDOMIZED CLINICAL TRIAL

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ABSTRACT

Aim: The current study was conducted to evaluate the effectiveness of Calcium, Phosphate, Xylitol and Provitamin D Panthenol containing Fluoride Gel on plaque bacterial count and salivary pH versus Conventional Fluoride Gel in high caries risk adult patients. **Methodology:** Thirty-two participants were treated without a prior dental prophylaxis. Unstimulated saliva was collected to measure pH using pH meter. Plaque was collected with sterile wooden toothpicks or removed by a dental floss from a single interproximal area and samples were cultured on the same day. After baseline plaque assessment for each participant, gel application procedure was carried out using commercially available fluoride applicator trays. The control group received conventional fluoride gel (colgate gel kam) (0.4% stable stannous fluoride gel). The intervention group received fluoride gel system contains calcium, phosphate, xylitol and provitamin D-panthenol (Fluor protector gel). Streptococcus mutans bacterial count in plaque and salivary pH were evaluated at base line, one week, 6 weeks and after 12 weeks follow up. **Results:** For interdental plaque bacterial count both gels showed a statistically significant reduction between the baseline and all follow-ups ($P<0.001$). While There was no statistically significant difference between both groups in bacterial plaque count ($p=1$). For salivary pH both groups showed a statistically significant increase between the baseline and all follow-ups ($P<0.001$). While There was no statistically significant difference between both groups ($p=0.280$). **Conclusion:** Both fluor protector gel and colgate gel kam resulted in plaque bacterial count reduction and an increase in salivary pH, Re-application of fluoride gel every 3 months is preferred for high caries risk patients for better plaque bacterial control.

KEYWORDS: Calcium, Phosphate, Xylitol and Provitamin D Panthenol containing Fluoride Gel, Conventional Fluoride Gel, Plaque bacterial count, Salivary pH.

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INTRODUCTION

Dental caries is one of the most common chronic diseases affecting population. Dental caries develops over time as a result of a complicated interaction between acid producing bacteria and fermentable carbohydrates, as well as host components such as teeth and saliva [1].

Caries and periodontal diseases can only occur if bacterial biofilms and dental plaque are present. *Streptococcus mutans* has been identified as the primary cause of dental caries and is involved in the production of dental plaque biofilms [2].

The use of fluoride/s (F) to prevent caries has been demonstrated to be a successful public health measure (PHM), and it is regarded as one of the ten biggest achievements of PH (potential hydrogen) in the twentieth century. Topical F (F toothpaste, mouthwashes, varnishes, gels, foams, and slow-release F devices) reduces enamel demineralization, promotes remineralization, and prevents bacteria in dental plaque from metabolizing sugar [3].

Xylitol is one of several non-sugar sweeteners approved for use in meals. Some research suggests that xylitol plays a unique, beneficial role in the prevention of dental caries. It has been proven that using xylitol reduces the number of *streptococcus mutans* in plaque. Furthermore, xylitol appears to have a variety of unique impacts [4].

Various strategies for managing dental caries have recently focused on disrupting the interaction between all of the risk variables known to play a role in dental caries. Dietary changes, proper oral hygiene, fluoride use, pit and fissure sealants, and the use of chemotherapeutic drugs are all examples of these interventions. [5].

Primary prevention should be based on the identification of common risk factors. Secondary prevention and treatment should focus on tracking the progression of caries in individuals over time, using a less invasive, tissue-preserving method [1].

Understanding how caries develops is crucial for developing risk assessment strategies, prevention, diagnosis and treatment. We believe that caries has numerous etiologies, but that the *Streptococcus mutans* play a critical role in many cases [6].

Hence, the objective of this study was to assess the clinical effects of calcium, phosphate, xylitol and provitamin D panthenol containing Fluoride gel on bacterial count of plaque and salivary pH change compared to conventional fluoride in high caries risk patients.

The null hypothesis of this study is that there is no difference between the effect of Fluoride gel containing calcium and xylitol (fluor protector gel) and conventional Fluoride gel (colgate gel kam) in bacterial load of plaque and salivary pH change.

MATERIALS AND METHODS

Caries risk assessment using ADA model:

Caries risk assessment is the organized process of evaluating protective and pathogenic factors and provides the foundation for selecting treatment interventions. The American Dental Association (ADA) convened a group of experts to develop an easy-to-implement caries classification system (CCS). The ADA CCS offers clinicians the capability to capture the spectrum of caries disease presentations ranging from clinically unaffected (sound) tooth structure to non cavitated initial lesions to extensively cavitated advanced lesions. The ADA CCS supports a broad range of clinical management options necessary to treat both non cavitated and cavitated caries lesions. The use of the ADA CCS offers standardized data that can be used to improve the scientific rationale for the treatment of all stages of caries disease.

Two types of fluoride gel were used in this study as follows:

1. Fluoridated gel with calcium, phosphate, xylitol and provitamin D-panthenol (*Fluor Protector Gel*, Ivoclar *Vivadent)*

2. Conventional fluoride gel (*Colgate® Gel-Kam® Preventative Treatment Gel)*

Application of fluoride gels

A total of 32 volunteer participants: 16 for the control group and the other 16 for the intervention group were treated without a prior dental prophylaxis. After baseline plaque assessment for each of the subjects, a gel application procedure was carried out for each of the subjects in the two groups. Commercially available fluoride applicator trays were used. Approximately 10 ml of either the fluor protector gel or conventional gel were placed. The trays were then inserted by the operator and held in place for 5-10 min on each of 10 consecutive days, administered in a double-blind procedure. **Figure (1).**

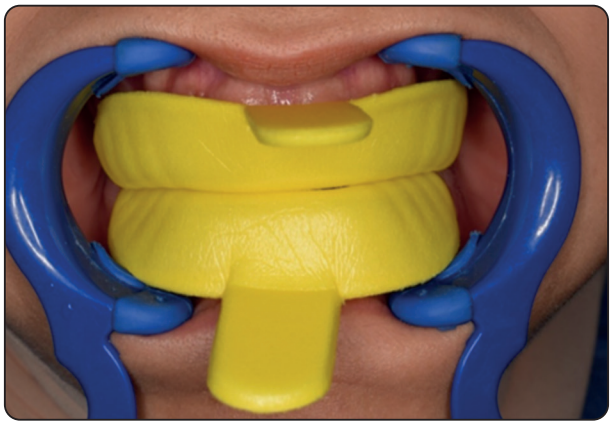

Fig. (1) Application of Fluoride Gel roughness

The randomization list was kept securely away from the operator and the participants to ensure no tampering with the random list. Each participant has chosen a sequentially numbered opaque sealed envelope containing the code. The assessor was responsible for keeping the list and handling the envelope to the operator. The number on the envelope was recorded in the participant's chart to ensure that the participants were assigned to the randomized group.

All procedures performed in this study, involving human participants, were in accordance with the ethical standards of Research Ethics Committee of Faculty of Dentistry, Cairo University (CREC) (Approval No.19617). This randomized controlled clinical study was held in Faculty of Dentistry, Cairo University, Egypt.

Outcome assessment:

1- Primary outcome: Assessing plaque bacterial count:

Plaque was collected with sterile wooden toothpicks or removed by a dental floss from a single interproximal area, i.e. the distal of the mandibular left second premolar and the mesial of the mandibular left first molar [(11)] and transferred to small capped vials containing saline. All samples were kept refrigerated until cultured on the same day. Plaque will be evaluated at baseline and after 1 week, 6 weeks, and 12 weeks in the morning before teeth brushing.

Microbiological procedures

The vials containing plaque were agitated on a Vortex test-tube mixer for 30 sec to dislodge the plaque from the toothpick and to obtain a homogeneous suspension. A micropipette was then used to collect the diluted plaque from the transport vial to make serial dilutions. The samples were then serially diluted (ten-fold) from 1:10 to $1:10^5$

A volume of 0.1 mL of each dilution was pipetted onto a plate containing agar medium supplemented with bacitracin, using the same pipette**.** Bacitracin is incorporated in mitis-salivarius as it is effective in inhibiting bacteria other than *S. mutans* when human dental plaque samples were cultured. The serially dilutions then were spread with a sterile bent, glass rod to obtain homogenous bacterial growth on the surface of the agar plate

All plates were then incubated anaerobically by Candle jar incubation for 2 days at 37°C.

Conventional CFU counting was used to count the colonies on the plates; typically, between 30 and 300 colonies per standard agar petri dish of 8cm in diameter result in optimal counting. To minimize the bacterial count, we employed log10 statistics. Because individual colonies cannot be distinguished, larger numbers can easily lead to underestimating [12].

2- Secondary outcome: Assessing Salivary pH:

Participants were instructed not to brush the morning before sampling. The participants were given bottled water to drink and were instructed to thoroughly rinse their mouths (without drinking water). The individual was requested to spit whole saliva 5 minutes following the oral rinse. The participants were instructed to stop talking and to lower their heads, allowing saliva to flow naturally to the front of their mouths. The participants were also instructed not to cough up mucous while saliva was being collected. A total of 5 mL of unstimulated saliva samples were taken [7]. The salivary samples were collected between 9:00 am and 11:00 am. The pH of the saliva was immediately measured to prevent any deterioration of the sample [8]. flow of saliva was expressed as ml/min. pH was measured using a pH meter which has a reading from 0 to 14 (acidic pH < 7 and alkaline pH > 7 and neutral = 7) [13]

The salivary pH value was measured using the pH meter (AD 11 pH meter). [9] Each time the electrode was dipped in the sample, it was gently dried entirely with new sterile filter papers. A hand-held electronic pH meter with a digital display was used to immediately measure saliva pH change. The pH sensitive electrode was placed in the tube containing the collected unstimulated saliva after calibration using the supplied standard solutions of pH 4.0 and 7.0 to measure the initial pH value within 30 seconds [10]

Statistical analysis:

Numerical data were tested for normality using Shapiro-Wilk test and were presented as mean and standard deviation values. Bacterial count data showed non-parametric distribution so it was analyzed using Mann-Whitney U test for intergroup comparisons and Freidman's test followed by Dunn's post hoc test for intragroup comparisons. Salivary pH change values were normally distributed so they were analyzed using independent t-test for intergroup comparisons and one-way repeated measures ANOVA followed by Bonferroni post hoc test for intragroup comparisons. The significance level was set at $p \leq 0.05$ within all tests. Statistical analysis was performed with IBM® SPSS® Statistics Version 26 for Windows.

RESULTS

Plaque bacterial count:

Regarding plaque bacterial count for both groups, they both have the same manner. The inter dental plaque bacterial count showed maximum decrease after 1 week, with further gradual increase till the last follow up after 12 weeks with significant difference between baseline and all follow-ups ($p<0.001$).

At the Baseline: Fluor Protector Gel (5.53±0.15) showed significantly higher mean value than Colgate Gel Kam (5.12±0.18) ($p=0.016$).

Then after 1 week: Fluor Protector Gel (3.33±0.25) showed a higher mean value than Colgate Gel Kam (3.25±0.17) yet the difference was not significant ($p=0.690$).

After 6 weeks: Colgate Gel Kam (3.89±0.12) showed higher mean value than Fluor Protector Gel (3.69±0.23) yet the difference was not significant ($p=0.222$).

And after 12 weeks: Colgate Gel Kam (4.86±0.14) showed significantly higher mean value than Fluor Protector Gel (4.56±0.17) ($p=0.032$).
Table (1)

TABLE (1) Mean and standard deviation (SD) values for plaque bacterial count (CFU/ml) in different groups

Follow-up	Plaque bacterial count (Mean±SD)		p-value
	Fluor Protector Gel	Colgate Gel Kam	
Baseline	5.53±0.15	5.12±0.18	**0.016***
1st week	3.33±0.25	3.25±0.17	**0.690ns**
6th week	3.69±0.23	3.89±0.12	**0.222ns**
12th week	4.56±0.17	4.86±0.14	**0.032***

; significant (p<0.05)** **ns; non-significant (p>0.05)

As for the intergroup comparison of percentage change of plaque reduction, Fluor Protector Gel showed a significantly higher mean value than Colgate Gel Kam in all the follow ups (p<0.001) except for the Baseline-1 week the difference was not significant (p=0.07). **Table (2)**

TABLE (2) Mean and standard deviation (SD) values for percentage change of reduction in plaque bacterial count (%) in different groups

Follow-up	Plaque bacterial count (Mean±SD)		p-value
	Fluor Protector Gel	Colgate Gel Kam	
Baseline-1 week	39.77± 2.94	36.61±1.67	**0.070ns**
Baseline-6 weeks	33.24±2.52	24.08±1.01	**<0.001***
Baseline-12 weeks	17.48±1.58	5.01±1.50	**<0.001***

The overall p-value was 1 (p=1). which means that there was no significant decrease in plaque bacterial count observed when comparing Flour Protector gel with colgate gel kam.

Data in **table (1,2)** show the results of the plaque bacterial count.

Salivary pH change

Data in **table (3)** show the results of the salivary pH.

TABLE (3) Mean and standard deviation (SD) values for salivary pH in different groups

Follow-up	Salivary pH (Mean±SD)		p-value
	Fluor Protector Gel	Colgate Gel Kam	
Baseline	6.50±0.16	6.46±0.15	**0.694ns**
1st week	7.54±0.11	7.38±.08	**0.035***
6th week	7.20±0.14	7.06±0.09	**0.098ns**
12th week	6.70±0.16	6.44±0.11	**0.018***

; significant (p<0.05)** **ns; non-significant (p>0.05)

Regarding salivary pH change for both groups, The salivary pH showed maximum increase after 1 week, with further gradual decrease till the last follow up with significant difference between baseline and all follow-ups

At the Baseline: Fluor Protector Gel (6.50±0.16) showed a higher mean value than Colgate Gel Kam (6.46±0.15) yet the difference was not significant (p=0.694).

Then after 1 week: Fluor Protector Gel (7.54±0.11) showed a significantly higher mean value than Colgate Gel Kam (7.38±.08) (p=0.035).

After 6 weeks: Fluor Protector Gel (7.20±0.14) showed a higher mean value than Colgate Gel Kam (7.06±0.09) yet the difference was not significant (p=0.098).

And after 12 weeks: Fluor Protector Gel (6.70±0.16) showed significantly higher mean value than Colgate Gel Kam (6.44±0.11) (p=0.018).

The overall p-value was 0.280 which means that Fluor Protector Gel showed higher mean value than Colgate Gel Kam yet the difference was not significant in increasing in salivary pH between Flour Protector gel comparing with colgate gel kam.

DISCUSSION

Fluoride is widely used nowadays and is being added to dentifrices, mouthwashes, gels and solutions for topical applications, and pit-and-fissures sealants. This is due to its cariostatic action and its ability to improve the remineralization of the enamel and dentin exposed to acid challenge in the oral environment [(14)].

Moreover, the addition of fluoride to gels and toothpaste has attracted the attention of dental researchers and clinicians, as these gels result in a large reduction in tooth decay in both permanent and deciduous teeth, [(15)]. These dental fluoride gels have been introduced aiming to inhibit dental caries, especially in patients with high caries risk, [(16)].

This anti-cavity action was first discovered in drinking water or through the use of fluoride supplements. Chronic ingestion of high fluoride concentrations in drinking water, on the other hand, has several drawbacks, including dental fluorosis, a cosmetic condition in which the teeth become mottled, and skeletal fluorosis in more severe cases [(17)]. To overcome these drawbacks, it is preferable to provide fluoride directly to the tooth via topical fluoride gel rather than ingesting it through fluoridated water [(17)].

Fluor Protector Gel contains calcium, phosphate, and fluoride, this leads to the development of a calcium fluoride layer which is additionally stabilized by phosphate ions, and the direct incorporation of calcium, fluoride, and phosphate ions into the tooth enamel. They contribute to the formation of fluorapatite. When a hydroxyl ion is replaced by a fluoride ion in hydroxyapatite, the enamel becomes more resistant to acidic assaults [(18)].

Xylitol has an anticariogenic effect, this can be attributed to its passive substitution of fermentable carbohydrates. Moreover, Xylitol was found to reduce the growth of *Streptococcus mutans* and inhibit caries by reducing the bacterial adherence to the tooth structure, Also Xylitol cannot be fermented by most oral microorganisms [(19)].

Furthermore, The provitamin D-panthenol relieves the pain in the gingiva and mucous membranes, leading to the enhancement of the oral cavity health [(20)]. Therefore, Fluoride + calcium + phosphate + Xylitol + Provitamin D-panthenol was selected in the present study. The commercial product of this product used in the current study was Fluor Protector Gel as it is the only commercial product of this combination available in the market

The participants were asked to avoid brushing and flossing their teeth for 24 hours before the visits since Plaque was permitted to form in this period of time [(21)]. And also they were asked to avoid eating, chewing gum, and drinking anything other than water for 1 hour before the visits as well to avoid the misreading of the pH measures [(22)].

Each patient's caries risk was assessed individually to help identify the factors linked to dental caries so that a cooperative preventative care plan could be created. The precise information received from a comprehensive assessment of caries risk aids the dentist in formulating treatment and preventive protocol for patients with dental disease and those who are at risk [(23)].

The caries balance concept states that the balance between pathogenic and protective variables determines the progression or reversal of dental caries. The caries risk assessment using the ADA model is a simple way to keep a record of information that will help the dentist determine the patient's caries risk [(24)].

After the salivary assessment, plaque samples were then collected using sterile wooden toothpicks or removed using dental floss from a single interproximal area since dental plaque tends to be highly accumulated on the interproximal surface [(25)]. Then all samples were kept refrigerated until cultured on the same day.

The gel application technique was carried out in both the control and intervention groups using commercially available fluoride applicator trays after baseline plaque assessment, as the efficacy of

topical fluoride is dependent on the frequency and duration of treatment. Topical fluoride diffuses into dental plaque, acting as a fluoride reservoir that can be released at a later time. Fluoride ingestion was shown to be considerably lower following the application of fluoride gel with a tray. A small amount of fluoride on the tray resulted in similar fluoride retention and less ingestion **.** [26]**.**

Intragroup analysis, the results of the present study show that for both groups there was a statistically significant reduction in the mean log CFU. This was in agreement with previous studies. [27] evaluated the effects of xylitol on *S. mutans* count in dental plaque, [28] who evaluated a direct inhibitory effect of xylitol on *S. mutans*, [29] who evaluated the effect of xylitol.

Sodium fluoride on *S. mutans*, [30] who investigated the impact of various fluoride concentrations, corresponding to various oral-care products, on *S. mutans* metabolic activity and biofilm development. But the results were in disagreement with [31] who stated that the use of 1,500 ppm fluoride toothpaste, lacks the ability to produce an antimicrobial effect on bacteria in dental plaque. However, comparing both groups, there was no significant difference observed. This is in agreement with [32] who stated that the differences were not statistically significant in the mean CFU of *S.mutans* between the intervention group containing fluoride and xylitol in combination and the control group containing fluoride only

Fluoride has a combination of direct and indirect actions on bacterial cells, some of which may have a major impact on the acid-producing bacteria in dental plaque. The pathogenicity of bacteria is one of the factors that contribute to the reduction of *S. mutans* in dental plaque. NaF reduced at least two major virulence factors in *S. mutans* biofilm cells, with a fluoride concentration of 190 ppm required to inhibit bacterial acid generation [33].

Another factor is the frequency of application of the fluoride gel. In high-risk patients, a daily mouth rinse with 0.05 % stannous fluoride for 1 minute was generally suggested to prevent dental caries by reducing S. mutans in dental plaque. However, in this study, the daily treatment of stannous fluoride(0.4%) for 10 min was chosen based on the hypothesis that if the treatment time is increased, the anti-biofilm effects of stannous fluoride would be supported [33].

Plaque does not ferment xylitol. Because xylitol is non-fermentable, it has a nonspecific effect and does not promote bacterial growth. Also, xylitol can cause neutralization of low pH by increasing the buffer capacity of the saliva and by increasing the clearance of fermentable carbohydrates from the oral cavity [28].

Regular use of xylitol can cause the xylitol-resistant mutants to shed more easily into the saliva from the plaque [34].

According to the results of this study, both groups have shown an equal percentage of reduction in *S. mutans*. Therefore, The addition of xylitol to fluoride did not affect the decrease of *S. mutans,* This is because the most suitable form of delivery vehicle for xylitol is proposed to be chewing gum [35]. According to our research xylitol addition to fluoride has not shown a significant effect on the count of *S. mutans*, when compared with 0.4% stannous fluoride as the main ingredient in the control group.

The results of the second outcome show that for both groups there was a statistically significant increase in the_salivary pH. This was in agreement with previous studies, [36] who evaluated the effect of fluoride containing toothpaste on the salivary pH change, [37] who evaluated the correlation between salivary pH and the use of mouth rinses containing fluoride.

But the results were in disagreement with [38] who reported that the usage of oral hygiene products containing NaF did not affect salivary pH. And when comparing both groups, there was no significant difference observed

Saliva's defensive mechanisms can be classified into two types: physical and chemical. Saliva's physical defense actions include flushing and displacing bacteria from their natural habitat. Salivary pH and buffer are two endogenous chemical protection mechanisms that keep the caries balance from shifting toward demineralization. Xylitol is known to reduce the *S. mutans* levels and also improve the pH and buffer capacity of saliva. Various research in the literature,[39,40] have confirmed this.

According to this study, Salivary pH rises considerably after both gel placement. The effect of fluoride on salivary pH could be related to a reduction in *Streptococcus mutans* or carbohydrate metabolism, Bacterial glucose metabolism results in an acid generation, Fluoride lowered acid generation by inhibiting carbohydrate metabolism of oral streptococci. When fluoride levels are high, salivary pH rises as well [36].

It should be pointed out that this clinical study had some limitations due to the lack of clinical reference data that would allow for a discussion on this subject. As a result, these findings are provided as novel findings that can be discussed further in the context of the association between clinical long-term usage of NaF-containing products and saliva pH [38].

By the end of the discussion, our null hypothesis was accepted that there is no significant difference between the amount of plaque bacterial reduction and raising salivary pH of fluor protector gel and Colgate gel Kam in high caries risk patients.

CONCLUSIONS

Within the limitations of this study, it could be concluded that:

Both fluor protector gel and Colgate gel Kam resulted in plaque bacterial count reduction and an increase in salivary pH.

There is no significant difference between the amount of plaque bacterial reduction and raising salivary pH of fluor protector gel and Colgate gel Kam in high caries risk patients.

Conflict of Interest:

The authors declare no conflict of interest.

Funding:

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors

Ethics:

This study protocol was approved by the ethical committee of the faculty of dentistry- Cairo university on: 8-12-2019.

REFERENCES

1. Selwitz, R. H., Ismail, A. I., & Pitts, N. B. (2007). Dental caries. The Lancet, 369(9555), 51-59.
2. Tong, Z., Tao, R., Jiang, W., Li, J., Zhou, L., Tian, Y., & Ni, L. (2011). In vitro study of the properties of Streptococcus mutans in starvation conditions. Arch. Oral Biol, 56(11), 1306-1311
3. Šket, T., Kukec, A., & Artnik, B .(2017) .The history of public health use of fluorides in caries prevention. Zdr. Varst, 56(2), 140-146.
4. Maguire, A., & Rugg-Gunn, A. (2003). Xylitol and caries prevention—is it a magic bullet? Br. Dent. J, 194(8), 429-436.
5. Sajjan, P., Nagesh, L., Sajjanar, M., Reddy, S., & Venktesh, U. (2013). Comparative evaluation of chlorhexidine varnish and fluoride varnish on plaque S treptococcus mutans count–an in vivo study. J Dent Hyg, 11(3), 191-197.
6. Banas, J. A., & Drake, D. R. (2018). Are the mutans streptococci still considered relevant to understanding the microbial etiology of dental caries. BMC Oral Health, 18(1), 1-8.
7. Belstrøm, D., Holmstrup, P., Bardow, A., Kokaras, A., Fiehn, N.-E., & Paster, B. J. (2016). Comparative analysis of bacterial profiles in unstimulated and stimulated saliva samples. J. Oral Microbiol, 8(1), 30112.
8. Baliga, S., Muglikar, S., & Kale, R. (2013). Salivary pH: A diagnostic biomarker. J. Indian Soc. Periodontol, 17(4), 461-465.
9. Aripin, D., Suwargiani, A. A., Wardani, R., Susilawati, S., Jasrin, T. A., Dewi, W., & Sasmita, I. S. (2020). The Relationship between the salivary pH, flow rate, and the

number of oral Streptococci in elementary school age children. J. Int. Dent. Med. Res, 13(1), 327-331.

10. Kitasako, Y., Burrow, M., Stacey, M., Huq, L., Reynolds, E., & Tagami, J. (2008). Comparative analysis of three commercial saliva testing kits with a standard saliva buffering test. Aust. Dent. J, 53(2), 140-144.
11. Villa, O., Ramberg, P., Fukui, H., Emilson, C.-G., Papanikolaou, G., Heijl, L., & Birkhed, D. (2018). Interaction between chlorhexidine and fluoride in a mouthrinse solution—a 4- day and 6-week randomized clinical pilot study. "Clin. Oral Investig., 22(3), 1439-1448
12. Wan, A., Seow, W., Walsh, L., & Bird, P. (2002). Comparison of Five Selective Media for the Growth and Enumeration of Streptococcus Mutans. Aust. Dent. J, 47(1), 21-26.
13. Rajesh, K., Zareena, S. H., & Kumar, M. A. (2015). Assessment of salivary calcium, phosphate, magnesium, pH, and flow rate in healthy subjects, periodontitis, and dental caries. Contemp Clin Dent, 6(4), 461.
14. Nehme, M., Parkinson, C., Zero, D., & Hara, A. (2019). Randomised study of the effects of fluoride and time on in situ remineralisation of acid-softened enamel. Clin. Cosmet. Investig. Dent, 23(12), 4455-4463.
15. Marinho, V. C., Worthington, H. V., Walsh, T., & Chong, L. Y. (2015). Fluoride gels for preventing dental caries in children and adolescents. Cochrane Database Syst. Rev, (6).
16. Petersen, P. E. (2008). World Health Organization global policy for improvement of oral health--World Health Assembly 2007. Int Dent J, 58(3), 115-121
17. Aoun, A., Darwiche, F., Al Hayek, S., & Doumit, J. (2018). The Fluoride Debate: The Pros and Cons of Fluoridation. Prev Nutr Food Sci 3)23 ,)171-180 ,
18. Chadwick, B. L., Roy, J., Knox, J., & Treasure, E. T. (2005). The effect of topical fluorides on decalcification in patients with fixed orthodontic appliances: a systematic review. Am. J. Orthod. Dentofac. Orthop, 128(5), 601-606
19. Gul, P., Akgul, N., & Seven, N. (2018). Anticariogenic potential of white cheese, xylitol chewing gum, and black tea. Eur. J. Dent, 12(2), 199-203.
20. Benaiges, A., & Guillén, P. (2007). 8.5. Botanical Extracts. Analysis of cosmetic products, 345-363
21. Zheng, Y., Zhang ,M., Li, J., Li, Y., Teng, F., Jiang, H., & Du, M. (2018). Comparative Analysis of the Microbial Profiles in Supragingival Plaque Samples Obtained From Twins With Discordant Caries Phenotypes and Their Mothers. Frontiers in Cellular and Infection Microbiology, 8(361).
22. Pachori, A., Kambalimath, H., Maran, S., Niranjan, B., Bhambhani, G., & Malhotra, G. (2018). Evaluation of changes in salivary pH after intake of different eatables and beverages in children at different time intervals. Int. J. Clin. Pediatr. Dent, 11(3), 177.
23. Fontana, M., & Zero, D. T. (2006). Assessing patients' caries risk. J. Am. Dent. Assoc, 137(9), 1231-1239.
24. Gomez, F., Crystal, Y., Ng, M. W., Tinanoff, N., & Featherstone, J. (2010). Caries risk assessment, prevention, and management in pediatric dental care. Gen. Dent, 58, 505-517.
25. Otsuka, R., Nomura, Y., Okada, A., Uematsu, H., Nakano, M., Hikiji, K.,Momoi, Y. (2020). Properties of manual toothbrush that influence on plaque removal of interproximal surface in vitro. J. Dent. Sci, 15(1), 14-21.
26. Seyedakhavan, P., Sayahpour, S., Momeni, H., & Kharazi, M. (2017). Effect of fluoride gel and foam on salivary fluoride concentration. j. res. dent. maxillofac. sci, 2(2), 16-22.
27. Thaweboon, S., Thaweboon, B., & Soo-Ampon, S. (2004). The effect of xylitol chewing gum on mutans streptococci in saliva and dental plaque. J. Trop. Med. Public Health, 35(4):1024-7
28. Janakiram, C., Kumar, C. D., & Joseph, J. (2017). Xylitol in preventing dental caries: A systematic review and meta-analyses. J Nat Sc Biol Med, 8(1), 16
29. Subramaniam, P., & Nandan, N. (2011). Effect of xylitol, sodium fluoride and triclosan containing mouth rinse on Streptococcus mutans. Contemp Clin Dent, 2(4), 287
30. Nassar, H. M., & Gregory, R. L. (2017). Biofilm sensitivity of seven Streptococcus mutans strains to different fluoride levels. J. Oral Microbiol, 1328265 ,(1)9 .
31. Lynch, R. M., Navada, R., & Walia, R. (2004). Low-levels of fluoride in plaque and saliva and their effects on the demineralisation and remineralisation of enamel; role of fluoride toothpastes. Int. Dent. J, 54, 304-309.
32. Giertsen, E., Emberland, H., & Scheie, A. A. (1999). Effects of mouth rinses with xylitol and fluoride on dental plaque and saliva. Caries Research, 33(1), 23-31.
33. Pandit, S., Kim, J.-E., Jung, K.-H., Chang, K.-W., & Jeon, J.-G. (2011). Effect of sodium fluoride on the virulence factors and composition of Streptococcus mutans biofilms. Arch. Oral Biol, 56(7), 643-649.

34. Nayak, P. A., Nayak, U. A., & Khandelwal, V. (2014). The effect of xylitol on dental caries and oral flora. Clin. Cosmet. Investig. Dent, 6, 89.

35. Perala, S. R., & Bhupathiraju, P. (2016). Efficacy of Four Fluoride Mouth Rinses on Streptococcus mutans in High Caries Risk Children - A Randomized Controlled Trial. J. Clin. Diagnostic Res, 10(9), ZC56-ZC60.

36. Sarkar, A., Pal, D., Adak, A., Saha, S., Sarkar, S., & De, A. (2018). The changes of salivary ph before and after brushing with fluoride containing toothpaste and without toothpaste in 6 to 12 years old children in west bengal, J. Dent. Sci, 3(1):18-23.

37. Bobu, L., Vasluianu, R., Baciu, E. R., Bulancea, B., & Murariu, A. (2019). Study on the Correlation between Salivary pH and the Use of Mouthrinses in Young Adults. REVISTA DE CHIMIE, 70(3),1085-1088.

38. Frese, C., Wohlrab, T., Sheng, L., Kieser ,M., Krisam, J., & Wolff, D. (2019). Clinical effect of stannous fluoride and amine fluoride containing oral hygiene products: A 4-year randomized controlled pilot study. Scientific Reports, 9(1), 7681.

39. Maguire, A., & Rugg-Gunn, A. (2003). Xylitol and caries prevention—is it a magic bullet? Br. Dent. J, 194(8), 429-436

40. Tanzer, J., Thompson, A., Wen, Z., & Burne, R. (2006). Streptococcus mutans: fructose transport, xylitol resistance, and virulence. J. Dent. Res, 85(4), 369-373.