ASSESSMENT OF INHIBITION OF BIOFILM FORMATION AND PLAQUE BACTERIAL COUNT OF FLUORIDE VARNISH CONTAINING CHLORHEXIDINE AND CETYLPYRIDINIUM CHLORIDE (CPC) VERSUS CONVENTIONAL FLUORIDE VARNISH AMONG HIGH CARIES RISK PATIENTS: RANDOMIZED CLINICAL TRIAL

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ABSTRACT

Aim: The current study was conducted to compare and evaluate the effects of a new Fluoride varnish containing Chlorhexidine (CHX) and Cetylpyridinium Chloride (CPC) and a conventional Fluoride varnish on dental plaque and streptococcus mutans count in high caries risk patients.

Methodology: Thirty-Four high caries risk patients received randomly two types of varnishes, either Fluoride varnish containing CHX and CPC (Cervitec F) or conventional Fluoride varnish (Flour Protector) as an active control. Dental plaque index (PI) and digital image analysis (AI) to disclosed plaque as well as mutans streptococci count recording were performed at baseline before the application and at 2nd, 4th, 12th, and 24th week of the study. Statistical analysis was done using ANOVA tests & t-test and Wilcoxon signed-rank test where significance level was set at $P \leq 0.05$.

Results: For the Plaque index and image analysis of dental plaque, both varnishes showed a statistically significant reduction between baseline and all follow-ups. For plaque bacterial count, both varnishes showed a statistically significant reduction between the baseline and all follow-ups. While the Cervitec F varnish achieved a statistically significant reduction compared to the Fluor Protector group.

Conclusion: (1) Both Conventional Fluoride varnish and Fluoride varnish with CHX and CPC can decrease bacterial load and plaque accumulation. (2) Fluoride varnish with CHX and CPC achieved more reduction in streptococcus mutants count compared to the Conventional Fluoride varnish group. (3) Re-application of vanishes every 3 months is preferred for high caries risk patients for better plaque and bacterial control.

KEY WORDS: Chlorohexidine, Cetylpyridinium Chloride (CPC), Sodium fluoride varnish, Plaque index, Image analysis, plaque bacterial count.

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INTRODUCTION

Dental plaque is considered to be a primary etiological factor for most common oral diseases. It is highly specific, and its specificity depends on the microorganisms that colonize it. Streptococcus mutans (S. mutans) was discovered to be the most common microbe responsible for dental caries out of all the studied microorganisms. When bacteria are left undisturbed on tooth surfaces or adjacent plaque retaining sites, the bacteria will form a biofilm and reproduce to optimize the chance for survival*(Abo Bakr et al., 2021).*

Preventive dentistry, thus, is the only solution to the problem by which most of the common dental diseases can be prevented. Antibacterial agents have long been included in the formulations of oral care products such as mouthwashes, toothpastes, and varnishes to prevent biofilm formation on teeth surfaces and dental caries. Fluoride and chlorhexidine (CHX) are two antibacterial agents that are routinely used. Fluoride is the most commonly used anticaries agent that have significant caries reducing potential by strengthening the enamel, also fluoride reduces plaque growth and activity *(Achmad et al., 2021) .*

Chlorhexidine has been the gold standard among antiplaque and antibacterial agents used in dentistry over the previous few decades. It is effective against a wide range of pathogenic bacteria *(Lipták et al., 2018).* Among dental researchers, the use of chlorhexidine for caries prevention has been a controversial issue. Chlorhexidine varnishes were found to be the most effective in reducing mutans streptococci, according to various studies. While the evidence for using various chlorhexidine modes or a combination of chlorhexidine and fluoride therapy for caries prevention has been described as "suggestive but incomplete".

In recent years, the use of the chemical antimicrobial agents as Cetylpyridinium chloride (CPC) has attracted some attention, due to its enhanced therapeutic benefits and low side effects (tooth dis-

coloration, ulcers, gingival irritation), its usage may be advised for extended periods. Cetylpyridinium chloride (CPC) is a cationic quaternary ammonium chemical with strong antibacterial properties against oral microorganisms. CPC has been proposed for use in a variety of dental products, including toothpastes, mouthwashes, varnishes, and orthodontic adhesives *(Langa et al., 2021) .*

In this respect, a combination of fluoride, CHX, and CPC has demonstrated favorable properties; moreover, in the presence of fluoride, the affinity of CHX for hydroxyapatite increases *(Elkerbout et al., 2019).* Comparative analysis of these active agents upon both qualitative and quantitative changes in plaque microflora in similar clinical settings is not reported in the literature. Thus, this clinical trial assessed the effect of a combination of CHX, CPC, and Fluoride in one varnish. Cervitec F varnish is a system containing 1400 ppm fluoride from ammonium fluoride in a varnish base with ethanol and water as solvents. In addition, the varnish contains nearly 0.3% chlorhexidine and 0.5% Cetylpyridinium chloride.

MATERIALS AND METHODS

In this randomized controlled clinical trial, the variables were two Fluoride varnishes, Conventional Fluoride varnish (*Fluor Protector, Ivoclar Vivadent - Schaan Liechtenstein*) as a control and Fluoride varnish containing Chlorhexidine (CHX), and Cetylpyridinium Chloride (CPC) (*Cervitec F, Ivoclar Vivadent - Schaan Liechtenstein*) as an intervention. 34 adult participants clinically diagnosed to be at high caries risk were selected and assigned in two groups after randomization and each group has 17 patients according to sample size calculation. Each generated random number represented assigning either intervention or comparator to each patient in a randomization that was kept secure away from the operator, outcome assessor, and participants to ensure no tampering

with the random list. Each participant chose a sequentially numbered opaque sealed envelope after signing the written informed consent, and the number on the envelope was be recorded in the patient chart to ensure that the participant is assigned to the randomized group. Then the operator opened the envelope and did the treatment to the participant as assigned on the card. 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), and approval no. 19735. This randomized controlled clinical study was held in Faculty of Dentistry, Cairo University, Egypt. The participants, the assessors and statistician were blinded to the material assignment while the operator was not in order to apply both interventions following the manufacturer's instructions.

1. Eligibility criteria:

The following inclusion and exclusion criteria were selected.

1.1. Inclusion criteria of the participants: (Alavi and Yaraghi, 2018)

- Patients should be aged between 18 and 40 years with a good general state of health and no signs of periodontitis.

- Patients had a recorded high bacterial count after caries risk assessment. ($\geq 10^5$CFU)

- No use of antiseptic mouth rinses or antibiotic treatment within 1 month prior to the start of the trial.

1.2. Exclusion criteria of the participants:

- Patients less than 18 years or more than 40 years old Patients with disabilities, systemic disease, or severe medical conditions.

- Patients with severe or active periodontal disease.

- Antibiotic treatment within 1 month prior to the start of the trial.

2. Interventions:

Prior to the application of varnish, base line Plaque index and plaque bacterial count were assessed, oral prophylaxis was carried out for subjects. The teeth were then be isolated with cotton rolls and saliva ejector and dried with a gentle blow of air for 30 seconds using a triple-way air syringe. The required amount of the varnish was dispensed into a glass dappen dish and a thin layer will be applied using a suitable single-use brush supplied by the manufacturer. Varnish was allowed to dry, and after one minute, the cotton rolls were removed. The treatment was performed with a single application of the varnishes on the vestibular and lingual surfaces of all teeth (**Paul et al., 2014**) .

2.1 Post-operative instructions:

Participants were instructed to (***Chiba et al., 2019***)

- Avoid eating and drinking for 3 hours.

- Avoid brushing or flossing for 24 hours.

3. Outcome assessment:

Each group was assessed five times (T), where T0 represents baseline patients assessment, T1 represents 2nd week patients assessment, T2 represents 4th week patients assessment, T3 represents 12th week patients assessment, and T4 represents 24th week patients assessment.

3.1. Primary outcome: plaque index:

The primary outcome was plaque assessment using Silness and loe Plaque index. After chewing disclosing tablets, plaque was evaluated for 6 teeth (16,12,24,36,32 and 44). These teeth were evaluated at 4 sites (mesiobuccal, mid-buccal, distobuccal, and lingual). Each of the four surfaces of the teeth is given a score from 0-3. And the index for each patient was obtained by summing the indices of the 6 teeth and then divided by six. (***Chiba et al., 2019***) .

3.2. Secondary outcome: "Digital photography and image analysis":

The secondary outcome was plaque assessment using "Digital photography and image analysis". Because of difficulties in the standardization of intra-oral photography and computer analysis limitations, only the upper and lower six anterior teeth were considered in image analysis. *(Rosa and Elizondo, 2015)* . Photoshop software was used to isolate anterior teeth from the rest of the image. And Using image j software, the entire visible tooth area was automatically measured in pixels. The image analysis steps and measurement technique can be summarized as follow:

Step 1: Photoshop software was used for the segmentation of teeth outline using the semiautomatic outline selection tool. In that way, teeth were isolated from the rest of the image. After that, the areas with dental plaque stained in red were automatically detected with the color range command and highlighted with blue color, and then separated from the rest of the image.

Step 2: Using image j software, the entire visible tooth area was automatically measured in pixels. The stained area in each tooth was automatically measured in pixels, and then calculated as % of the total teeth area using the following equation:

Stained area % =

$$\frac{\text{Sum. of dental plaque stained area (pixels)}}{\text{Total tooth area (pixels))}} \times 100$$

3.3. Tertiary outcome: "Plaque bacterial count":

The tertiary outcome was the streptococcus mutants bacterial count in interdental plaque. Plaque samples were collected with a sterile wooden toothpick from the interproximal sites between the first molar and second premolar. Samples were homogenized on a vortex for 30 seconds then serially diluted. 100µl from different dilutions were transferred onto Mitis Saliverous culture media and incubated at 37°C, anaerobically using candle jar. After 48 h of the incubation period, Streptococcus mutants colonies appeared on the culture plate and The colony-forming units were counted manually. *(Villa et al., 2018)* .

4. Statistical analysis:

Statistical analysis of the results was performed using two-way ANOVA test followed by t-test for inter-group comparison, and one way ANOVA test followed by post hoc test for intragroup comparison, Wilcoxon signed-rank test to comparing the ranks of viability % of two groups. The $p < 0.05$ was considered statistically significant (95% significance level). Shapiro Wilk test was used for testing the normality of data, and all bacterial data were compiled and logarithmically transformed in SPSS to normalize the variance distribution.

- Data were analyzed using the statistical software SPSS (version 25, IBM Co. USA).

Calculation of Streptococcus Viability %=

$$\frac{\text{mean CFU at time t}}{\text{mean CFU of Baseline}} \times 100$$

RESULTS

Regarding plaque index results, for both groups, the Plaque index showed a maximum decrease after 2 weeks, with further gradual increase till the last follow up with significant difference between baseline and all follow-ups. As for the intergroup comparison Flour Protector group showed a higher mean value than the Cervitec F group, yet the difference was not statistically significant. The overall p-value was 0.749 which means that there was no significant decrease in plaque index observed when comparing Flour Protector with Cervitec F.

Regarding Image Analysis results, for both groups, Image analysis of dental plaque showed a maximum decrease after 2 weeks, with further gradual increase till the last follow up. With significant difference between baseline and all

follow-ups. As for the intergroup comparison, Flour Protector group showed a higher mean value than the Cervitec F group, yet the difference was not statistically significant (Table 2). The overall p-value was 0.735 which means that both varnishes have almost the same efficiency to decrease plaque retention.

And finally, Regarding Plaque bacterial count for both groups, The inter dental plaque bacterial count showed maximum decrease after 2 weeks, with further gradual increase till the last follow up. With significant difference between baseline and all follow-ups

As for the intergroup comparison, Flour Protector group showed a higher mean value than the Cervitec F group, yet the difference was not statistically significant at all time intervals except the 4th week there was a statistically significant difference in plaque bacterial count when comparing the two

groups Favoring the intervention (P=0.027) (Table 3). The overall p-value was 0.04 which means there are statistically significant differences between the two groups in CFU counts. So, the results of this study revealed that the use of Cervitec F varnish achieved a reduction in streptococcus mutants count more than what was seen in the Fluor Protector group

According to Wilcoxon signed-rank test to compare the ranks of viability % of two groups, the best performance (the least viability% of the streptococcus colonies) was evident after the 2nd and 4th week of Cervitec F (C) application followed by the 2nd and 4th week of Flour Protector (F) application. Then gradual increase after 12th and 24th week of application of both Cervitec F and Flour Protector simultaneously (P-value=0.03). That confirmed the efficiency of the Cervitec F varnish.

TABLE (1): Comparative evaluation of mean plaque index between two groups

Group	Plaque index results (mean ± SD)					P-value Intragroup
	Baseline	2nd week	4th week	12th week	24th week	
Control	1.95±0.41a	1.05±0.33c	1.17±0.21c	1.58±0.33b	1.71±0.36b	0.001
Intervention	2.28±0.42x	0.98±0.09y	1.13±0.14y	1.49±0.16z	1.68±0.07z	0.003
P value intergroup	0.224 ns	0.645 ns	0.693 ns	0.599 ns	0.839 ns	

-Means with different superscript are statistically significant different at P ≤ 0.05

- P-value significant at P ≤ 0.05.   ns: non-significant (p>0.05)

TABLE (2) Comparative evaluation of Image Analysis results between two groups

Group	Image analysis % of tooth area covered with DP (mean ± SD)					P-value intragroup
	Baseline	2nd week	4th week	12th week	24th week	
Control	61.73±6.327a	19.92±2.143c	29.45±3.852c	41.53±4.988b	50.74±5.329b	0.005
Intervention	72.67±7.326w	14.64±2.986z	19.84±1.644z	33.51±3.217y	48.47±5.941x	0.023
P value intergroup	0.067 ns	0.417 ns	0.064 ns	0.059 ns	0.094 ns	

- Means with different superscript are statistically significant different at P ≤ 0.05

- P-value significant at P ≤ 0.05.   ns: non-significant (p>0.05)

TABLE (3) Comparative evaluation of Plaque bacterial count results between two groups

Group	Mean Log10 CFU (mean ± SD)					P-value intragroup
	Baseline	2nd week	4th week	12th week	24th week	
Control	4.72±0.142a	2.75± 0.268c	3.02±0.198c	3.58±0.166b	3.87±0.073b	0.000
Intervention	4.78±0.137x	2.51±0.227z	2.65±0.252z	3.35±0.219y	3.64±0.247y	0.001
P value intergroup	0.051 ns	0.156ns	0.027	0.083ns	0.070ns	

-Means with different superscript are statistically significant different at P ≤ 0.05
- P-value significant at P ≤ 0.05.   ns: non-significant (p>0.05)

TABLE (4): Comparing the Viability% for both groups (Flour Protector and Cervitec F).

	Viability %	
	Flour Protector	Cervitec F
Baseline	100	100
2 Weeks	58.26	52.51
4 Weeks	63.98	55.44
12 Weeks	75.85	70.08
24 Weeks	81.99	76.15

Fig. (1): Bar chart represents Wilcoxon Signed Ranks Test for the Viability % of the two groups at different experimental periods.

DISCUSSION

Dental caries is a complex process in which the colonization of the tooth surfaces by bacterial plaque is the main prerequisite for the development of the disease. Routine mechanical plaque control as tooth brushing is widely recognized as the mainstay for the prevention of oral biofilm-associated dental diseases. Due to the lack of effective use of mechanical plaque control, patients could additionally benefit from chemotherapeutic antiplaque agents that can serve as adjuncts to traditional mechanical plaque control and interfere with biofilm composition and metabolism. Antiplaque agents can reduce the rate of new plaque formation, reduce or eliminate existing plaque, restrict pathogenic microflora growth, and prevent the development of virulence factors *(Jafer et al., 2016)*.

Over time Fluoride has emerged as a main preventive agent for caries due to its ability to decrease acid formation in some bacterial species in dental plaque. Fluoride ions also promote the formation of fluorapatite in enamel in the presence of calcium and phosphate ions produced during enamel demineralization by plaque bacterial organic acids. This may be the major mechanism of action of fluoride ions in preventing enamel demineralization. Hence conventional fluoride varnish was used as a control material in the current study *(Goldberg, 2017)* .
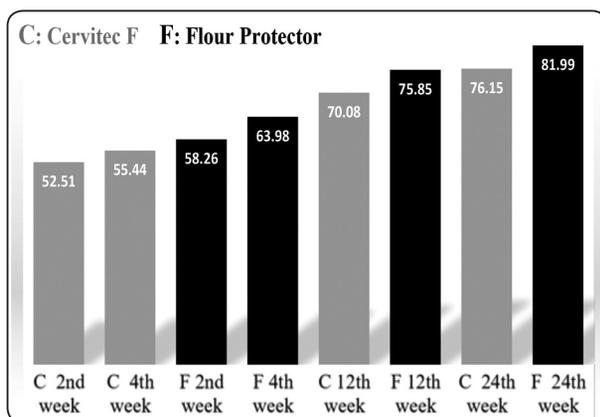
Chlorhexidine has proved to be a potent antimicrobial agent particularly against S. mutans in saliva and dental plaque. Because it binds to glycoproteins by reverse electrostatic binding, it stays on the oral surfaces. Its substantivity (ability to maintain therapeutic activity for a long time) is attributed to its adsorption onto tooth surfaces, pellicle, plaque, and mucous membranes*(Taghizadeh et al., 2021)*. Additionally, in smaller concentrations, it exhibits hydrophilic–hydrophobic properties as well as a bacteriostatic action, which hinders membrane transport and allows its light molecules to enter into the offending microbe. Furthermore, in the presence of fluoride, the affinity of CHX for hydroxyapatite increases thus reducing the effective concentration of CHX needed. In this respect, a combination of low concentrations of CHX and NaF has demonstrated favorable properties. The addition of fluoride to CHX also has been shown to inhibit caries development by providing an adjunctive beneficial remineralization effect to the CHX *(Elkerbout et al., 2019)*.

Cetylpyridinium chloride (CPC), despite its great antibacterial activity against oral microorganisms, has only been employed in a few types of dental varnishes. It has been demonstrated that increasing the amount of CPC retained in the biofilm improves the antibacterial action. This may be attributed to the ability of CPC to adsorb on to coated pellicle. Their mechanism of action relies on the interaction of the hydrophilic part of the CPC molecule with the bacterial cell membrane, causing disruption, modification of bacterial cell metabolism, growth inhibition, and ultimate cell death *(Pandit et al. 2015)*.

Based upon the mechanism of action of CHX and CPC, which is complementary to the well-known mechanism of action of fluoride, A Fluoride varnish system that contains Fluoride, Chlorhexidine (CHX), and Cetylpyridinium Chloride (CPC) was used in this study to assess its effect on plaque accumulation and plaque bacterial count compared to conventional fluoride varnish. The study aimed to test the effect of two varnishes in real-life situations hence this study has tried to test the selected two varnishes under field conditions and the results can be attributed to whole varnish rather than a single active ingredient.

In the research field, the use of disclosing agents and subsequent image software analysis can be employed as an improved plaque quantification method, overcoming the limitations of traditional plaque indices such as variability between examiners. When compared to traditional clinical indices, automated planimetric analysis enables for more sensitive and objective plaque localization and quantification, as well as high discriminating power, allowing for the detection of even little changes in plaque area. *(Rosa and Elizondo, 2015)*. Previous studies have shown that planimetric approaches are more precise, objective, sensitive, and reproducible than traditional indices and that they can detect even modest changes in plaque area.

The result of the present study shows that for both conventional Fluoride varnish and Fluoride varnish with CHX and CPC there was a statistically significant reduction in the mean of dental plaque between baseline and all follow-ups in both plaque index records and image analysis for dental plaque. This was in agreement with previous study by *Sehgal et al. (2018)* who used CHX varnish over 3 and 6 months.

On intergroup comparison, Fluoride varnish containing CHX, and CPC was found to be more effective than conventional Fluoride varnish, but the difference was not significant. This is consistent with previous in vivo studies by *Alavi et al (2018) & Chiba et al (2019)*, which showed that the combination of fluoride and CHX varnish decreases plaque retention. The reason could be attributed to CHX's ability to reduce bacteria-causing plaque accumulation on the tooth surface.

However, this was in disagreement with *Yoo et al. (2014) &Pandit et al. (2015).* The authors suggested that the properties of 0.05% CPC when added to routine oral hygiene treatment influence the attachment of dental plaque on the tooth surface, but in the present study, there was no overall significant difference between two varnishes on dental plaque and the effects were little and variable. This could be attributed to the difference in delivery method or the frequency of application*.*

Regarding plaque bacterial count, the result of the current study revealed that both varnishes achieved a statistically significant reduction in the mean Log CFU between baseline and all follow-ups. This was in agreement with *Badjatia et al. (2017)* who evaluated the effects of fluoride varnish on S. mutans count over 6 months, *Jentsch et al. (2014)* who evaluated the antibacterial activity of different formulations of a chlorhexidine varnish in vitro and in vivo, *Liptak et al. (2016)* who evaluated CHX varnish effect on S. mutans count over 6 months, and *Chiba et al. (2019)* who evaluated the effect of chlorhexidine and fluoride varnishes on the levels of Streptococcus mutans over 30 days.

There was a significant overall difference observed when comparing conventional Fluoride varnish and Fluoride varnish with CHX and CPC favoring the second one (Cervitec F). This is in line with *Sajjan et al. (2013), Gokhale (2017), & Pocha et al. (2018) .* Fluoride may have a specific effect on the metabolism and acid tolerance of oral bacteria as well as an initial bactericidal effect on S. mutans, but the sustainability effect might be lacking compared to chlorohexidine. On the other hand, chlorhexidine has proved to be a better antimicrobial agent against S. mutans. Chlorhexidine attaches to the glycoproteins by reversal electrostatic binding and thus gets retained on to the oral surfaces.

Maximum effect was evident in 2nd week for both groups, this initial effect is due to the phenomenon called 'burst effect'. Both varnishes are bacteriostatic, not bactericidal due to their low concentrations *(Paul et al., 2014) .* Hence, the antibacterial effect decreased considerably after the 4th week, with a subsequent significant increase in bacterial count over the rest of follow-up period. This could be attributed also to the early loss of varnish *(Baygin et al., 2014).* or the fact that prolonged suppression of S. mutans cannot be achieved by a one-time application of varnish, and more frequent applications may be necessary to achieve the inhibitory effect *(Narayan et al., 2017) .*

However, it might be noticed the conflict in the results between the studies carried out by **Fouad et al. (2013),** and our study. The author stated that with low concentrations of chlorhexidine mutans streptococci may not be killed effectively and proliferate and return to their original numbers within a few weeks. And **Narayan et al. (2017)** who evaluated the efficacy of Cervitec plus varnish and Flour Protector. on S. mutans count, found that S. mutans count in the Fluoride varnish group was the least when compared to the CHX varnish. This can be attributed to the fluoride deposited on the teeth by the fluoride varnish which later leached out and resulted in an inhibitory effect on the plaque bacteria. The authors suggested that in patients with cariogenic dietary habits and poor oral hygiene, a low concentration of chlorhexidine is not effective in reducing S. mutans count.

It is important to emphasize that there are divergences among the results of several studies, probably due to the multiple factors involved and to methodological differences such as different concentrations, frequency of applications, exposure time or varnish remnant, and the number of treated teeth *(Chiba et al., 2019) .*

Thus, the null hypothesis that there is no difference between the effect of Fluoride varnish containing Chlorhexidine and Cetylpyridinium Chloride (CPC) and Sodium Fluoride varnish on

plaque accumulation was accepted, and regarding their effect on bacterial load of plaque was rejected. In this study, we examined interproximal plaque after treatment with both varnishes which is more representative for the efficacy of materials disregarding oral hygiene measures carried out by patients. And this would explain the difference in results between the reduction of dental plaque and the effect on interdental bacterial count.

The results of our study are consistent with previous studies' results, supporting the finding that a combination of fluoride and chlorhexidine may be the most effective preventive protocol for high caries risk patients, which shows many advantages such as easy application and safe method without side effects of high concentration of CHX *(Baygin et al., 2014)* .

CONCLUSIONS

Under the limitation of the current study the following conclusion can be mentioned:

- Both Conventional Fluoride varnish and Fluoride varnish with CHX and CPC can decrease bacterial load and plaque accumulation.

- Fluoride varnish with CHX and CPC achieved a more reduction in streptococcus mutants count compared to the Conventional Fluoride varnish group.

- Re-application of varnishes every 3 months is preferred for high caries risk patients for better plaque and bacterial control.

RECOMMENDATIONS

1. Further clinical trials with multiple applications of the Chlorhexidine and Cetylpyridinium Chloride containing varnishes.

2. Maintaining good oral hygiene is the most important prophylactic measure in high caries risk patients.

REFERENCES

1. Alavi, S. and N. Yaraghi (2018). "The effect of fluoride varnish and chlorhexidine gel on white spots and gingival and plaque indices in fixed orthodontic patients: A placebo-controlled study." Dent Res J (Isfahan) 15(4): 276-282.

2. Achmad, H. (2021). The Impact of Using Fluoride in Pediatric Dentistry: A Systematic Review. Annals of the Romanian Society for Cell Biology, 2816-2839.

3. Abo Bakr, R. A., Tawfick, M. M., Mostafa, Z. A., & Abdulall, A. K. (2021). Virulence traits-based behavior of Streptococcus mutans bacteria from dental plaque and dental caries conditions. Microbial Biosystems, 6(1), 75-85.

4. Badjatia, S., R. G. Badjatia, K. Thanveer and A. C. Krishnan (2017). "Effects of fluoride varnish on Streptococcus mutans count in saliva." Int. J. Pediatr. Dent. 10(1): 62.

5. Baygin, O., T. Tuzuner,.7 A. Kusgoz, A. C. Senel, M. Tanriver and I. Arslan (2014). "Antibacterial effects of fluoride varnish compared with chlorhexidine plus fluoride in disabled children." Oral Health Prev Dent 12(4): 373-382.

6. Chiba ,F. Y., C. A. S. Garbin, T. A. Saliba, A. J. Í. Garbin and S. A. S. Moimaz (2019). "Effect of Chlorhexidine and Fluoride Varnishes on Streptococcus mutans Count and Dental Plaque Index." J. HEALTH Sci. 21(4): 371.

7. Elkerbout, T., D. Slot, C. Van Loveren and G. Van der Weijden (2019). "Will a chlorhexidine-fluoride mouthwash reduce plaque and gingivitis?" Int. J. Dent. Hyg. 17(1): 3-15.

8. Fouad, W., A. L. J. Am and E. Mh (2013). Clinical Evaluation of the Anti-Bacterial Effect of Fluoride and Chlorhexidine Varnishes. Egypt. Dent. J. 59(3): 2489:2501

9. Gokhale, N. (2017). Comparison of the Effect of Fluoride Varnish, Chlorhexidine Varnish and Casein Phosphopeptide- Amorphous Calcium Phosphate (CPP-ACP) Varnish on Salivary Streptococcus mutans Level: A Six Month Clinical Study. J. Clin. Diagnostic Res.: JCDR, 11(8), ZC53.

10. Goldberg, M. (2017). "Fluorides: Double Sword Implicated In caries Prevention and Fluorosis." SF Dent Oral Res J 1(2).

11. Jafer ,M., S. Patil, J. Hosmani, S. H. Bhandi, E. P. Chalisserry and S. Anil (2016). "Chemical Plaque Control Strategies in the Prevention of Biofilm-associated Oral Diseases." J. Contemp. Dent. 17(4): 337-343.

12. Jentsch, H., F.-R. Eckert, K. Eschrich, S.-I. Stratul and S. Kneist (2014). "Antibacterial action of Chlorhexidine/

thymol containing varnishes in vitro and in vivo." Int. J. Dent. Hyg. 12(3): 168-173.

13. Langa, G. P. J., Muniz, F. W. M. G., Costa, R. D. S. A., da Silveira, T. M., & Rösing, C. K. (2021). The effect of cetylpyridinium chloride mouthrinse as adjunct to tooth-brushing compared to placebo on interproximal plaque and gingival inflammation—a systematic review with meta-analyses. Clinical Oral Investigations, 25(2), 745-757.

14. Liptak, L., N. Barsony, S. Twetman and M. Madlena (2016). "The effect of a chlorhexidine-fluoride varnish on mutans streptococci counts and laser fluorescence readings in occlusal fissures of permanent teeth: A split-mouth study." Quintessence Int 47(9): 767-773.

15. Lipták, L., K. Szabó, G. Nagy, S. Márton and M. Madléna (2018). Microbiological Changes and Caries-Preventive Effect of an Innovative Varnish Containing Chlorhexidine in Orthodontic Patients. Caries Res, 52(4), pp.272-278.

16. Narayan, A., S. Satyaprasad, S. Anandraj, S. R. Ananda, P. A. Kamath and S. Nandan (2017). "Comparison of efficacy of three chemotherapeutic agents on Streptococcus mutans count in plaque and saliva: A randomized controlled triple blind study." J Indian Soc Pedod Prev Dent 35(2): 174-180.

17. Pandit, S., J. N. Cai, J. E. Jung, Y. S. Lee and J. G. Jeon (2015). "Effect of brief cetylpyridinium chloride treatments during early and mature cariogenic biofilm formation." Oral Dis. 21(5): 565-571.

18. Paul, S., S. Baranya Shrikrishna, E. Suman, R. Shenoy and A. Rao (2014). "Effect of fluoride varnish and chlorhexidine–thymol varnish on mutans streptococci levels in human dental plaque: a double-blinded randomized controlled trial." Int. J. Paediatr. Dent. 24(6): 399-408.

19. Pocha, S. R., D. K. Kumar, M. Dhanya and K. Sudhakar (2018). "Effects of sodium fluoride solution, chlorhexidine gel and fluoride varnish on the microbiology of dental plaque–A randomised controlled trial." J. Indian Assoc. Public Health Dent. 16(2): 109.

20. Rosa, G. M. and M. L. Elizondo (2015). "New portable system for dental plaque measurement using a digital single-lens reflex camera and image analysis: Study of reliability and validation." J Indian Soc Periodontol 19(3): 279.

21. Sajjan, P., L. Nagesh, M. Sajjanar, S. Reddy and U. Venktesh (2013). "Comparative evaluation of chlorhexidine varnish and fluoride varnish on plaque Streptococcus mutans count – an in vivo study." Int. J. Dent. Hyg. 11(3): 191-197.

22. Sehgal, A., S. Shetty and N. P. Jose (2018). "Efficacy of Chlorhexidine Varnish In Patients Undergoing Multibracketfixed Orthodontic Treatment: A Controlled Clinical Study." Biomed. Pharmacol. J. 11(2): 945-950.

23. Taghizadeh, F., & Fakhari, E. (2021). Effect of Chlorhexidine Containing Fluoride on Dental Plaque, Gingivitis, and Tooth Discoloration: A Randomized Clinical Trial. Avicenna Journal of Dental Research, 13(2), 67-71.

24. 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.

25. Yoo, C.-S., S.-K. Chae, H.-G. Lee, C.-H. Kim, D.-K. Kim, W.-G. Kim, K.-E. Kim, H.-K. Ahn, J.-E. Kim and Y.-J. Oh (2014). "The inhibition effects of cetylpyridinium chloride in dentifrices on plaque formation: clinical test." Int. J. Clin. Prev. 10(1): 9-14.