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EVALUATION OF NANOHYDROXYAPATITE VERSUS POVIDONE-IODINE MOUTHWASHES ON SALIVARY STREPTOCOCCUS MUTANS

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ABSTRACT

Objectives: This study was conducted to evaluate the antimicrobial effect of mouthwashes containing nanohydroxyapatite (Nno-HAp) and povidone-iodine (PVP-I) in comparison with chlorhexidine (CHX) on *Streptococcus mutans* in saliva. **Subjects and Methods:** Thirty children having high caries index aged between 6-12 years were included in the study following specific inclusion criteria and then grouped randomly into three main groups based on the used mouthwash (Chlorohexidine; Povidone-iodine; Nanohydroxyapatite). Two Saliva samples were collected pre-& post 15 days mouthwash rinsing from each child. Another saliva sample was taken after 15 days of mouthwash discontinuity. The samples were transported immediately to the bacterial lab for culturing and counting. The efficacy of mouthwashes were assessed. **Results:** After 15 days of mouth-rinsing, all tested mouthwashes revealed a significant reduction in *Streptococcus mutans* count. CHX mouthwash showed the highest antibacterial effect followed by PVP-I and Nano-Hap with a high significant difference between them. However, after 15 days of discontinuity, it was found that there was a significant difference between chlorohexidine and Nano-hydroxyapatite groups only. **Conclusion:** Although Mouthwashes containing PVP-I and Nano-HAp showed a high antimicrobial effect against *streptococcus mutans*, however, CHX mouthwash is still the most effective against *Streptococcus mutans*.

KEYWORDS: Chlorhexidine, Nanohydroxyapatite, Mouthwash, Povidone-iodine, *Streptococcus mutans*

INTRODUCTION

Inadequate oral health of children is generally accompanied by the presence of uncontrolled and active caries lesions [(1)]. Dental caries can be defined as an infectious disease characterized by localized destruction of hard tooth structures by the action of specific microorganisms[(2)]. The metabolic byproducts of these specific microorganisms within plaque are considered the etiologic factor for dental caries [(3)].

Streptococcus mutans is a gram-positive, anaerobic cocci and the most causative microorganism for dental caries [(4)]. Usually children with a higher caries rates suffer from high *Streptococcus mutans* salivary count, therefore, controlling or reducing this microbial via using chemotherapeutic agents is found to be useful in marked decrease in caries indices especially in children [(2,4)].

Chlorohexidine (CHX) is the commonest chemotherapeutic rinsing solution that is used to control the oral microorganisms, and is considered the gold standard rinsing solution with the reported

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potential to reduce the salivary *Streptococcus mutans* count due to its cationic nature [5,6]. However, it has a major disadvantage of tooth staining after a long period of use [6].

Also, Povidone-iodine (PVP-I) is one of the commonest chemotherapeutic agents that used as a disinfectant solution and used also as a rinsing solution [7]. It has various advantages namely; low cytotoxicity, lack of microbial resistance, the broad spectrum of anti-microbial activity, and ability to penetrate through biofilms [7,8].

On the other hand, nanohydroxyapetite (Nano-Hap) usually resembles the composition of the mineralized tooth structure and was used first to remineralize the demineralized tooth structures [9]. However, it was firstly introduced in preventive dental practice via its incorporation in toothpaste, and mouthwashes to reduce or mask tooth sensitivity [10]. However, only few studies focused on its antimicrobial effect [11].

Thus, the present study was directed to evaluate clinically the antimicrobial effect of mouthwashes containing PVP-I and Nano-HAp against salivary *Streptococcus mutans*, in comparison to the gold standard CHX in children.

SUBJECT AND METHODS

This clinical study was carried out on thirty children aged from 6-12 years. Children enrolled in this study were selected from Outpatients clinic of the Pedodontics and Oral Health Department, Faculty of Dental Medicine, Al-Azhar University (Cairo, Boys). The sample size for the current study was determined based on the results of Bharathi et al (2018) [12] study.

Ethical consideration

This study was carried out after approval of ethical committee, Faculty of Dental Medicine, Al-Azhar University, Boys, Cairo (EC Ref No.320/345/08/09/19). All parents will sign an informed written consent form (Attached copy) before treatment beginning.

The included children in the present study had high caries index and were systemically free with no previous history of antibiotic, inflammatory or steroid therapy for at least three weeks before starting this work. The children were grouped randomly into three equal groups based on the used mouthwash; group 1: children used 0.12% CHX; group 2: children used 0.1% PVP-I; and group 3: children used 5% Nano-HAp. The mouthwashes were tested to determine their potential against salivary *Streptococcus mutans* after 15 days of use and after further 15 days of discontinuity and compared to the baseline count before mouth rinsing.

Intervention:

Preoperative assessment:

The involved children were clinically examined and a sampling frame was prepared of those who fulfilled the inclusion criteria.

Patient instructions and saliva sample collection:

Children were asked to give the baseline saliva samples (S1) by spitting in a sterilized tube. The unstimulated saliva samples were collected from each child in each group at least 1 ml per sample. All subjects were instructed to do regular tooth brushing twice daily using the modified Bass technique and make sure that the brushing was correct, standardized, and effective. Each child was given 150 ml (3 bottles) of the mouthwash. Each bottle contains 50 ml to use 10 ml of the mouthwash for each application per day at morning and swish it in all quadrants of the mouth for 2 minutes for 15 days. [13].

The subjects were instructed not to eat or rinse for the next 30 minutes and frequent reminders through telephone where given to supervisors to ensure compliance and not to take any antibiotics without reference to the operator. At the end of 15 days with mouth rinse therapy, all subjects were asked to rinse their mouth with deionized water for 1 minute before sampling. Then the second unstimulated saliva sample (S2) was collected from each child. For the next 15 days, all subjects

were adjusted to discontinue the mouthwashes and maintain the regular tooth brushing twice daily, then, another unstimulated saliva sample (S3) was collected from each subject [12,13].

Microbiological analysis:

All collected saliva samples were transported, under complete aseptic condition, as soon as possible to the microbiological lab at the regional center for microbiology and biotechnology (RCMT) (Al-Azhar University, Cairo, Egypt). The collected samples were prepared and cultured on the selective media "Mitis Salivarius Agar with bacitracin". The plates were incubated in an anaerobic chamber for 7 days at 37°C. Then, the *Streptococcus mutans* organisms were identified and the total number of colonies on the incubated plates were digitally counted by the digital colony counter and expressed as CFU/ml [13].

Statistical analysis:

The collected data was revised, coded, tabulated, and analyzed using SPSS program software, version 22.0. Pearson Chi-Square test was applied to gauge the difference between categorical data. Paired t-test and ANOVA were used to compare between sample means for quantitative data with normal distribution. The statistical significance level was set at 5% ($p \leq 0.05$ is considered statistically significant).

RESULTS

The results of the current study revealed that at the baseline; the saliva samples showed no statistically significant difference in Streptococcus *mutans* count among all tested and control groups. While after 15 days of mouth-rinsing, all tested mouthwashes revealed a highly significant reduction in *Streptococcus mutans* count. However, after discontinuity for another 15 days although it was found that there was a high significant increase of streptococcal counts in all groups still less than baseline bacterial counts with a highly significant difference (Table 1).

TABLE (1) *S. mutans* counts (CFU/ml) at the three intervals periods in all groups.

Character	Interval	Min.	Max.	Mean ±SD	Sig.
Chlorohexidine	Base-line	4800	5700	5220±308.4	P1<0.001* P2<0.001* P3<0.001*
	After rinsing	2100	2500	2265±137.54	
	After discontinuity	4500	5200	4840±211.87	
Povidone-iodine	Base-line	4800	5700	5135±268.79	P1<0.001* P2<0.001* P3<0.001*
	After rinsing	2500	3000	2760±159.51	
	After discontinuity	4600	5200	4885±178.03	
Nano-hydroxyapatite	Base-line	4700	5700	5180±322.49	P1<0.001* P2<0.001* P3<0.001*
	After rinsing	3100	3700	3330±226.32	
	After discontinuity	4850	5400	5055±178.65	

* *Significant at* $\mathbf{P} \leq 0.05$ ** *High significant difference at* $\mathbf{P} \leq 0.01$

$\mathbf{P_1}$: *Difference between the base line bact. Count and after 15 days of mouthwash rinsing.*

$\mathbf{P_2}$: *Difference between the base line bact. Count and after 15 days of mouthwash discontinuity*

$\mathbf{P_3}$: *Difference between after 15 days of mouthwash rinsing and after 15 days of mouthwash discontinuity*

On comparing the *S. mutans* counts in all group at the different intervals it was found that there is no significant difference at the baseline interval. While at 15 days post rinsing Moreover, the CHX mouthwash showed the highest microbial reduction (56.61%) followed by PVP-I (46.25%) and Nano-Hap (35.71%) with highly significant difference between all groups. However, although there are increase of bacterial counts after the third interval the study showed that there was a significant difference between chlorohexidine and Nano-hydroxyapatite groups only (Table 2).

TABLE (2) Comparison of S. mutans counts in the three groups at different intervals.

S. mutans count (CFU/ml)		Chlorohexidine N=10	Povidone-iodine N=10	Nano-hydroxyapatite N=10
At the base-line	mean±SD	5220±308.4	5135 ± 268.79	5180± 322.49
	Sig.	P1=0.533	P2=0.768	P3=0.741
15 days after rinsing	mean±SD	2265±137.54	2760± 159.51	3330± 226.32
	Sig.	P1<0.001*	P2<0.001*	P3<0.001*
15 days after discontinuity	mean±SD	4840± 211.87	4885± 178.03	5055± 178.65
	Sig.	P1=0.858	P2=0.045*	P3=0.138

One Way ANOVA test,; significant at $p \leq 0.05$.*

P_1: Difference between Chlorohexidine &Povidone-iodine.

P_2: Difference between Chlorohexidine& Nano-hydroxyapatite.

P_3: Difference between Povidone-iodine & Nano-hydroxyapatite.

Pairwise comparison was done by Post Hoc Tukey test.

DISCUSSION

The antibacterial effect of CHX, PVP-I and Nano-HAp mouthwashes as chemotherapeutic solutions on *Streptococcus mutans* levels in the saliva of children with high caries index was investigated in this study. However, the actual controlling of *Streptococcus mutans* infection in the enrolled children was fulfill in two phases-caries the control by restoration of the existent cavities followed by chemotherapeutic medication [5].

Mitis salivarius agar media was chosen in this study as a selective medium for culturing and counting *Streptococcus mutans* microorganisms[14]. Since the addition of bacitracin to the Mitis salivarius agar media allows *Streptococcus mutans* to grow and form colonies and inhibit the growth of most other oral bacteria [14,15].

CHX was selected as a control mouth-rinsing agent because it is considered as the standardized chemotherapeutic agent that is able to reduce the *Streptococcus mutans* count in saliva, hence decreasing the accompanied dental caries [6]. A lower concentration of CHX (0.12%) was elected to minimize its harmful side effects while maintaining its benefits [16].

PVP-I as an oral rinsing solution has no adverse effect when used with young children for long periods. Hence it was chosen as a tested mouth

rinse to compare its effect with CHX against *Streptococcus mutans*. Furthermore, PVP-I was used with 0.1% iodine concentration as a common gargle formulation for oral care applications [17].

Nano-HAp was incorporated into products for oral care such as mouthwash and dentifrices to minimize tooth sensitivity as well as for the goal of endorsing the hard tooth structures remineralization [18]. However, it's the antibacterial potential of Nano-HAp was nearly evaluated [19]. Therefore, this study assessed the ability of mouthwashes containing Nano-HAp against *Streptococcus mutans*. Moreover, in the current study the mouthwash containing Nano-HAp with a concentration of 5% was chosen to avoid the use of maximum concentration and its side effect [18].

According to the results of Piovano et al., [20]; 15 days of experimental period was sufficient to significantly reduce the *streptococcus mutans* count. Therefore, in this study, the period of 15 days was elicited as a suitable experimental period. Also, the enrolled children in this study were aged between 6-12 years, because they can easily rinse their mouth without swallowing the mouthwash to avoid swallowing reflex [21]. Moreover, the unstimulated saliva samples were favored because it reverses the caries risk experience accurately[22]. Additionally, mouthwashes were used in standardized manner since each subject in each group was asked to use 10 ml of each mouth wash for 15 days for 2 minutes twice daily [23].

The results of this study detected no statistically significant differences in the *streptococcus mutans* count among all groups before the use of the tested mouth rinses. Moreover, the results revealed that CHX mouth rinse significantly reduce the salivary *streptococcus mutans* count after rinsing for 15 days by 56.6%. This is because the cationic nature "positive charges" of the CHX molecule which binds with the outer microbial cell membrane which is negatively charged resulted in increasing its permeability and hence led to its kill [16]. These results came compatible with the results of Mamta et al. who reported that the CHX showed a bacterial reduction after 14 days of treatment by 25.72%[24] .

Also, it was found that the use of PVP-I mouth rinse was able to significantly reduce the salivary *streptococcus mutans* count after rinsing for 15 days by 46.3%. This due to the high chemical reactivity of iodine molecules that make it able to attack microbes via oxidizing its vital structures namely; proteins, nucleic acids, as well as membrane components [17]. This result came compatible with Koburger-Janssen TM, Eickmann M, Zorn J who reported that the 0.2% povidone iodine showed a microbial reduction by 30% [25].

Moreover, the use of mouth rinse containing Nano-HAp reduced significantly salivary *streptococcus mutans* count by 35.7% because of the anti-adhesive capacity of Nano-HAp which prevents the adhesion of new bacteria [26]. However there is nearly no studies investigating the antibacterial effect of Nano-Hap as a mouthwash, but in general this result came in agreement with Zhou L, Wong HM, Li QL who demonstrated that is the Nano-Hap was recommended as antibacterial releasing agent because of the possibility of its ability for delivering a high an antibacterial agent concentration locally[27] and agree with Ionescu et al reported that the Nano-Hap has antibacterial effect as a toothpaste[28].

These results came in disagreement with the results of Nakagawa et al, who reported that the PVP-I showed a superior result compared to chlorhexidine mouth wash [29] .

However, the results of this study revealed that after 15 days of discontinuity of using the all tested mouth rinses there was a significant increase in the salivary *Streptococcus mutans* count. This could explain by an important property called "substantively" which is defined as the ability of mouthwash to adsorbed and sustain into the surfaces of mucosa for a longer period just more than the time it is present in the mouth and it was affected by the solution pH, concentration, temperature and time of holding in mouth [30].

CONCLUSION

Based on the results of the current study, it could be explored that the use of mouthwashes containing PVP-I and Nano-HAp has antimicrobial effect against *Streptococcus mutans*. However, CHX mouthwash is still the most effective against *Streptococcus mutans*. Mouthwashes containing PVP-I and Nano-Hap need more modification and investigation to be more effective.

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