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ORIGINAL ARTICLE

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Mutans streptococci growth on glass ionomer incorporated with chlorhexidine: in-vivo study

Crescimento de *Estreptococos mutans* em ionômero de vidro incorporado com clorexidina: estudo in vivo

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ABSTRACT

Background: glass ionomer is one of the most frequently used restorative materials for primary teeth restoration. It has been in use for more than 30 years. Their restoration usefulness is preferential compared to other restorations due to their fluoride release and recharge, chemical adhesion to the structure of the dentin and their range of uses. Increasing the antibacterial efficacy of restorative materials is one of the primary goals to decrease the incidence of recurrent caries. Chlorhexidine is the gold standard antibacterial agent in dentistry. **Objectives:** the objective of this study is to evaluate the antibacterial effect of Chlorhexidine incorporated with glass ionomer on *Streptococcus mutans*. **Methods:** Thirty Children between ages ranged 6-9 years old were selected to participate in this study. Children with bilateral caries in lower second primary molars affecting the occlusal and proximal surfaces without pulpitis were included in the study. All cavities were divided into two groups; group (A) restored with Glass Ionomer and group (B) restored with Glass Ionomer Chlorhexidine mixture. The sound proximal surfaces in all cavitated teeth acted as a control. After one month, two months and three months' plaque samples were obtained and *Streptococcus mutans* counts were calculated. **Results:** The number of SM taken from sound proximal surfaces for all groups were not changed significantly in whole periods of study. At the all-time interval, the mean log10 of SM in group B was lower than group A and the difference was statistically significant. There is a significant difference in the mean log10 of SM in group B between the 1St month and the 3rd month. **Conclusion:** The growth of SM was found to be higher in the sound tooth than in GI groups and in GI group was higher than in CHX- GI mixture up to three months.

KEYWORDS

Chlorhexidine; Glass Ionomer; *Streptococcus mutans*.

RESUMO

Antecedentes: o ionômero de vidro é um dos materiais restauradores mais utilizados na restauração de dentes decíduos. É usado há mais de 30 anos. Sua indicação como material restaurador em comparação a outros baseia-se nas propriedades de liberação e recarga de flúor, adesão química à estrutura da dentina e sua variedade de usos. Aumentar a eficácia antibacteriana de materiais restauradores é um dos principais objetivos para diminuir a incidência de cárie recorrente. A clorexidina é o agente antibacteriano padrão-ouro em odontologia. **Objetivos:** o objetivo deste estudo é avaliar o efeito antibacteriano da Clorexidina incorporada ao ionômero de vidro no *Streptococcus mutans (SM)*. **Métodos:** Trinta crianças entre 6 e 9 anos foram selecionadas para participar deste estudo. Crianças com cárie bilateral nos segundos molares decíduos inferiores que afetavam as superfícies oclusal e proximal sem pulpite foram incluídas no estudo. Todas as cavidades foram divididas em dois grupos; grupo A, restaurado com Ionômero de Vidro e grupo B, restaurado com mistura de Ionômero de Vidro /Clorhexidina. As superfícies proximais sadias em todos os dentes cavitados atuavam como controle. Após um mês, dois e três meses, foram obtidas amostras de placa e as contagens de *Streptococcus mutans* foram realizadas. **Resultados:** O número de SM retirado da superfície proximal sadia para todos os grupos não foi alterado significativamente nos períodos do estudo. No intervalo de todos os tempos, o log10 médio da SM no grupo B foi menor que no A e a diferença foi estatisticamente significante. Há uma diferença significativa no log10 médio da SM no grupo B entre o 1º mês e o 3º mês. **Conclusão:** O crescimento da SM mostrou-se maior no dente sadio do que nos grupos A; e no grupo A foi maior que no grupo B até três meses.

PALAVRAS-CHAVE

Clorexidina; Ionômero de vidro; *Streptococcus mutans*.

INTRODUCTION

Many studies and documents have concluded caries lesions that develop around restorations are the most commonly reported reason for restoration replacement in primary teeth, especially when there is no compliance as child patient [1-4].

Recurrent caries was the basis to the extent of the cavo-surface margin to a self-cleansable location where the toothbrush might have had access to the plaque. Since the only recognized way to prevent caries at that time was tooth brushing [5].

Caries lesion mostly was seen on the proximal surface of the primary teeth, where the caries progression in this area seems to be faster than on occlusal surfaces [6-7].

Streptococcus mutans are essential for the initiation and advancement of caries, and lactobacillus acidophilus is frequently present in superficial and deep caries in large numbers. Streptococcus mutans and Lactobacillus acidophilus are often considered as the two most important cariogenic bacteria associated with dentine caries [8].

The ability of restorative material to resist secondary caries attack and micro-leakage at its margins can be achieved by the improvement of restorative material properties and will largely determine whether restoration will succeed or fail [9].

In the last century, many new modern filling products have been created to be resistant to secondary caries as it has shown some antimicrobial activity, The most significant thing is their potential to release fluoride and bind with the prepared tooth surface [10,11].

There is moderate strength of evidence for a positive association between Glass ionomer and the prevention of caries lesions only in the margins of occluso-proximal restorations of primary teeth [12].

Chlorhexidine is a strong antiplaque agent that also has an outstanding antimicrobial property. It is a wide antimicrobial spectrum and can be regarded as a boon to maintaining oral health as a whole. As well it has been shown to control the decay of the tooth with promising outcomes. It is efficient against a wide range of Gram-positive bacteria, Gram-negative bacteria and fungi [13-15].

Because of its enhanced susceptibility relative to other oral microorganisms, chlorhexidine is one of the most appropriate efficient and secure agents in decreasing *Streptococcus mutans* [16].

Several researches have shown that adding chlorhexidine to a glass ionomer has resulted in a regeneration that has improved its antibacterial characteristics over glass ionomers alone [17-22].

In the present study, the effect of Chlorhexidine on the growth of *Streptococcus mutans* when added to the Glass Ionomer was evaluated.

MATERIAL AND METHODS

Thirty Children between ages ranged 6-9 years old were selected to participate in this study from Outpatient Clinic in Pediatric Dentistry Department, Faculty of Dentistry, Assuit dental College; Assuit Egypt. Children with bilateral caries in lower second primary molars affecting the occlusal and proximal surfaces without pulpitis were included in the study. A consent form was signed by the children's parents before the study. Complete medical and dental history was obtained for each of the selected children and was subjected to cavity preparation then restoration.

Restoration

All cavities were divided into two groups:

Group A: cavities in right second primary molars were restored by Fuji II LC (GC America Inc.) glass ionomer.

Group B: cavities in left second primary molars were restored by Chlorhexidine- Glass Ionomer Fuji II LC.

Preparation of Chlorhexidine Glass Ionomer mixture was performed by adding 0.01 ml of chlorhexidine gel 2% Gluco-CHeX (*PPH Cerkamed, Stalowa Wola, Poland*) to resin-modified glass ionomer capsule which contains 0.10 ml net volume of mixed cement after dispensing on cleaned sterilized glass slap. The mixture was loaded to the cavity after cleaning the cavity with distilled water then dried with air for 10 seconds All restorations were cured for 30 seconds according to manufacture instructions.

All children received general oral hygiene instructions and were oriented to brush their teeth 3 times a day after meals using a toothbrush and a fluoride-containing dentifrice supplied by the researchers throughout the experimental period. No other fluoride sources were used.

Microbiological analysis

After one month the plaque samples were pooled by sterilized toothpick hold by sterilized hemostat. Before the sample had been taken the whole surface of the tooth washed with water and air-dried for 10 seconds. The tip of the toothpick was holed toward proximal surfaces between filling material and enamel for 10 seconds (A1 or B1 subgroups), and the tip of another toothpick was holed toward sound proximal surfaces in restored molars (A2 or B2 subgroups) [23].

The procedure was repeated at the interval of two months, and three months. Colonies with *mutans streptococci* (MS) characteristics were transferred to tubes containing thioglycollate (Difco Laboratories Inc., Detroit, MI, USA) and incubated at 37° C for 24 h for biotyping. The growth of MS colony-forming unit (CUF) was verified after the incubation period, and the following tests were performed for biochemical identification: fermentation of mannitol, sorbitol, raffinose and melibiose, resistance to bacitracin, hydrolysis of arginine and sculin, production of H_2O_2, and sensitivity to 2.0 IU bacitracin. Biofilm samples were spread on 15x100 mm sterile test tubes containing 4 to 5 glass beads and 2.0 mL phosphate buffer saline (PBS). biofilm samples were vortexed for 2 and 1 min, respectively, for microbial desorption, and submitted to ten-fold serial dilutions (10^{-5}). After that, 50 mL of each dilution was plated equidistantly on the SB-20M culture medium and incubated under the candle jar system at 37° C for 48 to 72 hours. The number of (CFU) *per* milliliter of biofilm was counted, and biotyping of colonies with MS characteristics were performed, the *Streptococcus mutans* colony-forming units (CFU) were done by standard, or viable, plate count method [24]. At the end of the incubation period, all of the Petri plates containing between 30 and 300 colonies were selected. The colonies on each plate were counted. A Quebec colony counter was used. (CFU) per milliliter was calculated by dividing the number of colonies by the dilution factor multiplied by the amount of specimen. The original data measured in CFU were transformed in log10 for statistical analysis and are reported as log (CFU)/ml.

Statistical methods

SPSS version 12.0 was used for data management and data analysis. *Streptococcus mutans* counts were transformed to log values to be normally distributed (avoid high variability). The analysis was done on log values and description was made by the mean and standard deviation. Repeated measures ANOVA was done to elicit time effect within each group and to verify if there is any difference in the rate of drop of bacterial count over time between the two groups what is called time and group interaction. Chi-square and Fisher's exact test was used for comparing proportions among 2 study groups. P-value is significant at 0.05 levels.

RESULTS

This in vivo study was conducted to evaluate the growth of *Streptococcus mutans* on resin-modified Glass Ionomer restorative material and chlorhexidine-glass Ionomer

mixture. Plaque samples were collected from;

The proximal surface of GI only restorations (group A1), (n = 30).

The sound proximal surfaces of teeth restored with GI only (group A2), (n = 30).

The proximal surfaces of teeth restored with GI CHX mixture (group B1), (n = 30).

The sound proximal surfaces of teeth restored with GI CHX mixture (group B2) (n = 30).

The samples from the sound proximal surface act as a control for both groups.

The number of SM taken from sound proximal surfaces for both groups (A2 and B2) were not changed significantly in whole periods of study (**Table I**).

The mean $\log_{10}$ of SM on GI (Group A1) was higher than the mean $\log_{10}$ of SM on GI with CHX (Group B1) after one month, two months and three months and the difference was found to be statistically significant (**Tables II**).

In group A: After one, two and three months the mean $\log_{10}$ of SM in group A1 was lower than in group A2 and the difference was statistically significant (**Table III**).

There is no significant difference in the mean $\log_{10}$ of SM in group A1 between the 1St month and the second month and between the 1st month and the 3rd month (**Table IV**).

In group B: After one, two and three months the mean $\log_{10}$ of SM in group B1 was lower than in group B2 and the difference was statistically significant (**Table V**)

There is no significant difference in the mean $\log_{10}$ of SM in group B1 between the 1St month and the second month, however, the difference was significate when comparing the 1st month with the 3rd month (**Table VI**) .

Table I - The mean, standard deviation (SD) values and results of paired t-test for comparison between $\log_{10}$ SM in sound proximal surfaces for both groups

Side / Period	Group A2(n=30)		Group B2(n=30)		*P*-value
	Mean $\log_{10}$	SD	Mean $\log_{10}$	SD	
1 month	4.51	0.04	4.52	0.02	0.3236
2 months	4.52	0.01	4.51	0.02	0.0527
3 months	4.51	0.02	4.51	0.01	1.0000

**: Significant at P ≤ 0.05*

Table II - The mean, standard deviation (SD) values and results of paired t-test for comparison between $\log_{10}$ SM in group A1 and Group B1

Side / Period	A1 GI (n=30)		B1 CHX (n=30)		*P*-value
	Mean $\log_{10}$	SD	Mean $\log_{10}$	SD	
1 month	4.05	0.04	3.34	0.09	<0.001*
2 months	4.06	0.01	3.54	0.05	<0.001*
3 months	4.06	0.03	3.77	0.07	<0.001*

**: Significant at P ≤ 0.05*

Table III - The mean, standard deviation (SD) values and results of paired t-test for comparison between $\log_{10}$ SM in group A1 and Group A2 at different periods

Side / Period	A1 GI (n=30)		A2 (n=30)		*P*-value
	Mean $\log_{10}$	SD	Mean $\log_{10}$	SD	
1 month	4.05	0.04	4.51	0.04	<0.0001
2 months	4.06	0.01	4.52	0.01	<0.0001
3 months	4.06	0.03	4.51	0.02	<0.0001

**: Significant at P ≤ 0.05*

Table IV - The mean, standard deviation (SD) values and results of paired t-test for comparison between $\log_{10}$ SM in group A 1 at different periods

GI (n=30) / Period	A1 at 1 month		A1 at 2 months		*P*-value
	Mean $\log_{10}$	SD	Mean $\log_{10}$	SD	
1-2 months	4.05	0.04	4.06	0.01	0.1892
1-3 months	1 month		3 months		
	4.05	0.04	4.06	0.03	0.2778

**: Significant at P ≤ 0.05*

Table V - The mean, standard deviation (SD) values and results of paired t-test for comparison between log_{10} SM in group B1 and Group B2 at different periods

Side / Period	Group B1 (n=30)		Group B2 (n=30)		*P*-value
	Mean log_{10}	SD	Mean log_{10}	SD	
1 month	3.34	0.09	4.52	0.02	<0.0001
2 months	3.54	0.05	4.51	0.02	<0.0001
3 months	3.77	0.07	4.51	0.01	<0.0001

**: Significant at P ≤ 0.05*

Table VI - The mean, standard deviation (SD) values and results of paired t-test for comparison between log_{10} SM in group B 1 at different periods

CHX (n=30) / Period	B1 at 1 month		B1 at 2 months		*P*-value
	Mean log_{10}	SD	Mean log_{10}	SD	
1-2 months	3.34	0.09	3.54	0.5	0.0352
	B1 at 1 month		B1 at 3 months		
1-3 months	3.34	0.09	3.77	0.07	< 0.0001

**: Significant at P ≤ 0.05*

DISCUSSION

The present study was carried out to compare the *Streptococcus mutans* accumulation on Glass Ionomer that had been modified by the addition of chlorohexidine with Glass Ionomer alone used for cavity restoration. and did the addition of chlorohexidine result in a restorative material that had increased antibacterial properties over light-cured glass-ionomer alone.

It is well known that dental caries is bacterially based diseases. Dental bio-film is a significant factor in the incidence of dental caries that includes complicated structures composed of various microbial multi-species groups created on oral tissue. Tooth decay is primarily obtained from carcinogenic (particularly streptococci) organisms that engage in biofilm formation and produce subsequent cariogenesis. [25] *Streptococcus mutans* and other cariogenic bacteria may enter the GIC-dentin interfaces via micro-leakage at the tooth restoration interface to cause secondary caries, resulting in GIC loss and substitution [26].

The present study was carried out by the split-mouth technique where the Glass Ionomer- Chlorhexidine mixture was employed as a restorative material for carious primary molars on one side of selected children, while the conventional Glass Ionomer was used for carious primary molars in the other side of the same arch.

The most frequently cited cause for replacing the restoration was caries that developed around restorations which are more common in primary teeth, especially when there is no compliance as child patient. Primary molars have therefore been chosen in this research. [1-4]

While most caries lesions occur on the proximal surface in primary molars and caries progression in this area appears to be faster than on occlusal surfaces,6,7so in this these surfaces were selected in this study.

All children's parents were instructed to prevent their children from using any product containing chlorohexidine during the waiting time between visits so the only source for chlorohexidine around the restoration was from the modified glass ionomer to exclude any external effect.

It was designed as a split-mouth study to exclude the influence of individual patient characteristics and to obtain a more powerful estimate of treatment effect with smaller sample size. In oral health studies, the split-mouth design is common [27,28].

The present study showed that SM count was almost constant in all control samples from both groups (A2, B2) through all period of examination, in GI group; (group A1) SM count was slightly decreased than in control samples (A2) all period of examination which is statically significant but difference was statistically insignificant in the same group (A1) between one month, two months and three months. The possible explanation is

that the Higher levels of fluoride release were observed on the first day, decreasing rapidly in the second and third days, and decreasing gradually in the following days until a constant level of fluoride -release was reached [29].

After one-month, the SM count in group (B1) was less than SM count in group (A 1) and in control samples and this difference was statistically significant. as the control restoration was included with the tested restoration in the same oral cavity so the effect of adding chlorhexidine was clear.

The decreased antibacterial effect of CHX with time may be due to elution which might result in material loss or as a result of the formation of insoluble salts with the glass-ionomer which suggested by Ribeiro and Ericson (1991) [8]. On the other hand, secondary caries may be prevented for a long time that's because the microenvironment of the restoration still has a sufficient level of CHX.

However, Forss H et al found that the fluoride concentration of plaque on or adjacent to glass ionomers is increased and the percentage of plaque *mutans streptococci* decreases even after one month [30].

On the other hand, several clinical studies showed that the fluoride concentrations released in vivo from old GI and RMGI are not high enough to affect the plaque levels of the caries-associated bacteria *mutans streptococci* and suggested that the antimicrobial activity occurs only in the initial phase and is not responsible for a long-term anti-cariogenic property [31-33].

Chlorhexidine is well known antibacterial agent and the short term clinical study by Mishra et al. examined the mixture of glass ionomer – chlorhexidine suggested that a significant increase in antibacterial effect of the mixture than glass ionomer alone [34].

However long term in vitro study claimed that chlorhexidine gluconate 2.5% showed great antibacterial activity up to 30 days and significantly decreased after 50 days [35].

Bellis et al investigated the long-term release of soluble chlorhexidine added to glass ionomer and found that the addition of a GIC with CHX paste resulted in cement releasing soluble chlorhexidine in a dose-dependent manner for more than 14 months. He used sustained-release CHX chlorhexidine–hexametaphosphate instead of CHX gluconate used in the current study [36].

After two and three months SM count in group (B1) was less than SM count in group (A1) and in control samples and this difference was statistically insignificant.

The results of our study were in accordance with Huiyi Yan et al. [37] who tested the antibacterial effect of the Glass Ionomer - Chlorhexidine mixture and concluded that CHX was continuously released, and anti-biofilm ability was maintained up to 30 days.

CONCLUSIONS

Incorporation of CHX to GI filling material may decrease SM growth than GI alone for up to three months.

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