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EFFICACY OF ATRAUMATIC RESTORATIVE TREATMENT USING GLASS IONOMER CONTAINING CHLOROHEXIDINE: IN VIVO AND IN VITRO STUDY

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ABSTRACT

Introduction: The atraumatic restorative treatment (ART)considered a trusted and effective approach to the control of carious lesions.Since the ART approach using glass ionomer (GICs) the use of antimicrobial agents(chlorhexidine) in combination with restorative materials are being developed for reducing the frequency and severity of secondary caries. hence, it is preferable to keep an optimum chlorhexidine concentration to maintain its antimicrobial effect without affecting GICs' mechanical performance.

Aim of the study: This study was planned to evaluate the antibacterial activity and mechanical properties of the glass ionomer containing different concentrations of chlorhexidine.

Material and methods: *In vivo* study: Sixty children aged 7-9 years old were included in this studywith at least onecavitated dentin carious lesion in occlusal surfacesin primary teeth.these children were randomly divided into three equal treatment groups. **Group I**: atraumatic restorative treatment approach using a conventional glass ionomer cement. **Group II**: atraumatic restorative treatment approach using glass ionomer containing1% chlorhexidine. **Group III:** atraumatic restorative treatment approach using glass ionomer containing 2% chlorhexidine. A sample was taken from the affected dentine using a sterile sharp small-size hand excavator at baseline and after 7 days. All samples were inoculated for the selective isolation of mutans streptococci, and lactobacilli.

***In vitro* study:** Fifteen cylindrical specimens for each group were prepared and tested for their compressive strength, diametral compressive strength and microhardness.

Results: The percentage reduction in mean and standard deviation (SD) of mutans streptococci and lactobacilli between treatment groups revealed that there were statistically significant differences between three groups (P= 0.01, P= 0.02).Tukey post hoc test revealed that the main difference was between glass ionomer containing chlorohexidine and conventional glass ionomer while there was no statistically significant difference between glass ionomer containing 1% and 2% chlorohexidine. The comparison of the mechanical properties among the three groups in term of compressive strength, diametral compressive strength and microhardness showed no statistically significant differences between conventional glass ionomer and glass ionomer containing 1% or 2% chlorohexidine.

Conclusion: Glass ionomers containing chlorhexidine displayed superior antibacterial activity than conventional glass ionomers without affecting the mechanical properties of glass ionomer.

KEY WORD: glass ionomer cement, chlorhexidine, antibacterial, mechanical properties .

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INTRODUCTION

Despite the marked decline in the level of dental caries and the continuous advance of the preventive methods, the caries disease still has a high prevalence within certain populations. These vulnerable populations include children from low income families, poorly educated and/or geographically isolated communities in particular.[1,2]

The atraumatic restorative treatment (ART) which based on the philosophy of minimal intervention and consists of removing the infected dentin by manual instruments, followed by filling the cavity with adhesive restorative material, preferably the glass ionomer cement (GIC), considered a trusted and effective approach to the control of carious lesions in these children.[3,4]

However, dental hand instruments alone do not remove carious dentin as effectively as rotary burs, and cariogenic bacteria can survive under GIC restorations for up to 2 years. Consequently, cavities treated by ART may have residual infected dentin, and if a GIC is unable to arrest the carious process, the restoration could fail.[5]Therefore many studies investigating the use of antimicrobial agents in combination with restorative materials for reducing the frequency and severity of secondary caries are being developed *in vitro*[6-8] and *in vivo*[9].

Among the different antimicrobial agents used to control dental microorganisms, chlorhexidine (CHX) has been considered as one of the most effective and safe substances. It presents a wide spectrum of activity against gram positive bacteria, especially mutans streptococci, gram negative, aerobic and facultative anaerobic bacteria, yeasts and fungi.[10]

However, when antibacterial materials are incorporated to the GICs; alterations of the physical and mechanical properties of the restorative materials were reported[11]. Consequently, increasing chlorhexidine concentrations above 5 %, significantly increases the antimicrobial activity of GIC; but the material tends to deteriorate rapidly as a restorative material [12].

Owing to the fact that chlorhexidine does not contribute to the formation of the glass ionomer network, high amounts of chlorhexidine would weaken the scaffold and compromise the mechanical properties of GICs. Since the ART approach using GICs is indicative for use in posterior teeth with high occlusal load, hence, it is preferable to keep an optimum chlorhexidine concentration to maintain its antimicrobial effect without affecting GICs' mechanical performance [13-15]. Hence, this study was planned to evaluate the efficacy of atraumatic restorative treatment using glass ionomer containing chlorohexidine with different concentrations in terms of its antibacterial activity and mechanical properties.

MATERIAL AND METHODS

In vivo study

The present study was designed as a randomized controlled clinical trial. The study sample was randomly selected from children aged 7-9 years old attending Pedodontic clinic, Faculty of Dentistry, Tanta University. sixty children were included in this study according to the following criteria[16]:(1) healthy people with at least one cavitated dentin carious lesion in occlusal surfaces in primary teeth and(2) and informed written consent was obtained from the parents. People having tooth cavities with expected pulpal involvement and those medically compromised were excluded.

Restorative Materials Used

The experimental glass ionomer was prepared by incorporating chlorhexidine diacetate (Sigma Aldrich, Steinheim, Germany) into the powder of the control glass ionomer at 1% and 2% (w/w). The control material was a conventional restorative glass ionomer (Fuji IX, GC, Tokyo, Japan).

Allocation of the test material to the individual was done on an alternating basis. The start of the allocation sequence was based on the outcome of a flip of a coin.

Group assignment:

Sixty children were randomly divided into three equal treatment groups, composed of 20 children each, according to the type of material used as follows:

Group I: Atraumatic restorative treatment approach using a conventional glass ionomer cement.

Group II: Atraumatic restorative treatment approach using experimental glass ionomer cement prepared by the incorporation of chlorhexidine diacetate into the powder of conventional glass ionomer at 1 % (w/w) [6].

Group III: Atraumatic restorative treatment approach using experimental glass ionomer cement prepared by the incorporation of chlorhexidine diacetate into the powder of conventional glass ionomer at 2% (w\w) [6].

Clinical procedures

The treatment procedure followed those described by ***Massara et al. 2002*** [17]. The teeth were isolated with cotton wool rolls. The cavity opening was enlarged if needed with a sterile hatchet. The cavity walls were then cleaned using sterile small-size excavators.

In order to assess the effect of the experimental material, a baseline sample from affected dentine were taken using a sharp small-size sterile excavator. The extent and depth of the cavity was recorded using an endodontic file. The cavity was restored with either test or control material but without conditioning the tooth surfaces.

Patients were recalled after 7 days. Using the previously recorded cavity depth and extension as reference points, the restoration was removed with the aid of a high-speed diamond bur until the deepest part was reached. The remaining part of the restoration was removed with a sterile hand excavator.

Thereafter, a sample was taken from the affected dentine using a sterile sharp small-size hand excavator. The cavities were completely cleaned by hand excavation and restored using the normal ART procedure.

The collected samples were placed in preweighed microcentrifuge tubes (Elkay, Costelloe, Co. Galway) containing 1 ml of reduced transport fluid (RTF) [18], fortified with 20%(v/v) fetal calf serum and 15% (v/v) glycerol. During collection, all dentine samples were kept cool in a closed cooling box, containing frozen ice packs. The microbiology samples were transported to the laboratory where tubes were placed in the freezer and processed within 6 h of return. At the laboratory the tubes were reweighed and the final weight was calculated to estimate the mass of the dentine sample in milligrams per milliliter of RTF.

The dentine samples were inoculated on MSB agar[19] for growth of streptococcus mutans, Rogosa SL agar[20] for growth of Lactobacilli. Aerobic cultivation of microorganisms was done in an incubator at 37˚C, whereas anaerobic cultivation was done in an anaerobic jar from which oxygen was removed and a mixture of 90% hydrogen and 10% carbon dioxide was introduced and incubated at 37°C for 48 hours. After two days, the number of colony forming units (CFU) was counted on electronic colony counter machine.

In vitro study

Evaluation of the compressive strength

Five cylindrical specimens for each group were prepared using plastic molds with inner diameter of 4 mm and 6 mm height according to American Dental Association (ADA) no. 30 [21] The powder and liquid (P/L ratio 3.6: 1, according to the manufacturer's instruction) were dispensed for each experimental group on a mixing pad and mixed using an agate spatula for 30 seconds [22]. The mold was overfilled with a plastic filling instrument. and covered with

glass plates[23]. All the specimens were stored at 37 ± 1°C and 95 ± 5% relative humidity for 1 hour. After that period, the specimens were finished with wet 1200-grit silicon carbide paper to flatten the surfaces, then removed from their molds, and stored at 37 ± 1°C in distilled water for 24 hours. [24]. Prior to testing, the diameter of the specimens were measured using a micrometer screw gauge (Mitutoyo, Kawasaki, Japan). Then the specimens were placed with the flat ends up between the plates of the universal testing machine (Zwickmachine, Z010, Zwick GmbH &Co., Ulm, Germany). Compressive strength (CS) of the material was tested by applying load along the long axis of the specimen with cross head speed of 1 mm/min until fracture of the specimen occurs. The maximum value of the force (F) till fracture was measured in Newtons and diameter (d) was applied in the formula :

$CS = (4F)/(\pi d^2)$,

to find out the compressive strength value in Megapascal (MPa). [25]

Evaluation of the diametral compressive strength

Five cylindrical specimens (4mm diameter- 2 mm hight) per group were prepared as mentioned before in the previous test using a split Teflon mold. Prior to testing diametral compressive strength, the diameter and thickness of each specimen was determined using a micrometer gauge. The specimens were placed on the universal testing machine so that the diameter of the specimen coincided with the direction of the compressive force. The specimens were then loaded in compression to fail at a crosshead speed of 1 mm/min.[23]The maximum force applied when the specimens fractured was recorded, and the diametral compressive strength was calculated in N/mm2 (MPa) according to the equation [26]:

$DTS = 2F/pdt$,

where **F** is the failure load, **d** the diameter, and **t** the thickness of the specimen.

Vickers microhardness test

Five cylindrical specimens (4mm diameter- 2 mm height) per group were prepared as mentioned before in the Compressive strength test using a split Teflon mold. Microhardness measurements were carried out on the top surface of the specimens with a digital microhardness tester (Durimet microhardness tester Leitz, Wetzlar, Germany). This test was performed with100g load applied for a dwell time of 15s using a diamond indenter. Five indentations were performed for each specimen. . The length of the diagonals of each indentation was measured. . The Vickers hardness number(VHN) is obtained using the following equation:

$H = 1854.4 \times P\ d^2$

Where H is Vickers hardness in kg/mm^2, P the load in grams and d the length of the diagonals in μm [27]. The mean VHN of the five readings of each specimen as well as the overall mean VHN for each subgroup was then calculated.

RESULTS

The mean weight of affected dentine in milligrams collected from carious teeth showed no statistically significant difference between the three test materialsat baseline and day 7 as illustrated in table 1.

TABLE (1) The mean weight (mg) and standard deviation (SD) of affected dentine at baseline and day 7.

Groups	**Day 0**	**Day 7**
	mean ± SD	mean ± SD
GIC	26.0± 2.8	25.6± 2.1
GIC/CHX 1%	25.7± 2.2	22.4± 30.1
GIC/CHX 2%	25.0± 1.98	26.5± 2.1
ANOVA (F)	**2.68**	**2.34**
P- value	**0.37**	**0.39**

GIC = Glass ionomer; GIC/CHX = glass ionomer containing chlorhexidine.

Table 2 showed a comparison between the mean number of mutans streptococci and lactobacilli (CFU/mg) within each group at baseline (day 0) and on day 7. There was significant decrease in the number of mutans streptococci (P= 0,029 and 0.02) and lactobacilli(P= 0.03and P=0.007) in cavities restored by glass ionomer containing 1% and 2% chlorohexidine while cavities restored by conventional glass ionomer showed no statistically significant differences in mean number of two bacteria at baseline and day 7.

The comparison of the percentage reduction in mean number and standard deviation (SD) of mutans streptococci and lactobacilli between treatment groups revealed that there were statistically significant differences between three groups (P= 0.01, P= 0.02) as shown in table 3. Tukey post hoc test demonstrated that the main difference was between glass ionomer containing chlorohexidine and conventional glass ionomer while there was no statistically significant difference between glass ionomer containing 1% or 2% chlorohexidine as shown in table 4.

TABLE (2) The mean and standard deviation of mutans streptococci and lactobacilli(CFU/mg) at baseline (0) and on day 7within treatment groups.

Groups	**S. mutans**				***L. acidophilus***			
	Day 0	**Day 7**	**T test**	**P value**	**Day 0**	**Day 7**	**T test**	**P value**
GIC	1.89±0.37	1.48±0.10	0.433	0.63	2.05±0.71	1.59±0.34	0.577	0.58
GIC/CHX 1%	1.95±0.24	0.95±0.07	3.21	0.029*	1.73±0.2	0.87±0.02	3.54	0.03*
GIC/CHX 2%	2.11±0.38	0.78±0.03	4.36	0.02*	2.05±0.4	0.88±0.027	5	0.007*

TABLE (3) The percentage reduction in mean and standard deviation (SD) of mutans streptococci and lactobacilli (CFU/mg) between treatment groups.

Groups	**S. mutans**		***L. acidophilus***	
	mean ±SD	**%**	mean ±SD	**%**
GIC	1.06±0.021	22	0.46±0.003	22
GIC/CHX 1%	1 ±0.01	51	0.86±0.009	50
GIC/CHX 2%	1.33±0.06	63	1.17±0.03	57
ANOVA (F)	**209.56**		**325.98**	
P- value	**0.01**		**0.02**	

Table (4) Inter group comparison by Tukey post hoc test

Groupscompared	S. mutans		*L. acidophilus*	
	Mean difference	P	Mean difference	P
1 versus 2	0.59	0.043	0.4	0.038
1 versus 3	0.92	0.017	0.71	0.02
2 versus 3	0.33	0.61	0.31	0.58

Table 5. Comparison of the mechanical properties among the three groups

Groups	Compressive strength	Diametrical compressive strength	Vickers microhardness test
GIC	221.1±14.1	12.89±3.24	63.68±9.34
GIC/CHX 1%	213.55±10.85	12.77±2.99	62.75±8.98
GIC/CHX 2%	211.62±18.67	11.84±4.16	60.43±8.56
ANOVA (F)	**2.27**	**1.98**	**1.67**
P- value	**0.08**	**0.18**	**0.21**

The comparison of the mechanical properties among the three groups in term of compressive strength, diametral compressive strength and microhardness showed no statistically significant differences between conventional glass ionomer and glass ionomer containing 1% or 2% chlorohexidine as demonstrated in table 5.

DISCUSSION

The ART is one of minimal-intervention approaches in which demineralized tooth tissues are detached by manual instruments and restored with adhesive restorative materials.[3] Nowadays, ART not only restricted to places where electricity is absent but it is also acknowledged in modern clinical settings for anxious patients and young children, as the sound and pressure caused by rotary instruments is absent and local anesthesia is not needed.[28]

The widespread use of glass ionomer in modern dentistry especially in ART restoration depended on its capability of releasing fluoride, which reduce the number of remaining bacteria in cavities in addition to remineralization of softened dentine. However, even after the removal of infected dentin and adequate sealing, viable bacteria have been found in the remaining affected dentine after different periods of evaluation. [29]

Many in vitro studies[6-9,18 ,29] have been carried out by adding chlorhexidine to glass ionomer cement to increase its antimicrobial effect against the remaining microorganism under the restoration, but there were limited in vivo studies[9,25]. So, this study carried out to investigate whether a glass ionomer containing chlorhexidine in different concentration inhibited the growth of microorganisms left in affected dentine under a restoration more than a conventional glass ionomer. Also, if the incorporation of chlorohexidine compromised the mechanical properties of the glass ionomer.

In this study the bacteria used to test the efficacy of the modified cement were S. mutans and Lactobacilli. S. mutans is the most cariogenic bacteria survive and grow in low pH environments. While Lactobacilli have been found to be the most resistant organisms to the inhibitory effects of GIC. The agar diffusion method was used because it is relatively inexpensive and this test is ideal for a large number of samples which can give rapid result.[30]

The amounts of affected dentine collected at baseline and on day 7 were not significantly different. This suggests that a similar mass of dentine was sampled at each collection.

The number of bacteria were reduced significantly in cavities restored with glass ionomer containing chlorohexidine after 7 days while in cavities restored with conventional glass ionomer the reduction in the number of bacteria was not statically significant. The percentage of reduction in bacteria was higher in the two test groups rather than the control group. These findings agreed with several studies demonstrated that the incorporation of chlorhexidine (CHX) to GICs in various concentrations (1–5%) had a significant antibacterial effect [29,8,22&6].

The greater antibacterial effect of GIC+CHX could be suggested by the fact that chlorhexidine could penetrate deep into tubules, sealing them and presumably enabling long term release resulting in a larger reduction of microorganisms [31].

The ability of restorative dental materials to withstand functional forces is an important requirement for their long-term clinical performance. To be accepted clinically, modified materials must provide superior antimicrobial activity and display comparable physical and mechanical properties such as surface tensile and compressive strength when compared with conventional materials.[32,33]

The mechanical properties of the two-tested material in term of indirect tensile strength and hardness were slightly lower than the conventional glass ionomer but not statistically significant (P= 0.08, 0.18, 0.21) respectively. This reduction due to CHX does not contribute to the formation of the glass ionomer network, and therefore,high amounts of CHX would weaken the scaffold and compromise the physical properties. This finding agreed with investigators who highly recommend the usage of the CHX diacetate, particularly between 1% and 5% final concentrations to obtain optimum antibacterial effectswithout jeopardizing the basic physical properties of theGICs.[34,8,12&29]

In conclusion, for clinical use of GIC with CHX, the best option is the addition of CHX at a concentration up to 2%, since this combination increased the antibacterial activity without changing the physical and mechanical properties of the material

CONCLUSION

Within the limitation of this study we can conclude that glass ionomers containing chlorhexidine displayed superior antibacterial activity than conventional glass ionomers without affecting its mechanical properties.

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