BACTERIOLOGIC EVALUATION OF RESIDUAL DENTIN AFTER CHEMO-MECHANICAL CARIES REMOVAL IN PRIMARY TEETH.

Elshamy S H, Taha S E, Abd ellatif S, Eldokky N A

ELshamy S H (Demonstrator in pediatric and community Dentistry Department, Faculty of Oral and Dental Medicine, Cairo University)
Taha S E(Professor in pediatric and community Dentistry Department, Faculty of Oral and Dental Medicine,Cairo University)
Abd ellatif S(Professor in Microbiology and Immunology Department, Faculty of Medicine , Cairo University)
Eldokky N A (Assistant Professor in pediatric and community Dentistry Department, Faculty of Oral and Dental Medicine,Cairo University)
KEY WORDS: Chemo-mechanical caries removal, Papacárie, Residual dentin

ABSTRACT

Objectives: the aim of this study was to to evaluate the residual dentin after chemo-mechanical caries removal using (Papacárie) as regard to bacteria present (by bacteriological study in vivo study) and remaining affected dentin (by caries detector dye in vitro study) in primary teeth.
Materials and methods: Bacteriological study:A total of thirty healthy children had primary molar teeth showing opened carious lesions were selected. The carious lesions were filled with the Papacári. The softened carious dentin scrapped by blunt excavator.. A sharp probe was used to confirm that the cavity were free of decay. Two application was used to take a swab from each carious cavity before and after application of Papacárie to identify colonies of Streptococcus mutans and Lactobacilli . cultivation occured in different selective bacterial media for 72 hours at 37°C. MSB medium was selected for cultivation of Streptococcus mutans, While MRS medium was selected for cultivation of Lactobacilli. Stereomicroscopic study:A total of ten primary molar teeth with opened carious lesion were selected. The teeth were grouped into two equal groups after sectioning of teeth in the mesio-distal longitudinal plane as following: I) caries removal (Papacárie). II)caries removal by round bur. In each group Caries detector dye was used as a confirmatory objective method to detect residual caries. This dye distinguishes between active and in-active carious lesion in dentin The teeth were checked for remaining caries under stereomicroscope.. Data were collected and statistically analyzed.

Results: Chemo-mechanical caries removal Papacárie significant reduce Streptococci mutan count and Lactobacilli count in opened accessible carious cavity of primary molar teeth. In vitro study the remaining carious area after removal of caries with bur was significantly lower than after caries removal with Papacárie.
Conclusion: From the result of this study we can conclude that Papacárie Remove soft dentin caries efficiently from cavitated primary teeth as . Reduce significantly the residual cariogenic bacteria regarding Streptococcus mutans and Lactobacilli. but it was less effective than the low speed round bur in complete caries removal.

1.Introduction

The principle of removal of sound tooth structure for extension, prevention, and retention is now being revised. Also, the improvement of chemically adhesive bonding systems lead to conceptual change in cavity designs and development of new technologies for cavity preparation It includes the following different techniques: Air abrasion, Sono abrasion, Laser and chemomechanical caries removal (CMCR) ***(1).***

Chemo mechanical Caries Removal involves the application of a solution that selectively softens the carious dentin, thus facilitating its removal. This limits the removal of sound tooth structure, the cutting of open dentinal tubules, pulpal irritation and pain, compared with conventional mechanical methods, in such way it may eliminate or reduce the need for drilling and local anesthesia, which could be more accepted and comfortable for the patient ***(2,3,4)***.

Around 1970, the need for an alternative to conventional rotary led to a research by ***Habib et al. (5)*** who studied the effect of sodium hypochlorite, which is a non-specific proteolytic agent on the removal of carious material from dentine. Sodium hypochlorite itself however was too corrosive for use on healthy tissue and very un-stable. So they decided to incorporate it into Sorensen's buffer (which contains glycine, sodium chloride and sodium hydroxide) in an attempt to minimize this problem. Quite fortuitously, a reaction occurred which resulted in a product which was more effective in removal of carious dentine than a saline placebo. This involved the chlorination of glycine to form N-Monochloroglycine (NMG) and the reagent subsequently became known as GK 101 and marketed in 1972 as first CMCR agent (***6).***. In subsequent studies, they found that the system was more effective if glycine was replaced by amino butyric acid; the product then being N-monochloroaminobutyric acid (NMAB) also designated GK-101E in 1975. The mechanism of action of NMG and NMAB on collagen is still unclear and knowledge of the chemistry of chlorination of amino acids and their effects is still very limited. Originally it was thought that the procedur einvolved chlorination of the partially degraded collagen in the carious lesion and the conversion of hydroxy proline to pyrrole-2-carboxylic acid**(*5)***. Mediteam in Sweden continued to work on a system and the latest CMCR reagent known as carisolv hit the headlines in January, 1998. Carisolv gel was a 2-component mixture. Equal parts of the two were mixed to form the active gel substance. One of the components primarily contained three amino acids (glutamic acid, leucine and lysine) and sodium hydroxide. The other fluid contained the reactive hypochlorite com-ponent (NaOCl) Though carisolv is the most successful agent, it has

its own share of disadvantage which includes extensive training and customized instrument which increases the cost of the solution. Because of these, there was a restriction in its application. To overcome these disadvantages of carisolv system, a new reagent was developed in Brazil. In Brazil 2003, Formula eacao by Sao Paulo, first time introduced papain gel as papacarie for chemomechanical caries removal agent (***7).***Papacarie is a national product; patented, registered and approved by ANVISA in Brazil. Its main components are papain, chloramine and toluidine blue

Papacarie is a biocompatible gel with antibacterial properties that eliminates the need for anesthesia, removes only the compromised tissue, and preserves the healthy tissue better. The formation of a smear layer is not observed after using the gel. The gel combines an atraumatic treatment with antibacterial properties without affecting healthy tissue and causing pain. Papacarie was evaluated in vitrofor cytotoxicity in fibroblasts culture at different concentrations (2, 4,6, 8 and 10%). It was concluded that for its development, any of the papain concentrations was feasible and Papacarie was safe, not cytotoxic in vitrofibroblast culture, and it is biocompatible to the oral tissues ***(8)***

Ammari and Moliterno, in 2005(9) compared between two products Carisolv and Papacárie for the chemo-mechanical caries removal. It was stated that the two products have the same indications for use and the same mechanism of action . They produced a similar selective proteolytic effect on infected dentin. Regarding their application technique, the Carisolv requires special kit of curettes while with Papacárie, any curette could be used***. El Kimary in 2011(10)*** evaluated the effectiveness of Papacárie and Carisolv on the residual cariogenic bacteria in dentin of primary teeth in comparison to traditional hand excavation. carious dentin samples of forty five primary teeth were taken. All samples were cultured anaerobically on different agar plates. Colony forming unites were determined for total bacterial counts, Streptococcus mutans and Lactobacilli. Papacárie was found to be more effective in caries removal and caused more reduction in bacterial count.

The aim of this study was to:Evaluate the residual dentin after chemo-mechanical caries removal using (Papacárie) as regard to bacteria present (by bacteriological study in vivo study) and remaining affected dentin (by caries detector dye in vitro study) in primary teeth.

2.Materials and methods:

Bacteriological study:

A total of thirty healthy children had primary molar teeth showing opened carious lesions were selected for bacteriological study. The carious lesions were filled with the Papacárie allowing it to work for 30 seconds, the gel turned turbid with debris. The softened carious dentin was scrapped away by blunt excavator. The gel was re-applied several times until it reached an unchanged light color. A sharp probe was used to confirm that the cavity were free of decay. The gel was removed with a cotton pellet soaked with water after which the cavities appeared glossy in appearance.Teeth were verified as being caries free by the conventional visual and tactile criteria; where there was no discoloration in the cavities and the explorer passed smoothly over

the walls of the cavities After caries removal, cavities were restored using composite resin according to manufacture‘s instructions.

Two sterile cotton application was used to take a swab from each carious cavity before and after application of Papacárie to identify colonies of Streptococcus mutans and Lactobacilli .The swabs were immediately transferred to sterile tube filled with 2 mm of Brain Heart Infusion broth for no more than 30 min, before cultivation in different selective bacterial media for 72 hours at 37°C. MSB medium was selected for cultivation of Streptococcus mutans, While MRS medium was selected for cultivation of Lactobacilli.

Stereomicroscopic study:

A total of ten primary molar teeth with opened carious lesion were selected for this study. The teeth were grouped according to type of carious removal technique into two equal groups after sectioning of teeth in the mesio-distal longitudinal plane using a disk rotating a low speed as following:

Group I) caries removal by chemo-mechanical agent (Papacárie).

Group II)caries removal by round bur

In each group all the teeth were checked for remaining caries using the same clinical criteria mentioned before. Caries detector dye was used as a confirmatory objective method to detect residual caries. This dye distinguishes between active and in-active carious lesion in dentin

The teeth were checked for remaining caries under stereomicroscope.

3.Results

I- RESULTS OF THE BACTERIOLOGICAL STUDY:

The present study included thirty healthy children aged 4-5 years, each child had one carious primary molar tooth.

Bacterial counts

a) For the Streptococcus mutans colonies:

In the present study the mean and standard deviation of $\log_{10}$ values of Streptococcus mutans colonies counts were 1.65 ± 0.59 before application of Papacárie and 0.76 ± 0.67 after application of Papacárie, **Table (1).Fig(1)** There was a statistically significant reduction in mean $\log_{10}$ values of Streptococcus mutans colonies counts after application of Papacárie (*P*-value < 0.001).

b) For the Lactobacilli colonies :

In the present study the mean and standard deviation of $\log_{10}$ values of Lactobacilli colonies counts were 1.10 ± 0.95 before application of Papacárie and 0.51 ± 0.79 after application of Papacárie, **Table (2). Fig(2)** There was a statistically significant reduction in mean $\log_{10}$ values of Lactobacilli colonies counts after application of Papacárie (*P*-value < 0.001).

II-RESULTS OF THE STERIOMICROSCOPIC STUDY:

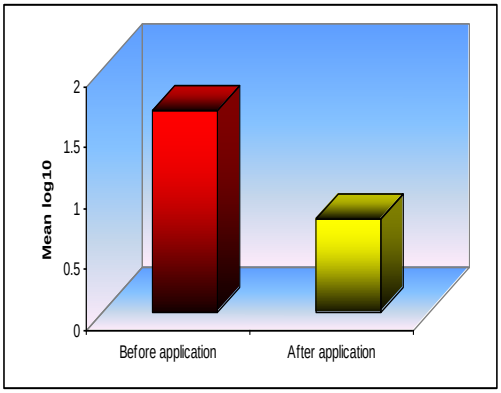
In the present study the percentage of remaining carious surface area which are detected by caries detector dye under stereomicroscope was calculated as: The mean and standard deviation of remaining carious surface area percentage were 16.46 ± 18.28% after removal of caries with Papacárie and 0.65 ± 1.16% after removal of caries with round bur**Table(3).Fig (3)**The mean Percentage of carious surface area after removal of caries with round bur was statistically significantly lower than Percentage of carious area after removal of caries with Papacárie (*P*-value < 0.001).

Table (1):The means and standard deviation (SD) values for the comparison between log_{10} values of Streptococcus mutans colonies counts before and after application of Papacárie.

CFU* of Streptococcus mutans before application of Papacárie		CFU of Streptococcus mutans after application of Papacárie		*P*-value
Mean log_{10}	±SD	Mean log_{10}	±SD	
1.65	0.59	0.76	0.67	<0.001**

**CFU: Colony forming units.*

***: Significant at P ≤ 0.05*



Fig(1): Mean log_{10} values of Streptococcus mutans colonies counts before and after application of Papacárie

Table(2):The means and standard deviation (SD) values for the comparison between log_{10} values of Lactobacilli colonies counts before and after application of Papacárie.

CFU* of Lactobacilli before application of Papacárie		CFU of Lactobacilli after application of Papacárie		*P*-value
Mean log_{10}	±SD	Mean log_{10}	±SD	
1.10	0.95	0.51	0.79	<0.001**

**CFU: Colony forming units.*

***: Significant at P ≤ 0.05*

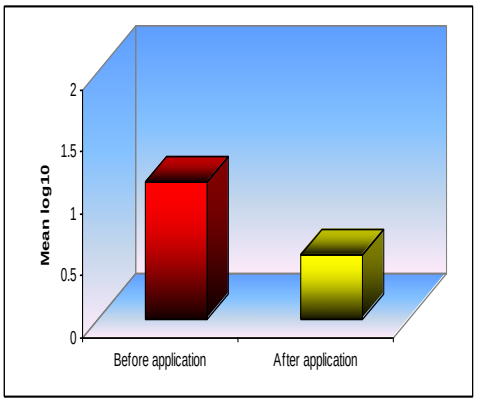


Fig (2): Mean log_{10} values of Lactobacilli colonies counts before and after application of Papacárie

Table (3):The means and standard deviation (SD) values for the comparison between Percentage of remaining carious surface area after caries removal with Papacárie and round bur (in vitro study).

Remaining carious surface area after application of Papacárie		Remaining carious surface area after removal of caries with round bur		*P*-value
Mean	±SD	Mean	±SD	
16.46	18.28	0.65	1.16	<0.001*

**: Significant at P ≤ 0.05*

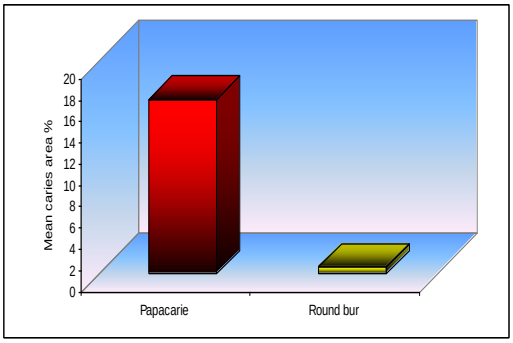


Fig (3): Mean Percentage of remaining carious surface area after caries removal with Papacárie and round bur in vitro study

4.Discussion

Bacteriological analysis was one of the method of assessment chosen in the study to test the effectiveness of Papacárie in the total caries removal by monitoring reduction in the cariogenic bacterial counts responsible for the occurrence of dental caries as the presence of bacteria has been considered by many investigators as an accurate indicator of infected carious dentin ***(10,11,12).*** Streptococcus mutans was chosen due to its initiative role in caries process and its ability to dominate the oral microflora under condition of stress**.** Lactobacilli was selected in the current study due to its important role in the progression of dentinal caries. Lactobacilli were first determined from the infected dentin, and then from the top layers of the residual dentine after caries removal either by conventional or chemo-mechanical methods ***(12,13,14,15, 16 17,18).***

Caries detector dye was used in this in vitro study, as dye can be applied to carious lesion and specifically stains infected dentin which was a very useful clinical agent in detecting caries. It aid in differentiating heavily infected dentin from sound dentin during carious tissue removal, this is in agreement with ***(19).***. In each group, all the remaining dentin were checked for the remaining caries using caries detector dye as an confirmatory objective method to detect residual caries, this was in agreement with ***(20,21)*** though they used different caries detector dye (methyl red) to evaluate efficiency of Carisolv to remove infected dentin.

The result of the current in vivo study, (table 1) showed a significant reduction of Streptococcus mutans count after caries removal in opened accessible carious cavity of primary molar teeth, this finding is in agreement with ***(10,22).*** As Papacárie showed antimicrobial activity, this is in agreement with ***(22).***

The result of the current in vivo study, (table 2) showed a significant reduction of Lactobacilli count after caries removal in opened accessible carious cavity of primary molar teeth , this finding is in agreement with ***(10,22).*** As Papacárie showed antimicrobial activity, this is in agreement with ***(22).***

Papain is the main ingredient of Papacárie, which is a cysteine protease, hydrolase enzyme present in papaya (*Carica papaya*), which has significant antibacterial activity against both

gram-positive and gram-negative bacteria. Also it has bactericidal and bacteriostatic properties ***(23).*** In addition Papacárie contain chloramine which is compound that contain chlorine and ammonia, they have bactericidal and disinfectant properties ***(24,25).*** Toluidine blue was added as a coloring agent to Papacárie, It is a powerful antimicrobial agent that fixes itself to the bacterial wall ***(8)***, this may explain the antimicrobial activity of Papacárie .

The results in Table (3) showed that the remaining carious area (which detected with caries detector dye) after removal of caries with a low speed round bur was significantly lower than the remaining carious surface area after caries removal with the chemo-mechanical method using Papacárie. This result was in agreement with ***(2 ,26,27)*** who investigated a different chemo-mechanical method using Carisolv. However the results disagree with ***(28, 29,30)*** who reported that no caries were detected in all the teeth treated by either the chemo-mechanical caries removal and conventional hand excavation. According to the results obtained in this study, the chemo-mechanical caries removal method was concluded to be an effective method in reduction of the residual cariogenic bacteria. However, Papacárie leave more remaining caries surface area than the drilling method.

Anderson and Charbeneau in 1985 and Kidd et al in 1993(31,32) reported that the staining of dentin by caries detector dye does not correspond with the microbiological status of the dentin. This may explain the difference results obtained in this study between the in vivo bacteriological study and the in vitro stereomicroscopic study in evaluation of the residual dentin after using Papacárie as a chemo-mechanical caries removal method.

5.Conclusions

From the result of this study we can conclude that Papacárie: Remove soft dentin caries efficiently from cavitated primary teeth as verified by the visual and tactile criteria. Reduce significantly the residual cariogenic bacteria regarding Streptococcus mutans and Lactobacilli.Was less effective than the low speed round bur in complete caries removal.May serve as a feasible practical easy and safe alternative to conventional methods of caries removal in opened carious cavities in very young uncooperative children.

6.Acknowedgement

I would like to thank my **professors** and all the **staff members of the Pediatric and Community Dentistry Department**, Faculty of Oral and Dental medicine, Cairo university, for their kindness, help, support, understanding and cooperation.

7. References

1.Beeley J, YipHK and Stevenson AG. (2000) Chemochemical caries removal: a review of the techniques and latest developments.Br Dent. J.;188: 427-431.

2.Cederlund A, Lindskog S and Blomlof J. (1999) Efficacy of Carisolv-assisted caries excavation. Int J Periodontics Restorative Dent.Oct;19(5):464-9.

3.Nadanovsky P, Cohen Carneiro F and Souza de Mello F.(2001) Removal of caries using only hand instruments: a comparison of mechanical and chemomechanical methods. Caries Res. Sep-Oct; 35 (5): 384-389.

4.Pereira SA, Silva LR, Piccinini DPF, Santos EM and Bussadori SK. (2004) Comparison antimicrobial potential in vitro between two materials for the Chemomechanical caries removal. In: 21ª AnnualReunionSBPqO, Águas de Lindóia. Net abstract.Pesqui Odont Bras.; 18: 78.

5. Habib CM, Kronman J, Goldman M (1975). A chemical evaluation of collagen and hydroxyproline after treatment with GK-101 (Nmonochloroglycine). Pharm. Thera Dent., 2: 209-215.

6.Goldman M and Kronman JH. (1976)A preliminary report on a Chemo-mechanical means of removing caries. J. Am. Dent. Assoc.;93: 1149-1153.

7.Bussadori SK, Castro LC and Galvγo AC. (2005) Papain gel a new chemomechanical caries removal agent. J Clin Pediatr Dent.;30:115-9.

8.Silva LR, Motta LJ, Reda SH, Façanha RAAA and Bussadori SK.(2004)Papacárie®- a new system for a chemo mechanical caries removal –case report. Rev Paul Odontol ;XXVI:4-8.

9. ***Ammari MM and Moliterno LFM.(2005)***Chemo-mechanical caries removal: current evidences.RBO.;62:125-7.

10. ***El Kimary E. (2011)***Effectiveness of Two Chemo-mechanical Caries Removal Methods on Residual Bacteria inDentin of Primary Teeth. Master thesis, Pediatric Dentistry and Public Health Department, Faculty of Dentistry, Alexandria University.

11.Lennon AM, Buchalle W, Switalski L and Stookey GK.(2002)Residual caries detection using visible fluorescence. Caries Res.;36:315-9.

12.Azrak B, Callaway A, Grundheber A, Stender E and Willershausen B.(2004)Comparison of the efficacy of chemo-mechanical caries removal (Carisolv™) with that of conventional excavation in reducing the cariogenic flora. Int J Pediatr Dent.; 14:182-91.

13.Kohler B, Andreen I and Johnsson B.(1988)The earlier the colonization by mutans streptococci, the higher the caries prevalence at 4 years of age. Oral Microbiol Immunol.; 3:14-7.

14.Salonen L, Allander L, Branthall D and Hellden L.(1990)Mutans Streptococci, Oral Hygiene and Caries in an Adult Swedish Population. J Dent Res.;69(8):1469-1475.

15.Bowden GH. (1996)Mutan streptocooci and chlorhexidine. J. Can. Dent. Assoc. ;62(9): 703-7.

16. ***Alamoudi N, Farsi N, Faris J, Masoud I, Merdad K and Meisha D.(2004)***Salivary characteristics of children and its relation to oral microorganism and lip mucosa dryness.J Clin Pediatr Dent; 28(3):239-248.

17.Law V and Seow WK.(2006)A longitudinal collected study of factors associated with mutans streptococcus infection and caries lesion initiation in children 21-72 months old. J Clin Pediatr Dent.; 28(1):58-65.

18. ***Olak J, Mandar R, Karjalainen S, Soderling E and Saag M.(2007)***Dental health and oral Mutans streptococcus in 2-4 years old Estonian children. Pediatr Dent.; 17(2):92-7.

19.***Kelsey WP, France SJ, Blankenau RJ, Cavel WT and Barkmeier WW. (1981)***

Caries as a cause of restoration replacement: a clinical survey.

Quintessence Int Dent Dig. Sep;12(9):971-4.

20. ***Ekstrand KR, Ricketts DN, Kidd EA, Qvist V and Schou S.(1998)***

Detection, diagnosing, monitoring and logical treatment of occlusal caries in relation to lesion activity and severity: an in vivo examination with histological validation.

Caries Res.;32(4):247-54.

21.El Khodary HM.(2005)An In Vitro Study to Evaluate The Effectiveness of Mechanical and Chemo-Mechanical Methods used for Removal of Soft Carious Dentine in Primary Teeth. Master thesis, Pedodontics and Community Dentistry Department, Faculty of Dental Medicine, (Girls branch) ,AL-Azhar University.

22.Motta LJ, Bussadori SK, Guedes CC, Reda SH and Santos EM.(2005)
In vitro evaluation of the antimicrobial activity of two materials used for chemical and mechanical removal of carious dentin: Carisolv™ And Papacárie ®. Arquivos Em Odontologia.;41:273-368.

23. ***Dawkins G, Hewitt H, Wint Y, Obiefuna PC and Wint B. (2003)***Antibacterial effects of Cracia Papaya fruit on common wound organisms. West Indian Med J.;52:290-2.

24.White GC .(1992)Handbook of Chlorination and Alternative Disinfectant, 3rd ed.; Van Nostrand Reinhold, New York

25. ***Maragakis GM, Hahn P and Hellwig E.(2001)***Chemo-mechanical caries removal: a comprehensive review of the literature. Int Dent J.; 51:291-9.

26. ***Splieth C, Rosin M and Gellissen B.(2001)***Determination of residual dentine caries after conventional mechanical and chemomechanical caries removal with Carisolv. Clin Oral Investig. Dec;5(4):250-3.

27.Prabakar AR, Taranjot KAUR and Basappa N.(2009)Comparative evaluation of Carisolv in removal of carious dentin in primary molar teeth: in vitro study.
Pesq Brasem Odontoped Clin Integr, João Pessoa.;9;77-80.

28.Ericson D, Zimmerman M, Raber H, Gotrick B, Bornstein R, Thorell J.(1999) Clinical evaluation of efficacy and safety of a new method for chemo-mechanical removal of caries.*CariesResearch*;**33**: 171–177.

29. ***Fure S, Lingström P and Birkhed D.(2000)***Evaluation of Carisolv for the chemo-mechanical removal of primary root caries in vivo. Caries Res.; May-Jun;34(3):275-80.

30.El Kashlan HI. (2003)A Clinical And Laboratory Evaluation Of A Chemo-Mechanical Method Of Caries Removal In Primary Teeth. PhD thesis, Faculty of Dentistry, Alexandria University.

*31.****Anderson MH and Charbeneau GT.(1985)***A comparison of digital and optical criteria for detecting carious dentin. J Prosthet Dent.; May;53(5):643-6.

32.Kidd EA, Joyston-Bechal S and Beighton D.(1993)The use of a caries detector dye during cavity preparation: a microbiological assessment.Br Dent J. Apr 10;174(7):245-8.