Comparison of the Efficacy of Chemomechanical Caries Removal (Papacarie - A Papain Gel) and Conventional Excavation in Reducing Cariogenic Flora: An In Vivo Study

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Abstract:
Background: Aim of this study was to compare the efficacy of chemomechanical caries removal (CMCR) (Papacarie) with that of conventional excavation in reducing the count of the cariogenic flora checked on blood agar plates.

Materials and Methods: A total of 40 molar teeth (n = 40) were divided into two groups of 20 teeth each. In the first group, caries was removed using Papacarie gel and in second group caries was removed using dental bur. Caries samples were collected in sterile vials containing 1 ml of saline before and after application of Papacarie gel and dental bur. Excavated dentin samples were vortexed in microbiological lab followed by serial dilution and inoculation on blood agar aerobically for 24 h, and total viable counts (TVCs) were determined and expressed as colony forming units per sample. The data obtained was tabulated and subjected to statistical analysis.

Results: The statistical analysis of data using Mann–Whitney U-test, and Wilcoxon signed matched pairs test showed that there was mean reduction of TVC of bacteria by both, conventional drilling and CMCR (Papacarie) methods. The mean reduction of TVC was 90.55% and 89.02% for the conventional and chemomechanical methods, respectively.

Conclusion: The results of this microbiological study show that efficacy of chemomechanical (Papacarie) removal of carious dentin is comparable to the result obtained by the conventional method.

Key Words: Blood agar plates, chemomechanical, colony forming units, Papacarie gel

Introduction
Dental caries is one of the most prevalent oral diseases of public health concern. Dental caries consists of two layers: Infected dentin and affected dentin, which differ in their microscopic structure as well as bacteriological characteristics. An effective system for caries removal should identify those layers, removing only the infected outer layer of carious dentin characterized by irreversible changes and bacterial invasion.

Minimal invasive dentistry adopts a philosophy that integrates prevention remineralization and minimal intervention for the placement and replacement of restorations. Minimal invasive dentistry includes air abrasion, atraumatic restorative technique, sono abrasion, laser, and chemomechanical caries removal (CMCR).

The chemomechanical method of caries removal was developed to preserve healthy dental tissue-like dentin and to make caries removal more comfortable to the patient. The chemomechanical method brings together, atraumatic characteristics of procedure and bacteriostatic action of caries removal chemical gel. In this method, ingredient of gel softens the predegraded collagen of carious lesion without pain and preserves adjacent healthy tissue.

In 1975, Habib et al. introduced a method using 5% sodium hypochlorite to remove carious lesion with the use of 5% sodium hypochlorite.

New GK-101 containing N-monochloroglycine has been developed. However, it is very slow in carious tissue removal. Caridex was later developed from formula made of N-monochloroglycine and amino butyric acid.

Despite its effectiveness Caridex is expensive, requires more solution and has a short shelf life.

Recently, Carisolv was introduced as a successor to the Caridex system. Carisolv consists of three amino acids: Lysin, leucine, and glutamic acid. It requires extensive training and customized instruments.

In 2003, a research project in Brazil by Bussadori et al. led to the development of a new formula for caries removal which was launched, in 2005, under the brand name Papacarie. Papain gel, the basic component of this product is responsible for its bactericidal, bacteriostatic, and inflammatory characteristics. Various in vivo and in vitro studies have been done using different CMCR agents, namely, 5% sodium hypochlorite, GK101, Caridex, and Carisolv. However, the literature on the efficacy of CMCR using Papain gel is scanty. Thus, the need to evaluate the recent material arose.
Materials and Methods

Source of data
This in vivo study was carried out at a private dental clinic in Bangalore, Karnataka, India. Informed written consent was obtained from patients. 40 patients (n = 40) aged between 15 and 40 and who had molars with broad cavitated occlusal lesions showing brown and softened dentin were selected. The study design, objectives, potential benefits, and methodology were explained to the patients.

Armamentarium and material
1. Basic dental diagnostic and operative instruments set up
2. Papacarie gel (Figure 1) (formulae Aeao, Sao Paulo, Brazil)
3. Sterile vial
4. Bacteriological incubator
5. Inoculation loop
6. Blood agar plates
7. Composite
8. Glass ionomer Fuji IX

Method of collection of samples
a. Sample size: 40 permanent molars with occlusal caries showing brown and softened dentin (n = 40).
b. Teeth selection criteria: (1) Patients of age group 15-40 years, (2) molars with open carious lesions with dentin involvement but not involving pulp. Exclusion criteria employed was molars with interproximal caries, mobile teeth, arrested caries, restored teeth, non-vital teeth, no carious lesion, etc.

Method of study
Teeth were divided into two groups according to the method of caries removal.
Group A: The test group was subjected to removal of caries using a chemomechanical agent (Papacarie).
Group B: The control group was subjected to removal of caries using conventional drilling method using dental burs.

Clinical procedure
Group A: Caries removal using chemomechanical method (Papacarie gel) (Figure 2a-d)
According to manufacturer’s instructions, the Papacarie® gel syringe was brought to room temperature. Following rubber dam isolation, the first sample from superficial carious lesion was removed with the help of sterile spoon excavator. Then, carious cavity was filled with Papacarie gel for 40-60 s. The fresh gel was clear in appearance, after application, it denatured the structured collagen fibers of carious lesion and became turbid. Then, softened dentin was removed with a spoon excavator. The gel was applied till it no longer turns turbid. Cavity was examined by visual inspection and an explorer to assess caries removal. The remaining gel was removed with sterile cotton pellet soaked in water. The first and second samples were transferred to sterile vials containing 1 ml of saline for microbiological evaluation.

Group B: Caries removal using conventional drilling method
Caries removal was carried out using sterile round bur on micro motor handpiece at slow speed (6000-8000 rpm) without water spray. Dentin samples were taken before and after drilling using same sampling procedure as with CMCR.

After caries removal, dentin was considered caries free using optical and tactile criteria. Then, dentin samples were transferred to sterile vial containing 1 ml of saline for microbiological evaluation (Figure 2e).

Restoration of cavity
After caries removal by either method, cavities were restored using Glass Ionomer Fuji IX, composite resin, or silver amalgam according to manufacturer’s instructions (Figure 2f).

Microbiological cultivation and evaluation
Dentin samples of the both groups were processed in the microbiological laboratory within 2 h of collection. Here, samples were vortexed for 30 s to dislodge the bacteria from dentin. The samples were then serially diluted to obtain a $10^4$ dilution, and a sterile loop full of sample (0.1 ml) was collected and cultured with aseptic technique onto blood agar.
plates by streaking method. Then, the plates were incubated aerobically for 24 h. Using colony counter, the total viable count (TVC) was determined and expressed as colony forming units (CFU) per ml of sample (Figures 3 and 4).

**Statistical analysis**
The data obtained was tabulated and subjected to statistical analysis using Wilcoxon signed matched pairs test for pairwise comparison and Mann–Whitney U-test to find out the significant difference between two independent groups. The statistical assessment was carried out using Statistical Package for Social Sciences software 20.0 version.

**Results**
The difference between the pre-treatment sample score and post-treatment sample score found to be statistically significant in both groups ($P < 0.05$).

1. TVC (CFU/ml) before and after treatment of carious molars with conventional drilling method was $73.9 \times 10^4$ and $6.7 \times 10^4$, respectively, with 90.55% reduction
2. TVC (CFU/ml) before and after treatment of carious molars with CMCR (Papacarie) method was $70.4 \times 10^4$ and $4.5 \times 10^4$, respectively, with 89.02% reduction.

The TVC was reduced to $6.73 \times 10^4$ CFU/ml following conventional dental bur method and $4.56 \times 10^4$ CFU/ml after CMCR (Papacarie) method shown in Table 1 and Graph 1.

- Comparison of before and after treatment TVCs (CFU/ml in $10^4$) in conventional drilling method by Wilcoxon matched pairs test, ($n = 20$). $P < 0.05$ shown in Table 2 and Graph 2
- Comparison of before and after treatment TVCs (CFU/ml in $10^4$) in CMCR (Papacarie) method by Wilcoxon matched pairs test, ($n = 20$). $P < 0.05$ shown in Table 3 and Graph 3.

**Discussion**
Infected dentin is soft in consistency and highly infected by bacteria, and collagen fibers are partially degraded and cannot be remineralized and an inner layer of affected dentin which is hard and leathery in consistency which is demineralized with intact collagen fibers and can be remineralized.

Dentin color and hardness are not good parameters to indicate end point in removing carious dentin. Thus, the self-limiting caries removal therapy is the best alternative to ensure complete removal of all bacteria, which should be the goal when treating a carious lesion to prevent recurrence of decay or eventual pulpal damage. A chemomechanical caries removing system acts by

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**Table 1**: Comparison of conventional drilling (dental bur) and chemomechanical caries removal (Papacarie) methods with respect to total viable counts (CFU/ml in $10^4$) before and after treatment by Mann–Whitney U-test ($n=20$).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Methods</th>
<th>Mean</th>
<th>SD</th>
<th>Median</th>
<th>Sum of ranks</th>
<th>U-value</th>
<th>Z-value</th>
<th>P-value</th>
</tr>
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<tbody>
<tr>
<td>Before</td>
<td>Dental bur method</td>
<td>73.92</td>
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<td>395.00</td>
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<td>Papacarie method</td>
<td>70.38</td>
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<td>23.50</td>
<td>425.00</td>
<td>185.00</td>
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<tr>
<td>After</td>
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<td>6.73</td>
<td>10.69</td>
<td>1.69</td>
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<tr>
<td></td>
<td>Papacarie method</td>
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<td>1.35</td>
<td>400.50</td>
<td>190.50</td>
<td>0.2570</td>
<td>0.7972</td>
</tr>
</tbody>
</table>

SD: Standard deviation, CFU: Colony forming unit

**Table 2**: Comparison of before and after treatment total viable counts (CFU/ml in $10^4$) in conventional drilling (dental bur) method by Wilcoxon matched pairs test ($n=20$).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean</th>
<th>SD</th>
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<th>SD difference</th>
<th>Percentage of change</th>
<th>Z value</th>
<th>P value</th>
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<tbody>
<tr>
<td>Before</td>
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<td>119.74</td>
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</tbody>
</table>

*P<0.05. SD: Standard deviation, CFU: Colony forming unit
Chemomechanical Caries Removal vs. Conventional Excavation… Kulkarni G

It is basically comprised of Papain, chloramines, toluidine blue salts, thickening vehicle, which together are responsible for the Papacarie bactericidal, bacteriostatic, and anti-inflammatory characteristics.

Papacarie acts by breaking the partially degraded collagen molecules. The action of Papacarie causes cleavage of the polypeptide chains and hydrolysis the crosslinks of collagen. Right after the degradation, oxygen is freed, this explains the appearance of bubbles on the surface during a clinical procedure. The Papain chemical agent was found to have no ability to affect the sound collagen fibers in the inner affected and normal dentin as papain can digest only dead cells because infected tissues lack or do not show alpha 1-anti trypsin which inhibits protein digestion.

The Papacarie gel used in this study is simple to store in refrigerator and simple to use without any discomfort to the patient.

The bacteriological analysis was the method of assessment chosen in this study to test the effectiveness of both dental bur and Papacarie for the completeness of caries removal. The presence of bacteria has been considered by many investigators to be an accurate indicator of carious dentin.

For all tested groups in this study, complete caries removal was assessed by criteria such as catch of the probe when passed over the caries free dentin. The color of the Papacarie gel was a clinical indicator of complete caries removal when the blue Papacarie gel no longer turned turbid. According to the clinical criteria, they effectively removed carious tissue leaving the dentin surface hard on probing.
In this study, dislodging bacteria from dentin samples was done by Vortexing. The cultures in this study were processed aerobically and inoculated on blood agar plates to determine TVCs.

Reductions in TVC by both methods of caries removal were significant according to data analysis shown in tables and graphs.

After caries removal in this study, all cavities were restored with Fuji IX Glass Ionomer and composite resin and in cases of deep carious lesions calcium hydroxide (Dycal) cement.

This clinical procedure is efficient for caries removal. CMCR method by Papacarie gel seems to be an adequate alternative to the conventional rotary instruments method.

Conclusions
1. The CMCR method (Papacarie gel) significantly reduced the cariogenic bacteria
2. Both methods of caries removal (CMCR with Papacarie and conventional bur method) produced a statistically significant reduction in the TVC
3. Papacarie gel appears to be more feasible than the conventional method as it reduces the need for local anesthesia and the use of drill. It is not technique sensitive.

References