AN IN-VIVO COMPARATIVE EVALUATION OF THE EFFICACY OF TWO DIFFERENT PAPAIN BASED CHEMO-MECHANICAL CARIES REMOVAL AGENTS IN PRIMARY MOLARS

**ABSTRACT**

**Background:** Dental caries is one of the severe diseases which leads to irreversible loss of tooth structure. Caries removal means of rotary is quick but, also may damage healthy tooth structure. Chemomechanical caries removal agent is an excellent alternative in removing caries, by not only reducing bacterial count but also remineralizing tooth structure.

**Material and method:** A total of 40 primary molar teeth (n = 40) were divided into two groups of 20 teeth each. In the first group, caries was removed using Brix 3000 and in second group caries was removed using V-Carie - Solve. Caries samples were collected in sterile vials containing 1ml of saline before and after application of CMCR gel. Excavated dentin samples were vortexed in microbiological lab followed by inoculation on blood agar aerobically for 24 hrs, and total viable count (TVCs) were determined and expressed as colony forming units per sample.

**Result:** Mean reduction of TVC of bacteria was seen in both the group, but was more significant in Brix 3000.

**Conclusion:** The advent of Papacarie® gel has revolutionized the chemomechanical caries removal process and has proved to be a highly effective and excellent product with added advantage of maximum preservation of healthy tooth tissue, especially in cases of deep dentinal caries.

**INTRODUCTION**

Dental caries is one of the most prevalent chronic diseases of people worldwide; individuals are susceptible to this disease throughout their lifetime. It continues to affect a significant portion of the world population and treatment of the decay is associated with pain by many patients. Although, there has been a substantial reduction of the prevalence of caries in industrialized countries, this disease lingers to be widespread in the world. Once it has become installed, it is of fundamental importance to use conservative procedures that simultaneously prevent lesion progress and minimize healthy tooth structure wear.

The techniques used in carious dentine removal have developed since GV Black, in 1893, initially proposed the principle of 'extension for prevention' in the operative treatment of carious lesions. But the disadvantages of using burs as conventional caries removal system include (i) unpleasant perception created by drilling, (ii) frequent requirement of local anaesthesia injected using syringes, (iii) pressure effects on the pulp caused due to drilling, (iv) thermal effects on the pulp caused due to drilling, (v) excessive loss of sound tooth structures due to removal of uninfected dentin. Thus, in order to preserve the healthy dental tissues as well as to provide comfort for the patients a new method of caries removal, called the chemo-mechanical caries removal system was developed. According to Banerjee et al., the chemo-mechanical method is an effective alternative for caries removal because it brings together (i) atraumatic characteristics and (ii) bactericide and bacteriostatic action. The method was created so that an active ingredient would soften the pre degraded collagen of the lesion without pain or undesirable effects to adjacent healthy tissues.

In 1975, Habib et al., introduced a method using 5% sodium hypochlorite to remove carious tissues, which proved to be toxic and aggressive to adjacent healthy oral tissues. Thus, a modified formula was developed known as GK-101, composed of 5% sodium hypochlorite to which sodium hydroxide, glycerine and Ni-monomethylglycine were added. Though, it was more effective than the hypochlorite alone but was very gentle in carious tissue confiscation. Further research brought, Caridex™ and Carisolv™, which required extensive training, customized instruments which increase their cost.

Papacarie®, a research product from Brazil, basically comprised of papain, chloramines, toluidine blue, salts, thickening vehicle, which together brought bactericidal, bacteriostatic and anti-inflammatory characteristics. In 2016, a new papain based material has been introduced in Argentina, named Brix 3000, obtained from leaves of clove oil. Clove oil is a natural analgesic and anti-septic, used in dentistry primarily for its soothing effect. Clove is opulent in minerals such as calcium, hydrochloric acid, iron, phosphorous, Sodium, Potassium, Vitamin A and Vitamin C. The high levels of eugenol contents in clove essential oil are responsible for its strong biological and antimicrobial activities. Both eugenol and clove essential oil, phenolic compounds, can denature proteins and act in response with cell membrane. Phospholipids changing their permeability and inhibiting bacterial growth. The palliative effects of eugenol containing clove essential oil has been attributed to its ability to subdue prostaglandins and other inflammatory mediators such as leukotrienes.

V-Carie - Solve is another gel based product from papaya extract, rich in papain, added with clove oil, which is in a blend of therapeutic essential oils. Clove oil is a natural analgesic and anti-septic, used in dentistry primarily for its soothing effect. Clove is opulent in minerals such as calcium, hydrochloric acid, iron, phosphorous, Sodium, Potassium, Vitamin A and Vitamin C. The high levels of eugenol contents in clove essential oil are responsible for its strong biological and antimicrobial activities. Both eugenol and clove essential oil, phenolic compounds, can denature proteins and act in response with cell membrane. Phospholipids changing their permeability and inhibiting bacterial growth. The palliative effects of eugenol containing clove essential oil has been attributed to its ability to subdue prostaglandins and other inflammatory mediators such as leukotrienes.

Brix 3000 and V-Carie - Solve have been used effectively as chemomechanical caries removal agents by selectively dissolving carious dentin, while preserving healthy dentin, thus minimizing the use of rotary instruments. Despite of their proven effectiveness scant attention has been shown in the literature. Thus, the aim of the current study was to evaluate and to compare the efficacy of two different papain based chemo-mechanical caries removal agents, i.e., Brix 3000 and V-Carie - Solve in reducing the cariogenic bacterial flora.

**MATERIALS AND METHODS**

Prior approval of this in-vivo study was taken from ethical clearance committee. The study group consisted of 40 healthy children (22 males, 18 females), with occlusal caries involving dentin, aged 3 to 9 years visiting the department for restorative dental treatment.
Occlusal carious lesions were examined by an experienced clinician, visually and via periapical radiographs to assess the depth of the lesions and rule out any pathology. Dental examinations were carried out on a dental chair under standardized conditions after an oral prophylaxis.

Only children exhibiting definitely positive or positive behavior according to Frankl's behavior rating scale were included so they could provide appropriate responses to the operator's queries. Each child had at least one primary molar, with occlusal carious lesion, clinically and radiographically judged to extend at least 2 mm into the dentin (measured by a World Health Organization periodontal probe placed into the cavity depth without pressure), but not involving the pulp, and have a minimum cavity opening diameter of 1.5 mm for easy access to the lesion estimated using digital vernier caliper. (Figure 1) Children with a history of spontaneous pain, any painful sensitivity to cold, or any clinical or radiographic evidence of periapical/interradicular pathology were excluded from the study.

The children were randomly allocated into two groups: Group A (Brix 3000) and Group B (V- Carie - Solve). According to manufacturer's instructions, both Brix 3000 and V- Carie - Solve gel syringes were brought to room temperature before using. In both groups, the initial samples from the superficial carious lesion were taken under rubber dam isolation before the use of chemo-mechanical agents. The individual samples were then transferred into sterile vials that contain 1 ml of sterile isotonic saline for microbiological analysis.

In Group A, Brix 3000 gel was applied on to the dentinal carious lesions with a blunt spoon excavator. The lesions were completely covered by the gel for 2 mins, following which the soft dentin was gently excavated using spoon excavator, with pendulum movement and without pressure. The gel was reapplied until its light color remained unchanged, without washing or rinsing the cavity between gel applications. A dark color indicated that the decomposition of the decayed tissues was still in progress. Cavity was observed by visual inspection and an explorer to assess caries removal. (Figure 2) The residual gel was removed with sterile cotton pellet soaked in water. The first and second samples were transferred to sterile vials containing 1ml of saline for microbiological evaluation.

Similarly, in Group B, caries excavation was carried out using V-Carie - Solve, as per the manufacturer's instructions. The carious cavities were filled with Papacarie gel, using an applicator tip, and allowed to act for 30 to 60 seconds. The gel appeared darker in color during the process of decomposition of the decayed tissue. The decayed dentin, thus softened, was scraped away with the non-cutting edge of a sharp spoon excavator in a pendulum motion without applying pressure. The gel was reapplied until the surface feels hard.

Before and after the caries removal in each method, the dentin samples of the both groups were processed in the microbiological laboratory within 2 hours of collection. (Figure 3)The samples were vortexed for 30 seconds 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. (Figure 4) Then, the plates were incubated aerobically for 24 hours. Using colony counter, the total viable count (TVC) was determined and expressed as colony forming units (CFU) per ml of sample.

Concerning the microbiological analysis, in the two groups before and after the removal of the carious dentinal tissue, a statistical significant reduction in the total bacterial count was found for each method. However, the reduction in the total bacterial count for the Brix 3000 group was slightly more compared with that of V-Carie – Solve (Table 3).
Table 1: Comparison of (CFU/ml in 10³) before and after treatment in Brix 3000 with Wilcoxen matched pairs test (n=20)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean ±SD</th>
<th>Mean difference</th>
<th>SD Difference</th>
<th>Percentage of change</th>
<th>Z value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before</td>
<td>94.12±13.66</td>
<td>21.50</td>
<td>30.73</td>
<td>79.26</td>
<td>2.14</td>
<td>0.004*</td>
</tr>
<tr>
<td>After</td>
<td>112.36±154.61</td>
<td>140.34</td>
<td>36.21</td>
<td>79.26</td>
<td>2.88</td>
<td>0.05*</td>
</tr>
</tbody>
</table>

*indicates statistical significance at p<0.05

Table 2: Comparison of (CFU/ml in 10³) before and after treatment in V-Carie – Solve with Wilcoxen matched pairs test (n=20)

<table>
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Table 3: Comparison of reduction in colony forming units (CFU/ml in 10³) between two groups before and after treatment with Mann–Whitney U-test (n=20)

<table>
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<tr>
<th>Variable</th>
<th>Groups</th>
<th>Mean</th>
<th>Median</th>
<th>Sum of ranks</th>
<th>Z value</th>
<th>P value</th>
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</thead>
<tbody>
<tr>
<td>Before</td>
<td>Brix 3000</td>
<td>94.12</td>
<td>136.24</td>
<td>21.50</td>
<td>100.00</td>
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<tr>
<td>After</td>
<td>V-Carie - Solve</td>
<td>112.36</td>
<td>154.61</td>
<td>17.00</td>
<td>395.50</td>
<td>0.002*</td>
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<tr>
<td>Before</td>
<td>V-Carie - Solve</td>
<td>14.42</td>
<td>24.14</td>
<td>5.25</td>
<td>158.00</td>
<td>0.05*</td>
</tr>
<tr>
<td>After</td>
<td>V-Carie - Solve</td>
<td>22.31</td>
<td>35.54</td>
<td>7.60</td>
<td>370.00</td>
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DISCUSSION
The principle of extension for prevention in the operative treatment of carious lesion was proposed by GV Black in 1893. However, the demand of removing sound enamel and dentin has been dramatically changed over the years. Today, operative dentistry focuses on cavity design, outline and selecting filling materials. Thus, in the last few decades, a growing interest has been noticed to develop towards alternative minimally invasive techniques like air abrasion, lasers, ultrasonic, ozone therapy and others. It is obvious that most of the mentioned techniques are trying to achieve a conservative approach for removing dental caries. This issue stimulated the development of a clinical approach, known as atraumatic restorative treatment (ART) that involves excavation and removal of the gross caries with hand instruments and subsequently restoring the cavities with adhesive restorative material with bacteriostatic effect. Combination of the mechanical means of caries removal using hand instruments and use of chemical solvents resulted in the development of a new method of caries removal called as the chemomechanical caries removal method. This innovative method appears to be efficient in removing soft, infected dentine without altering the healthy dental tissue. Thus in present study this approach was used to remove carious lesion in primary molars.

The micro flora is one of the main causative factors in caries initiation and progression, therefore, it is essential to reduce the microbial counts in carious lesions. Visual and tactile methods are used to determine caries removal, which is considered to be complete based on resistance in carious lesions. Visual and tactile methods are used to determine caries removal, which is considered to be complete based on resistance in carious lesions. Int J Paediatr Dent 2004;14:182-91.

REFERENCES

CONCLUSION:
Chemomechanical caries removal agents may not be able to replace the use of rotary instruments (drills) for caries removal, but can be used as an alternative treatment in many cases especially in children (who required multiple restorations, very young children and those who have difficult behavior) It is a simple method and does not need any effort or training.

1. The Chemomechanical caries removal method (Papacarie gel) significantly reduced the cariogenic bacteria
2. Brix 3000 is more efficient than V- Carie – Solve in reducing total viable count of bacteria
3. Papacarie gel appears to be more feasible as it reduces the need for local anesthetics and has excellent bactericidal and bacteriostatic effect.

Table 4: Comparison of reduction in colony forming units (CFU/ml in 10³) before and after treatment with Mann–Whitney U-test (n=20)

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<td>17.00</td>
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