*Journal of International Oral Health 2016; 8(7):760-766*

*Received: 21st January 2016 Accepted: 08th April 2016 Conflict of Interest: None*

*Source of Support: Nil*

**Original Research**

Doi: 10.2047/jioh-08-07-04

# Microbiological Assessment of Carious Dentine using Chemomechanical Caries Removal and Conventional Hand Excavation in Primary and Permanent Teeth: A Clinical Study

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***How to cite the article:***

Modimi KV, Siddaiah SB, Chikkanarasaiah N, Rucha V, Abubakar SB, Dinraj K, Syed SS. Microbiological assessment of carious dentine using chemomechanical caries removal and conventional hand excavation in primary and permanent teeth: A clinical study. J Int Oral Health 2016;8(7):760-766.

***Abstract:***

**Background:** This study was done to assess the effectiveness of caries removal using chemomechanical agent (Papacarie Duo) and conventional hand excavation methods in primary and permanent molars on the residual cariogenic bacteria, analyzing the time required for the procedure and the need for anesthesia.

**Materials and Methods:** The present *in vivo* study was conducted on 30 children of the age groups between 4 and 8 years. Using a split-mouth design, the teeth were randomly allocated into two groups, Group I – Papacarie Duo and Group II – hand excavation method. The groups were further subdivided into Group IA and Group IIA for primary molars and Group IB and Group IIB for permanent molars. The dental caries samples before and after caries removal from each group were taken to the Department of Microbiology and processed to check for the growth of *Streptococcus mutans* and *Lactobacillus*. The colony forming units were counted using a digital colony counter, and the results were subjected to statistical analysis. The degree of discomfort during caries excavation was evaluated and the time taken for complete removal of caries was recorded using a digital stopwatch.

**Results:** The mean total viable count of *S. mutans* and *Lactobacillus* showed considerable reduction after caries removal in both Papacarie Duo and the hand excavation groups. The difference in the mean values for *S. mutans* between the two groups was found to be statistically significant ($P = 0.001$). However, the difference was not statistically significant for *Lactobacillus* ($P = 0.91$). The mean time required for the Papacarie Duo gel was lesser than hand excavation method. The degree of discomfort was significantly higher in the hand excavation group compared with that of Papacarie Duo group.

**Conclusion:** The chemomechanical method (Papacarie Duo) is more effective in the reduction of both *S. mutans* and *Lactobacillus* from the carious dentine of primary and permanent molars than the hand excavation method.

***Key Words***: Chemomechanical caries removal, dental caries, pain scale, Papacarie Duo

## Introduction

Dental caries is one of the most prevalent diseases of mankind.[1] Although there has been as substantial reduction in the prevalence of caries in industrialized countries, this disease continues to be widespread in the world.[2] Black in 1893 proposed the principle of "extension for prevention" in the operative treatment of carious lesions.[3] With the advent of adhesive materials and greater understanding of the disease process, we are moving into the era of "minimal intervention dentistry!" As soft and wet carious dentin lesion harbor significantly more bacteria than hard and dry lesions, clinicians are commonly advised to remove carious dentin to the level where it is firm.[4]

Traditional methods of carious tissue removal have been substituted by new methods comprising of lasers, air abrasion, ultrasound, and chemomechanical removal. The chemomechanical caries removal (CMCR) systems have renewed interest by selectively removing carious dentine but avoiding painful and unnecessary removal of sound dentin.

The CMCR was carried out by Kronmann *et al*., in 1975, using 5% sodium hypochlorite.[5,6] Later, it was replaced by Caridex in the year 1980. In 1998, Mediteam in Sweden introduced new CMCR reagent known as Carisolv™. Carisolv, a newer CMCR agent, bought about minimal removal of sound tooth structure, reduced risk for pulp irritation, less pain, and increased patient comfort.[7]

In the year 2003, a research project in Brazil developed a new chemomechanical agent Papacarie to promote minimal invasive methods for caries removal and also its use in public health. It is basically comprised of papain, chloramines, toluidine blue, salts, and thickening vehicle.[8] The new version of this product is Papacarie Duo™ released in 2011 and has the

same efficacy plus a number of additional properties, such as a longer shelf life and no need for refrigerated storage. The gel has also greater viscosity allowing more precise placement and less wastage during the procedure.

There are no gold standard criteria for determining when a cavity is caries free. Therefore, in defining the efficacy of the new methods of excavation and restoration, studies on the bacteriological content of the dentine lesion are important.[9]

Hence, the aim of the study was to assess the effectiveness of caries removal using chemomechanical agent (Papacarie Duo) and conventional hand excavation methods in primary and permanent molars on the residual cariogenic bacteria, analyzing the time required for the procedure and the need for anesthesia.

## Materials and Methods

This *in vivo* study was conducted on 30 children of the age groups 4-8 years visiting Department of Pedodontics and Preventive Dentistry, Rajarajeshwari Dental College and Hospital, Bengaluru. Using a split-mouth design, 30 primary molars and 30 permanent first molars with the presence of broad-cavitated lesions extending into the dentin were selected. Teeth with pulpal and periapical pathologies, multi-surface caries lesions excluded from the study. And also, children on antibiotic therapy or any other systemic disorder were not included in the study. Periapical radiographs were taken to confirm the extent of the lesion. A written informed consent was taken from the patients' parent/guardian prior to the procedure.

### *Sample preparation*

Teeth were randomly divided into two groups of 30 each:

- Experimental Group I (30 teeth) – Papacarie Duo
- Experimental Group II (30 teeth) – Hand Excavation.

Both the experimental groups (Group I and Group II) were further randomly subdivided into the following groups:

- Experimental Group IA (15 teeth) – Primary molars treated by chemomechanical caries removal (Papacarie Duo)
- Experimental Group IB (15 teeth) – Permanent first molars treated by chemomechanical caries removal (Papacarie Duo)
- Experimental Group IIA (15 teeth) – Primary molars treated by hand excavation method of caries removal
- Experimental Group IIB (15 teeth) – Permanent first molars treated by hand excavation method of caries removal.

All clinical procedures were done under complete isolation using rubber dam and saliva ejector. Topical anesthesia was applied around all the teeth to be isolated. No local anesthesia was administered as it would alter the pain perception of the patient unless it was necessary.

### *Base line sample*

Before sampling, the outermost layer of carious dentin was removed with a sharp, sterile excavator and discarded to avoid contamination. Sampling was performed using a sharp, sterile excavator. One scoop of carious dentin was taken with the same sized excavator for all cases to standardize the size of the sample as much as possible. The amount of dentin removed was sufficient enough to cover the surface of the excavator. Samples were immediately placed in a sterile vial containing phosphate-buffered saline and transported to the Department of Microbiology for processing.

### *Caries removal in Group IA and IIA*

The cavity was filled with Papacarie gel which was allowed to work for 60 s. On initial application, Papacarie gel was clear, gel then turned opaque or turbid with necrotic dentin. The softened debris was then scraped away with a blunt excavator. The gel was reapplied whenever turbidity appeared, this darkish discoloration indicating that the process of breakdown of the decayed tissue was still in progress. Application of

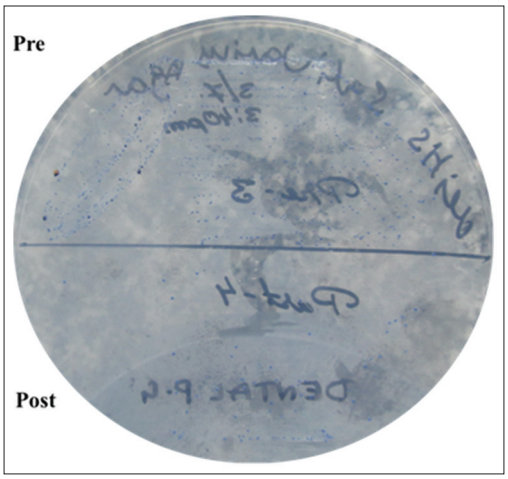



**Figure 1:** Growth on Mitis Salivarius-Bacitracin agar plates in pre- and post-intervention – Hand excavation.

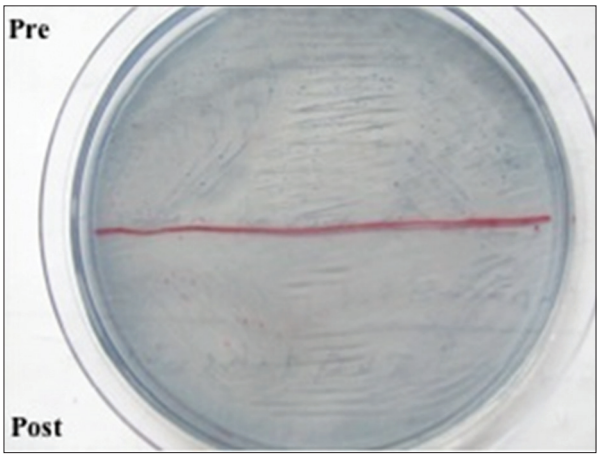



**Figure 2:** Growth on Mitis Salivarius-Bacitracin agar plates in pre- and post-intervention – Papacarie Duo.

Papacarie Duo was repeated until no change in color was noted. Later, the cavity was tested according to visual and tactile clinical criteria. Finally, the remaining gel was removed with sterile cotton pellets soaked in water, and the cavity was repeatedly washed until the gel is completely removed. The time taken for entire caries removal was recorded using a digital stopwatch.

### *Caries removal in Group IB and IIB*

The carious tissue was completely removed from the cavity by the hand excavation method using a sterile sharp hand excavator. The cavity was tested to be caries-free according to visual and tactile clinical criteria. The time taken for the caries removal was recorded using a digital stopwatch.

Evaluation criteria for complete removal of caries (Ericson, 1999):[10]

1. The visual criteria - absence of any discoloration
2. The tactile criteria - the smooth passage of a dental explorer with no tug back or catch sensation.

### *Second dentine samples*

In all the groups, after complete caries removal, each of the cavities washed and dried with saline and sterile dry cotton pellets. The second sample was taken using a sterile, sharp excavator. To obtain a sufficient amount of sound dentin after caries removal, at least some visible dentin particles were removed from both the floor and the walls of the cavity (Figures 1 and 2, Tables 1 and 2, Graph 1). Dentin samples were then placed in sterile vials containing transporting media. The cavity outline was adjusted with burs; any undermined enamel around the cavity walls was removed with a small round bur. The teeth restored with composite resin according to the manufacturer's instructions (Figures 3 and 4, Tables 1 and 2, Graph 2).

### *Degree of discomfort during caries excavation*

The degree of discomfort during caries excavation was evaluated using the visual analog scale given by Whaley and Wong (1987) which is composed of six facial expression scores: 0 = no hurt, 1 = hurts little bit, 2 = hurts little more, 3 = hurts even more, 4 = hurts whole lot, and 5 = hurts worst. Visual analog scale of faces was presented to the patient after the restoring the teeth with the following question: "If you compare yourself to this face right now, which face would represent you the most?" The child would then point to the corresponding face that best represented their degree of pain or discomfort. Scores of visual analog scale of faces were recorded in all groups and were subjected to statistical analysis for assessment of patient acceptance.

### *Microbial cultivation and evaluation*

Immediately after removal, the samples were transferred into the sterile vials with a cap and processed in the laboratory for microbiological investigation. The samples were diluted vertoxed and then plated on two different agar plates. Mitis Salivarius and Bacitracin agar and Rogosa SL agar (HiMedia) were the culture media to determine *Streptococcus mutans* and *Lactobacillus,* respectively. Then, the numbers of colonies were counted using a digital colony counter.

## Results

The mean *S. mutans* count for both the study groups Group I – Papacarie Duo and Group II – hand excavation method during pre-intervention was $1.54 \times 10^5$, $1.58 \times 10^5$ colony forming units (CFUs)/ml and post-intervention was $0.0020 \times 10^5$, $0.0034 \times 10^5$ CFUs/ml, respectively, with a $P = 0.001$. Statistically, significant reduction in *S. mutans* count was seen in both the study groups. The mean *Lactobacillus* count for both the study groups Group I – Papacarie Duo and Group II – hand excavation method during pre-intervention was $0.0067 \times 10^5$, $0.0068 \times 10^5$ CFUs/ml and post-intervention was $0.47 \times$

**Table 1: Comparison of mean *Streptococcus mutans* colony forming units/ml between the two study groups, Group I – (Papacarie Duo) and Group II – (Hand excavation method) during pre- and post-intervention period using Student's unpaired *t*-test.**

| Period | Group | *N* | Mean | SD | SEM | Mean | 95% CI of the differ | | *t* | df | *P* value |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Lower | Upper | | | |
| Pre-intervention | Group I | 30 | 1.54 | 0.12 | 0.02 | −0.04 | −0.11 | 0.03 | −1.086 | 58 | 0.28 |
| | Group II | 30 | 1.58 | 0.16 | 0.03 | | | | | | |
| Post-intervention | Group I | 30 | 0.0020 | 0.0013 | 0.0002 | −0.0014 | −0.0022 | −0.0006 | −3.386 | 58 | 0.001* |
| | Group II | 30 | 0.0034 | 0.0018 | 0.0003 | | | | | | |

*Statistically significant. CI: Confidence interval, SD: Standard deviation, SEM: Standard error of mean

**Table 2: Comparison of mean *Lactobacillus mutans* colony forming units/ml between the two study groups, Group I – (Papacarie Duo) and Group II – (Hand excavation method) during pre- and post-intervention period using Student's unpaired *t*-test.**

| Period | Group | *N* | Mean | SD | SEM | Mean | 95% CI of the differ | | *t* | df | *P* value |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Lower | Upper | | | |
| Pre-intervention | Group I | 30 | 0.47 | 0.28 | 0.05 | 0.04 | −0.10 | 0.18 | 0.564 | 58 | 0.58 |
| | Group II | 30 | 0.43 | 0.25 | 0.05 | | | | | | |
| Post-intervention | Group I | 30 | 0.0067 | 0.0029 | 0.0005 | −0.0001 | −0.0015 | 0.0013 | −0.117 | 58 | 0.91 |
| | Group II | 30 | 0.0068 | 0.0027 | 0.0005 | | | | | | |

CI: Confidence interval, SD: Standard deviation, SEM: Standard error of mean

$10^5$, $0.43 \times 10^5$ CFUs/ml. A reduction was seen in the number of CFUs/ml of *Lactobacillus*; however, the difference was not statistically significant ($P = 0.91$).

The time taken for caries removal using Papacarie Duo was lesser at 9.07 ± 1.41 min when compared to the hand excavation method (Table 4). During hand excavation, five patients complained of pain and required anesthesia. The stopwatch was not stopped during the application of anesthesia, which led to the higher time duration at 14.94 ± 0.75 min in the hand excavation group (Table 4). In the CMCR group, no cases received anesthesia while in the conventional group, five cases were administered anesthesia with significant difference between the two groups ($P = 0.04$).

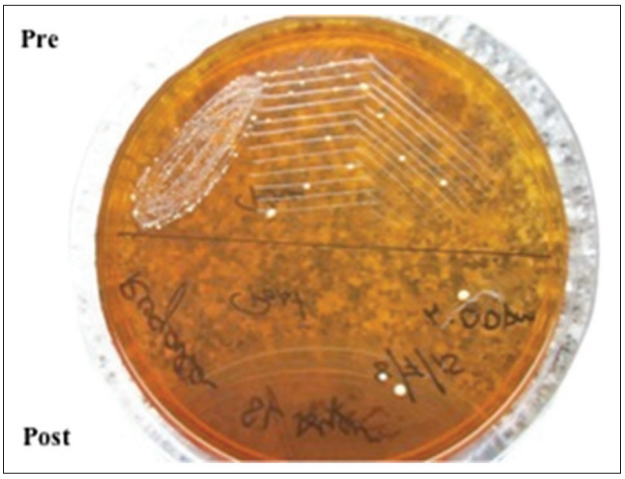



**Figure 3:** Growth on Rogosa SL agar plates in pre- and post-intervention – Hand excavation.

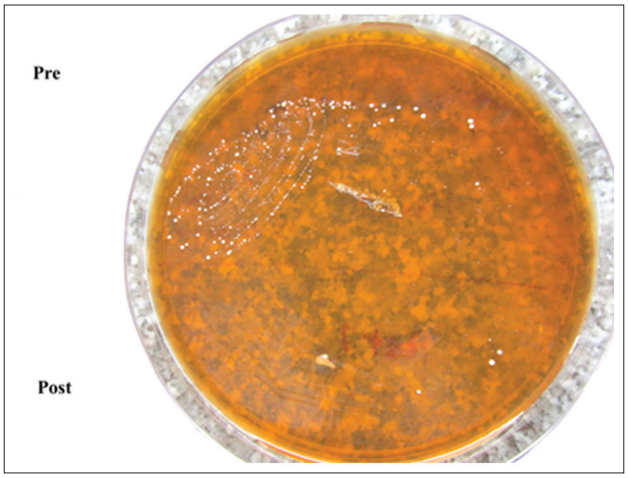



**Figure 4:** Growth on Rogosa SL agar plates in pre- and post-intervention – Papacarie Duo.

## Discussion

In dental practice, before restoring the cavity, the soft and irreversibly demineralized dentinal tissue is removed. The clinical criteria for a cavity to be caries free differ around the world. The process of cavity preparation involves removal of most of the microorganisms along with the necrotized dentinal tissue; however, the prepared cavity cannot be termed bacteria free. The clinical criteria for indication of endpoints of excavation may not always be reliable in the present day. Studies by Banerjee *et al.* concluded that the complete removal of all the infected dentin is not possible.[3] Thus, a 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. CMCR techniques gained interest in dental research due to their concept of tissue preservation while removing denatured dentinal collagen.[11] As this procedure involves removal of carious dentin only without any painful damage to healthy surrounding structures, the need for a local anesthetic solution is avoided. Our study was conducted to assess the efficacy of Papacarie Duo a CMCR agent in primary and permanent teeth. As the microflora is the main etiological factor, it is essential to reduce the microbial flora thus bacteriological analysis was the method of assessment chosen in this study to test the effectiveness of the two methods of caries removal hand excavation and Papacarie Duo. Very few studies were performed comparing the efficacy of Papacarie Duo gel on the microflora of caries affected primary and permanent teeth. It should be emphasized that primary teeth have a less mineralized dentin than permanent teeth. In our study, the mean total viable count of *S. mutans* and *Lactobacillus*

**Table 3: Comparison of mean time taken to perform the specific procedure between the two study groups, Group I – (Papacarie Duo) and Group II – (Hand excavation method) using Student's unpaired *t*-test.**

| Group | *N* | Mean | SD | SEM | Mean | 95% CI of the differ | | *t* | df | *P* value |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Lower | Upper | | | |
| Papacarie Duo group | 30 | 9.07 | 1.41 | 0.26 | −5.87 | −6.46 | −5.29 | −20.181 | 58 | <0.001* |
| Hand excavation group | 30 | 14.94 | 0.75 | 0.14 | | | | | | |

*Statistically significant. CI: Confidence interval, SD: Standard deviation, SEM: Standard error of mean

**Table 4: Comparison of the proportion of patients requiring anesthesia among the two study groups, Group I – (Papacarie Duo) and Group II (Hand excavation method) using Fischer's exact test.**

| Group | *N* | Mean | SD | SEM | Mean | 95% CI of the differ | | *t* | df | *P* value |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Lower | Upper | | | |
| Papacarie Duo group | 30 | 9.07 | 1.41 | 0.26 | −5.87 | −6.46 | −5.29 | −20.181 | 58 | <0.001* |
| Hand excavation group | 30 | 14.94 | 0.75 | 0.14 | | | | | | |

*Statistically Significant. CI: Confidence interval, SD: Standard deviation, SEM: Standard error of mean

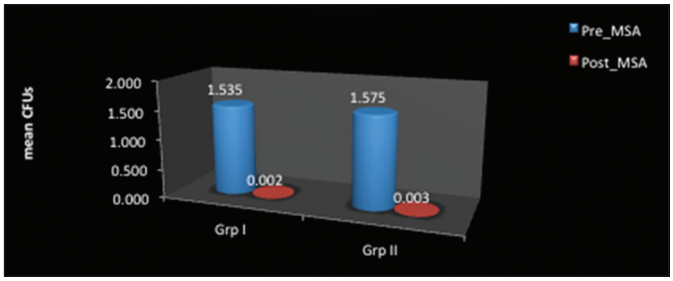
after caries removal with Papacarie Duo showed a statistically significant reduction in both primary and permanent teeth compared with hand excavation method. This is in accordance with a study done by Gupta *et al.*,[12] where it was concluded that Papacarie is an effective caries removal method clinically in both permanent and primary teeth. In our study, children selected belong to age group of 4-8 years. According to Mariri *et al.*, caries activity is high during this age group due to increased intake of sugars, starchy foods, and greater frequency of eating. Manji and Fejerskov[13] reported that the lower molars were the most severely affected teeth in the entire dentition than upper molars. Macek *et al.*[14] investigated the caries susceptibility of permanent teeth and found that molars were more susceptible than incisors, canines, or premolars; thus, in our study, primary molars and permanent first molars with broad-cavitated lesions extending to dentine were selected. To standardize all variables of the sample (e.g., shape and activity status of the lesions), a split-mouth design was followed with only occlusal carious lesions extending till the middle two-third of the dentin confirmed through periapical radiographs.

Complete caries removal was achieved by both methods (Papacarie Duo and hand excavation) that were evaluated visually by the absence of discoloration and tactile method by smooth passage of an explorer without any catch or a tug back sensation according to the criteria given by Ericson.[10] Kumar *et al.*[15] concluded that caries removal using Papacarie showed less destruction of dentinal tissues under light microscope. In a study conducted by Bussadori *et al.*,[16] it was found that a residual smear layer was left behind by the conventional method of caries removal using a stainless steel or a diamond bur while more preservation of dentinal tissue with more effective caries removal seen in case of Papacarie gel.

Flindt[17] demonstrated that Papain acts only in infected tissues because of the absence of plasmatic anti-protease called alpha-1 antitrypsin, which is present in sound tissues, and it inhibits protein digestion. The absence of the alpha-1 antitrypsin in infected tissues allows Papain to break the partially degraded collagen.[18] Toluidine blue is antimicrobial agent and water acts as a vehicle. In relation to the oral microbiota, evidence has shown that the acidogenic species mainly streptococci are strictly associated with the onset and the presence of dental caries.[16] Thus, with the help of each of its constituents, Papacarie achieves synergistic action that facilitates caries removal and increased antimicrobial properties.[19] The new version of Papacarie released in 2011 was denominated as Papacarie Duo which has the same efficacy against the growth of both Gram-positive and Gram-negative organisms, plus a number of additional properties, such as a longer shelf life and no need for refrigerated storage.[20]

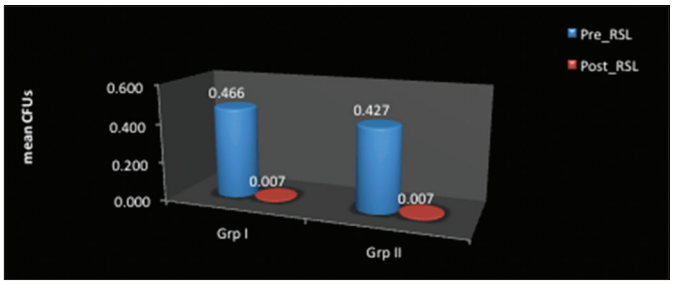
In our study, the mean total viable count of *S. mutans* after caries removal showed reduction. The difference in the mean between the two groups was found to be statistically significant.

| Period | Group I | Group II |
|---|---|---|
| Pre-MSA | 1.535 | 1.575 |
| Post-MSA | 0.002 | 0.003 |




**Graph 1:** Comparing the pre- and post-intervention mean colony forming units/ml of *Streptococcus mutans* between the two groups Group I (Papacarie Duo) and Group II (hand excavation).

| Period | Group I | Group II |
|---|---|---|
| Pre-RSL | 0.466 | 0.427 |
| Post-RSL | 0.007 | 0.007 |




**Graph 2:** Comparing the pre- and post-intervention mean colony forming units/ml of *Lactobacillus* between two groups Group I (Papacarie Duo) and Group II (hand excavation).

The mean total viable count of *Lactobacillus* after caries removal in the Papacarie group also showed reduction; however, the difference was not statistically significant ($P = 0.91$). A study by El-Tekeya *et al.*[9] concluded that the caries removal using Papacarie was more effective on the residual cariogenic bacteria of primary teeth against Carisolv and hand excavation methods. Further studies by Viral *et al.*[21] and Matsumoto[22] inferred that minimally invasive caries removal was achieved using Papacarie with significant reduction in the total microbial count especially *S. mutans* which is in accordance with the findings of the present study where Papacarie Duo showed bactericidal and bacteriostatic properties inhibiting both *S. mutans* and *Lactobacillus* species.

The mean time taken for carious removal by Papacarie Duo was 9.07 ± 1.41 min and for hand excavation method was 14.94 ± 0.75 min. It was observed that CMCR time was lesser than conventional caries removal which is in agreement with findings reported in a study carried out by Konde *et al.*[23] The time taken in the initial few cases was higher than the conventional hand excavation method. However,

with the development of expertise, the CMCR became less time consuming. According to Kotb *et al.*,[24] Papacarie was as effective as the drill in caries removal with no significant difference in operating time. However, previous investigations of CMCR method done by Ericson *et al.*,[11] Nadanovsky *et al.*[17] and Maragakis *et al.*[25] revealed the consumption of significantly higher time as compared with the conventional method. It has been stated that Papacarie Duo requires more than one application for its action to work. Carrillo *et al.*[25] reported that treatment duration of 9 min/tooth was seen using Papacarie gel. In our study, the mean time required for the Papacarie Duo gel, which was lesser than hand excavation method at 9.07 ± 1.41 min. None of the cases in the Papacarie Duo group required anesthesia while in hand excavation, five patients complained of pain and were administered local anesthesia. The digital stopwatch was not stopped during the application of anesthesia, which may have led to the higher time duration in the hand excavation group.

In the present study, visual analog scale of faces by Whaley and Wong (1987) was used to evaluate the pain sensation.[26] Pain scores during the Papacarie Duo method of caries excavation were 1.07 ± 0.25, and in the hand excavation method, it was 2.43 ± 1.19. The analysis of pain perception during caries removal in Papacarie Duo group was less painful when compared with the hand excavation method. The overall pain perception experienced by patients revealed higher comfort levels with chemomechanical method over the conventional hand excavation method. This result was found to be statistically significant ($P < 0.001$). This was in accordance with studies conducted by Silva *et al.*,[27] who stated that conventional method of caries removal was significantly more painful than the Papacarie method. The difference in the pain scores between the two methods could be attributed to the fact that Papacarie Duo acts on the carious dentin only avoiding the painful removal of sound tissue.

Some of the limitations of the techniques evaluated in this study are that hand excavation which is inappropriate for cutting enamel as most cavities are not large enough to allow proper access to carious dentin. This may also raise doubts in the minds of public as to whether this is really a satisfactory solution for the very unpleasant conventional treatment of decay. The second limitation encountered in this study was the smaller sample size; further research with larger sample sizes and longer follow-ups is recommended. Finally, the visual tactile methods were used as clinical criteria for indication of endpoints of excavation, which may not always be reliable in the present day.

## Conclusion

The chemomechanical method (Papacarie Duo) is more effective in the reduction of both *S. mutans* and *Lactobacillus* from the carious dentin of primary and permanent molars than the hand excavation method. The time taken for caries removal using chemomechanical method Papacarie Duo is comparatively lesser than the hand excavation method. Five children in the hand excavation group required local anesthesia; thus, the degree of discomfort in children was higher in hand excavation when compared to Papacarie Duo.

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