Antimicrobial efficacy of an indigenously prepared caries removing gel

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Abstract

Objective: The aim of this study was to assess the anti-microbial efficacy of an indigenously prepared caries removing gel, in primary molars. **Materials and Methods:** Twenty teeth with broad occlusal cavitated lesions that fulfilled the clinical and radiographic criteria formed the study group. These teeth were subjected to chemomechanical method of caries removal, using an indigenously prepared caries removing gel. Prior to and following caries removal, the dentin samples were analyzed for total viable count and lactobacilli count. **Results:** The percentage of reduction in the total viable count was 92.4% and in the lactobacilli count it was 94.1%, which was statistically highly significant. **Conclusion:** Removal of carious tissue with a caries removing gel, a natural plant extract, proved to be efficient, easy to perform, and comfortable for the patient.

Keywords: Chemomechanical caries removal, colony forming unit, caries removing agent, lactobacilli count, total viable count

Introduction

Chemomechanical caries removal is a technique of eliminating infected dentin via a chemical agent. This method of treatment has gained high acceptance especially among children and patients with dental anxiety.[1] It is a process that only removes the infected tissues, preserving the healthy dental structures, avoiding pulp irritation and patient discomfort.[2] The chemomechanical method was introduced in 1972, when a product named GK 101 was marketed.[3] However, due to its inherent aggressive effect on both carious and non carious lesion, a search for a new material was started which led to the development of Caridex in 1984.

Caridex™ contains n-monochloro DL-2 aminobutyric acid (NMAB), which reduces the aggressive effects on healthy tissues. Its clinical disadvantage was because of the prolonged time required for its action, shorter shelf-life, and its high treatment cost.[4] In the 1990s a newer chemomechanical caries removal gel - Carisolv was developed, containing three amino acids in its composition (leucine, lysine, and glutamic acid). The reaction of the three amino acids with sodium hypochlorite neutralized the aggressive effect on the sound tissues, allowing caries removal. Carisol was not a blockbuster mainly because it required extensive training and customized instruments, which increased the cost of the solution.[2] In 2008, a Brazilian study recommended the use of Papacarie in the chemomechanical removal of caries.[2]

With a similar idea, a search was undertaken by the authors to indigenously develop a chemomechanical agent that was naturally available, economical, antimicrobial, affecting only infected dentin and simple to use. A study was then undertaken which aimed to evaluate the efficacy of this indigenously prepared caries removing gel in reducing the total viable count and lactobacilli counts, which play an important role in the progression of dentinal caries.

Materials and Methods

Twenty -five children (11 females and 14 males) aged between four and eight years were selected. To be included in the study the subjects had to have one primary molar with a broad occlusal cavitated lesion, showing brown and softened dentin. A brief history was recorded and the teeth were subjected to clinical and radiographic evaluation prior to the study. Radiographically, the carious lesion had to be clearly visible as a radiolucency extending into, but confined to the outer dentin of the occlusal surface.[5] Teeth with interpoximal caries were excluded. Patients on an antibiotic regimen either on the day of the treatment or for at least two weeks prior to the study were also excluded.[5] From these 25 children, 20 teeth formed the study group.

Composition of the caries removing agent

The caries removing agent consists of an enzyme (papain), an anti-oxidant (D--α-tocopherol acetate), humectant (glycerine),

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Table 1: Evaluation based on the total viable count CFU / ml × 10^4 and Lactobacilli count (CFU / ml) x 10^4

Microbiological count	Pre-assessment	Post-assessment	% Change	Log reduction CFU / ml	*P* value
Total viable count CFU / ml ×10^4	77.44 ± 8.52 (61 – 89)	5.92 ± 1.97 (2 – 9)	92.4%	2.63 ± 0.40	t = 43.442 *P*<0.001**
Lactobacilli count (CFU / ml) ×10^4	30.48 ± 7.51 (18 – 43)	1.80 ± 1.08 (0 – 4)	94.1%	2.82 ± 0.52	t = 19.110 *P* < 0.001**

emulsifier (amylopectin), thickener (carbopol), preservative (propyl-p-hydroxybenzoate), coloring agent (green apple), and distilled water as a vehicle.

Clinical procedure[5]

The caries removing gel can be refrigerated and is required to be removed one hour prior to use. Following rubber dam isolation, an initial sample of the carious material was removed superficially from the lesion using a sharp sterile spoon excavator. It was immediately transferred into a sterile vial containing 1 ml of saline. With the help of another sterile spoon excavator the caries removing gel was placed in the cavity so as to cover the dentinal caries. After 40 seconds contact with carious dentin gently scraping of infected dentin was carried out using light pressure with the spoon excavator. On initial application the gel was clear, but gradually became opaque due to the debris removed from the carious lesion. The debris saturated gel was removed with a cotton pellet. Fresh gel was reapplied and carious dentin scraped using similar procedure, until the gel no longer turned opaque after scraping of dentinal surfaces.. A second sample of dentin was then taken from the cavity floor with another sterile spoon excavator and transferred to another sterile vial containing 1 ml of saline.

Microbial cultivation and evaluation[6]

The dentin samples were processed in the microbiological laboratory within one hour of collection. Each sample was vortexed for about 30 seconds in order to dislodge the bacteria from the dentin. The samples were then serially diluted to obtain 10^{-4} dilutions, and 0.1 ml of this dilution was inoculated onto two different agar plates. The Schaedler agar was used to determine the total viable count and Mitis Salivarius agar was used to determine the viable count of the lactobacilli. The plates were incubated anerobically at 37^0C for three days. Then, using a colony counter, the number of colonies was determined per sample and expressed as CFU / ml.

The data obtained was tabulated and subjected to descriptive statistical analysis. The Student t test (two tailed, dependent) was used to find the significance of the study parameters.

Results

Prior to caries removal, the mean count of the total viable bacteria was observed to be $77.44 \pm 8.52 \times 10^{-4}$ CFU / ml and it was $5.92 \pm 1.97 \times 10^{-4}$ CFU / ml after caries removal. The lactobacilli count was $30.48 \pm 7.51 \times 10^{-4}$ CFU / ml before caries removal and $1.80 \pm 1.08 \times 10^{-4}$ CFU / ml after caries removal [Table 1]. The percentage reduction for the total viable count was 92.4% and for the lactobacilli count it was 94.1%, which was statistically highly significant ($P < 0.001^{**}$) [Figure 1].

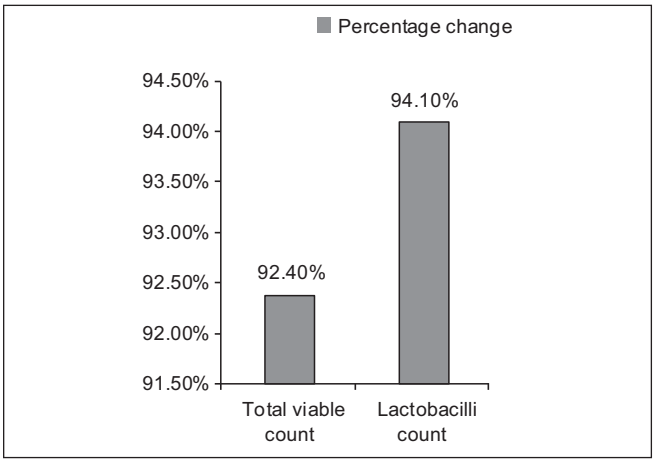

Figure 1 : Percentage reduction in the total viable count and lactobacilli count

Discussion

Conventional drilling is the most common clinical procedure for caries removal, but it generates pain, fear, discomfort, and anxiety in children.[6] The new chemomechanical caries removing method is desirable in Pediatric Dentistry, as it allows a minimal invasive technique to be applied.[7] This system also eliminates the use of anesthesia, painful symptoms, and unnecessary removal of the sound tooth structure, as only the carious dentin is removed and the painful removal of sound dentin is avoided.[8]

The dentinal carious lesion can be divided into two zones. An outer layer of infected dentin, in which the collagen fibers are partially degraded and cannot be remineralized, and an inner layer of affected dentin, which is partially demineralized with intact collagen fibers and cannot be remineralized. A chemomechanical caries removing system, acts by causing further degradation of the partially degraded collagen, in the infected dentin.[9] The mechanism of action of the caries removing gel used in this study appears to be similar.

The gel used in this study is a natural product consisting of an enzyme, papain, which is extracted from raw papaya fruit. Papain is proteolytic in nature, bacteriocidal, anti-inflammatory, and has whitening properties.[2] Papain acts only on damaged tissue due to the absence of the anti-plasmatic protease, alpha-1-anti-tripsine, which hinders its proteolytic action in tissues considered normal.[10] Papain is an enzyme similar to human pepsin, used in food technology, pharmaceuticals, and cosmetic industries.[2] Guzman and Guzman,1953,[11] performed clinical studies on patients with skin lesions caused by burns, observing that the enzymatic action of papain was considered excellent in areas with necrotic and purulent processes. Udok and Storojuk, 1981,[12] also verified that papain aided the cleansing of necrotic tissue and secretions, shortening the period of tissue repair. An anti-oxidant, D--α-tocopherol acetate, was added to reduce the oxidative stress produced by the bacteria. In addition, a humectant (glycerine), an emulsifier (amylopectin), a thickner (carbopol), and a coloring agent (green apple) were included. Propyl p-hydroxybenzoate, a natural substance found in many plants, was used as a preservative, and distilled water was used as a vehicle.

All primary teeth included in the present study showed broad occlusal cavitated lesions, with brown softened dentin. Although, mutans streptococci are mainly implicated in the initiation of caries, they are rarely the predominant species isolated from the carious dentin. The composition of the microflora is known to become more complex as the lesion progresses, and obligate anerobes, mainly Gram positive rods predominate, accounting for 70% of the total colony forming units / milliliter. Among these, the lactobacillus species are the principal isolates and play an important role in the progression of dental caries.[13] Thus, it is essential to determine both the total viable count and the lactobacilli count from the infected as well as the top layers of residual dentin, following caries removal.[14] Nowdays, a cavity is judged to be clinically caries-free according to the tactile, acoustic, and optical criteria. Several investigations could show that often a low number of residual microorganisms remain behind in clinically sound hard dentin in spite of a significant reduction in the bacterial count; however, this low number of bacteria is considered to be clinically acceptable by several authors.[15,16]

As with other chemomechanical caries removing agents, the gel used in this study was simple in application and caused no discomfort to the patient. No allergies were reported from any patients. It did not require the use of any special instruments for excavation of caries. Also, it is easy, economical to prepare, and and could be stored under refrigeration.

In our study, a significant reduction in both the total viable count and lactobacilli count was observed. This was in accordance with studies that reported a similar reduction in bacterial counts following the use of Carisolv.[5,14] Thus, the antimicrobial efficacy of this gel was comparable to Carisolv.

Conclusion

This study evaluated the antimicrobial efficacy of a new caries removing gel in the chemomechanical removal of caries. A significant reduction of 92.4% in the total viable count and 94.1% in the lactobacilli count was observed. Removal of carious tissue with a caries removing gel, a natural plant extract, proved to be efficient, easy to perform, and comfortable for the patient.

A patent with application no 2740 / CHE / 2008 and with a title 'papEdent — a painless gel' has been applied for this product with the Patent Office, Chennai, Government of India.

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