Histobacteriological evaluation of advancing front of carious dentin: Excavation done with and without using caries disclosing dye - A comparative in-vitro study

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Abstract

Background: Dental caries is the most common bacterial infection of the oral cavity. Early detection and complete removal of dental caries play a key role in the treatment and prognosis of dental caries. This study is aimed to evaluate histobacteriologically the advancing front of carious dentin: Excavation done with and without using caries disclosing dye.

Materials and Methods: Freshly extracted 100 permanent teeth with coronal and proximal caries were collected in a saline solution under aseptic conditions. Group 1 (n = 50) caries were excavated using caries detecting dye 1% acid red solution in propylene glycol method. Group 2 (n = 50) caries were removed by visual and tactile method. These teeth specimen are decalcified, processed, and stained for bacterial evaluation.

Results: The majority of the teeth (98%) in Group 1 showed the absence of bacteria, and only 10% of the teeth showed the absence of bacteria in Group 2.

Conclusion: Caries detecting dye is a useful adjunct and can supplement the conventional methods of caries detection in assessing the extent of caries in dentin.

Key words: Bacteria, caries disclosing dye, dental caries

Introduction

The oral cavity is said to be the mirror of health and the teeth are an integral part of it. Oral disease has been a problem for humans from the beginning of history and dental caries tops the list. A lot of research projects have focused on the pathogenesis, and prognosis of dental caries and have studied various modalities in caries prevention.1

Caries infection usually is an intermittent process, which may evolve through repeated phases of remission and recurrences. If unchecked, it may ultimately lead to complete destruction of the tooth. Early detection and complete removal of dental caries play a key role in the treatment and prognosis of dental caries. Today, we have a lot of techniques to detect dental caries such as direct visual inspection, indirect vision, auxiliary external illumination, transillumination, radiographs, direct digital radiography, electronic caries monitor, caries activity tests, and quantitative light induced fluorescence. The above-mentioned techniques are either not reliable or far too expensive for all dentists to afford.

In contrast to these aids, caries detector dyes are reliable, less expensive, and can be used effectively as a chair-side protocol.2

The possibility of caries detector dyes was originally developed in the 1970’s when basic fuchsin staining was used as a guide to the removal of the outer layer of infected unremineralized dentin in the carious lesion. There are many dyes such as methyl red, alizarin stain, 8-hydroxyquinoline, fluorescent dyes, carbolan green, coomassie blue, lissamine blue, 0.5% basic fuchsin, 1.0% acid red, which have been used for detecting caries, and was used to help the clinician to distinguish between affected and infected dentin during cavity preparation. There has always been concern about the safety of disclosing agents in terms of carcinogenicity, and 1% acid red in propylene glycol was introduced as a safe and effective alternative.2-4

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Different studies have been done on acid red dye, and it has been proved that it is more reliable in detection of caries than mere clinical judgment of complete removal of bacterially infected dentin and soft carious dentin.[4] The use of this dye allows a greater accuracy in removing only the tissue that is infected and irreversibly deteriorated.[5]

By routine traditional techniques most of the infected dentin is not excavated, therefore, the microorganisms present in the infected dentin are retained back after restoration. If nutrients are made available to these microorganisms either through marginal leakage or through diffusion from the pulp, these microorganisms would multiply in number and possibly lead to the renewal of carious process.[2]

The study aimed to evaluate histobacteriologically the advancing front of carious dentin when caries excavation was done with and without using caries disclosing dye.

**Materials and Methods**

Freshly extracted permanent teeth with coronal and proximal caries were collected in a saline solution under aseptic conditions. In this study, 100 carious teeth specimens were taken. Group 1 \((n = 50)\) caries were excavated using caries detecting dye 1% acid red solution in propylene glycol method. Group 2 \((n = 50)\) caries were removed by visual and tactile method.

In the visual method, the caries were removed by using a sterile #557 straight fissure carbide bur and spoon excavator. The surface of excavated dentin was examined under 2.5 magnifying lens [Figure 1]. In dye method, 1% acid red solution in propylene glycol was applied on the floor of the cavity for 10 seconds [Figure 2], and then excess dye was washed off from the dentin for an additional 10 s with a stream of water and the cavity was stained [Figure 3]. The red stained dentin was excavated completely with the sterile #557 straight fissure carbide from the dye stained specimens. The teeth were stained again with a caries detector solution, and any stained dentin was removed completely. This procedure was repeated until the walls of the cavity in the excavated part of the lesion no longer gets stained when the acid red solution is applied [Figure 4]. The surface of excavated dentin was examined under 2.5 magnifying lens.

Teeth of both the groups were stored in 10% neutral buffered formalin in individual air – tight glass specimen jar for 10–20 days at room temperature. A code number was assigned to each specimen. Each specimen was then transferred to a specimen jar and decalcified in 1N formic acid for at least 24 days. The formic acid solution was changed every 3 days.[9] The decalcification endpoint test was used to check for the completion of decalcification (calcium oxalate test).[7-9]

Calcium oxalate test was done to check the decalcification. Decalcified specimens were kept under running tap water for approximately 12 h for complete removal of the acid. Each decalcified crown was cut in half longitudinally using a new sterile #15 scalp blade for each tooth. Decalcified tissues were then processed, and blocks were prepared. The blocks were first trimmed, and then consecutive sections of 4.5 µm thickness were prepared. Five sections of each tooth were taken from both the groups and all sections from both the group teeth were stained with hematoxylin and eosin and Gram stain. Then, the slides were observed under a light microscope to check for the presence of microorganisms. The Chi-square test was used to confirm the difference in the microorganisms in the two groups.

**Results**

Group 1: Dye method: Of the 50 teeth processed, 48 teeth showed the absence of bacteria and 2 teeth showed the presence of bacteria [Figure 5].

Group 2: Visual method: Of the 50 teeth observed by this method, 5 teeth showed the absence of microorganisms and the remaining 45 teeth showed the presence of microorganisms using Gram stain [Figure 6]. There was a statistically significant difference in between the groups with regards micro-organism \((\chi^2 = 74.22, P = 0.000)\).

**Discussion**

Dental caries is a multifactorial disease. There are practically no geographic areas in the world whose inhabitants do not exhibit some evidence of dental caries. It affects persons of both genders, in all races, all socioeconomic strata, and every age group.[10] In India, the prevalence of dental caries is reported to be about 50–60%,[8]

Caries is the biggest challenge for dental clinicians all over the world and objectives of most of the dental procedures are directed toward successfully treating the same. The best way to tackle caries is to eradicate it. The single most significant contributing factor in caries epidemiology is sugar.[11,12] The enzymatic breakdown of sugar produces acids and plaque helps in holding these acids to the tooth surface. Anaerobic catabolism of carbohydrate predominates in plaque. Organisms such as streptococci and many lactobacilli, ferment sugar and produce lactic acid, which plays a role in the occurrence of caries.

The difficulty in detecting infected carious dentin accurately and reliably by tactile and visual examination is not new to us.[14] The success of treatment lies in first eliminating the existing carious lesion. For this cavity, preparation is done that attempts to remove all infected dentin prior to placing a restorative material. Traditionally the color and texture of dentin at the base of the cavity preparation served as a subjective indicator of caries penetration. Histological and bacteriological experiment performed to determine whether viable microorganisms remain on the dentinal surface at the termination of routine cavity preparation has shown the presence of microorganisms.[15] In order to overcome...
these difficulties, many techniques have been introduced, such as radiographs, direct digital radiography, electronic caries monitor, fiber optic transillumination, ultrasonic caries detector, caries activity tests, and quantitative light induced fluorescence, which is the latest of all.[7] In the author’s opinion, the above-mentioned techniques are sometimes not very reliable and practical, being far too expensive and not always feasible. Since the use of caries disclosing dye in caries removal is more promising, cheaper and less time consuming, it prompted us to undertake a study in this regard.

Dyes have a widespread use in medicine, biology, and dentistry. They have been used for clinical indication of affected dental tissue, improvement of diagnostic methods, enhancement of patient awareness, and information about specific processes. The concept of using caries detector dyes was originally developed in the 1970s, when basic fuchsin staining was used as a guide for the removal of the outer layer of infected unremineralizable dentin in carious lesions. However, its use has been discarded due to its carcinogenic potential. There are many dyes such as silver nitrate, methyl red, alizarin stain, carbolan green, 8-hydroxyquinoline, coomassie blue, lissamine blue, and fluorescent dyes, which have been used for detecting caries and are believed to help the clinicians to distinguish between the affected dentin and infected dentin during cavity preparation.[8] There has always been a concern about the safety of disclosing agents in terms of carcinogenicity. About 1% acid red in propylene glycol was introduced by Fusayama, Takatsu and Itoh, in 1979, as a safe and effective alternative.[2,3]
The present study uses 1.0% acid red in propylene glycol (i.e., caries detector) in identifying the infected dentin, and also to assess the depth of microbial invasion in the carious dentin. We compared the findings of presence or absence of microorganisms in carious teeth by caries detector dye method and visual method of caries removal. In the visual method, out of 50 carious teeth 45 showed the presence of bacteria, which was confirmed by gram staining, 5 teeth showed the absence of bacteria. In another 50 carious teeth, caries were removed after application of 1% acid red dye in propylene glycol. There was the absence of bacteria in 48 teeth and 2 teeth showed the presence of bacteria, which was confirmed by gram staining, and these results were statistically significant.

Our findings are in accordance with early workers namely Shimizu et al.,[14] Boston and Graver et al.,[6] Starr and Langenderfer,[53] and Maupomé et al.[96] However, Ansari et al.[3] suggests that the application of caries disclosing dye result in unnecessary removal of sound dentin, which is not in favor of our result. Shimizu et al.[14] used 1% acid red for removal of softened dentin in pulpless teeth and concluded that complete removal of infected dentin in pulpless teeth can be achieved by removing all the dentin that stains with a solution of 1% acid red. Boston and Graver et al.[6] carried out histological study of an acid red caries-disclosing dye in 20 extracted teeth and found that acid red staining and bacterial penetration were somewhat independent phenomena, and the deepest portion of a carious lesion may contain lower number of bacteria as compared to the most superficial stained dentin. Starr and Langenderfer[53] used caries-disclosing agent to improve dental residents’ ability to detect caries and proved that caries-disclosing dyes made from acid red are capable of distinguishing between infected and affected dentin, thereby aiding the clinician in the caries removal process. Boston and Graver[17] again carried out histobacteriological analysis of acid red dye-stainable dentin found beneath intact amalgam restorations and found 14 of 16 clinically sound amalgam restorations had microscopic evidence of presence of microorganisms in the subjacent dentin and 11 of the 14 specimens containing bacteria exhibited acid red dye-stainable dentin. Maupomé,[10] carried out an in-vivo diagnostic assessment of dentinal caries utilizing acid red and povidone-iodine dyes. A total of 221 cavities prepared by senior dental students were used, and 36.7% of teeth tested positive to either one of the two dyes. Prudent utilization of either acid red or povidone iodine appeared to be equally useful in assisting clinical decisions concerning cavity size while restoring dentinal lesions. Ansari et al.[3] carried out an in-vitro assessment of five different caries detector dyes, and concluded that none of the five dyes studied offers any real advantage and may lead to the unnecessary removal of sound dentin. Iwami et al.[18] carried out an in-vitro study for the clinical evaluation of carious dentin using colorimetry and assessed that the rate of bacterial detection in caries were inversely related to the lightness (intensity of staining) of the carious dentin stained with a caries detector dye.

Considering the economic scenario of our country, visual and tactile methods have remained the cornerstone for the detection of dental caries. These are slowly being replaced by newer techniques; unfortunately these techniques are either far too expensive or not very reliable. This is, when the attention turned to the caries detector dyes. Our work strongly confirms the earlier views of using caries detecting dye as a useful adjunct in the removal of caries as compared to the visual method alone. Thus, it can be concluded that use of caries detector dye can supplement the conventional methods of caries detection and in assessing the extent of caries in dentin. Further research can be done to evaluate the use of the dye in the in-vivo conditions.

References