Efficacy of Two Caries Detector Dyes in the Diagnosis of Dental Caries

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Abstract:
Objective: The aim of the present study was to evaluate the efficacy of two caries detector dyes in the diagnosis of dental caries.

Materials and Methods: Twenty extracted human posterior teeth without pulpal exposure were sectioned mesiodistally through the center of the lesions using a water-cooled disk. The tooth halves were randomly divided into two groups and treated with Caries Detector (CD) and Caries Check (CC) detector dyes. Access cavities were prepared followed by caries removal and dye application. All cavities were arbitrarily divided into two right and left sections and excavation of the stained areas was performed on the left parts, while the right sections remained untouched. Bacterial penetration into dentinal tubules was evaluated using Gram-stained decalcified sections under light microscopy. Sensitivity and specificity of both dyes were calculated.

Results: The sensitivity of CD and CC were 74% and 71%, respectively. The specificity obtained for both dyes was 100%.

Conclusion: Considering the low sensitivity of the dyes evaluated in the present study, it seems that they may not be reliable when used as the sole diagnostic technique for detection of carious lesions in posterior teeth.

Key Words: Dental Caries; Caries Detector; Propylene Glycols

INTRODUCTION
The main goal of treating dental caries is complete removal of carious tissues with maximum preservation of sound tooth structure and maintenance of pulp vitality [1]. Clinical assessment of dental caries is often based on color and dentin hardness which is considered to be completely subjective with low reproducibility [2,3]. Application of caries detector dyes to facilitate diagnosis of carious dentin was initially introduced by Fusayama in 1979 [4]. Several studies have shown that these dyes may cause false staining of healthy tooth walls leading to unnecessary removal of the intact dentin [5-7]. However, according to other investigations, dyes may be beneficial in detecting caries and when not used during cavity preparation, carious tissues may go undiagnosed by the clinician [8,9]. DeMarco et al [10] suggested that the presence of dye residues in cavity walls following removal of dental caries may lead to lower shear bond strength of composite to enamel. In contrast, another study showed that dye residues in cavity walls have no effect on restoration leakage [11]. Yazici et al [12] compared the efficacy of a laser fluorescence device (DIAGNOdent) and a caries-detector dye in identifying resi-
dual dentinal caries. They found specificities of 100% and 86% when using the caries detector dye and DIAGNOdent, respectively. It has been suggested that caries detecting dyes generally stain the demineralized organic matrix of dentin and not the bacteria and thus should be used with caution [4]. Caries detector dyes are composed of two components including a dye and a solvent mostly made of propylene glycol. It is believed that solvents with low molecular weight are able to penetrate deeper into permeable tissues as compared to those with high molecular weight. Based on this effect, a new caries detector dye, Caries Check (CC), containing 1% acid red in polypropylene glycol has been recently introduced. The high molecular weight of the solvent in Caries Check compared with propylene glycol present in routine dyes was claimed to prevent over-penetration of the dye into porous dentin tissues, inhibiting unnecessary dentin removal [13]. The aim of the present study was to evaluate the efficacy of Caries Check in detecting dental caries and to compare it with Caries Detector, which contains a low molecular weight glycol component.

MATERIALS AND METHODS
This was an experimental study performed on 20 permanent occlusal decayed teeth (12 molars and 8 premolars), which were extracted within the previous ten days due to periodontal problems. For this study, carious lesions with roughly similar dimensions (assessed by inspection) were selected and none of them had hypoplasia, abnormal discoloration, or pulp exposure. Following extraction, the teeth were immediately immersed in sterile distilled water and kept in the dark at 4°C according to previous studies [7]. The teeth were divided into two buccal and lingual halves using a water-cooled sterile diamond disk on a laboratory handpiece. Later, each half was randomly assigned to one of the two study groups based on the utilized caries detector dye and was properly labeled. Access cavities were prepared with a 008 sterile diamond fissure bur (Tizkavan, Iran) placed on a high speed handpiece with air-water spray. Carious tissues were removed by sterile #5 carbide round burs mounted on a low speed handpiece with compressed air. All cavity walls and surfaces of samples in Group 1 were stained with Caries Detector (Kurary, Medical Inc, Tokyo, Japan) for 10 seconds, and subsequently washed with water for another 10 seconds and finally air-dried. Each cavity was arbitrarily divided into two right and left parts with a CD marker and the stained area on the left section was removed by a sterile round bur. The colored parts on the right were left untouched and served as positive controls. Dye-stained tooth structure was completely removed from the left half so that no remaining dye was detected on the cavity walls. A similar method was applied to the samples in Group 2, except that the exposure time for CC (Nippon Shika Yakuhin Shimonosek, Japan) was 3 seconds. All procedures were carried out according to the manufacturer's instructions. The samples were transferred in formalin to the pathology laboratory of Qazvin University of Medical Sciences’ School of Dentistry. A decayed molar and an intact premolar (extracted for orthodontic purposes) accompanied the rest of the samples serving as positive and negative controls, respectively. After fixation, all teeth were demineralized in 10% nitric acid, which depending

| Assay   | Criterion Positive | Criterion Negative | Total |
|---------|--------------------|--------------------|-------|
| Positive| 20                 | 0                  | 20    |
| Negative| 7                  | 13                 | 20    |
| Total   | 27                 | 13                 | 40    |
on tooth hardness took about 1-2 weeks. The samples were then washed with tap water for 2 hours and placed in 10% formalin for 24 h. The teeth were embedded in paraffin and cut into 4µm-sections for microscopic examination. Deparaffinization in xylene was followed by rehydration in graded alcohol and Gram staining. For this purpose, all sections were stained in 1% crystal violet for 1 minute and rinsed with tap water. They were then treated with Lugol's Gram iodine for 40 seconds and rinsed with running water, acetone-ethanol (15 seconds) and water again. The sections were immersed in fuchsin for 30 seconds and washed in tap water [8]. Positive and negative controls were run simultaneously with the rest of the study samples. All stained sections were assessed under a light microscope (Olympus, Japan) by a single oral pathologist who was blind to the samples' identities. Dark red cylindrical shaped structures inside the dentinal tubules usually close to the surface of the specimen were considered as presence of bacteria. According to Lennon et al [14], samples with "single infected tubules or less" were considered as negative and those with two and more infected tubules were scored positive. These histologic scores were employed as the "gold standard" for calculation of the sensitivity and specificity of the studied dyes in detecting carious dentin. Differences between the two groups were assessed by \( \chi^2 \) test.

RESULTS

All stained areas on the right half of the samples in both experimental groups (100%) contained tubules with bacterial penetration. In addition, 65% of the areas on the left halves of the CD specimens were devoid of bacteria, while 35% demonstrated infected tubules. Based on these findings, the sensitivity and specificity of this dye were calculated as 74% and 100%, respectively (Table 1).

In the Caries Check group, 60% of the samples were free of contamination and 40% showed bacterial penetration in the dentinal tubules. The sensitivity obtained for this dye was 71% and the specificity was calculated as 100% (Table 2). The positive and negative control samples were found to be with and without bacteria, respectively. There was no significant difference between the two groups (P>0.05).

DISCUSSION

The present study showed that both CC and CD detector dyes have specificities of 100%, but sensitivities insufficient for complete removal of carious dentin. Hosoya et al [13] studied the efficiency of CC dyes on both deciduous and permanent teeth. They stated that the lower molecular weight and surface tension of propylene glycol found in CD along with its high diffusional property may lead to deeper penetration of the dye into sound dentin. In other words, over-staining of the caries-affected dentin underneath carious tissues may result in excessive removal of the intact tooth structure. Therefore, it was suggested that using polypropylene glycol, which has a higher molecular weight than propylene glycol may prevent unnecessary loss of sound dentin. The authors suggested clinical application of CC in permanent teeth due to the fact that it effectively eliminated tooth decays, preserved non-carious dentin and was significantly superior to CD. Itoh and Okiawa [15] also found a significant difference between the efficacy of CC and CD dyes in the diagnosis of dental caries.

**Table 2. Agreement between Caries Check (CC) assays and standard in detection of dental caries.**

| Assay | Criterion Positive | Criterion Negative | Total |
|-------|--------------------|--------------------|-------|
| Positive | 20 | 0 | 20 |
| Negative  | 8 | 12 | 20 |
| Total    | 28 | 12 | 40 |
and proposed that CC could be used as a guide to diagnose dental caries. The results reported by these two investigations were in contrast to those obtained in the present study. This discrepancy, at least in part, may be attributed to the different methods used in those studies. Both investigations employed a laser fluorescence device (DIAGNOdent) as their gold standard for detecting dental caries, whereas we based our diagnosis on the actual observation of bacteria in dentinal tubules. DIAGNOdent is considered to be an auxiliary tool in the assessment of tooth decay. It has been demonstrated that data acquired from this device essentially show the quantitative correlation between the degree of mineral loss and depth of carious lesions [16]. Similarly, several studies have indicated that the results of DIAGNOdent readings usually reflect the amounts of organic matrix rather than the mineral content of the tooth [16-18]. Thus, it seems that employing DIAGNOdent as a gold standard may not be as accurate as using light microscopy for detection of infectious dentinal tubules.

Lennon et al [14] claimed that the elimination of “every single bacteria” is of limited clinical importance before restoration; hence, in the current investigation, similar to their study, the presence of one single infected dentinal tubule was reported as negative. Their histologic results showed that the number of bacteria remaining in the dentinal tubules was higher in CD and Carisolv samples compared to that left after conventional excavation of caries [14]. In contrast, Lennon et al [19] in another study reported similar efficacy for the conventional caries-removal technique and caries detector dyes in the assessment of dental caries and eliminating bacterial infection. These two studies used different microscopic methods for determining bacterial infection of the carious dentin [14,19]. Streptococcus mutans is a cariogenic bacterium that plays a major etiologic role in human tooth decay and is commonly encountered in the oral cavity in the dental plaque, which is regarded as its natural ecosystem. Additionally, large numbers of different bacterial species like facultative and obligate anaerobic bacteria also reside in deeper lesions. The most accurate method for tracing microorganisms is considered to be polymerase chain reaction (PCR) [3]. Similar to a number of other studies [7,8] we used histochemical techniques with Gram stain for detection of microbial penetration, due to its feasibility, easiness and availability. However, a limitation of microscopic examinations is that histologic sections basically provide a two-dimensional representation of a three-dimensional tissue and can thus lead to possible errors, especially loss of suspected and infected cases [2]. Unfortunately, we did not have access to PCR analysis, which could have provided more reliable results regarding the presence of microorganisms in dentinal tubules.

Yazici et al [12] reported low sensitivity values for both DIAGNOdent and caries detector dyes and suggested development of new methods for better detection of residual carious tissues during cavity preparation. The low sensitivity of caries detector dyes found in the present study and previous investigations is indicative of caries residues in the prepared dental cavity due to failure of detecting the affected area. Undiagnosed dental caries that remain in the cavity are among the major causes of failure in dental restorations. Therefore, the exclusive application of dye as the only detecting agent in diagnosing dental caries is unreliable and needs further evaluation [12].

The findings of the present study indicated a specificity of 100% for both dyes and a lack of significant difference between the sensitivity of the new CC formulation and the commonly used CD dye. This implies that both dyes have the potential to detect sound dentin in all cases. Yazici et al [12] also found a specificity of 100% for this dye, which was in accordance
with the results obtained in the current study. Considering the easy performance and suitable cost of caries detector dyes, their application in diagnosing carious dentin could be a proper substitute to more complicated procedures such as Fluorescence Aided Caries Excavation (FACE), Diagnodent and Carisolve; if and when reliable evidence on the accuracy of these dyes is provided. Further investigations with a larger sample size and PCR evaluation of bacterial penetration are suggested to clarify the actual efficacy of these dyes.

CONCLUSION
Within the limitations of the present study, our results indicate that the current detector dyes are not reliable enough to be used as the sole diagnostic technique in detecting carious lesions.

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