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Evaluation of laser fluorescence in combination with photosensitizers for detection of demineralized lesions

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ABSTRACT

Background and Objectives: Early detection of caries plays an important role in its prognosis. This study sought to assess the efficacy of laser fluorescence alone and in combination with photosensitizers for detection of demineralized smooth-surface lesions of permanent teeth.

Materials and Methods: This in vitro experimental study was conducted on 60 extracted sound premolars. Four windows measuring 2 x 2 mm were created on each tooth. Forty out of 60 teeth were subjected to demineralization cycle. All teeth were then sectioned and subjected to DIAGNOdent Pen (Kavo, Biberach, Germany) alone and in combination with 0.2-mM tetrakis N-methylpyridyl porphyrin (TMPyP) fluorescent dye, 2% methylene blue and 2% new methylene blue photosensitizers. The results of histological analysis of sections served as the gold standard. The correlation of the results with the gold standard was assessed using the Pearson's correlation coefficient and receiver operating characteristic (ROC) curve. The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of the four techniques were evaluated as well.

Results: The correlation between the results of DIAGNOdent laser with TMPyP dye and the gold standard was significant ($P < 0.05$). Also, this technique had the highest sensitivity while DIAGNOdent laser alone had the highest specificity.

Conclusion: Laser fluorescence with TMPyP fluorescent dye has suitable efficacy for early detection of initial enamel lesions.

1. Introduction

Dental caries is the most common chronic disease of childhood with a prevalence rate five times that of asthma and seven times that of hay fever [1]. Tooth decay has a dynamic process comprised of demineralization and remineralization cycles that occur consecutively. White spot lesions develop when the lost mineral content of the enamel is no longer replaced and the balance between demineralization and remineralization is impaired. Formation of white spot lesions is the first step of caries development. These lesions are reversible at this phase and can be stopped by thorough oral hygiene and use of preventive measures [2–4].

Despite a significant reduction in prevalence of dental caries, it is still a common health dilemma. Early detection of caries is always challenging and ideal methods are not available to detect caries (high sensitivity) or ensure the soundness of dental structure (high specificity) [5].

To control dental caries, carious lesions must be detected early during their initial histological phases of development [6]. Visual assessment, tactile sense and radiography are the main and most commonly used methods for caries detection. However, these methods are subjective and have low reproducibility [7].

Visual assessment for caries detection maintains the integrity of the enamel layer over the subsurface lesion and allows for its remineralization. However, in this method, the demineralized lesion needs to be visually detected and may not be found due to insufficient drying or light reflectance. Moreover, lesions must have penetrated to the external third of the enamel in order to be detected by the naked eye. Such large decalcifications require longer periods of treatment for remineralization.

Caries detection using a dental explorer and tactile sense is obsolete and is no longer used in most developed countries because the tip of the

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explorer may damage the superficial, sound layer of enamel covering the subsurface lesion. Also, it may transfer the cariogenic bacteria from one area to another. Radiography is also incapable of early detection of lesions unless they extend to dentin. Increased popularity of conservative dentistry and introduction of methods to reverse the process of caries development led to attempts to find techniques for early detection of carious lesions [1]. Early detection of smooth-surface lesions increases their chance of non-invasive treatment. Several non-invasive methods have been introduced for early detection of caries [8–10].

DIAGNOdent pen was introduced for early detection of caries on occlusal and smooth tooth surfaces. It has been used for non-invasive and early detection of hidden, non-cavitated caries lesions. Using diode laser, this device irradiates red light within the visible spectrum with 638–655 nm wavelength, which are absorbed by the organic and mineral tooth content. The light is absorbed by teeth and creates infrared fluorescence (light photon with longer wavelength) [11,12]. The results are shown by numbers between 0–99. Values ≥20 and 25 indicate presence of carious lesion. Higher values indicate greater penetration depth of caries. Light absorbed by the tooth structure creates infrared fluorescence by the organic and mineral contents, and increased fluorescence indicates caries.

The exact mechanism of action of DIAGNOdent laser has yet to be fully understood. But two theories have been suggested. The first one discusses that when red light is irradiated to porosities caused by demineralization or hypomineralization of the tooth structure, fluorescent light is produced with a different wavelength. The second theory discusses that microbial metabolites especially porphyrins are responsible for increased fluorescence of carious teeth [9].

In the recent years, use of dyes with wavelengths close to the absorbance spectrum of DIAGNOdent laser led to the development of dye-enhanced laser fluorescence (DELF) technique. This technique is based on penetration of fluorescent dye into the initial carious lesion to enhance its detection by DIAGNOdent laser. In absence of dental plaque, DELF is a better diagnostic method than quantitative laser fluorescence for caries detection. Moreover, visual assessment of the amount of absorbed dye can effectively help in detection of interproximal subsurface lesions [13].

This study aimed to assess the efficacy of DIAGNOdent laser alone and DELF for detection of smooth-surface initial enamel lesions. Histological analysis served as the gold standard to assess the accuracy of results.

2. Materials and methods

This in vitro, experimental study was carried out on 60 sound first and second premolars freshly extracted for orthodontic reasons.

All debris and residual soft tissues were removed and the teeth were cleaned. Soundness of enamel was confirmed macroscopically in terms of absence of caries, restoration, cracks, erosion, hypocalcification or structural defects. The teeth were chosen using convenience sampling. The teeth were immersed in 0.2% thymol solution for 48 h [14] and stored in distilled water until the experiment to prevent dehydration.

Two windows measuring 2 x 2 mm were cut out of a piece of adhesive tape and the tape was placed on the cervical area of the buccal and lingual surfaces. Two windows measuring 2 x 2 mm were created on the cervical third of the buccal and another two on the cervical third of the lingual surface of the teeth as such [15,16]. Then, the entire crown was coated with nail varnish except for the windows at the cervical area of the buccal and lingual surfaces. After drying of varnish, the adhesive tapes were removed. The teeth were then coded and the four windows at the mesiobuccal, distobuccal, mesiolingual and distolingual area were coded as a, b, c and d.

Prior to demineralization, the four windows in all teeth were evaluated with DIAGNOdent pen (Kavo, Biberach, Germany). The displayed value was recorded as the baseline value. Tip A of DIAGNOdent was used for assessment of smooth surfaces. Calibration (for the purpose of standardization) was done using a piece of porcelain with a known fluorescence available in the kit. The tip was moved in a circular motion perpendicular to the longitudinal axis of the tooth. This was repeated for each window in triplicate and the mean value was recorded. After three repetitions, calibration was performed again.

Next, demineralization was induced in 40 out of 60 teeth. For this purpose, the samples were immersed in acidic hydroxyl ethyl cellulose (pH of 4.5) at 37 °C for 84 h [37, 38]. After demineralization, the four windows in each tooth were examined again with DIAGNOdent pen and the values were recorded.

The remaining 20 teeth were not subjected to demineralization (for assessment of specificity).

All teeth were then sectioned into four pieces. First, a mesiodistal section was made through the midline of crown and then a buccolingual section was made. For mesiodistal sectioning, a cutting machine was used (Accutom-50; Struers, Denmark). Other sections in buccal and lingual segments were made using a thin metal disc and a micromotor.

Each piece was then placed in a microtube containing saline and coded (from 1 to 60 for the tooth and a, b, c or d for the window). Thus, four samples from each tooth crown were evaluated. In this study, the samples were evaluated in four groups. One piece of each tooth was assigned to one group. Histopathological assessment (to serve as the gold standard) was performed on the group subjected to DIAGNOdent pen alone. Therefore, in this study, all materials and techniques were tested on all teeth and each tooth served as its own control as well.

The four groups in this study were as follows(Table 1):

Group one: Tetrakis N-methyl pyridinioporphyrin (TMPyP) was used in this group as fluorescent dye. One out of four sections of each tooth was assigned to this group. Thus, 40 demineralized and 20 sound tooth samples were present in this group. The samples in this group were immersed in 0.02 mM of TMPyP (Aldrich, Milwaukee, USA) for 60 s, were removed from the solution and rinsed with distilled water twice. They were then dried with air spray for 5 s [17,18]. The samples were then examined with DIAGNOdent and the displayed values were recorded.

Group two: Methylene blue was used in group two. The samples were immersed in 5 mL of 2% solution of methylene blue for five minutes. The samples were then rinsed and dried with air spray for 5 s. The samples were examined with DIAGNOdent and the displayed values were recorded.

Group three: New methylene blue was used in group three. The samples were immersed in 5 mL of 2% new methylene blue for five minutes, rinsed with water and dried with air spray for 5 s. The samples were then examined with DIAGNOdent and the displayed values were recorded.

Group four: No dye was used in this group. The samples were examined with DIAGNOdent and the displayed values were recorded.

2.1. Histopathological assessment

After examination of samples in group four with DIAGNOdent, they were subjected to histopathological assessment. For this purpose, they were mounted in auto-polymerizing acrylic resin such that the created window was completely exposed. After coding, they were sectioned by a cutting machine (Accutom-50; Struers, Denmark). A pathologist

Table 1
The four groups of the study.

	Number of demineralized sample	Number of sound sample	fluorescent dye
Group 1	40	20	TMPyP
Group 2	40	20	Methylene blue
Group 3	40	20	New methylene blue
Group 4	40	20	None

Table 2
Mean DIAGNOdent scores before and after demineralization in the groups (n = 40).

Group	Mean	Standard deviation	Standard error	Paired t-test statistic	P value
DIAGNOdent value of group 1 before demineralization	5.83	0.903	0.143	−27.362	0.000
DIAGNOdent value of group 1 after demineralization	16.80	2.544	0.402		
DIAGNOdent value of group 2 before demineralization	5.85	0.834	0.132	−23.840	0.000
DIAGNOdent value of group 2 after demineralization	16.53	2.873	0.454		
DIAGNOdent value of group 3 before demineralization	5.78	0.891	0.141	−18.798	0.000
DIAGNOdent value of group 3 after demineralization	16.90	3.678	0.582		
DIAGNOdent value of group 4 before demineralization	5.90	0.778	0.123	−21.425	0.000
DIAGNOdent value of group 4 after demineralization	16.70	3.236	0.512		

blinded to the group allocation of samples evaluated the specimens under a stereomicroscope at 50 magnification. Depth of carious lesion was determined in micrometers. Images were taken using VVmer software. The results of histopathological assessment served as the gold standard.

2.2. Statistical analyses

Data were analyzed using SPSS version 11 (SPSS Inc., IL, USA) via Pearson's correlation coefficient, paired *t*-test and receiver operating characteristic (ROC) curve. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of each method were also determined.

3. Results

Table 2 shows the mean DIAGNOdent values before and after demineralization in the groups. Paired *t*-test showed a significant difference in each of the four groups when comparing the DIAGNodent values before and after demineralization, and as shown in Table 2, DIAGNOdent values after demineralization were significantly greater than the baseline values (P = 0.000). It should be noted that only 40 (which were demineralized) out of 60 samples were evaluated in this analysis (to assess the accuracy of demineralization). The Pearson's correlation test was used to assess the correlation of histopathological gold standard with the results in groups 1–4. Table 3 shows the Pearson's correlation coefficients. As shown in Table 3, only the correlation between the histopathological result and DIAGNODent scores in group 1 was statistically significant (r = 0.963, P = 0.000) and other correlations were not statistically significant (P > 0.05).

Table 4 shows the sensitivity, specificity, PPV and NPV of the four methods tested in this study. As shown in Table 4, the highest sensitivity belonged to the use of laser with TMPyP dye while the highest specificity belonged to the use of laser alone. For further assessment, ROC curve was drawn (Fig. 1). The area under the curve for groups 1 (TMPyP) and 4 (laser alone with no dye) was found to be 1. The area was 0.55 for group 2 and 0.78 for group 3. The cutoff point to obtain the highest sensitivity and specificity was 16 for group 1 and 9 for group 4. In other words, for caries detection using TMPyP dye plus DIAGNOdent laser, values under 16 indicate absence of demineralization and values over 16 indicate presence of demineralization. In use of DIAGNOdent alone with no dye, values under 9 indicate absence of demineralization and values over 9 indicate presence of demineralization (Figs. 2–5).

Table 3
Pearson's correlation coefficients for the correlation of DIAGNOdent scores in groups 1–4 (n = 40) and the depth of caries determined histopathologically (gold standard).

	Value	LF TMPyP	LF MB	LF NMB	LF
Histopathological assessment	Correlation coefficient	0.963	−1.04	0.059	−0.66
	P value	0.000	0.525	0.718	0.688

LF TMPyP: DIAGNOdent score of group 1 after demineralization and using TMPyP fluorescent dye; LF MB: DIAGNOdent score of group 2 after demineralization and using methylene blue fluorescent dye; LF NMB: DIAGNOdent score of group 3 after demineralization and using new methylene blue fluorescent dye; LF: DIAGNOdent score of group 4 after demineralization.

Table 4
Sensitivity, specificity, positive predictive value and negative predictive value of the four methods tested in this study.

	Sensitivity	Specificity	False negative	False positive	NPV	PPV
Group 1	100%	85%	0%	15%	100%	93%
Group 2	100%	0%	0%	100%	0%	66.7%
Group 3	97.5%	0%	2.5%	100%	0%	66.1%
Group 4	87.5%	100%	12.5%	0%	80%	100%

NPV: Negative predictive value; PPV: Positive predictive value.

4. Discussion

Significant advances have been made in methods for caries detection in the past two decades [19]. An ideal method of caries detection must have high sensitivity and specificity in order to be able to differentiate sound and carious teeth [20]. False positive diagnosis is particularly unfavorable since it would lead to over-treatment. This study aimed to assess and compare the efficacy of DIAGNOdent laser pen with 655 nm wavelength alone and in combination with fluorescent dyes (DELF) for detection of initial enamel lesions. The results were compared with those of histopathological assessment as the gold standard. The results showed that DIAGNOdent laser was capable of detecting initial enamel lesions. In the current study, we histologically evaluated the samples and then the depth of demineralization was measured in micrometers. By doing so, we ensured that the demineralized lesions were initial. The mean depth of demineralization of samples was 50 µm, which was in accord with the definition of initial enamel lesions [21]. The values displayed by DIAGNOdent laser pen in this study were in agreement with the expected values. In all demineralized samples, the mean values shown by DIAGNOdent laser before and after demineralization were significantly different and the mean value after demineralization was significantly higher than the baseline value. Thus, it may be stated that DIAGNOdent laser is suitable for detection of smooth-surface enamel demineralization in permanent teeth. These results are in line with those of Bahrololoomi et al, [9] and Tomczyk et al [11].

In the current study, histopathological assessment served as the gold standard while some other researchers measured surface microhardness as the gold standard. Mendes et al, [8] and two other studies [22,23] showed that the correlation between the DIAGNOdent values and the depth of carious lesions determined histopathologically was greater than their correlation with surface microhardness value. Thus, histopathological assessment seems to be a more valuable index for comparison of the accuracy of results of diagnostic methods.

Fig. 1. Sound premolars collected, 2 × 2 mm adhesive tapes placed on cervical thirds, coated with nail varnish & demineralization was induced in 40 out of 60 of them.

Our findings showed that only group 1 (DIAGNOdent plus TMPyP) results had a significant correlation with histopathological findings. Since no previous study has evaluated the correlation of the results of DIAGNOdent laser plus methylene blue or new methylene blue with histopathological findings, our findings in this respect cannot be compared with any other study. The reason of using methylene blue and new methylene blue was that we wanted to find a cheap and accessible photosensitive supplant. Further studies are required in this respect to confirm or refute these findings. In the current study, 2% methylene blue was used while Puralibaba et al. [24] used 0.003% concentration of methylene blue in combination with DIAGNOdent for assessment of surface roughness of composite restorations and obtained favorable results. Future studies are required to assess the efficacy of using other concentrations of methylene blue for this purpose and evaluate the correlation of results with histopathological findings. Moriyama et al. [25] evaluated the efficacy of fluorescence-based techniques for detection of demineralization and remineralization of smooth surfaces in situ and indicated that the Spearman's correlation coefficient between depth of demineralization (determined by histopathological assessment) and use of DIAGNOdent laser alone was low and not significant, which was in agreement with our findings. However, Shi et al. [22] reported that the Spearman's correlation coefficient between the depth of carious lesions determined histopathologically and the DIAGNOdent values was significant. Such a controversy in the results of studies may be due to different methodologies. For example, Moriyama et al. [25] similar to our study, artificially demineralized sound teeth while Shi et al. [22] used naturally carious teeth.

Mendes et al. [18] evaluated the efficacy of DELF for detection of initial enamel lesions in smooth surfaces. They used TMPyP and PPIX dyes for this purpose. The accuracy of demineralization and its degree were assessed by spectrometry. They found that the results of DIAGNOdent laser plus TMPyP were significantly correlated with the actual amount of mineral loss. This finding was in line with our results. They also showed that the results of DIAGNOdent plus PPIX and DIAGNOdent alone had no correlation with the actual amount of mineral loss. In general, they reported that laser fluorescence with TMPyP dye was efficacious for detection of initial enamel lesions.

Many studies have evaluated the sensitivity and specificity of DIAGNOdent laser for detection of caries. In our study, use of DIAGNOdent laser plus TMPyP had the highest sensitivity while laser alone had the highest specificity. The sensitivity and specificity of DIAGNOdent laser in combination with methylene blue and new methylene blue were not favorable. The sensitivity and specificity of DIAGNOdent alone for detection of smooth-surface caries were 87.5%

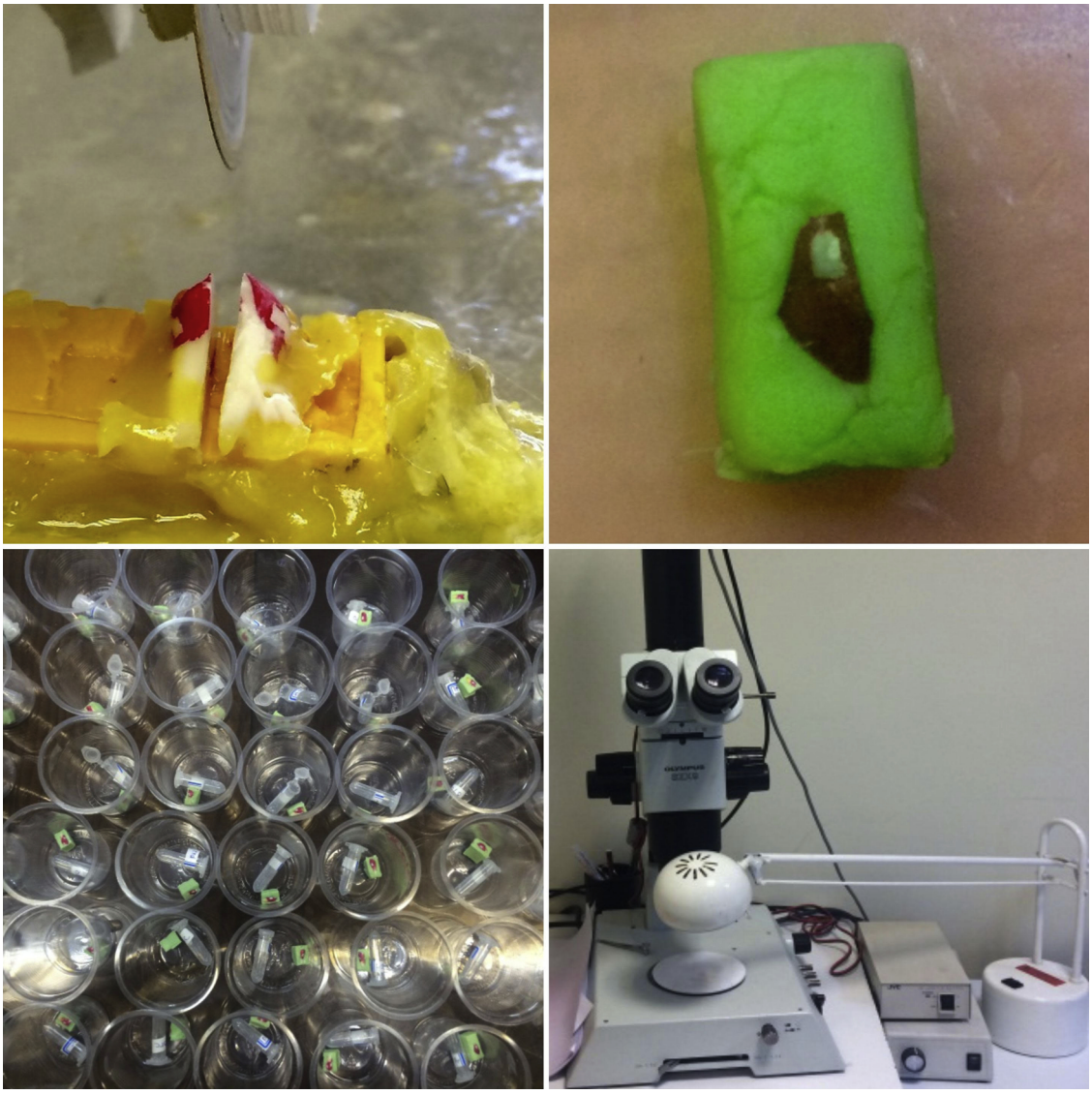

Fig. 2. All teeth sectioned in four pieces, slices mounted on acrylic blocks &then placed in a microtube containing saline and coded(ready to be cut by ground section machine).

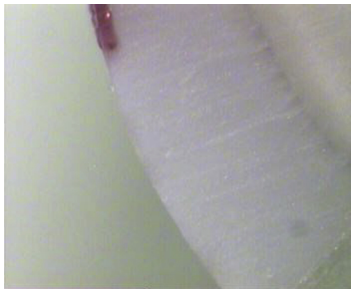

Fig. 3. Histopathologic section.

Fig. 4. Histopathologic section.

and 100%, respectively while the sensitivity and specificity of laser plus TMPyP for detection of smooth-surface caries was 100% and 85%, respectively. Considering the ideal sensitivity of DIAGNOdent plus TMPyP, it may be stated that this technique is ideal for this purpose.

Shi et al. [22] evaluated the reliability of DIAGNOdent laser for quantification of smooth-surface caries and showed that DIAGNOdent laser can be suitable for clinical diagnosis. They reported the sensitivity of 0.63 to 0.75 and specificity of 0.84 to 0.95 for detection of smooth-surface caries. These values indicated moderate sensitivity and specificity. Nonetheless, they stated that laser fl uorescence can be used as an adjunct for clinical practice.

Sound premolars were tested in our study. Further studies should focus on the occlusal surface of molar teeth to increase the generalizability of the results. Moreover, we artificially induced caries in teeth and evidence shows that significant differences exist between artificially induced and naturally occurring caries. Artificial creation of carious lesions requires a couple of days while in the clinical setting,

Fig. 5. Histopathologic section.

natural development of caries requires a much longer time. Thus, the surface zone of artificially induced caries is softer than the natural carious lesions [26]. However, in the current study, only initial demineralization of enamel surface was evaluated and the effects of confounders were minimal. In vitro studies on natural enamel caries are required to confirm the results of this study. Also, in situ and in vivo studies are recommended to obtain more accurate results. It should be mentioned that in oral environment and real carious lesions results can be different due to high load of microorganisms that can produce fluorescence.

5. Conclusion

Within the limitations of this in vitro study, the results indicated that laser fluorescence with TMPyP fluorescent dye has suitable efficacy for early detection of initial enamel lesions in permanent teeth.

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