SUMMARY

Background/Aim: Evaluating the performance of two fluorescence-based methods on the detection of occlusal carious lesions in permanent teeth, compared with visual inspection and radiographic examination was the aim of this study. Material and Methods: Occlusal surfaces of 150 extracted human permanent molars were examined by two researchers using the International Caries Detection and Assessment System II (ICDAS-II), FluoreCam (Daraza) and DIAGNOdent Pen® (KaVo). Standardized periapical radiographs were taken. The teeth were sectioned for histological validation and examined under 25x magnification under a stereomicroscope (Leica M27.5) using Downer criteria 0-4 as the gold standard. The correlation of diagnostic methods with histological analyses was evaluated using Spearman’s rank correlation coefficients (rho). Results: All teeth were scored from 0 to 6 for ICDAS-II. The highest correlation of the gold standard was found with ICDAS-II followed by FluoreCam and DIAGNOdent Pen®. The association strength of ICDAS-II, Radiography, FluoreCam and DIAGNOdent Pen® with the histological evaluation was quite strong (p<0.05). Both fluorescence-based methods performed similarly. Conclusions: Radiograph is not specific for early carious lesions but may be used as an adjunct method for dentin caries. ICDAS-II classification is an accurate method for clinical examination of occlusal caries however additionally using fluorescence as a quantitative and visual examination is quite supportive and reinforcing especially for detecting enamel caries lesions.

Keywords: Dental Caries, Diagnosis, Fluorescence, Radiography

Introduction

Dental caries is considered the most common disease all over the world. Early diagnosis of caries is important for preserving natural tooth tissue. Minimal invasive dentistry includes early detection of caries, its arrest and remineralization before lesions expand. Through diagnosis of early caries lesions, tooth loss may even be prevented by appropriate treatments.

Dental caries progress continuously varying degrees from unseen changes at the enamel surface to total demolition of the tooth. Traditional methods of diagnosis include tactile examination by a probe and visual inspection which may remain insufficient to define the severity of a tooth decay. A subjective assessment of dental caries by clinicians leads to differences in diagnosis, clinical management and prognosis. ICDAS II system was introduced to achieve an objective standardised detection of caries lesion. This visual method determines the alterations that occurred on the tooth surface and the depth of the carious lesions. The ICDAS criteria have been found to be reliable and reproducible in the diagnosis of caries lesions and their longitudinal follow-up. However, additional diagnostic methods are needed to improve the detection of non-cavitated enamel lesions.

Radiography, commonly used in caries detection, is based on the principle that demineralized tooth tissues absorb X-rays insufficiently and show radiolucency...
compared to sound enamel and dentine tissues. With innovations made on dental films, in addition to clinical examination, radiography became a more sensitive method for the detection of proximal and occlusal lesions as well as allowing the calculation of the depth of caries. However, inadequate diagnosis of early enamel lesions, inability to determine the activity of a lesion, and false negative results on buccally/lingually positioned caries cause the seek for additional diagnostic methods. Apart from visual, tactile and radiographic examination, there are laser or light fluorescence-based devices for assessing dental caries. DIAGNOdent Pen is one of the non-invasive methods with the laser fluorescence principle. The laser fluorescence is emitted by the porphyrins released by cariogenic bacteria in carious lesions when subjected to 655 nm red light.

Quantitative light-induced fluorescence (QLF) assessment is another non-destructive diagnostic method for early caries detection. It is based on capturing the auto-fluorescence loss in enamel and dentinal tissues. The fluorescence grade decreases at the position of the carious lesion due to mineral loss. Early caries lesions can be detected and the fluorescence loss of the lesion be quantitatively evaluated in comparison with the fluorescence radiance level of sound enamel. Differences in fluorescence radiance of the lesion can be monitored over time and lesion development can be measured. This method has been successfully applied on smooth and occlusal surfaces. The FluoreCam system (Therapeutic Technologies, Inc., Indiana, USA), based on the QLF principle is a portable device consisting of a hand-held instrument with software. This device uses Fluorescence Enamel Imaging (FEI) technology for diagnosis, quantitative assessment, and monitoring of early caries lesions. Enamel tissues demonstrate fluorescence radiance at specific light wavelengths because of their mineral content. Due to the semi-translucent structure of enamel, enamel emits different levels of fluorescence in different densities. Through the developments of caries detection systems the sensitivity and specificity in diagnosis of early caries lesions has improved over time. Thus, early carious lesions may be prevented and also remineralized.

This study aimed to evaluate the compatibility of two fluorescence-based techniques in diagnosing occlusal caries in permanent teeth, compared with ICDAS II as the visual inspection and the radiographic examination for histological validation.

Material and Methods

Study design and sample preparation

The study was performed in conformance with the Declaration of Helsinki ethical guidelines. The ethical approval was received on 17.03.2021 with the protocol number 09.2021.414. One hundred fifty permanent human molars and premolars, freshly extracted for periodontal or orthodontic reasons from adult patients were selected for this study. Teeth that were sound or comprised of carious lesions on occlusal surfaces were included in the study while teeth with cracks, fractures, restorations, and enamel defects were excluded.

The teeth were kept in saline solution at 20°C until the examination time. Before the examination, the teeth were cleaned with a dental scaler, and any calculus or residues were removed and numbered for identification purposes. Then the occlusal surfaces were photographed with a digital camera (Nikon D7100, Nikon, Tokyo, Japan) at a standardized process. During the examinations, the teeth were stored in distilled water.

Visual examination (ICDAS II)

Two dentists with five years of experience using the ICDAS II classification inspected the occlusal surfaces of teeth and recorded the caries codes following the ICDAS II criteria suggested by the ICDAS Coordinating Committee. The researchers performed caries diagnosis methods under good light and using a periodontal probe without knowing in which group the teeth were. The researchers assessed the teeth when moist and dried for 5 sec, then kept them in distilled water.

DIAGNOdent Pen® device Examination

The occlusal surfaces of teeth were assessed by two examiners using the DIAGNOdent Pen® (SN: 12-2004608, KaVo, Biberach, Germany) device concerning the manufacturer’s instructions. After attaching the cylindrical tip for the occlusal surfaces, the calibration of the device was done using a reference block. For the examination, the teeth were dried gently with an air syringe, and the sound side of each tooth was measured for calibration to determine the 0 value. Then the tip of the device was put on the occlusal site where the lesion was located. The depth of the lesion is indicated by the intensity of the fluorescence. Three measurements for each tooth were taken by two examiners and the mean values were recorded. DIAGNOdent Pen measures the intensity on a scale of 0–99. The obtained values were grouped by the classification of Lussi and Helweg. The suggested cut-off points were used to evaluate as follows: 0–13, sound dental tissues; 14–20, lesions in the outer half of the enamel; 21–29, lesions in the inner half of the enamel; >30 dentinal caries.

Radiographic examination

Standardized periapical radiographs of the teeth were taken using an X-ray (Dürr Dental, Germany) and films (Dürr Dental, Size 2) at 70 kV, 8 mA with an exposure time of 0.12 sec. After processing the films with an automatic X-ray film developer (Dürr Dental, Germany) the radiographs were inspected using an X-ray viewer.
(Dürr Dental, Germany). The occlusal surfaces were evaluated by two examiners as follows: no radiolucency (0), radiolucency in enamel (1), radiolucency in the outer half of dentin (2) and radiolucency in the inner half of dentin (3). The cut-off score for dentinal lesions was determined as 2.

**FluoreCam System examination**

FluoreCam device (Daraza, Therametric Co., Indianapolis, USA) based on Fluorescence Enamel Imaging (FEI) technology was used to take fluorescence images of the occlusal areas. Eventually, the density of enamel can be measured with FEI technology, by measuring its fluorescence when exposed to certain light wavelengths. The handpiece of FluoreCam radiates a high-intensity light with a filtered wavelength of 410 nm to induce fluorescence of the enamel and capture the image of the lesion with a CCD camera. The computer including the FluoreCam software records and analyses

![Figure 1. FluoreCam imaging of teeth scored as ICDAS II code 3, 4 and 5; respectively.](image)

**Histological examination (Validation)**

The histological analyses of the teeth were taken as the gold standard to validate the visual and two fluorescence examinations. After cutting the teeth through the central region of the lesion with a diamond disc (IsoMet 1000, Buehler, ABD) the sections were polished using 400, 600, and 1200 grit silicon carbide paper and analysed under the Stereo-microscope (Leica M27.5) at 25X magnification. Images were scored following the Downer Histological Classification System by both examiners independently. Radiographic, digital photographic and histological images of teeth according to ICDAS II codes were shown in Figure 2.

![Figure 2. Radiographic, digital photographic and histological examination of teeth according to ICDAS II codes](image)

**Data processing and statistical analysis**

Statistical analyses were done using Stata 15.1 (StataCorp, 4905 Lakeway Drive College Station, Texas 77845 USA). The cutoff points were grouped under the same category, D0 (sound), D1 (incipient enamel caries), D2 (deep enamel caries), and D3 (dental caries), to interpret the performance of the methods. Inter-observer reproducibility was analysed with ICC analysis for two observers. The Receiver Operating Characteristic (ROC) analysis method was used to calculate the area under the curve (AUC). The relationship between all the systems was evaluated using the Spearman rank correlation (rho) and the level of significance was p<0.05.

**Results**

Occlusal surfaces of a total of 150 teeth were examined. The distribution of the teeth according to ICDAS II criteria was code 0: 15, code 1: 30, code 2: 30, code 3: 30, code 4: 15, code 5: 15, code 6: 15. The distribution of teeth according to ICDAS II criteria was shown in Table 1.
Table 1. Classification of teeth according to visual criteria using ICDAS II criteria.

<table>
<thead>
<tr>
<th>ICDAS 0 (N (%))</th>
<th>ICDAS 1 (N (%))</th>
<th>ICDAS 2 (N (%))</th>
<th>ICDAS 3 (N (%))</th>
<th>ICDAS 4 (N (%))</th>
<th>ICDAS 5 (N (%))</th>
<th>ICDAS 6 (N (%))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of teeth N=150 (100 %)</td>
<td>15 (10%)</td>
<td>30 (20%)</td>
<td>30 (20%)</td>
<td>30 (20%)</td>
<td>15 (10%)</td>
<td>15 (10%)</td>
</tr>
</tbody>
</table>

Table 2. Optimal cut-off points for Radiography, ICDAS II criteria, DIAGNOdent Pen and FluoreCam devices under the same groups.

<table>
<thead>
<tr>
<th>(Scores)</th>
<th>Histology</th>
<th>Radiography</th>
<th>ICDAS II</th>
<th>FluoreCam</th>
<th>DIAGNOdent</th>
</tr>
</thead>
<tbody>
<tr>
<td>D0 Sound</td>
<td>D 0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0-13</td>
</tr>
<tr>
<td>D1 Incipient enamel caries</td>
<td>D 1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>14-20</td>
</tr>
<tr>
<td>D2 Deep enamel caries</td>
<td>D2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>21-29</td>
</tr>
<tr>
<td>D3 Dentin caries</td>
<td>D 3</td>
<td>3</td>
<td>3-4-5</td>
<td>3</td>
<td>30-99</td>
</tr>
</tbody>
</table>

Following the end of all test methods, to evaluate the performance of each method statistically, the scoring of all methods were collected in 4 scores as D0 (sound), D1 (incipient enamel caries), D2 (deep enamel caries), D3 (dentin caries). Table 2. presents the cut-off points combined under the same groups for all methods.

The histological examination of the 150 permanent teeth by observer 1 revealed that 24 teeth (16 %) were sound (D0), 55 teeth (36.67 %) had incipient enamel lesions (D1), 29 teeth (19.33%) had deep enamel lesions (D2), and 42 teeth (28%) had dentin lesions (D3) (Table 3). Observer 2 revealed that 25 teeth (16.67 %) were sound (D0), 56 teeth (37.33 %) had incipient enamel lesions (D1), 26 teeth (17.33%) had deep enamel lesions (D2), and 43 teeth (28.67%) had dentin lesions (D3) (Table 3).

Table 3. Distribution of caries extension after histological examination by 2 observers using Downer’s histological classification.

<table>
<thead>
<tr>
<th>Histologic Score</th>
<th>Frequency observer 1</th>
<th>Percentage (%) observer 1</th>
<th>Frequency observer 2</th>
<th>Percentage (%) observer 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (D0)</td>
<td>24</td>
<td>16.00</td>
<td>25</td>
<td>16.67</td>
</tr>
<tr>
<td>1 (D1)</td>
<td>55</td>
<td>36.67</td>
<td>56</td>
<td>37.33</td>
</tr>
<tr>
<td>2 (D2)</td>
<td>29</td>
<td>19.33</td>
<td>26</td>
<td>17.33</td>
</tr>
<tr>
<td>3 (D3)</td>
<td>17</td>
<td>11.33</td>
<td>17</td>
<td>11.33</td>
</tr>
<tr>
<td>4 (D3)</td>
<td>25</td>
<td>16.67</td>
<td>26</td>
<td>17.33</td>
</tr>
<tr>
<td>Total</td>
<td>150</td>
<td>100.00</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The correlation of the methods with each method was calculated using Spearman’s rank correlation coefficients (rho) and the results were shown in Table 4. The highest correlation was found between the ICDAS-II system and the gold standard (histological evaluation) followed by the correlation between the FluoreCam and the gold standard. The lowest correlation was recorded between radiography and FluoreCam. In general, the lowest correlation was found for radiographic examination with other methods.

The association strength of visual examination using the ICDAS-II system, Radiography, FluoreCam and DIAGNOdent Pen® with the histological evaluation was found very strong. Area Under Curve (AUC) values were obtained by comparing all methods with the histological examination (Table 5). In general, DIAGNOdent Pen showed the highest while the Radiography demonstrated the lowest Az (area under ROC curve) values. When comparing areas under ROC curves, differences between values were found statistically significant (p<0.001). Graphic representation of ROC curves comparing all techniques with the histological validation is shown in Figure 3.

Table 4. The correlation of diagnostic methods with each one was evaluated using Spearman’s rank correlation coefficients (rho)

<table>
<thead>
<tr>
<th>Histological evaluation</th>
<th>Visual evaluation (ICDAS-II)</th>
<th>FluoreCam</th>
<th>Radiographic examination</th>
</tr>
</thead>
<tbody>
<tr>
<td>Obs. 1</td>
<td>Obs. 2</td>
<td>Obs. 1</td>
<td>Obs. 2</td>
</tr>
<tr>
<td>DIAGNOdent Pen</td>
<td>0.9019</td>
<td>0.9035</td>
<td>0.9099</td>
</tr>
<tr>
<td>Radiographic examination</td>
<td>0.8470</td>
<td>0.8521</td>
<td>0.8553</td>
</tr>
<tr>
<td>FluoreCam</td>
<td>0.9107</td>
<td>0.8809</td>
<td>0.8933</td>
</tr>
<tr>
<td>Visual evaluation (ICDAS-II)</td>
<td>0.9530</td>
<td>0.9516</td>
<td></td>
</tr>
</tbody>
</table>

Obs.: Abbreviation for observer.
The area under curve values of diagnostic methods for 150 permanent teeth

<table>
<thead>
<tr>
<th>Method</th>
<th>AUC</th>
<th>AUC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Observer 1</td>
<td>Observer 2</td>
</tr>
<tr>
<td>Visual evaluation (ICDAS-II)</td>
<td>0.9680</td>
<td>0.9687</td>
</tr>
<tr>
<td>Radiography</td>
<td>0.9360</td>
<td>0.9246</td>
</tr>
<tr>
<td>FluoreCam</td>
<td>0.9680</td>
<td>0.9687</td>
</tr>
<tr>
<td>DIAGNOdent Pen®</td>
<td>0.9872</td>
<td>0.9861</td>
</tr>
</tbody>
</table>

According to the calculation made for absolute acceptance with the two-way random-effect model, the average ICC correlation coefficient for 150 samples evaluated by both observers with 5 different methods was calculated as 0.941 (with 95% Confidence interval 0.885-0.966) (p<0.001). According to the intraclass correlations (ICC) analyses by using the two-way random-effects model for absolute agreement; Observer 1 had an average ICC coefficient of 0.941 (95% Confidence interval 0.885-0.966), and individual ICC of 0.763 (95% CI 0.607-0.850) for 5 distinct methods and n=150 observations (p<0.001). Observer 2 had an average ICC coefficient of 0.941 (95% Confidence interval 0.885-0.966), and an individual ICC of 0.761 (95% CI 0.606-0.849) (p<0.001) (Table 6).
Discussion

In modern dentistry, many options are available for preventive treatment such as the use of remineralizing agents like fluorides, fissure sealants and infiltration of caries. For the treatment decision, dentists should carefully detect sound dental tissue as well as initial enamel caries to give preventive treatment. The ICDAS-II and fluorescent-based methods have demonstrated their performance in detecting dental lesions in many studies. In this study, the capacity of the diagnostic methods alone at giving information about lesion stages has been investigated to differentiate sound surfaces, enamel or dentin caries. The fluorescence systems being highly reproducible make it possible to re-diagnose the lesions, so the treatment decision would be the same when clinicians use these methods at different times even if the user changes. In the present study, the ICC values for the fluorescent methods were high for inter-examiner reproducibility and the results were comparable to the several studies.

Nowadays, since minimally invasive treatments are preferred by both patients and dentists, early diagnosis of occlusal caries has become more important. Fluoresce methods used in detecting smooth surface lesions at an early stage are preferable because they are non-invasive, painless and practical to use. For daily clinical use, it is helpful to use techniques that detect and assess caries lesions at early stages. ICDAS II was represented as a reliable and reproducible method to detect early lesions and changes in long-term follow-up. In the present study, ICDAS II codes were confirmed to have the highest diagnostic accuracy. Diniz et al. found the strongest correlation between the ICDAS and Treatment Decision (0.85) in their study. They suggested the ICDAS as the primary criteria for assessing the tooth surface before making a treatment plan. They also indicated that ICDAS is reliable for early caries diagnosis while presenting only a moderate correlation with histology. Some previously conducted studies also stated a moderate correlation between the ICDAS and the histological examination. However, Ekstrand et al. reported that there is a strong correlation between histological and visual examination in detecting occlusal caries, similar as ours.

The radiographic examination is considered to be inaccurate in detecting early enamel lesions, however, some studies reported that radiographs might add information to the visual examination about caries progress, including hidden caries. Bhumireddy et al. indicated that ICDAS II showed better accuracy than digital radiographs in the diagnosis of caries within the enamel, and both tools were equally effective in the detection of dentinal lesions. The results of our study showed that the visual method showed the highest correlation to the gold standard followed by Fluorescent methods, FluoreCam and DIAGNOdent Pen. However, radiographic examination showed the lowest correlation. The area under the ROC curve expresses diagnostic accuracy which might be easily interpreted. The results of the present study demonstrated that the area under the ROC curve (Az) for DIAGNOdent was highest (0.98) followed by FluoreCam and visual examination (0.96), and the lowest for radiographic evaluation (0.93) indicating better performance of the fluorescent methods and visual examination (Table 5). Rodrigues et al. found the results for the ICDAS criteria (0.75) and for the radiographic examination (0.71) on detecting occlusal lesions and showed a strong correlation (0.74) between the ICDAS and radiographic examination.

Jablonski – Momeni et al. have investigated in vitro the performance of ICDAS II and DIAGNOdent in the detection of occlusal caries on permanent teeth. They stated that ICDAS II had better diagnostic precision than DIAGNOdent and the sensitivity of ICDAS II was 0.91 at the D1 stage. Castillo et al. pointed out that ICDAS and DIAGNOdent are reproducible methods showing similar performance in the diagnosis of occlusal lesions in dentin. Moreover, DIAGNOdent might detect incipient enamel lesions at a higher rate than ICDAS, but with low specificity. They added that using DIAGNOdent as an additional method for the evaluation of incipient occlusal lesions in permanent molars is arguable. Having more experience in the use of visual caries diagnosis systems might have influenced these results. Moreover, the risk of false-positive results in laser fluorescence might have caused a lower diagnostic accuracy compared to visual examination.

Similar to our results Jablonski-Momeni et al. showed ICDAS II had excellent AUC as well as high sensitivity and specificity at different diagnostic thresholds. The same authors also demonstrated Bitewing radiographs had the weakest correlation compared to other methods. Our results showed similar radiographic evaluation with the lowest ICC correlation coefficient compared to all methods used in this study as Diniz et al. also indicated that the visual examination using ICDAS showed better performance than the radiographic examination for detecting occlusal caries. Our findings (correlation to the gold standard) indicate ICDAS-II may have a high potential in detecting occlusal lesions, and other diagnostic methods especially the fluorescence devices, may give additional information, thus dentists may plan the treatment more accurately.

The FluoreCam device with its intraoral camera, takes images of teeth which show different fluorescence on sound and carious tissues. Yanıkoglu et al. demonstrated that FluoreCam can be used to detect enamel caries on smooth surfaces. Similarly,
examiner agreement, demonstrated that the examiners showed excellent inter-repeatability, indicating absolute agreement. Our results showed that radiography is inadequate for detecting occlusal caries. The visual examination and fluorescent methods were highly correlated with the gold standard.

Marczuk-Kolada et al. compared the efficacy of the ICDAS II with two fluorescent methods (DIAGNOdent Pen and VistaCam iX) on the diagnosis of occlusal caries on permanent teeth and suggested that diagnostic methods using fluorescence might be used adjunct to visual caries detection methods. The results of the present study demonstrated that the association strength of visual examination (ICDAS-II), Radiographic evaluation, FluoreCam and DIAGNOdent Pen® with the histological evaluation was very strong. Hence, combining different detection methods might be beneficial and the best option for the diagnosis of caries lesions on occlusal surfaces, which is supported by the findings of this study, and also indicated by others. In the current study, the ICC values for the ICDAS II, Fluorescence methods and radiographic examination were high for inter-examiner repeatability, indicating absolute agreement. Our results demonstrated that the examiners showed excellent inter-examiner agreement; indicating that the examiners had similar levels of clinical experience.

Conclusions

Our results showed that radiography is inadequate for initial carious lesions however may be beneficial to use as an adjunct method for dentin caries. The visual ICDAS-II classification is an accurate method for occlusal caries detection yet the fluorescence devices performed well concerning the diagnosis of occlusal caries in permanent teeth compared with other methods. A combination of methods would be the best option for the diagnosis of caries on occlusal surfaces.

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