CLINICAL EVALUATION OF DIAGNOSTIC METHODS COMBINATION (VISUAL EXAMINATION, LASER FLUORESCENCE AND DIGITAL RADIOGRAPHY) IN DETECTION OF OCCLUSAL CARIES

Anita Bajsman*[1], Amra Ahmić[1], Selma Zukić[1], Amila Zukanović[2], Selma Jakupović[3]

[1] Department for Dental Morphology, Dental Anthropology and Forensic Dentistry, Faculty of Dentistry, University of Sarajevo, Sarajevo, Bosnia and Herzegovina

[2] Department for Preventive and Paediatric Dentistry, Faculty of Dentistry, University of Sarajevo, Sarajevo, Bosnia and Herzegovina

[3] Department for Dental Pathology and Endodontics, Faculty of Dentistry, University of Sarajevo, Sarajevo, Bosnia and Herzegovina

*Corresponding author

Anita Bajsman, PhD
Faculty of Dentistry,
University of Sarajevo
Bolnička 4a
71000 Sarajevo
Bosnia and Herzegovina
Phone: +387 33 407 838
e-mail: abajsman@sf.unsa.ba

ABSTRACT

Occlusal surface is the most difficult for performing reliable caries detection on it. Traditional visual-tactile diagnostic methods do not show high sensitivity and specificity, and there are efforts to overcome this disadvantage by using conventional, digital radiography, as well as numerous non-invasive techniques for quantification of demineralization. Despite a large number of studies comparing the effectiveness of individual diagnostic methods in caries detection, there are still not enough studies investigating effectiveness of various combinations of diagnostic methods.

The aim of this paper is to examine whether there is a difference between individual diagnostic methods (visual examination, laser fluorescence and digital radiography) regarding their sensitivity and specificity; and combination of these diagnostic methods in diagnosis of occlusal caries on permanent molars.

Material and methods: The sample comprised 140 permanent molars. Teeth were inspected visually, and the appearance of the lesion was classified according to UniViSS criteria, than by use of laser fluorescence (DIAGNOdent 2095), and by using digital retro-coronary radiographs. Validation method was cavity opening.

Results: Values of sensitivity and specificity of the methods were calculated. After that, the same values from the combination of the specified diagnostic methods were calculated as well.

Conclusion: By using a combination of diagnostic methods, an increase in sensitivity and specificity values is achieved with respect to the values of the same parameters from the individual diagnostic methods.

Key words: occlusal caries detection, UniViSS, laser fluorescence, digital radiography

Introduction

Dental caries represents demineralization of dental tissue caused by acidogenic bacteria in the dental biofilm [1]. Caries affects individuals of all ages, different cultural, ethnic and socio-economic origin [2].

Over the past decades, a decrease in caries prevalence in the world population level has been noted. It is believed that the increased use of fluoride is largely responsible for changing the model and progression of the dental caries. It is accepted that, despite the undeniable contribution, the large capacity of remineralisation of hard dental tissues by fluoride can "mask" the dentin caries. It is believed that 50 to 60% of occlusal fissures are affected by caries. The occlusal surface is the most difficult for performing reliable caries detection due to complexed morphology of this surface. Hence, discussing over the difficulties regarding initial caries detection is constant thus reinforcing interest for the research of these lesions [3, 4, 5, 6, 7].

In the eighties of the twentieth century, a new type of lesion - a hidden caries - began to be discussed. Hidden caries is a subtype of the occlusal caries, and is defined as the occlusal dentin caries visible on radiographic images, while the visual inspection shows enamel appearing intact or minimally perforated. The pathophysiological model of hidden caries initiation is based on the strengthening and remineralisation of the outer layers of enamel by topical fluoridation method. Cariogenic bacteria penetrates into the enamel through minimal cavitation of the enamel surface. When they reach dentin that is softer and contains more organic substances, their progression is easier. At the same time, the enamel passes through the process of remineralisation thus closes the path of bacterial entry. Apart from the minimal cavitation, the cariogenic bacteria penetrates through the enamel strips (lamellae) [8, 9].

Therefore, the detection of hidden caries is difficult and it is necessary to use a combination of diagnostic methods to set the diagnosis. An ideal diagnostic method needs to be safe for patient and therapist thus enabling early stage lesion detection. It needs to be objective, quantitative, non-invasive and inexpensive.

The use of visual examination is not always sufficient for diagnosing caries, and probing, which is commonly used, can cause trauma. Hence, there is a need to establish non-traumatic, non-invasive techniques that can diagnose occlusal caries accurately [10]. The criteria for ideal diagnostic method are to have a high sensitivity value, and also to be highly specific. Traditional visual-tactile diagnostic methods are not fully able to achieve such criteria [3, 11].

In addition to visual and visual-tactile methods, caries diagnostic methods include conventional radiography, digital radiography, and non-invasive demineralization quantification techniques, including methods based on laser or light fluorescence, electrical impedance measurement, fibber optic and digital fibber optic trans- illumination (FOTI and DIFOTI), videoscope. Even though results are promising, clinical use of quantitative methods is still limited [6, 12].

Although there are numerous studies comparing efficacy of single diagnostic method in occlusal caries diagnosis, there are still relatively small number of studies investigating the effectiveness of various combinations of diagnostic methods. This type of research should be focused on in vivo conditions [6, 13, 14].

Visual systems for caries diagnosis

There is a large number of visual systems for describing the carious process propagation on dental surfaces (Ekstrand et al., Rickets et al., Nyvad et al., ICDAS, ICDAS II) [11, 14, 15, 16, 17, 18]. All these systems, in addition to the undisputed advantages, also show some deficiencies. Nelson et al. [17] point out that there is still no standard system for detection and evaluation of caries universally accepted among researchers. There are several caries detection systems in use trying to describe and diagnose the caries process.

Wishing to overcome the deficiencies of visual systems for the detection and description of caries, a group of authors created Universal Visual Scoring System (UniViSS) (Picture 1.) for occlusal and smooth surfaces lesions. This system is designed to compensate for the disadvantages of existing visual diagnostic systems, to meet the contemporary requirements set for caries detection / diagnosis and to be flexible. The system can be used without limitation in patients of all age groups, under field, clinical and laboratory research. UniViSS uses a three-step diagnostic procedure for a detailed classification of the complex clinical appearance of the caries lesion. The first step is the assessment of lesion severity, the second is the estimation of discoloration, and the third step is an assessment of the activity. The system is universally applicable and adaptable to the clinical conditions [19, 20].

Several authors [6, 14] suggest that, as far as the visual inspection is followed using some of the auxiliary methods, the precision of the diagnosis of occlusal caries is improved.

Laser fluorescence

Evaluation of fluorescence stimulated by laser or infrared light gives us possibility to distinguish between healthy and carious hard dental tissue. The basis of fluorescence of healthy enamel consists of inorganic components of the tissue, and to a lesser extent, organic too. In carious dental tissue, porphyrins (products of bacterial metabolism) are considered responsible for fluorescence. The method of laser fluorescence was developed primarily for the purpose of detecting coronary caries, especially pits and fissure caries. It showed that this method is characterized by good precision and reproducibility, even better than radiographic examinations [3, 6, 21].

DIAGNOdent, designed for performing laser light fluorescence examination, produces laser light that is absorbed by both inorganic and organic substances in hard dental tissues as well as oral bacterial metabolites. The light of the higher wavelengths in the caries presence is reemitted, and the changes are registered with the digital numeric scale. It is considered that DIAGNOdent represents useful diagnostic tool combined with visual inspection, primarily for long-term caries evaluation and estimation of the preventive interventions outcome, since the carious process can be quantitatively measured this way [7].

The function of DIAGNOdent is based on the concept of fluorescence stimulation, using laser light. Device has a laser diode that producing a red light wavelength of 655 nm, applied by the user to the dental surface, and a long filter with transmission higher than 680 nm as a detector. The light is transmitted to the occlusal surface of the tooth by a fibber optic beam. Upon reaching the occlusal surface, the light goes through the enamel and dentin. Red light has the ability to penetrate deeper into the hard dental tissue, and therefore we can be able to detect fluorescence even in the carious dentine beneath the visually healthy enamel. Another fibber-optic beam (filter) absorbs the beam of reflected fluorescent light. In order to ignore the ambient light of larger wavelengths that also pass through this filter, the laser diode beam is modulated. That is why the filter only registers light that has the same modulation characteristics. A numeric value (0 to 99) is designated to changes caused by demineralisation, and it is presented on device display. It is considered that if numerical value is higher, the propagation of the caries is deeper. When the laser illuminates hard dental tissues, light is absorbed by the organic and inorganic substances present in hard tissue pores, as well as oral bacterial metabolites, most likely porphyrins, which show some fluorescence after red light excitation. For this reason, dental tissues emits fluorescence after red laser light application. Carious tissue shows increased intensity of fluorescence compared to healthy tissue, and therefore there is a significant difference in fluorescence values between carious and healthy tissue. The display of device shows two values - the value of the current position of the measuring probe ("moment") and the maximum value for the whole surface ("peak") [3, 5, 22, 23, 24, 25, 26, 27].

DIAGNOdent proved to be a useful additional diagnostic tool for occlusal caries detection, especially combined with visual inspection [28].

Radiological caries diagnosis

Radiographic methods are useful in the detection of approximal caries, but have little value in occlusal caries detection. Therefore, a clinical evaluation of caries should always precede radiological examination. Radiography shows some limitations: it cannot distinguish between active and arrested lesions, or small lesions with cavitation and non-cavitated lesions. The depth of the lesion is difficult to assess accurately using radiographic images. Retrocoronary radiographic images cannot detect early caries lesions [4].

Radiological diagnosis of the initial occlusal lesion poses a problem, because of morphological and structural characteristics of the occlusal surface. Only one third of initial carious lesions are diagnosed radiologically. The lesions with minimal progression in dentine are properly diagnosed in two thirds of cases. The presence of caries on the occlusal surfaces is often very difficult to determine radiologically due to the presence of filling materials and because of the morphological characteristics of the occlusal surface - fissures and pits, and superposition of the enamel [29, 30].

Due to the lack of precision in early enamel occlusal lesions detection, the use of retro-coronary radiographic images for that purpose has been the subject of debate for a long time. The value of this diagnostic method is again being analysed due to its value in the diagnosis of hidden caries. Computer-assisted analysis of images for diagnosing caries with digital radiographs has been investigated since the 1980s. Digital records can be manipulated later thus changing certain image features. This process is called "image processing" and has the appropriate software support. The ability to process a picture is perhaps the most important advantage of digital radiography, as this way the information contained in the digital image becomes more acceptable to the human eye [31, 32, 33].

Evidence suggests, but this is far from making the ultimate conclusion, that some digital radiographic methods may provide a slightly better sensitivity compared to conventional x-ray at both approximal and occlusal surfaces [13].

The aim of this paper is to examine whether there is a difference in the sensitivity and specificity of the individual diagnostic methods (visual examination, laser fluorescence and digital radiography) and the same values of the combination of these diagnostic methods in the detection of occlusal caries on permanent molars.

Material and methods

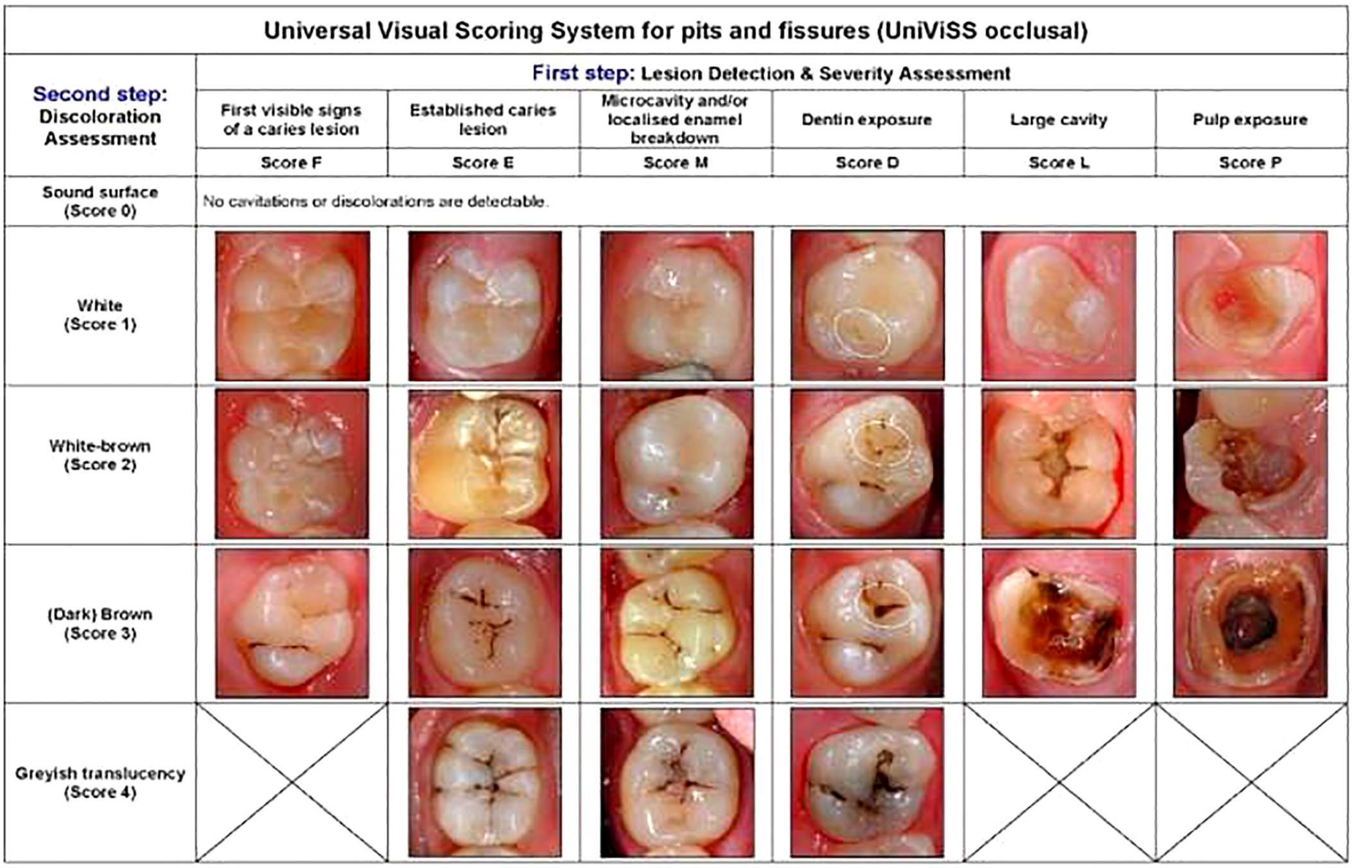
This study was approved by the Ethical Committee of the Faculty of Dentistry, University of Sarajevo.

The study involved patients older than 16 years, who had been admitted to the Department of Dental Pathology and Endodontics at the Faculty of Dentistry, University of Sarajevo. The study included 64 examinees. The study sample consisted of 140 permanent molars. Inclusive and exclusive criteria for teeth enrolled in this study are described in detail in research of Bajsman et al [34]. Each tooth involved in the study had to have occlusal surface that met one of three criteria:

1. Occlusal fissure system where a discoloured brown or black area without cavitation is clinically observed.
2. Grey discoloration derived from carious dentine underneath non-cavitated enamel.
3. Obvious cavitation on the occlusal surface.

During visual examination, the examinee was positioned in dental chair, and the examination was performed using artificial light, on wet and compressed air dried-up surfaces, using dental mirror without enlargement. Occlusal surfaces designated for the examination were cleaned by rotating brush and water, and dried by compressed air. The appearance of the fissure system on the occlusal surface is classified according to the UniViSS scoring system (Figure 1). If there were multiple demineralization areas on the occlusal surface, the most demineralized area was examined. Visual inspection was always performed first, to reduce the possibility of bias by other diagnostic methods.

For laser fluorescence examination, DIAGNOdent 2095 (KaVo, Biberach, Germany) was used. After thorough cleaning with a rotating brush, without use of prophylactic paste, occlusal surface was thoroughly washed and dried by compressed air. After that, tooth was isolated by cotton rolls. Measuring probe A was used, because it is designated for testing on occlusal surface. Calibration of the device using ceramic standard was performed prior to using the appliance in each patient. After calibration, value of healthy spot fluorescence on buccal surface of the tooth is measured - individual calibration (ideally, the middle third of the buccal surface) after light drying by compressed air. If there were multiple demineralization areas on occlusal surface, the most demineralized area was examined. The top of the measuring probe is set to the selected area, and a slight rotation around the longitudinal axis is performed until maximum value is registered. This



Picture 1. Universal Visual Scoring System for pits and fissures (UniViSS occlusal) [19]

tilting of instrument at the test site is necessary to register the fluorescence of fissure wall inclination. The highest value was recorded. Each test site was measured three times by described procedure, and the average of these three measurements was considered as a definite value. A linear scale used in the research of Lussi and associates [35] was used in this research.

For all teeth in which at least one of the two previously applied diagnostic methods speaks for the presence of carious lesion, a retro-coronary digital radiography was performed, using De Götzen xgenus® digital device (De Götzen S. r. l. Via Roma, 45-21057 Olgate Olona (VA)-Italy, software version 1.30.113). Xgenus® digital system uses CCD sensor. Although, according to the manufacturer's recommendations, sensor size 2 is used in trans canine sector, and also for the retro-coronal technique, we decided to use sensor size 1 in this study. The reason for this decision was difficulty in positioning sensor in patient's oral cavity due to stiffness, sharp edges and angles as well as inability of the patient to lead maxillary and mandibular arch to occlusal contact. The analysis of retro-coronary radiovisiographic images were performed on a computer monitor (diameter 17 "). The radiographs were analysed as processed, in terms of manipulation with contrast or brightness of image. The choice of software tools was arbitrary. The most commonly used tools were "contrast", "border enhancement", "zoom", "equalization".

After the analysis of radiographic images, each examined tooth was assigned a score, (Table 3.), according to research of Pereira et al. [14].

Validation of the collected data was performed by cavity opening. For ethical reasons, operative treatment was performed when the results of two diagnostic methods were in favour of the presence of lesion in the vicinity of the enamel-dentinal border, or dentinal caries. The depth of the lesion propagation was recorded using World Health Organization's graduated probe, as the distance between the deepest point of the cavity and enamel surface, according to several researches [5, 36, 37, 38]. After diagnostic procedures and removal of carious tissue, each tooth was assigned a score (Tables 1., 2., 3), according to research of Heinrich-Weltzien et al. [38].

Cavity opening was followed by restorative procedure. All diagnostic procedures, validation and restorative treatment were carried out by one researcher.

Results

The sample in this clinical study consisted of 140 permanent molars in 64 respondents who met the inclusion criteria. For the purposes of statistical data processing, UniViSS scores were transformed into four ordinal values, namely:

0 – no caries

1 - opacity/discoloration visible after drying (F1, F2, F3)

2 - opacity/discoloration visible without drying (E1, E2, E3, E4)

3 - localised enamel defect/microcavity (M1, M2, M3, M4)

4 - dentine exposure (D1, D2, D3, D4, L1, L2, L3, P1, P2, P3).

It was considered that the values 1 and 2 represent enamel caries, and values 3 and 4 caries in dentine.

Data were analysed using SPSS 15.0. 0. for Windows, and MedCalc 9.5.0.0.

In 3 cases, although the appearance of the occlusal lesion categorized according to UniViSS was in favour of the lesion in the enamel, it was found that caries were not present. In 18 cases, UniViSS agrees with the validation method for caries in dentine under the enamel-dentinal border (Table 1.).

In 110 cases, DIAGNOdent and validation method agree for the presence of dentinal caries. In only 3 cases DIAGNOdent spoke against the presence of caries, and the caries was present in enamel (1) and dentine (2) (Table 2.).

Table 1. Cross-tabulation of UNIViSS and validation method

		UniViSS				Total
		1 – opacity/ discoloration visible after drying	2 – opacity/ discoloration visible without drying	3 – localised enamel defect/ microcavity	4 – dentine exposure	
Validation method	0 – no caries	3	0	0	0	**3**
	1 – enamel caries	11	6	0	2	**19**
	2 – caries in the outer half of dentin	20	35	18	25	**98**
	3 – caries affects more than half of dentinal depth	2	2	6	10	**20**
Total		**36**	**43**	**24**	**37**	**140**

Table 2. Cross-tabulation of DIAGNOdent and validation method

		DIAGNOdent			Total
		0 – no caries	1 – enamel caries	2 – dentinal caries	
Validation method	0 – no caries	3	0	0	3
	1 – enamel caries	1	2	16	19
	2 – caries in the outer half of dentin	2	6	90	98
	3 – caries affects more than half of dentinal depth	0	0	20	20
Total		**6**	**8**	**126**	**140**

Table 3. Cross-tabulating of digital radiographs and validation method

		RVG				Total
		0 – no caries	2 – radiolucency in the inner half of enamel depth	3 – radiolucency in the outer half of dentinal depth	4 – radiolucency in the inner half of dentinal depth	
Validation method	0 – no caries	3	0	0	0	3
	1 – enamel caries	14	0	5	0	19
	2 – caries in the outer half of dentin	22	1	75	0	98
	3 – caries affects more than half of dentinal depth	2	0	15	3	20
Total		**41**	**1**	**95**	**3**	**140**

Table 4. Spearman's correlation coefficient

			UniViSS	**DiagnoDent**	**RVG**	**Validation method**
Spearman's rho	UniViSS	Correlation Coefficient	1,000	,242**	,150	,409**
		Sig. (2-tailed)	.	,004	,077	,000
		N	140	140	140	140
	DiagnoDent	Correlation Coefficient	,242**	1,000	,200*	,277**
		Sig. (2-tailed)	,004	.	,018	,001
		N	140	140	140	140
	RVG	Correlation Coefficient	,150	,200*	1,000	,457**
		Sig. (2-tailed)	,077	,018	.	,000
		N	140	140	140	140
	Validation method	Correlation Coefficient	,409**	,277**	,457**	1,000
		Sig. (2-tailed)	,000	,001	,000	.
		N	140	140	140	140

**Correlation is significant at the 0.01 level (2-tailed).
*Correlation is significant at the 0.05 level (2-tailed).

Although digital radiographs excluded caries lesions, enamel caries was present in 14 cases, caries in the outer half of dentinal depth was present in 22 cases, and caries that affects more than half of dentinal depth was present in 2 cases. In 75 cases, digital radiographs and validation method was in agreement (Table 3.).

Correlation of UniViSS values and validation method values is positive and moderate, at a confidence level of $p < 0.01$. Correlations of validation method values and DIAGNOdent results, as well as correlation of validation method values and digital radiograph score values are positive moderately at the significance level of $p < 0.01$. A weak positive correlation exists between values of DIAGNOdent and digital radiograph score values, at the significance level of $p < 0.05$ (Table 4.).

To calculate sensitivity and specificity of visual inspection with UniViSS, UniViSS codes 1 and 2 are interpreted as enamel caries (D2), and UniViSS codes 3 and 4 as dentinal caries (D3).

Observing the obtained results in the light of the abovementioned facts (Table 5), it can be seen that the values of visual inspection sensitivity (UniViSS) and digital retro coronal radiographs sensitivity are somewhat lower at level of enamel caries. Specificity values of these two procedures are relatively high, at enamel caries level. Laser fluorescence showed the highest sensitivity and specificity values at enamel

Table 5. Sensitivity values (SE), specificity values (SP), positive predictive values (PPV), negative predictive values (NPV) and values under ROC curve (AUC) for UniViSS, laser fluorescence – DIAGNOdent (LF) and digital retro coronal radiography (RVG) for enamel caries (D2) and dentinal caries (D3)

			D2		
	SE (95% CI)	**SP** (95% CI)	**PPV** (95% CI)	**NPV** (95% CI)	**AUC** (95% CI)
UniViSS	**0.42** (0.20 – 0.67)	**1.00** (0.31 – 1.00)	**1.00** (0.63 – 1.00)	**0.21** (0.05 – 0.51)	**0.711** (0.480 – 0.881)
LF	**0.95** (0.74- 0.99)	**1.00** (0.31- 1.00)	**1.00** (0.81 – 1.00)	**0.75** (0.20 – 0.96)	**0.974** (0.800 – 0.987)
RVG	**0.26** (0.09 – 0.51)	**1.00** (0.31 – 1.00)	**1.00** (0.48 – 1.00)	**0.18** (0.04 – 0.43)	**0.632** (0.402 – 0.824)
			D3		
	SE (95% CI)	**SP** (95% CI)	**PPV** (95% CI)	**NPV** (95% CI)	**AUC** (95% CI)
UniViSS	**0.80** (0.70 – 0.87)	**0.64** (0.41 – 0.83)	**0.91** (0.83 – 0.96)	**0.41** (0.25 – 0.59)	**0.751** (0.664 – 0.826)
LF	**0.92** (0.85 – 0.96)	**0.27** (0.11 – 0.50)	**0.85** (0.77 – 0.91)	**0.43** (0.18 – 0.71)	**0.600** (0.507 – 0.688)
RVG	**0.78** (0.68 – 0.85)	**0.77** (0.55 – 0.92)	**0.94** (0.86 – 0.98)	**0.44** (0.28 – 0.60)	**0.773** (0.688 – 0.844)

caries level. Considering AUC values, laser fluorescence also showed the best values, and its ability to discriminate between diseased and healthy cases is very good. For other two diagnostic methods the AUC values are in the range of weak and good.

At the level of dentinal caries, visual inspection sensitivity and digital radiographs sensitivity values are higher and are followed by the consequent and logical discrete decrease in specificity values. The sensitivity value of the laser fluorescence is somewhat lower at this diagnostic level, compared to the sensitivity value at the level of enamel caries. The observed sample shows a decrease in the value of laser fluorescence specificity. Possible cause is the selected diagnostic threshold for dentinal caries (DIAGNOdent reading >20), which could lead to overestimation and higher number of false positive results. Probably by choosing a higher diagnostic threshold (DIAGNOdent reading ⩾ 30) the situation would be different. However, the aforementioned diagnostic threshold in this study was chosen regarding caries incidence in our population, and the discrete overestimation of the results does not represent mistake. In populations with low caries incidence it is quite appropriate and it is recommended to select a higher diagnostic threshold [2]. The AUC values at dentine level for all three diagnostic methods range from weak to good.

For the combination of diagnostic methods the sensitivity and specificity values were calculated according to the following formulas [39]:

Sensitivity of test combination = 1 – (1 – sensitivity of test 1) x (1 – sensitivity of test 2)

Specificity of test combination = 1 – (1 - specificity of test 1) x (1 – specificity of test 2).

Results are presented in Table 6.

Compared to the sensitivity and specificity of the individual diagnostic methods, it is evident that combinations of diagnostic methods provide higher values of the above mentioned parameters on both diagnostic thresholds. At the enamel caries, the lowest sensitivity value belongs to combination of UniViSS and digital radiography, but the value of specificity for all three combinations at the enamel caries level is very high. Digital radiography was not able to register carious changes in enamel that did not affect the enamel-dentinal border, and therefore its performance in combination with UniViSS was not satisfactory either. However, because of high sensitivity of laser fluorescence, sensitivity of digital radiography in combination with laser fluorescence is much better.

Table 6. Sensitivity (SE) and specificity (SP) for combinations of diagnostic methods: UniViSS and laser fluorescence (UniViSS + LF), UniViSS and digital retro coronal radiography (UniViSS + RVG) and laser fluorescence and digital retro coronal radiography (LF + RVG), for enamel caries (D2) and dentinal caries (D3)

D2		
	SE	SP
UniViSS + LF	0.97	1.00
UniViSS + RVGL	0.57	1.00
F + RVG	0.96	1.00
D3		
	SE	SP
UniViSS + LF	0.98	0.74
UniViSS + RVGL	0.96	0.92
F + RVG	0.98	0.83

At the dentinal caries level, sensitivity values of combinations of diagnostic methods are high. But, specificity values in combinations with laser fluorescence are somewhat lower. Possible explanation is again the diagnostic threshold for dentinal caries detection by laser fluorescence, which could lead to false positive results.

Discussion

Künisch et al. 19, 20 evaluated UniViSS performance on extracted third molars with histological validation. For enamel caries, recorded values of sensitivity and specificity were 1.00 and 0.58. For detection of caries in dentin, sensitivity value was 0.63, and specificity 0.98. Taking into account the suggestion that the sum of sensitivity and specificity values should be approximately 1.6 in order for the diagnostic method to be accepted for practical use, these values illustrate the potentials of UniViSS. Considering the reproducibility UniViSS, the authors believe that the UniViSS score for discoloration is more difficult to reproduce in relation to the scores ICDAS II. In our sample, at diagnostic threshold D2, the sensitivity and specificity sum was 1.42 (SE = 0.42, SP = 1.00) and at diagnostic threshold D3 - 1.44 (SE = 0.80, SP = 0.64). These differences could be based on the fact that our research was in vivo, and that detailed histological evaluation of recorded scores was not possible for ethical reasons. The recorded results, regardless of the above mentioned differences, are comparable to the results of Künisch and associates.

In clinical trials, it is important to choose the diagnostic thresholds in connection with registered laser fluorescence values, which should in fact reflect the depth of propagation of caries lesion. Generally speaking, the values recommended for in vivo testing are somewhat higher than the values recommended by the manufacturer as well as the values recorded in the in vitro studies.

Sensitivity and specificity values for laser fluorescence depend heavily on diagnostic thresholds for different stages of caries progression. The use of different values of diagnostic thresholds could be the reason for divergence between results of laser fluorescence examination in various researches. Depending on what is expected of the diagnostic method, selecting the appropriate diagnostic thresholds is of crucial importance. If the method is associated with another method characterized by a high specificity value, such as a visual examination, then a scale of diagnostic thresholds emphasizing the sensitivity of the laser fluorescence should be considered. When choosing a high-value diagnostic threshold, device shows high specificity, at the expense of sensitivity. At lower values of diagnostic thresholds, sensitivity value is higher and specificity value is lower [25, 40]. So, it is possible to conclude that the selected numerical value of the DIAGNOdent reading for dentinal caries was reason of lower laser fluorescence specificity in our research.

Khalife et al. [39] investigated sensitivity and specificity of DIAGNOdent in diagnosis of occlusal caries at different values of diagnostic thresholds in vivo. At diagnostic threshold 20 (dentinal caries) corresponding to diagnostic threshold in this study, the sensitivity value was 0.97 and the specificity 0.15. These values are comparable to the results of our research. On the sample of the aforementioned research, the diagnostic threshold between 35 and 40 proved to be adequate as it provided the appropriate specificity values for the population with lower risk for caries in the area with good fluoridation. Lower values of specificity in this range of diagnostic thresholds would lead to false positives if DIAGNOdent was used as the only diagnostic tool. Regardless of the values shown by DIAGNOdent, the authors explicitly recommend it as an auxiliary tool in combination with visual and radiographic examination.

Our results are not in accordance to Antonnen's results [41]. Antonnen combined visual examination and laser fluorescence on permanent molars at diagnostic threshold> 30 for dentinal caries to distinguish between healthy / inactive and active / caries in the dentine, and recorded sensitivity value of 0.75, and specificity value of 0.82. Explanation of these differences is the selection of different diagnostic thresholds of DIAGNOdent numerical values for dentinal caries.

Costa and associates [5] evaluated DIAGNOdent performance in dentinal caries detection on occlusal surfaces without cavitation, in clinical conditions. They compared the results obtained with the results of a visual examination (scored as "without caries", "enamel caries" and "caries in dentine") and conventional retro coronal radiographs. The visual inspection showed sensitivity value 0.50, and specificity value 0.95. For conventional retro-coronal radiography, the sensitivity value was 0.26, and specificity 0.94. For laser fluorescence the sensitivity value was 0.93, specificity 0.75. These results are partially in accordance with the results of our research, in terms of sensitivity and specificity of visual examination and digital radiography at the level of enamel caries. In conclusion, the authors suggest the use of laser fluorescence in combination with visual inspection in order to reduce false positives.

The idea of comparing native and processed digital retro coronal radiographs was actually based on Wenzel and Fejerskov [42], who compared the diagnosis of suspected carious lesions on occlusal surfaces of extracted third molars using visual examination, conventional radiography, digital radiography with border enhancement, and digital radiography with contrast manipulation. In their research, the best diagnostic performance was demonstrated by digital radiography with contrast manipulation (SE = 0.71, SP = 0.85, PPV = 0.91, NPV = 0.59) being consistent with the results of our research at the level of dentinal caries. Analysing combinations of methods, the best performance was shown by a combination of visual examination, conventional radiography and digital radiography with contrast manipulation, although this combination also resulted in a rise of false positive results.

Findings of Pereira et al. [43] stated that manipulation of dental radiographic images brings no difference in diagnostic accuracy. Additionally, dentists seem to use the possibilities of improving digital radiographs very differently in clinical practice. If this improvement is not used properly, it can actually reduce diagnostic precision.

Künisch et al. [44] suggest the use of retro coronal radiography and laser fluorescence as a second or third choice method in all unclear cases additional to clinical examination. Chu et al. [45] evaluated the occlusal caries diagnosis on decidual and permanent molars using conventional methods (visual examination - Ekstrand criteria and conventional retro coronal radiography) and laser fluorescence. The gold standard was cavity opening. At diagnostic threshold D2, sensitivity of visual examination in their research was 0.89, and specificity 0.44, not being consistent with our results, while at D3 sensitivity was 0.66 and specificity 0.75, being comparable to the results of our research. Sensitivity of retro coronal radiography on the D2 and D3 diagnostic thresholds was 0.13 and specificity was 1.00, being comparable to our results. At diagnostic threshold D2, sensitivity of laser fluorescence was 0.95 and specificity was 0.11 (our study recorded a significantly higher specificity of 1.00), and at the D2 and D3 thresholds, sensitivity ranged from 0.56 to 0.75 and the specificity ranged from 0.71 to 0.89, not being consistent with our results. For the combination of visual inspection and laser fluorescence, sensitivity values ranged from 0.55 to 0.72 and a specificity values ranged from 0.86 to 0.94. In our study, sensitivity value of the combination of visual inspection and laser fluorescence is 0.98, and specificity is 0.74. The possible explanation for such differences is the composition of the sample, since their sample is made up of both decidual and permanent molars, and the degree of mineralization of hard dental tissues of decidual molars is weaker than the permanent ones, and the greater content of the organic component can affect the values of DIAGNOdent reading. The authors conclude that caries detection based on a combination of those two methods provides a satisfactory levels of specificity and sensitivity. That combination can be acceptable for diagnosing occlusal caries in the dentine.

Heinrich-Weltzien and associates [37] clinically evaluated visual examination, conventional retro coronal radiography and laser fluorescence methods on permanent molars. Cavity opening was a gold standard, and values of diagnostic thresholds for caries in enamel and dentine by laser fluorescence were identical to values in this study. Visual examination was preformed according to Ekstrand criteria. Sensitivity for visual examination was 0.25, and specificity was 1.00. Sensitivity of laser fluorescence was 0.93, and specificity was 0.63. Sensitivity of conventional retro coronal radiography was 0.70, and specificity was 0.96. The authors did not explicitly state the diagnostic threshold for analysing the performance of used methods. If we compare their results with our results at diagnostic threshold D3, our research showed a higher sensitivity value of visual examination, and lower value of specificity of laser fluorescence. The results based on radiographic method are comparable to our findings, taking into account that digital retro coronal radiography with image processing was used in our research. In conclusion, the authors pointed out that DIAGNOdent reading above 20 as a diagnostic threshold for dentine caries should be considered a sensitive marker in everyday practice. The authors believe that the basic advantage of a combined diagnostic procedure is that it combines visual inspection as a high specificity method and laser fluorescence as a high sensitivity method.

Souza-Zaroni et al. [6] investigated various combinations of occlusal caries detection methods on a sample of permanent molars (in vitro study). The research involved the use of visual examination, laser fluorescence, and retro coronal radiography, using histological validation as a gold standard. Three groups of researchers (undergraduate students, postgraduate students and professors) participated in the research. Analysing the results of all research groups, the authors conclude that individual methods show lower performance compared to the combination of methods, which is entirely in accordance with the results of our research.

Conclusion

Using a combination of diagnostic methods, an increase in sensitivity and specificity values is achieved with respect to the values of the same parameters of the individual diagnostic methods. Consequently, it is advisable to use a combination of diagnostic methods, especially in unclear cases, as well as in cases of initial lesions and their monitoring.

References

1. Lukacs JR, Largaespada LL. Explaining sex differences in dental caries prevalence: saliva, hormones and „life-history“etiologies. American Journal of Human Biology 2006;18:540-555.
2. Young DA, Featherstone JDB, Roth JR. Curing the silent epidemic: Caries management in the 21st century. Journal of Californian Dental Association 2007;10:681-685.
3. Barberia E, Maroto M, Arenas M, Cardoso Silva C. A clinnival study of caries diagnosis with a laser fluorescence system. Journal of American Dental Association 2008; :572-579.

4. Baelum V. What is an appropriate caries diagnosis? Acta Odontologica Scandinavica 2010;68:65-79.
5. Costa AM, De Paula LM, Baretto Bezerra AC. Use of DIAGNOdent for diagnosis of non-cavitated occlusal dentin caries. Journal of Applied Oral Science 2008;16:18-23.
6. Souza-Zaroni WC, Ciccone JC, Souza-Gabriel AE, Ramos RP, Corona SAM, Palma-Dibb RG. Validity and reproducibility of different combinations of methods for occlusal caries detection: An in vitro comparison. Caries Research 2006;40:194-201.
7. Angnes G, Angnes V, Grande RHM, Battistella M, Dourado Loguercio, Reis A. Occlusal caries diagnosis in premanent teeth: An in vitro study. Brazilian Oral Research 2005;19:243-248
8. Zadik Y, Bechor R. Hidden occlusal caries – Chalenge for the dentist. New York State Dental Journal 2008;4:46-50.
9. Walker BN, Makinson OF, Peters MCRB. Enamel crakcs. The role of enamel lamellae in caries initiation. Australian Dental Journal 1998;43:110-116.
10. Kamburoğlu K, Şenel B, Yüksel SP, Özen T. A comparison of the diagnostic accuracy of in vivo and in vitro photostimulable phosphor digital images in the detection of occlusal caries lesions. Dentomaxillofacial Radiology 2010;39:17-22.
11. Ismail AI, Sohn W, Tellez M Amaya A, Sen A, Hasson H, Pitts NB. The International Caries Detection and Assesment System (ICDAS): An integrated system for measuring dentaln caries. Community Dentistry and Oral Epidemiology 2007;35:170-178.
12. Jablonski-Momeni A, Stachniss V, Ricketts DN, Heinzel-Gutenbrunner M. Reproducibility and accuracy of the ICDAS-II for detection of occlusal caries in vitro. Caries Research 2008;42:79-87.
13. Bader JD, Shugars DA, Bonito AJ. Systematic reviews of selected dental caries diagnostic and management methods. Journal of Dental Education 2001;10:960-968.
14. Pereira AC, Eggertsson H, Martinez-Mier EA, Mialhe FL, Eckert GJ, Zero DT. Validity of caries detection on occlusal surfaces and treatment decisions based on results from multiple caries-detection methods. European Journal of Oral Sciences 2009;117:51-57.
15. Ekstrand KR. Improving clinical visual detection – Potential for caries clinical trials. Journal of Dental Research 2004;83 (Spec Iss C):C67-C71.
16. Fyffe HE, Deery C, Nugent ZJ, Nuttal NM, Pitts NB. In vitro validity of the Dundee Selectable Threshold Method for caries diagnosis (DSTM). Community Dentistry and Oral Epidemiology 2000;28:52-58.
17. Nelson S, Eggertson H, Powel B, Mandelaris J, Ntragatakis M, Richardson T, Feretti G. Dental examiners consistency in applying the ICDAS criteria for a caries prevention community trial. Community Dental Health 2011;28:238-242.
18. Künisch J, Berger S, Goddon I, Senkel H, Pitts N, Heinrich-Weltzien R. Occlusal caries detection in permanent molars according to WHO basic methods, ICDAS II and laser fluorescence measurements. Community Dentistry and Oral Epidemiology 2008;36:475-484.
19. Kühnisch J, Goddon I, Berger S, Senkel H, Bücher K, Oehme T et al. Development, methodology and potential of the new Universal Visual Scoring System (UniViSS) for caries detection and diagnosis. International Journal of Environmental Research and Public Health 2009;6:2500-2509.
20. Kühnisch J, Bücher K, Henschel V, Albrecht A, Garcia-Godoy H, Mansmann U et al. Diagnostic performance of the Universal Visual Scoring System (UniViSS) on occlusal surfaces. Clinical Oral Investigations 2011;15:215-223.
21. Dičak J, Tarle Z, Knežević A. Vizualno-taktilna detekcija karijesa u usporedbi sa laserskom fluorescencijom. Acta Stomatologica Croatica 2007;41:132-141.
22. Lussi A, Megert B, Longbottom C, Reich E, Francescut P. Clinical performance of a laser fluorescence device for detection of occlusal caries lesions. European Journal of Oral Sciences 2001;109:14-19.
23. Tam LE, McComb D. Diagnosis of occlusal caries: Part II. Recent diagnostic technologies. Journal of the Canadian Dental Association 2001;8:459-463.
24. Lussi A, Hibst R, Paulus R. DIAGNOdent: An optical method for caries detection. Journal of Dental Research 2004;83(Spec Iss C):C80-C83.
25. Braga MM, Mendes FM, Imparato JCP, Rodrigues CRMD. Effect of cut.off points on performance of

laser fluorescence for detecting occlusal caries. The Journal of Clinical Pediatric Dentistry 2007;32:33-36.
26. Matošević D, Tarle Z, Miljanić S, Meić Z, Pichler L, Pichler G. Laserski inducirana fluorescencija porfirina u karijesnoj leziji. Acta Stomatologica Croatica 2010;44:82-89.
27. Matošević D, Tarle Z, Miljanić S, Meić Z, Pichler L, Pichler G. Detekcija porfirina karijesne lezije pomoću fluorescencije inducirane ljubičastim laserom. Acta Stomatologica Croatica 2010;44:232-240.
28. Pinheiro IVA, Medeiros MC, Ferreira MA, Lima KC. Use of laser fluorescence (DIAGNOdent®) for in vivo diagnosis of occlusal caries: a systematic review. Journal Of Minimum Intervention In Dentistry 2008;1:45-51.
29. Bloemendal E, de Vet HCW, Bouter LM. The value of bitewing radiographs in epidemiological caries research: a systematic review of the literature. Journal of Dentistry 2004;32:255-264.
30. Forner Navarro L, Llena Puy MC, Garcia Godoy F. Diagnostic performance of radiovisiography in combination with a diagnosis assisting program versus conventional radiography and radiovisiography in basic mode and with magnification. Med Oral Pathol Oral Cir Bucal 2008;13:261-265.
31. Parks ET, Williamson GF. Digital radiography: An overview. The Journal of Contemporary Dental Practice 2002;3:23-39.
32. Van der Stelt PF. Better imaging: The advantages of digital radiography. Journal of American Dental Association 2008;139(6 supplement):7S-13S.
33. Gormez O, Yilmaz HH. Image post-processing in dental practice. European Journal of Dentistry 2009;3:343-347.
34. Bajsman A, Haračić A, Vuković A, Zukić S. Analysis of concordance between clinical and radiographical depth of occlusal caries lesions – a pilot study. Stomatološki vjesnik 2016; 5 (1-2):33-40.
35. Kidd EAM, Fejerskov O. What constitutes dental caries? Histopathology of carious enamel and dentin related to action of cariogenic biofilms. Journal of Dental Research 2004;83(Spec Iss C):C35-C38.
36. Chu CH, Lo ECM, You DHS. Clinical diagnosis of fisure caries with conventional and laser-induced fluorescence techniques. Lasers in Medical Science 2010;25:355-362.
37. Zaidi I, Somani R, Jaidka S. In vivo effectiveness of laser fluorescence compared to visual inspection and intraoral camera for detection of occlusal caries. Indian Journal of Dental Sciences 2010;2:15-20.
38. Heinrich-Weltzien R, Weerheijm KL, Künisch J, Oehme T, Stösser L. Clinical evaluation of visual, radiographic and laser fluorescence methods for detection of occlusal caries. Journal of Dentistry For Children 2002;69:127-132.
39. Parikh R, Mathai A, Parikh S, Sekhar GC, Thomas R. Understavding and using sensitivity, specificity and predictive values. Indian Journal of Ophtalmology 2008;56:45-50.
40. Khalife MA, Boynton JR, Dennison JB, Yaman P, Hamiltom JC. In vivo evaluation of DIAGNOdent for the quantification of occlusal dental caries. Operative Dentistry 2009;34:136-141.
41. Anttonen V. Laser fluorescence in detecting and monitoring the progression of occlusal dental caries lesions and for screening persons with unfavourable dietary habits [disertation]; University of Oulu, Faculty of Medicine, Oulu, 2007.
42. Wenzel A, Fejerskov O. Validity of diagnosis of questionable caries lesions in occlusal surfaces of extractes third molars. Caries Research 1992;26:188-194.
43. Pereira AC, Eggertsson H, Moustafa A, Zero DT, Eckert GJ, Mialhe FL. Evaluation of three radiographic methods for detecting occlusal caries lesions. Brazilian Journal of Oral Sciences 2009;2:67-70.
44. Künisch J, Dietz W, Stösser L, Hickel R, Heinrich-Weltzien R. Effects of dental probing on occlusal surfaces – a scanning electron nicroscopy evaluation. Caries Research 2007;41:43-48.
45. Chu CH, Lo ECM, You DHS. Clinical diagnosis of fisure caries with conventional and laser-induced fluorescence techniques. Lasers in Medical Science 2010;25:355-362.