

Influence of examiners' experience on the reproducibility of different gold standard techniques and histological criteria for validation of the diagnosis of occlusal caries lesions

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Summary. The aims of this study were to evaluate the influence (1) of the examiner experience and (2) three histological classification criteria on the reproducibility of two gold standard techniques (non-dye or dye) for validation of the diagnosis of occlusal caries. This study comprised a sample of 210 digital images of 105 permanent teeth (105 rhodamine B dyed and 105 dye-free hemisections) and six examiners. Images were evaluated on a laptop computer and categorised according to three different histological classification criteria (proposed by Ekstrand, Lussi or Downer) and repeated in order to allow reproducibility calculation. For data analysis, the six participants were divided into two groups: G1: examiners with previous experience in histological evaluation and G2: examiners with no experience in histological evaluation. Statistical significance was set at 0.05. Results. The mean intra-examiner reproducibility values in G1 were higher than G2 in all variables. Intra-examiner reproducibility was lower for the dye-free technique in both groups. The mean values of inter-examiner reproducibility in G1 ranged from 0.60 to 0.68, and in G2 values ranged from 0.34 to 0.69. Conclusion. It can be concluded that: (1) the examiners' experience seems to influence the reproducibility of the two gold standard techniques studied and reproducibility tends to be lower when the

dye-free technique is used, and (2) the histological classification criterion does not appear to influence the reproducibility for validation of the diagnosis of occlusal caries.

Key words: Dental caries, Diagnosis, Caries detection, Reproducibility of tests

Introduction

Caries detection methods should be evaluated for their performance (validity and reproducibility) (Hintze et al., 1995). For validity determination, it is necessary to consider the proportion of correct results, taking into account the reference diagnosis (gold standard). A validation method should be accurate, reproducible and independent from the diagnostic method under evaluation (Hintze et al., 1995).

True validation in a clinical situation would only be achieved if a non-destructive method existed to reliably assess mineral loss *in vivo*; therefore, *in vitro* studies are conducted using the available gold standard methodologies: microradiographies, polarized light microscopy, surface microhardness measurement (Ten Bosch and Angmar-Månsson, 2000) and inspection by microscope of dental sections using dye coloration (rhodamine B or fuchsine) (Lussi, 1993; Lussi et al., 1999, 2006; Lussi and Hellwig, 2006) or the analysis of the simple cut surface without any procedure (Downer,

1975; Ekstrand et al., 1997; Huysmans and Longbottom, 2004; Rodrigues et al., 2008a,b, 2009).

In addition to the gold standard methodologies, the different proposed types of histological classification can be used as a scoring system of either absence or presence of demineralization, and to define the extent of mineral loss (Downer, 1975; Ekstrand et al., 1997; Lussi et al., 1999). Thus, there may be a variation in results depending on the parameters used for the validation of caries lesions.

Since it is not possible to assess which methodology and histological classification criteria provides more accurate results, data on the gold standard techniques used for validation of the diagnosis of caries lesions is scarce. Rodrigues et al. (2012), in order to elucidate the relationship between gold-standard techniques, compared 4 techniques: (1) rhodamine B dye; (2) fuchsin dye; (3) microradiography and (4) analysis of the cut of dental surface in the microscope without previous procedure. The authors used the histological classification criteria proposed by Lussi et al. (1999). The results showed good agreement between dye techniques, while using the microscope without a prior procedure classified the lesions as deeper lesions.

Because of the different validation methodologies, it is important to evaluate their inter-examiner reproducibility and correlations. Oliveira et al., (2012) evaluated the reproducibility of seven different validation methods for occlusal caries diagnosis in primary molars: magnifying glass, stereomicroscope, photographs, slide projections of photographs, stereomicroscope slide projections and projections of polarised light microscope slides. The authors observed moderate inter-examiner reproducibility for all validation methods, except for the indirect assessment by slide projection.

In general, studies on the validity of caries detection methods clarify in their methodologies the importance of knowledge and practice routine of the examiners for the use of those methods. However, few of them report the examiners' experience for the application of a histological classification system according to the gold standard technique recommended in the study (Apostolopoulou et al., 2009; Kuhnisch et al., 2009; Ekstrand et al., 2011; Kavvadia et al., 2012; Seremidi et al., 2012; Soviero et al., 2012; Teo et al., 2014; Castilhos et al., 2016; Singh et al., 2016), leading to different results.

Thus, with the understanding that histological validation of dental caries is a subjective practice, this study aimed to evaluate the influence (1) of the examiners' experience and (2) three histological classification criteria on the reproducibility of two gold standard techniques (non-dye or dye) for validation of the diagnosis of occlusal caries lesions.

Materials and methods

The present study was carried out according to the

rules of the Local Ethics Committee (#1791.611). Prior to extraction, the patients were informed about the use of their teeth for research purposes, and their informed consent was obtained.

A convenience sample of 210 digital images of 105 permanent teeth (45 premolars and 60 molars) from the study by Diniz et al. (2012) were selected. In this study, clinical examinations were performed for occlusal caries detection on 88 patients, all of whom had at least one permanent posterior tooth with extraction indication for orthodontic reasons or due to periodontal diseases, without proximal, buccal or lingual caries lesions, restorations, pits and fissures sealants, hypoplasia or orthodontic band. The teeth were perpendicularly sectioned to the occlusal surface, near the site previously selected to perform the histological evaluation predicted in the study. A hemisection of each tooth was photographed with a digital camera (EOS Rebel XSi, Canon, Tokyo) with a 100mm lens, stained with 0.1 saturated rhodamine B solution, and photographed again, resulting in a total of 210 digital images. Standardized digital photographs were used in this investigation, as previously described in a construct validation study of a dental caries criteria (de Souza et al., 2012).

A convenience sample of six examiners was selected for this study: (G1) three professors involved with Cariology and Diagnosis studies (J.A.R., T.F.N and M.B.D.) with previous experience in histological evaluation of tooth slices, according to the gold standard techniques evaluated, through review of relevant knowledge and practical assessments, and (G2): three post-graduate dental students (C.S.S., R.S.O. and P.H.C.) with no previous experience in histological evaluation. Each of the 210 digital images was independently evaluated by all six examiners on a 14.1-inch LCD laptop screen, with no modification of size, color, contrast, brightness or magnification during photograph examinations. These images were classified according to three criteria of histological classification:

Downer (1975): No enamel demineralization or opacity on small surface area (0); Demineralization limited to outer half of the enamel layer (1); Demineralization involving the inner half of enamel to amelodentin junction (2); Demineralization involving external half of dentin (3); Demineralization involving the inner half of dentin (4).

Lussi et al. (1999): Caries free (0); Caries extending to halfway through the enamel (1); Caries extending into the inner half of enamel (2); Caries penetrating the dentine-enamel junction, but limited to the outer half of the dentine (3); Caries involving the inner half of the dentine (4).

Ekstrand et al. (1997): No demineralization in enamel or a small surface area with opacity (0); Demineralization limited to external half of enamel (1); Demineralization involving between 50% of enamel and external third of dentin (2); Demineralization involving middle third of dentin (3); Demineralization involving internal third of dentin (4).

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The methodology of the image evaluations was composed of 6 steps: (1) Evaluation of 105 digital images of dye-free hemisections according to the criteria proposed by Downer (1975); (2) Evaluation of 105 digital images of dye-free hemisections according to the criteria proposed by Ekstrand et al. (1997); (3) Evaluation of 105 digital images of dye-free hemisections according to the criteria proposed by Lussi et al. (1999); (4) Evaluation of 105 digital images of rhodamine B dye hemisections according to the criteria proposed by Downer (1975); (5) Evaluation of 105 digital images of rhodamine B dye hemisections according to the criteria proposed by Ekstrand et al. (1997); and (6) Evaluation of 105 digital images of rhodamine B dye hemisections according to the criteria proposed by Lussi et al. (1999). The examiners repeated each of the evaluations so that reproducibility could be calculated. Thus, in all, 12 independent analyses were performed. To avoid possible biases, the order of evaluation for each examiner was defined randomly and inserted in a table. The interval between each evaluation was 24 hours (Jablonski-Momeni et al., 2010), for a total of 12 days of evaluation.

Statistical analysis

For data analysis, the six examiners were divided into two groups: G1: examiners with previous experience in histological evaluation of tooth slices, and

G2: examiners with no experience in histological evaluation.

The intra-examiner reproducibility of each group, for each variable (criteria of histological classification and hemisections with or without rhodamine B dye), was expressed as the mean of the unweighted Cohen's kappa values presented by each examiner independently. Similarly, an unweighted Cohen's kappa test was used to assess inter-examiner agreement. The inter/intra-examiner reproducibility values were classified according to Landis and Koch (1977) as: (1) non-existent: <0; (2) poor agreement: 0-0.19; (3) slight agreement: 0.20-0.39; (4) moderate agreement: 0.40-0.59; (5) substantial agreement: 0.60-0.79 and (6) almost perfect agreement: 0.80-1.

A Mann-Whitney U test was used to verify possible differences according to the examiners' experience for each of the variables. The data were analysed in the SPSS program (Statistical Package for the Social Sciences) version 22.0, and the significance level was set at 0.05.

Results

Intra-examiner reproducibility mean values for G1 were higher than G2 considering all variables, with substantial and almost perfect agreement for G1 and moderate and substantial agreement for G2 (Table 1). Intra-examiner reproducibility was lower for the dye-free technique for both groups.

The mean kappa values for inter-examiner reproducibility in G1 ranged from 0.60 to 0.68 (substantial agreement). For G2, values ranged from 0.34 (slight agreement) to 0.69 (substantial agreement) (Tables 2,3).

When evaluating possible differences for each of the variables according to the examiners' experience, the results showed a statistically significant difference between the evaluations of the examiners with previous experience and examiners with no experience in histological evaluation in 29.6% of the cases (Table 4). There was similar agreement between the examiners regarding the depth of the lesions. Cases in which the disagreement among the examiners was greater were when the lesions were on the enamel-dentin junction.

Table 1. Mean of intra-examiner reproducibility values of each group for each variable (unweighted Kappa - K).

Histological classification	G1 (With experience) K	G2 (With no experience) K
Dye-free technique		
Lussi et al., 1999	0.81	0.56
Ekstrand et al., 1997	0.78	0.56
Downer, 1975	0.79	0.72
Rhodamine B dye technique		
Lussi et al., 1999	0.83	0.75
Ekstrand et al., 1997	0.84	0.79
Downer, 1975	0.87	0.79

Table 2. Inter-examiner reproducibility values for G1 (with previous experience) for each variable (unweighted Kappa values) and mean values of inter-examiner reproducibility.

	Histological classification	AxB	AxC	BxC	Mean
Dye-free technique	Lussi et al., 1999	0.56	0.68	0.64	0.63
	Ekstrand et al., 1997	0.51	0.70	0.83	0.68
	Downer, 1975	0.51	0.72	0.56	0.60
Rhodamine B dye technique	Lussi et al., 1999	0.59	0.81	0.49	0.63
	Ekstrand et al., 1997	0.66	0.79	0.54	0.66
	Downer, 1975	0.75	0.73	0.50	0.66

Discussion

In vitro studies that have evaluated the validity of caries detection methods are common in the literature (Rodrigues et al., 2008a,b; Braga et al., 2009; Diniz et al., 2009, 2010). These studies used gold standard techniques (reference diagnosis) to calculate the values of sensitivity, specificity, accuracy and area below the ROC curve for each proposed method. However, few studies clarify in the methodology section the experience and reproducibility of the examiners to perform the histological classification (Apostolopoulou et al., 2009; Kuhnish et al., 2009; Ekstrand et al., 2011; Kavvadia et al., 2012; Seremidi et al., 2012; Soviero et al., 2012; Teo et al., 2014; Castilhos et al., 2016; Singh et al., 2016). Thus, this study proposed to evaluate the influence of examiners' experience on the reproducibility of different gold standard techniques (rhodamine B dye or dye-free hemisections) for validation of the diagnosis of occlusal

caries, since they are the most used methodologies for histological validation (Huysmans and Longbotton, 2004; Diniz et al., 2009, 2012; Goel et al., 2009; Rodrigues et al., 2011; Souza et al., 2012).

The digital images used in the present investigation were from a previous study by our research group (Diniz et al., 2012). It should be emphasized that in that study stereomicroscopy was used as the validation method. Although stereomicroscopy seemed to be the most trustworthy of the validation methods, compared to film radiography, microradiography and naked-eye inspection, for occlusal caries diagnosis (Hintze et al., 1995), a previous study showed good results using histology validation from digital photograph assessments (de Souza et al., 2012).

The results of the present study show the importance of the histological evaluation being performed by an experienced examiner, since the experience influenced the reproducibility of the examination variable. This

Table 3. Inter-examiner reproducibility values for G2 (with no experience) for each variable (unweighted Kappa values) and mean values of inter-examiner reproducibility.

	Histological classification	AxB	AxC	BxC	Mean
Dye-free technique	Lussi et al., 1999	0.44	0.50	0.44	0.46
	Ekstrand et al., 1997	0.28	0.59	0.15	0.34
	Downer, 1975	0.51	0.48	0.23	0.41
Rhodamine B dye technique	Lussi et al., 1999	0.48	0.69	0.38	0.52
	Ekstrand et al., 1997	0.67	0.68	0.72	0.69
	Downer, 1975	0.54	0.62	0.60	0.59

Table 4. Comparison of examiners according to each variable of the study.

	Histological classification	Histological technique	Examiners for G1			
			A	B	C	
Examiners for G2	D	Lussi et al., 1999	Rhodamine B dye	0.810	0.381	0.792
			Dye-free	0.471	0.003*	0.320
		Ekstrand et al., 1997	Rhodamine B dye	0.245	0.038*	0.640
			Dye-free	0.258	0.067	0.062
		Downer, 1975	Dye-free	0.063	0.835	0.033*
			Rhodamine B dye	0.054	0.522	0.072
	E	Lussi et al., 1999	Dye-free	0.010*	0.118	0.003*
			Rhodamine B dye	0.122	0.571	0.212
		Ekstrand et al., 1997	Dye-free	0.009*	0.590	0.003*
			Rhodamine B dye	0.003*	0.951	<0.001*
		Downer, 1975	Dye-free	0.054	0.874	0.029*
			Rhodamine B dye	0.001*	0.466	0.001*
F	Lussi et al., 1999	Dye-free	0.842	0.142	0.497	
		Rhodamine B dye	0.471	0.003*	0.320	
	Ekstrand et al., 1997	Dye-free	0.107	0.678	0.047*	
		Rhodamine B dye	0.265	<0.001*	0.788	
	Downer, 1975	Dye-free	0.674	0.068	0.449	
		Rhodamine B dye	0.331	<0.001*	0.265	

* statistically significant differences (Mann-Whitney U-test, $P < 0.05$).

result was expected because the examiners' experience is important to produce more reproducible results, that is, to reduce the variability of the procedures and increase the comparability of the exams, besides increasing the reliability of the obtained information (Susin and Rosing, 2000). Moreover, the choice of the gold standard technique should also be taken into account, because the intra-examiner reproducibility was lower when the dye-free technique was used, even when performed by an experienced examiner. This may be related to the greater difficulty in assessing the depth of the lesions when the dye-free technique is used, since the colour change provided by the reactive dentine may confuse the examiners. In the technique with the rhodamine B dye, the chance of error is lower, because the depth of the lesion is given by the presence or absence of dye, which penetrates the sites with altered dental tissue due to the carious process (Rodrigues et al., 2012).

The kappa values for intra-examiner reproducibility found in this study for G1 group—examiners with previous experience (substantial and near-perfect)—are in agreement with those reported by many studies that investigated the validity of caries lesion detection methods and histological validation, presenting reproducibility values of 0.77 to 1.00 (Apostolopoulou et al., 2009; Kuhnish et al., 2009; Ekstrand et al., 2011; Kavvadia et al., 2012; Seremidi et al., 2012; Soviero et al., 2012; Teo et al., 2014; Castilhos et al., 2016; Singh et al., 2016). It is important to note that, since most of the studies do not present information about the calibration of the examiners who performed the histological evaluation, methodological information about the histological validation process used in the studies is limited.

It is known that there are different approaches to classify the stages of caries lesions in tooth specimens, such as the inclusion or exclusion of the subdivision of the enamel and determining whether the dentino-enamel junction is involved (Oliveira et al., 2012). When comparing the reproducibility within each group for each of the three histological classification criteria studied, the results showed no differences. However, an *in vitro* study, which evaluated the accuracy of ICDAS in detecting caries lesions on occlusal surfaces, using histological classification criteria proposed by Ekstrand et al. (1997) and Lussi et al. (1999), the results presented a variation in terms of sensitivity and specificity when comparing the results according to the criterion of histological classification. The specificity when using the criterion of Ekstrand et al. (1997) ranged from 0.69 to 1.0, while with the criterion of Lussi et al. (1999) values ranged from 0.47 to 0.62 (Diniz et al., 2009). This shows that there may be variation in the results of the studies according to the chosen classification criteria, despite having presented similar reproducibility results.

Moreover, the results of the present study showed a statistically significant difference between the tests performed by experienced examiners and examiners with no previous experience. This is important because it

demonstrates that the results of a survey that does not present histological evaluation performed by an examiner with no knowledge and practical routine may be different from those found by a study in which histological evaluations are done by an experienced examiner for this purpose.

Thus, assuming the methodological limitations of the present investigation, such as the use of a convenience sample from a previous study, the results indicate that: (1) the examiners' experience seems to influence the reproducibility of the two gold standard techniques studied and reproducibility tends to be lower when a dye-free technique is used, independently of the examiners' experience, and (2) the histological classification criterion does not appear to influence the reproducibility for validation of the diagnosis of occlusal caries. Therefore, since reproducibility is essential to increase the reliability of the information obtained, it is suggested that there be a methodological control in studies using histological evaluations to validate the methods for caries detection.

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