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ORIGINAL ARTICLE

Comparison of diagnostic effects of infrared imaging and bitewing radiography in proximal caries of permanent teeth

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Abstract
This study aimed to compare the diagnostic efficacy of VistaCam iX intraoral camera system using infrared light and bitewing radiography for detection of proximal caries in permanent teeth. This in vitro study was performed on 108 teeth. The proximal surfaces of the teeth were examined for caries using ICDAS II criteria, bitewing radiography, and the Proxi head of VistaCam iX. The teeth were then sectioned and histologically analyzed (gold standard). Data were analyzed using SPSS version 25 via the correlation test at $P < 0.05$ level of significance. The overall and segmental sensitivity and specificity values were calculated for (1) the contact area and higher regions, (2) below the contact area to the cementoenamel junction (CEJ), and (3) below the CEJ. Radiography had the highest specificity. VistaCam had the highest overall and segmental sensitivity for enamel caries. Radiography had the highest segmental sensitivity for dentin. In region 1, VistaCam had the highest sensitivity and lowest specificity, and radiography and ICDAS II had the highest specificity and lowest sensitivity. In region 2, radiography showed the highest sensitivity and specificity. VistaCam had the lowest sensitivity and ICDAS II had the lowest specificity in this region. In region 3, VistaCam did not detect any caries and radiography had a better performance than ICDAS II. The specificity value was equal for both methods. VistaCam had the highest diagnostic efficacy among the three methods for caries in region 1; however, bitewing radiography had a superior efficacy in regions 2 and 3.

Keywords Bitewing radiography · Histology · Proximal · Infrared · Dental caries

Introduction

Early and correct detection of dental caries is an everyday challenge for dental clinicians. Dentists should be able to detect even the slightest changes in tooth structure and decide on a correct treatment plan. An ideal caries detection system should enable early detection of initial caries and enhance decision making regarding a suitable treatment plan. Such systems can pave the way towards the application of preventive, conservative, targeted, and cost-effective procedures [1].

Several methods have been suggested for detection of caries in proximal surfaces. Adjunct techniques such as bitewing radiography and fiber-optic transillumination can aid visual inspection in achieving more accurate results. Visual inspection using the naked eye often results in detection of less than 50% of occlusal caries; this value is even lower for proximal surfaces [2]. Since the detection of caries in interproximal surfaces is not often possible with the naked eye, radiography is often employed for detection of carious lesions and determination of their depth and extension in these regions [3, 4]. A combination of visual inspection and bitewing radiography has been approved as a standard method for detection of interproximal caries [5]. However, presence of radiolucencies in interproximal regions on bitewing radiographs does not necessarily translate to presence of clinical carious lesions; but,

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the deeper the radiolucent lesion, the higher the risk of cavitation would be [6].

In addition to visual methods of caries detection, fluorescence-based techniques are also regarded as suitable methods for caries detection [7, 8]. The mechanism of action of fluorescence-based techniques is based on the presence of chromophores in the enamel and dentin and their auto-fluorescence. These chromophores are also present in carious lesions and bacteria, and their fluorescence can be quantified by subtracting the fluorescence of a sound tooth surface from that of a carious tooth surface. DIAGNOdent pen and quantitative light-induced fluorescence are among the commonly used fluorescence systems for caries detection [9]. VistaProof is another fluorescence-based device comprised of a camera for detection of occlusal caries, which was marketed in 2007 [10]. VistaCam (Classic, CL and IX, Durr Dental, Bietigheim-Bissingen, Germany) is an intraoral fluorescence camera, which illuminates the tooth with 405 nm violet light and records the reflected light as a digital image. The reflected light with less than 495 nm wavelength is filtered and a green-yellow color with a 510-nm peak is illustrated for sound teeth. Bacterial metabolites have red fluorescence with a peak at 680 nm. DBSWIN software version 5.3 quantifies the reflected red and green light using a 0–3 scale. A new model of VistaCam (CL-IX) was recently introduced to the market, which is a wireless camera with a removable head and light-curing function. A replaceable Proxi head is also available as an accessory for VistaCam iX and VistaCam iX HD intraoral camera. It is also equipped with two infrared LED lamps (850 nm wavelength) in its optical system (5 mW output, 7×9 mm optical dimensions) that illuminate the enamel of the distal and mesial surfaces of adjacent teeth. An image is generated by activating the light capturing cycle and analyzed by the DBSWIN software or VistaSoft imaging system as a black-and-white image. The camera is placed on the tooth, and the images are captured to determine the caries score. The main advantage of fluorescence cameras such as VistaProof and VistaCam is that they can digitize and store data, which can further enhance communication with patient and treatment planning [11–13].

On the other hand, following introduction of a new device into the market, studies are required to assess and compare its efficacy with other available devices/techniques. Search of the literature by the authors yielded only one previous study on this topic. Jablonski-Momeni et al. showed that the efficacy of the Proxi head of VistaCam was comparable to that of bitewing radiography for detection of non-cavitated lesions [13]. Histological analysis is the gold standard for caries detection. However, Jablonski-Momeni et al. [13] did not compare their results with the gold standard. The effect of occlusogingival dimension of proximal caries on their detection was not evaluated either. Thus, a comprehensive study was required to evaluate these parameters. Therefore, this in vitro study was undertaken to assess and compare the efficacy of VistaCam iX, bitewing radiography and visual inspection for detection of enamel and dentin proximal caries.

Materials and methods

This in vitro, experimental study was carried out on 108 extracted human permanent molar and premolar teeth. Soft tissue residues and calculus were removed using a toothbrush and a scaler, and the teeth were disinfected by immersion in 2% sodium hypochlorite solution for 20 min. They were then stored in distilled water [14].

The inclusion criteria were molar and premolar teeth without enamel hypoplasia or fluorosis. Teeth with large proximal cavitated carious lesions with extensive tooth destruction were excluded and replaced. The selected teeth were coded.

Sample preparation

The teeth were mounted in putty impression material next to each other such that they were in contact at their marginal ridges to simulate their position in the oral cavity. Red dental wax was applied over the putty surface and at the gingival embrasures to simulate the gingiva and interdental papilla. Each pair of teeth was immersed in a container filled with distilled water after coding.

Visual inspection

The proximal surfaces of the teeth were examined by a restorative dentist using a dental mirror, explorer, and air spray, and the lesions were classified according to the ICDAS II criteria as follows:

Score 0: Sound surfaces
Score 1: Initial visible changes in the enamel
Score 2: Changes in the enamel that can be detected even when wet
Score 3: Enamel loss due to caries but without dentin exposure
Score 4: Dark shadow in dentin with/without enamel loss
Score 5: A cavitated carious lesion confined to exposed dentin
Score 6: An extensive cavitated carious lesion with exposed dentin

The samples were scored accordingly [15].

Radiography

Bitewing radiographs were obtained of teeth. The distance between the film and tooth was 0.5 cm, and the distance between the tube and film was 32 cm. X-ray was irradiated tangent to the proximal surface using Kodak2200 intraoral x-ray system (Eastman, Kodak Co., Rochester, NY, USA) and size 2 Insight film (Eastman Kodak Company, Paris, France). The exposure time was 0.16 s and a CCX intraoral unit with 0.8 mm focal spot, 8 mA, 70 kVp, and aluminum filter was used. All films were processed on the same day using an automated film processor (Velopex, Extra-x, Medivance Instruments Ltd., London, UK, and NW107A) and fresh processing solution. The samples were coded as follows:

Score 0: No radiolucency
Score 1: Radiolucency confined to the outer half of enamel
Score 2: Radiolucency affecting the internal enamel surface and reaching the dentinoenamel junction.
Score 3: Radiolucency involving 50% of the external dentin surface
Score 4: Radiolucency involving the inner half of dentin with/without pulp involvement [15].

VistaCam iX

Photographs were obtained using a replaceable Proxi head with a temporary retainer. A protective shield was used for imaging of each tooth. The light system was positioned above the proximal region of the teeth, and photographs were captured according to the manufacturer's instructions using DBSWIN version 5.9.0 software.

Depth of lesions was determined independently on photographs and radiographs. A classification was used according to the manufacturer's instructions as follows:

0: No change in the enamel
IR 1: A wide bright band with wedge-shaped structures in dark translucent enamel. The lesion may extend to the dentinoenamel junction.
IR 2: A wide bright band with wedge-shaped structures passing the dentinoenamel junction [13]

Histological assessment

The teeth were then prepared for histological assessment. The teeth were serially sectioned (1-mm-thick sections) in the mesiodistal direction using a Mecatome (T201A, Presi, France). For each teeth, the deepest aspect of lesion (one section) was then evaluated under a stereomicroscope (SMZ800, Nikon, Tokyo, Japan) at × 10 magnification by a pathologist. The samples were histologically scored by an experienced examiner, who was otherwise not involved in the study, using the following classification [15]:

Score 0: no enamel demineralization or a thin opaque surface (no caries)
Score 1: enamel demineralization limited to the outer 50% of the enamel
Score 2: demineralization involving the inner 50% of the enamel, up to the dentinoenamel junction.
Score 3: demineralization involving of the outer 50% of dentin
Score 4: demineralization involving of the inner 50% of dentin

Statistical analysis

Data were analyzed using SPSS version 25. The sensitivity and specificity of the three methods were calculated. The correlation test was used to compare sensitivity and specificity of the methods at $P < 0.05$ level of significance. Sensitivity and specificity were separately calculated for each region. The overall sensitivity indicates the presence of caries in a specific region while segmental sensitivity indicates the penetration depth of caries in a specific region. Table 1 indicates the regions evaluated for caries in occlusogingival direction.

Results

A total of 108 proximal surfaces were evaluated in three regions (a total of 324 regions). Proximal surfaces were evaluated using ICDAS II criteria, bitewing radiography, and VistaCam and were then analyzed histologically (gold standard). Table 2 presents the test results and the agreement between the results of histological assessment and other methods. Tables 3, 4, and 5 show the results for assessment of each region.

Region 1

The overall sensitivity of caries detection was 0.54 for radiography and ICDAS II. The overall sensitivity of caries detection was 1 for VistaCam. The difference in sensitivity among the methods was significant ($P < 0.001$).

The specificity of radiography and ICDAS II was 1. The specificity of VistaCam was 0.83. The difference in specificity was significant among the methods ($P = 0.008$).

The overall sensitivity of enamel caries detection was 0.5 for radiography and ICDAS II. This value was 1 for VistaCam. The difference among the methods was significant in this respect ($P < 0.001$). The segmental sensitivity of enamel caries detection was 0.25 for radiography and ICDAS II and 0.5 for VistaCam. The difference in this respect was significant among the three methods ($P = 0.013$).

Table 1 Regions evaluated for caries in occlusogingival direction

Area	Region
Contact area and higher	1
Below the contact area to the cementoenamel junction	2
Below the cementoenamel junction	3

The overall sensitivity of dentin caries detection was 0.55 for radiography and ICDAS II and 1 for VistaCam. This difference was also statistically significant ($P < 0.001$). The segmental sensitivity of dentin caries detection was 0.07 for ICDAS II, 0.42 for radiography, and 0.73 for VistaCam. This difference was significant among the three methods ($P < 0.001$, Table 6).

Region 2

Overall sensitivity for caries detection was 0.52 for ICDAS II, 0.56 for radiography, and 0.16 for VistaCam. The difference in sensitivity among radiography, ICDAS II, and VistaCam was significant ($P = 0.001$). However, the difference in sensitivity between radiography and ICDAS II was not significant ($P = 0.854$).

The specificity of radiography and VistaCam was 1 while the specificity of ICDAS II was 0.98. The difference among the methods was not significant in this regard ($P = 0.999$). The overall sensitivity for detection of enamel caries was 0.2 for all three methods. The segmental sensitivity for enamel caries detection was 0.2 for ICDAS II and VistaCam and 0 for radiography. This difference was significant ($P = 0.047$).

The overall sensitivity for dentin caries detection was 0.6 for ICDAS II, 0.65 for radiography, and 0.15 for VistaCam. The difference among VistaCam, ICDAS II, and radiography was significant ($P < 0.001$) while the difference between ICDAS II and radiography was not significant in this respect ($P = 0.802$).

The segmental sensitivity for dentin caries detection was 0.15 for ICDAS II, 0.6 for radiography, and 0 for VistaCam. The difference in this respect was significant between radiography and VistaCam and also radiography and ICDAS II ($P < 0.001$), but the difference between VistaCam and ICDAS II was not significant ($P = 0.097$, Table 7).

Table 2 Results of histological analysis compared to radiography, VistaCam, and visual inspection in general

Histology	ICDAS II							Radiography					VistaCam		
	0	1	2	3	4	5	6	0	1	2	3	4	0	1	2
0	244	1	0	0	0	0	0	245	0	0	0	0	235	9	1
1	0	0	2	0	0	0	0	0	2	0	0	0	0	0	2
2	8	0	0	1	2	0	0	8	0	0	3	0	4	5	2
3	12	3	1	4	0	0	0	14	0	1	5	0	8	3	9
4	20	11	5	4	4	0	2	15	0	6	18	7	17	10	19

Table 3 Results of histological analysis compared to radiography, VistaCam, and visual inspection for caries in the contact area and higher

Histology	ICDAS II							Radiography					Vistacam		
	0	1	2	3	4	5	6	0	1	2	3	4	0	1	2
0	62	0	0	0	0	0	0	62	0	0	0	0	52	9	1
1	0	0	2	0	0	0	0	0	2	0	0	0	0	0	2
2	4	0	0	0	2	0	0	4	0	0	2	0	0	4	2
3	8	2	0	2	0	0	0	8	0	1	3	0	0	3	9
4	9	7	4	3	3	0	0	9	0	4	12	1	0	7	19

Region 3

VistaCam. This difference was significant among the three methods ($P = 0.036$). The difference between ICDAS II and VistaCam was not significant ($P = 0.97$). The specificity of radiography and ICDAS II was 1. The overall sensitivity for dentin caries detection was 0.12 for ICDAS II and 0.37 for radiography. This difference was statistically significant ($P = 0.036$).

The segmental sensitivity of dentin caries detection was 0 for ICDAS II and 0.25 for radiography. This difference was statically significant ($P = 0.042$, Table 8).

Overall assessment

The overall sensitivity for caries detection was 0.49 for ICDAS II, 0.53 for radiography, and 0.63 for VistaCam. This difference was not significant ($P = 0.086$). The specificity was 0.99 for ICDAS II, 1 for radiography, and 0.96 for VistaCam. This difference was not significant either ($P = 0.824$). The overall sensitivity for enamel caries detection was 0.38 for ICDAS II and radiography and 0.69 for VistaCam. This difference was significant among the three

Table 4 Results of histological analysis compared to radiography, VistaCam, and visual inspection for caries in the area below the contact point to the cementoenamel junction

Histology	ICDAS II							Radiography					VistaCam		
	0	1	2	3	4	5	6	0	1	2	3	4	0	1	2
0	82	1	0	0	0	0	0	83	0	0	0	0	83	0	0
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	4	0	0	1	0	0	0	4	0	0	1	0	4	1	0
3	2	1	1	2	0	0	0	4	0	0	2	0	6	0	0
4	6	3	1	1	1	0	2	3	0	1	5	5	11	3	0

Table 5 Results of histological analysis compared to radiography, VistaCam, and visual inspection for caries in the area below the cementoenamel junction

Histology	ICDAS II							Radiography				
	0	1	2	3	4	5	6	0	1	2	3	4
0	100	0	0	0	0	0	0	100	0	0	0	0
3	2	0	0	0	0	0	0	2	0	0	0	0
4	5	1	0	0	0	0	0	3	0	1	1	1

methods ($P = 0.001$). Segmental sensitivity for enamel caries detection was 0.23 for ICDAS II, 0.15 for radiography, and 0.38 for VistaCam. The difference in sensitivity among the methods was significant ($P = 0.037$). Segmental sensitivity for dentin caries detection was 0.09 for ICDAS II, 0.45 for radiography, and 0.42 for VistaCam. The difference among ICDAS II, radiography, and VistaCam was significant ($P = 0.048$), but the difference in sensitivity of VistaCam and radiography was not significant ($P = 0.9$).

The overall sensitivity for dentin caries detection was 0.51 for ICDAS II, 0.56 for radiography, and 0.62 for VistaCam. The difference in this regard was not significant among the three methods ($P = 0.058$, Table 9).

Discussion

Proximal caries is often not easily detectable in visual inspection. Bitewing radiography is a diagnostic modality with higher sensitivity than visual inspection and moderate to high specificity for detection of enamel and dentin caries [16, 17]. This technique, however, has shortcomings such as overlapping of proximal surfaces on radiographs [17]; moreover, presence/absence of cavitation in proximal surfaces of the posterior teeth is not well detectable on bitewing radiographs.

According to a previous study, a suitable method for caries detection must have at least 75% sensitivity and over 85% specificity [18]. Considering the conservative approach of contemporary dentistry and its emphasis on preservation of sound tooth structure, it is important to be able to detect sound

Table 6 Sensitivity and specificity of radiography, VistaCam, and visual inspection for caries detection in the contact area and higher

L = 1	ICDAS II	Radiography	Vistacam
Overall sensitivity	0.54	0.54	1
Overall enamel sensitivity	0.5	0.5	1
Segmental enamel sensitivity	0.25	0.25	0.5
Overall dentin sensitivity	0.55	0.55	1
Segmental dentin sensitivity	0.07	0.42	0.73
Specificity	1	1	0.84

Table 7 Sensitivity and specificity of radiography, VistaCam, and visual inspection for caries in the area below the contact point to the cementoenamel junction

L = 2	ICDAS II	Radiography	VistaCam
Overall sensitivity	0.52	0.56	0.16
Overall enamel sensitivity	0.2	0.2	0.2
Segmental enamel sensitivity	0.2	0	0.2
Overall dentin sensitivity	0.6	0.65	0.15
Segmental dentin sensitivity	0.15	0.6	0
Specificity	1	1	1

teeth. Thus, the Proxi head of VistaCam intraoral camera was used as a new, non-invasive diagnostic modality for detection of proximal caries and its efficacy for this purpose was compared to that of bitewing radiography and visual inspection. The replaceable Proxi head has been produced as an accessory to VistaCam iX and VistaCam iX HD intraoral cameras. It enhances the penetration of irradiated infrared (850 nm) light into the enamel; this light is severely scattered by the enamel and dentin lesions. The goal behind the production of the Proxi head according to the manufacturer is to enhance the detection of caries even in initial phases of development with no need for tooth exposure. It seems to be a safer technology for caries detection compared to radiography. Photographs are obtained from the occlusal surface of teeth, and thus, they are not exactly comparable to bitewing radiographs [13].

In the current study, for further assessment, proximal areas were divided into three regions of contact area and higher, below the contact area to the cementoenamel junction and below the cementoenamel junction. Also, the overall and segmental sensitivity values were calculated. One strength of this study was conduction of histological assessment to serve as the gold standard. However, in vitro design was among the limitations of this study, which cannot completely simulate the oral clinical conditions such as presence of saliva.

In this study, the overall sensitivity (without differentiating enamel and dentin) of ICDAS II, radiography, and VistaCam was not significantly different but the overall sensitivity of VistaCam was slightly lower than that of the other two methods. This is due to false-positive cases. Photographs taken of some teeth in completely dark room by irradiation of infrared light revealed bright areas, which can indicate

Table 8 Sensitivity and specificity of radiography, VistaCam, and visual inspection for caries detection in the area below the cementoenamel junction

L = 3	ICDAS II	Radiography
Overall sensitivity	0.12	0.37
Segmental sensitivity	0	0.25
Specificity	1	1

Table 9 Overall sensitivity and specificity of radiography, VistaCam, and visual inspection

General	ICDAS II	Radiography	VistaCam
Overall sensitivity	0.49	0.53	0.63
Overall enamel sensitivity	0.38	0.38	0.69
Segmental enamel sensitivity	0.23	0.15	0.38
Overall dentin sensitivity	0.51	0.56	0.62
Segmental dentin sensitivity	0.09	0.45	0.42
Specificity	0.99	1	0.96

presence of caries according to the manufacturer's instructions. Enamel cracks were also noted in these teeth. However, histological analysis showed no evidence of caries. It seems that enamel cracks can result in false-positive results due to light refraction in cracked areas. On the other hand, initial caries, primary demineralization, and cracks may not be detectable on radiographs [17], which was also confirmed by the current results. Tassery et al. in a review study on different methods of caries detection reported that the specificity of VistaCam was lower than that of bitewing radiography and visual inspection [19]; this finding was in agreement with the current results regarding interproximal surfaces.

The overall sensitivity of enamel caries detection of VistaCam was significantly higher than that of ICDAS II and radiography. However, the overall sensitivity of dentin caries detection was not significantly different among the three methods. The segmental sensitivity of enamel caries in VistaCam was significantly higher than that of ICDAS II, and that of ICDAS II was significantly higher than that of radiography. Also, the difference in segmental sensitivity of ICDAS II with radiography and VistaCam was significant. However, the difference between VistaCam and radiography was not significant. According to the results, it seems that radiography is superior to the other two methods in detection of depth of dentin caries.

In the current study, the overall sensitivity of VistaCam for caries detection in region 1 was significantly higher than that of radiography and ICDAS II. However, the specificity of VistaCam was significantly lower than that of radiography and ICDAS II. The overall and segmental sensitivity for enamel and dentin caries detection in VistaCam were significantly higher than those of radiography and ICDAS II. Jablonski et al. compared VistaCam and VistaProof for occlusal caries detection and found that both devices had suitable performance for detection of occlusal caries in different phases of progression [11].

In contrast to our results, Jablonski-Momeni et al. reported that the level of agreement between the Proxi head of VistaCam and bitewing radiography for detection of proximal caries, sound surfaces, and enamel caries was moderate and the two methods were not significantly different in this regard [13]. They did not perform histological analysis as the gold standard and did not mention the presence/absence of enamel cracks, which may explain the difference in the results of the two studies.

In region 2, the overall sensitivity of caries detection by VistaCam was significantly higher than that of radiography and ICDAS II. However, the specificity of the three methods was almost the same. The overall sensitivity of enamel caries detection was also the same for all three methods. The segmental sensitivity of enamel caries detection by ICDAS II and VistaCam was the same and significantly higher than that of radiography. In other words, the former two methods better visualized the progression of caries. The overall sensitivity of dentin caries detection by radiography and ICDAS II was significantly higher than that of VistaCam. The segmental sensitivity of dentin caries was 0.15 for ICDAS II, 0.6 for radiography, and 0 for VistaCam. The difference between radiography and VistaCam and ICDAS II was significant but the difference between VistaCam and ICDAS II was not significant. No similar study is available on this region but it appears that radiography had the highest efficacy among the three methods in this region because anatomical areas do not often limit caries detection by radiography.

In region 3, the overall sensitivity of radiography was significantly different from the sensitivity of the other two methods but the difference between VistaCam and ICDAS II was not significant. The overall and segmental sensitivity for dentin caries detection by radiography were significantly higher than those by ICDAS II. In the current study, the Proxi head had no diagnostic ability in region 3. It seems that for carious lesions below the cementoenamel junction, a large portion of sound tooth structure is located between the head of the device and carious lesion, which prevents the light from reaching lower areas and masks the carious lesion. Thus, it remains undetected. However, in case of occlusal caries, the carious lesion is closer to the camera and is easier to detect.

According to the current findings, it seems that radiography is more efficient than the other tested methods for detection of caries extended to the dentinoenamel junction. Thus, bitewing radiography is efficient for both occlusal and proximal caries detection [17].

Conclusion

In the current study, the overall sensitivity and specificity values were 0.49 and 0.99 for ICDAS II, 0.53 and 1 for radiography, and 0.63 and 0.96 for VistaCam, respectively, and no significant difference was noted among the three methods in sensitivity and specificity. VistaCam seemed to be the best method for detection of primary enamel lesions, and visual inspection ranked second. Radiography did not provide optimal results. However, it should be noted that presence of enamel cracks can yield false-positive results in use of VistaCam. The overall sensitivity for dentin caries detection was not significantly different among ICDAS II, radiography, and VistaCam. The efficacy of radiography was the highest for determination of depth of dentin caries, followed by VistaCam and ICDAS II. The difference among ICDAS II, radiography, and VistaCam was significant but the difference in sensitivity of VistaCam and radiography was not significant. Due to high precision of the Proxi head of VistaCam for detection of proximal caries, it can be used as an adjunct for decision-making regarding cavity preparation or remineralization treatment to provide patients with the most suitable treatment.

Future studies are recommended to evaluate the diagnostic accuracy of fluorescence-based systems and bitewing radiography for detection of proximal caries in permanent teeth in vivo compared to the gold standard. Also, the efficacy of the Proxi head of VistaCam should be compared with other novel techniques for caries detection.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any study with human or animal subjects. In addition, this study was approved by the Ethical Committee of Tehran University of Medical Sciences, International Campus. (No.1396.4241).

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