UNIVERSIDADE ESTADUAL DE CAMPINAS

FACULDADE DE ODONTOLOGIA DE PIRACICABA

FÁBIO LUIZ MIALHE

Cirurgião Dentista

DETECÇÃO DE LESÕES CARIOSAS EM SUPERFÍCIES OCLUSAIS: AVALIAÇÃO DE DIFERENTES TECNOLOGIAS

Tese apresentada à Faculdade de Odontologia de Piracicaba da Universidade Estadual de Campinas, para obtenção do título de Doutor em Odontologia - área de concentração em Cariologia.

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Orientador:

Prof. Dr. Antonio Carlos Pereira

Banca Examinadora:

Prof. Dr. Antonio Carlos Pereira
Prof. Dr. Carlos Wagner de Araújo Werner
Prof. Dr. Eduardo Hebling
Prof[a]. Dr[a]. Gláucia Maria Bovi Ambrosano
Prof. Dr. José Roberto de Magalhães Bastos

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SUMÁRIO

RESUMO

O presente trabalho, composto por 3 estudos, teve como objetivo geral avaliar "in vitro" a eficácia e a reprodutibilidade de diferentes métodos diagnósticos para lesões cariosas em superfícies oclusais. A amostra dos estudos foi composta por 96 molares permanentes. O objetivo do 1º estudo foi comparar o desempenho dos sistemas de radiografia digital CDR (Schick Technologies, EUA) e Sidexis (Sirona, Bensheim, Alemanha) com o método radiográfico convencional, utilizando os resultados do exame histológico como método de validação. Dois examinadores realizaram todos os exames. Concluiu-se, a partir do cálculo da área abaixo da curva ROC para os métodos avaliados, que não houve diferenças estatisticamente significantes entre o desempenho do filme radiográfico convencional em relação aos dois sistemas digitais CCD para a detecção de lesões cariosas em superfície oclusais, porém, houve diferenças entre os dois últimos métodos avaliados. O objetivo do 2º estudo foi comparar o desempenho de 5 métodos de diagnóstico, ou seja, (1) o exame clínico-visual, (2) o radiográfico convencional, (3) o aparelho QLF (Inspektor Research System BV, Amsterdam, Holanda), (4) o aparelho ECM (LODE, Groningen, Holanda) e, (5) o aparelho DIAGNOdent (Kavo, Biberach, Alemanha) para a detecção de lesões cariosas em supefícies oclusais, comparado ao exame histológico. Os resultados demonstraram que o aparelho DIAGNOdent apresentou valores superiores de sensibilidade, especificifidade, acurácia e área abaixo da curva ROC (Az) em relação aos outros métodos avaliados. Conclui-se, portanto, que este método pode ser um importante adjunto aos exames convencionais nos casos em que há dúvidas quanto a higidez de uma fissura que não apresenta uma imagem radiolúcida ao exame radiográfico. O objetivo do 3º estudo foi comparar o desempenho de quatro métodos de diagnóstico em detectar e estimar a profundidade das lesões cariosas em superfícies oclusais em função dos resultados obtidos a partir de três métodos de validação, ou seja, o exame estereomicroscópio, a microrradiografia transversa e a microscopia confocal de varredura a laser e analisar a correlação entre eles. Concluiu-se, pelos resultados obtidos, que o desempenho dos métodos de diagnóstico não foi influenciado pelos resultados dos métodos de validação sendo que todos eles podem ser considerados fidedignos para se avaliar o desempenho dos métodos de diagnóstico avaliados.

Abstract

The present thesis, composed by 3 studies, had as general objective to evaluate " in vitro" the efficacy and the reproducibility of different diagnostic methods for carious lesions on occlusal surfaces. The sample of the studies was composed by 96 extracted permanent molars. The objective of the 1st study was to compare the performance of the digital radiographic systems CDR (Schick Technologies, USA) and Sidexis (Sirona, Bensheim, Germany) with the conventional radiographic method for the detection of carious lesions in occlusal surfaces, using the histologic examination as validation method. Two examiners accomplished all the exams. It was concluded, starting from the calculation of the area below curved ROC for the evaluated methods, that there were not statistically significant differences between the performance of the conventional film and the two CCD digital systems for the detection of carious lesions in occlusal surfaces. However, it was found differences between the two last evaluated methods. The objective of the 2nd study was to compare " in vitro " the performance of 5 diagnostic methods, that is, (1) the clinical-visual exam, (2) the conventional radiographic examination, (3) the QLF device (Inspektor Research System BV, Amsterdam, Holland), (4) the ECM device (LODE, Groningen, Holland), and (5) the DIAGNOdent device (Kavo, Biberach, Germany) for the detection of carious lesions in occlusal surfaces. The results demonstrated that the DIAGNOdent device presented higher values of sensibility, specificity, accuracy and area below curved ROC (Az) in relation to the other methods. Therefore, it was concluded that this method can be an important adjunct to visual-radiographic examination when there is doubt in relation to the state of fissures. The objective of the 3rd study was to compare the performance of four diagnostic methods to detect and estimated the depth of the carious lesions in function of the results obtained from stereomicroscopy, transversal microradiograph and confocal laser scanning microscopy exams and to analyze the correlation among them. It was concluded, based on the obtained results that the performance of the diagnostic methods was not influenced by the results of thevalidation methods and all they can be considered trustworthy to evaluate the performance of the diagnosis methods.

1. Introdução

Atualmente, a detecção e a estimação da profundidade do comprometimento tecidual das lesões cariosas podem ser encaradas como um desafio para o cirurgião-dentista. Alterações na macromorfologia das mesmas têm sido relatadas em vários estudos, atribuídas, em grande parte, ao maior contato das populações com os compostos fluoretados, fazendo com que as lesões não-cavitadas, ou em estágios iniciais de desenvolvimento, sejam, atualmente, mais prevalentes do que as lesões cariosas cavitadas (Lussi, 1991; Ismail et al., 1992; Wenzel, 1993; Bratthal et al., 1996; Ismail, 1997; Clarkson & Mcloughlin, 2000).

Acompanhando esse processo, verificou-se também, que as superfícies oclusais, apesar de representarem apenas cerca de 12% do total das superfícies dentárias presentes na cavidade bucal, são as mais freqüentemente afetadas pelo processo carioso (Brunelle & Carlos, 1982; Ripa et al., 1988; Pereira et al., 1995; Mejàre et al., 1998; Hugoson et al., 2000; Mejàre et al., 2004). A maior susceptibilidade ao início e ao desenvolvimento da doença em tais superfícies é atribuída, em grande parte, à sua configuração anatômica que favorece a estagnação de bactérias e alimentos e ao fato de o efeito preventivo dos fluoretos, nessas superfícies, ser considerado menor, se comparado às superfícies lisas (Brunelle & Carlos, 1982; Carvalho et al., 1989; Milicich, 2000; Hopcraft & Morgan, 2003).

A dificuldade em detectar-se corretamente a presença e a extensão das lesões cariosas nas superfícies oclusais, por meio dos métodos de diagnóstico tradicionais, como os exames visual/tátil e o radiográfico, é bem documentada na literatura (Ricketts et al., 1995; Atrrill & Ashey, 2001; Bader et al., 2002).

A fim de melhorar-se a qualidade da imagem obtida, através da manipulação do tamanho, do brilho e do contraste da imagem, sistemas de radiografias digitais foram desenvolvidos e introduzidos no mercado. Enquanto nas técnicas radiográficas convencionais a imagem radiográfica é recebida em um filme ou em uma combinação de filme-écran, nos sistemas de radiografias digitais, o filme radiogrãfico é substituído por um receptor de imagens digitais (Wenzel, 1998, 2000; Macdonald, 2001; Parks & Williamson, 2002). Atualmente, existem basicamente dois tipos de sistemas disponíveis para essa finalidade: o sistema CCD (sigla em inglês que significa Charge Couple Device) e o sistema de armazenamento de fósforo.

Os sistemas CCD são assim designados por utilizar o dispositivo CCD para a captação da imagem. O CCD é um chip de silício que possui em seu núcleo, numerosos fotodiodos, correspondendo, cada um, a um pixel, que se traduz na menor unidade de informação da imagem; o conjunto destes fotodiodos forma a matriz digital do fóton-detector. Quando uma energia eletromagnética, como por exemplo, a radiação X interage no pixel, uma carga elétrica é criada e armazenada no seu interior. O total de carga armazenada é proporcional à energia incidente nos mesmos. Após a exposição, essa carga é removida e cria-se um sinal analógico de saída, cuja voltagem é proporcional à carga sucessiva de cada pixel. O sensor é conectado a um computador por meio de um cabo e, através de um software que converte o sinal analógico em digital, a imagem capturada fica quase imediatamente disponível para a interpretação no monitor do computador (Wenzel , 2000; Oliveira, 2001; Parks & Williamson, 2002). Esse tipo de técnica também é denominado radiografia digital direta.

As figuras 1,2,3 e 4 apresentam dois sistemas de radiografia digital que utilizam a tecnologia CCD.

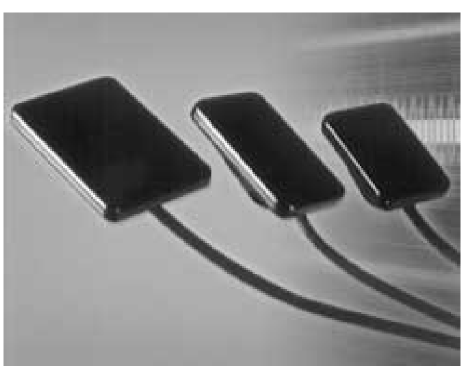


Fig. 1:
Sensores do sistema de radiografia digital CDR® (Shick Technologies, USA) (www.schicktech.com)

Fig. 2:
Componentes do sistema digital CDR®
(www.schicktech.com)

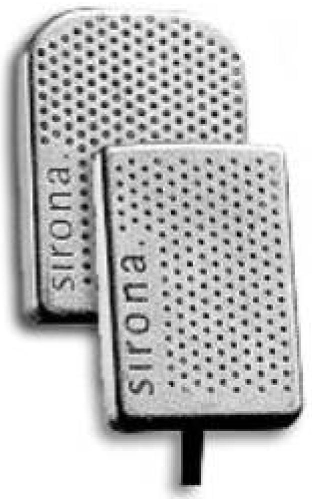


Fig. 3:

Sensores do sistema digital Sidexis®

(Sirona Dental Systems, Benshein, Germany)

(www.sirona.com)

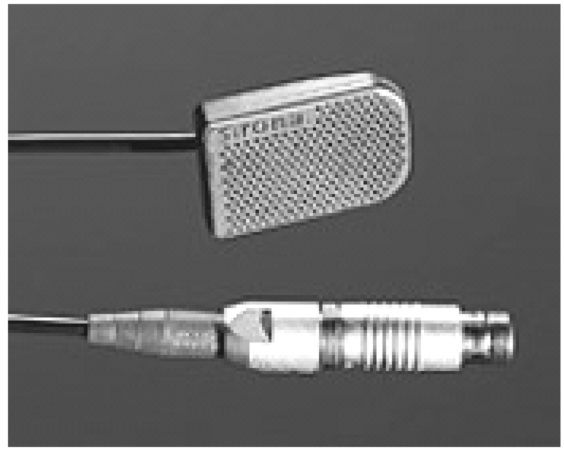


Fig. 4:

Componentes do sistema digital Sidexis®

(www.sirona.com)

Nos sistemas que utilizam placas de fósforo, uma imagem latente é criada em uma placa que contém sais de fósforo e uma característica deste sistema é não possuir um cabo acoplado ao computador. Após a placa ser irradiada, uma significante quantidade de energia é absorvida ficando temporariamente armazenada nos cristais de fósforo. Os elétrons aí aprisionados são liberados pela estimulação realizada por meio de um feixe de laser que apresenta um comprimento de onda específico. Para a leitura da informação contida na placa, deve-se introduzi-la em um scanner apropriado, onde, por meio de um fino feixe de laser de He-Ne, é efetuada a varredura na superfície da placa (figura 5). A energia liberada da placa é detectada no tubo fotomultiplicador e convertida em sinais elétricos, que produzem uma voltagem proporcional à intensidade da luz emitida. O sinal analógico formado é, então, convertido em digital por meio de um conversor, e é enviado ao computador para ser exibido na tela. (Wenzel, 2000; Oliveira, 2001).

A radiografia digital fornece algumas vantagens fundamentais, ou seja, os sistemas alcançam uma redução significativa na dose de radiação aplicada e as imagens são, ou quase, que imediatamente disponibilizadas, dispensando o processamento do filme e a utilização de produtos químicos. Também se podem realizar nas imagens medições de tamanho e de comprimento, bem como alterações de brilho e de contraste, por meio de uso de softwares específicos.

Figuras 5 a 7: exemplo de sistema de radiografia digital (Digora® -Soredex,USA) que utiliza a tecnologia de placa de fósforo.

Fig. 5: sensores do sistema DIGORA® (Soredex, USA) (www.soredex.com)

Fig. 6: Escaner à laser para a leitura da imagem latente no sensor (www.soredex.com)

Fig. 7: Visão geral dos componentes do sistema DIGORA® (www.soredex.com)

Durante os últimos anos, vários sistemas de radiografia digital foram introduzidos no mercado, porém poucos fabricantes fornecem informações sobre a capacidade dos seus equipamentos em detectar lesões cariosas. Aliado a esse fato, poucos estudos na literatura têm avaliado a capacidade de tais equipamentos em detectar e em quantificar a profundidade das lesões cariosas em superfícies oclusais. Portanto, faz-se necessária a realização de mais estudos que avaliem o desempenho destes equipamentos a fim de se determinarem os méritos de cada um e de comparar seus desempenhos com os métodos radiográficos convencionais.

Dessa forma, a fim de se avaliarem essas características, foi realizado o estudo "**Evaluation of three radiographic methods performance in the detection of occlusal caries lesions**" (Capítulo 1).

A necessidade de se diagnosticar com precisão as lesões cariosas, tem estimulado o desenvolvimento de novos métodos, baseados em diferentes princípios. Dentre eles, podemos citar os métodos que utilizam o princípio da indução da fluorescência tecidual. Quando a luz interage com os tecidos dentários de várias formas, ela pode ser dispersa, transmitida ou absorvida. Quando um fóton é absorvido pelo tecido, a energia luminosa pode ser transformada em calor ou pode causar a fluorescência tecidual. Neste último caso, a energia proveniente do fóton de luz excita os átomos teciduais, impulsionando seus elétrons para um estado de energia mais alta. Nesse salto quântico "para cima" o átomo salta por sobre vários estados de energia intermediários. Ao relaxar, o átomo pode realizar vários saltos menores, emitindo fótons com energias menores. Uma vez que a energia liberada no fóton emitido a cada salto quântico durante o processo, é menor do que a energia total original do fóton de luz, são emitidos fótons de freqüências mais baixas a do que, originalmente incidiu sobre o tecido. O comprimento de onda proveniente do processo de fluorescência, portanto, é diferente daquele que incidiu inicialmente sobre a superfície dentária (figura 8). Portanto, quando o tecido dentário absorve a luz de uma determinada cor, emite luz de uma cor diferente. Tal fenômeno de emissão de luz, parte do material quando em estado de relaxamento, recebe o nome de fluorescência (Angmar-Mansson e ten Bosch, 2001). Métodos de diagnóstico como o QLF (Quantitative Light-induced Fluorescence) e o DIAGNODent (Laser fluorescente) e a microscopia de varredura confocal a laser baseiam-se na detecção da fluorescência tecidual induzida (Duschner et al., 1995; Angmar-Mansson e ten Bosch, 2001)

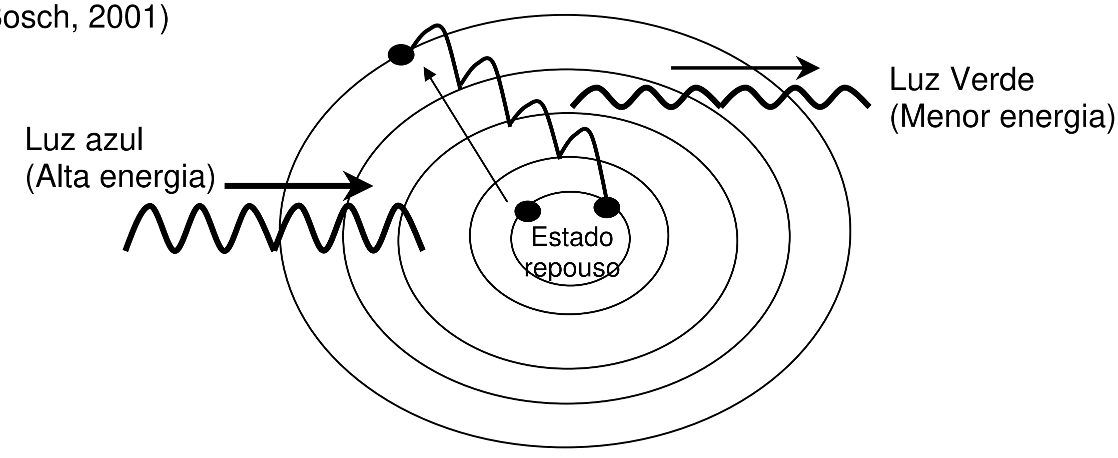


Fig. 8: Esquema representando o processo de indução da fluorescência tecidual

A causa da fluorescência do esmalte ainda não é muito certa. A maior parte da fluorescência tecidual é atribuída aos componentes orgânicos e às proteínas cromóforas, e a parte restante, aos cristais de apatita (Spitzer & ten Bosch, 1976). A desmineralização do tecido dental resulta na diminuição da autofluorescência, ou seja, da fluorescência natural do tecido. Muitos fatores podem contribuir para a diminuição da fluorescência tecidual nas lesões cariosas incipientes (Angmar-Mansson & ten Bosch, 2001) e entre eles, podemos citar estes:

1) A absorção da luz por volume tecidual é muito menor na lesão cariosa do que no tecido hígido, e, portanto, a fluorescência tecidual é muito mais fraca;

2) A fluorescência tecidual nas lesões cariosas é diminuída pelas mudanças no ambiente molecular dos cromóforos;

3) As proteínas cromóforas são removidas do tecido pelo processo carioso.

Dentre os métodos emergentes, que utilizam o princípio da fluorescência tecidual para detectar as perdas minerais do dentes, podem-se citar o QLF (Quantitative Light-induced Fluorescence) e o DIAGNOdent.

O sistema QLF (Quantitative Light-induced Fluorescence), consiste de uma microcâmera intra-oral, conectada a um microcomputador. A câmera emite uma fonte de luz visível, de 50 Watts de potência e de coloração variando entre o violeta e azul (comprimento de onda de 290 a 450nm), no local do exame, estimulando a fluorescência tecidual dentro do espectro da emissão amarelada (figuras 9 a 11). A fluorescência tecidual é captada por meio de um sensor presente na câmera e a imagem é visualizada através de um filtro de 520nm. A fluorescência tecidual induzida faz com que as lesões iniciais de mancha branca se manifestem como manchas escuras (figura 10) ao exame QLF. As imagens são processadas e analisadas por meio de um software especialmente desenvolvido para detector e analisar as perdas minerais (QLF, Inspektor Research Systems, Amsterdam, The Netherlands). A maioria dos estudos realizados com esse sistema avaliou sua capacidade de detectar e de quantificar as perdas minerais de processos cariosos localizados em superfícies lisas (Angmar-Mansson & ten

Bosch, 2001; Shi et al., 2001), mas poucos estudos foram realizados avaliando a capacidade do sistema em detectar lesões cariosas em superfícies oclusais (Angmar-Mansson et al., 1996; Ando et al., 2000).

Figuras 9 a 11: Sistema QLF e detecção da lesão cariosa pelo mesmo.

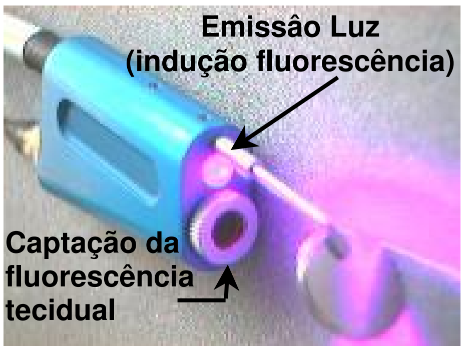
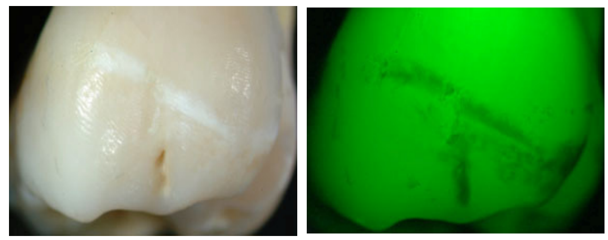


Fig. 9: Câmera do sistema QLF
(www.inspektor.nl)



Figs. 10 e 11: esmalte desmineralizado na superfície proximal detectado pelo exame visual e pelo sistema QLF
(www.inspektor.nl)

Um recente método, introduzido no mercado, para detectar e para quantificar a extensão da lesão cariosa, foi o aparelho de Laser Fluorescente da Kavo, denominado DIAGNOdent® (Kavo, Biberach, Alemanha) que utiliza um laser de diodo com comprimento de onda de 655nm (âmbito vermelho do espectro visível) e 1mW de potência. O aparelho constitui-se de uma unidade compacta alimentada por cinco baterias e apresenta uma ponteira que contém feixes de fibras ópticas, na qual um feixe central emite a luz laser e oito feixes periféricos captam a fluorescência tecidual induzida pela radiação laser. Alterações teciduais patológicas resultam em alterações no padrão de fluorescência, as quais o aparelho capta e mensura. No painel frontal existem dois visores digitais que indicam os valores medidos numa escala de 00 a 99. O valor à esquerda indica o valor atual medido (“momento”), e o visor à direita indica o valor máximo medido, ou pico, durante o ciclo de medições (figuras 12 e 13).

Figuras 12 e 13: Aparelho DIAGNOdent® (Kavo,Biberach, Germany)

Fig. 12: Aparelho DIAGNOdent®
(www.kavo.com)

Fig. 13: Medição da higidez tecidual por meio do aparelho DIAGNOdent®
(www.kavo.com)

Os estudos realizados até o momento com o aparelho revelaram que o mesmo apresenta altos valores de sensibilidade e de especificidade (Milicich, 2000; Shi et al., 2000; Attrill & Ashey, 2001; Lussi et al., 2001; Pereira et al., 2001).

O esmalte íntegro apresenta uma pequena quantidade de água e de íons livres em sua estrutura cristalina, o que lhe dá características de baixa condutância elétrica. Durante o processo carioso, os cristais de apatita são desmineralizados e suas dimensões reduzidas, causando aumento dos espaços intercristalinos. Quando estes são preenchidos com água e íons, provenientes da saliva, aumentam a condutância elétrica do dente, permitindo que uma corrente elétrica percorra com maior facilidade o interior da estrutura dentária (figuras 14 e 15). Dessa forma, quanto maior o grau de desmineralização do dente, maior a quantidade de fluido salivar que poderá estar presente por entre os cristais de apatita, fazendo com que a condutividade elétrica do tecido dental seja aumentada.

Figuras 14 e 15: representação esquemática da condutância elétrica no esmalte íntegro e no esmalte desmineralizado

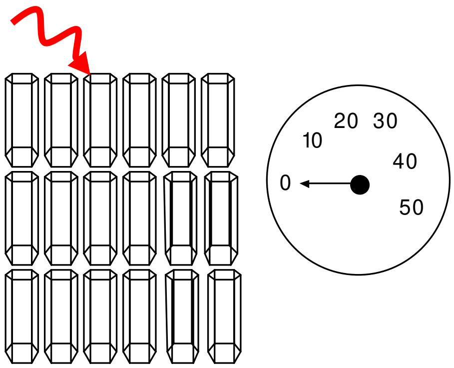


Fig. 14: baixa condutância elétrica quando o esmalte está íntegro

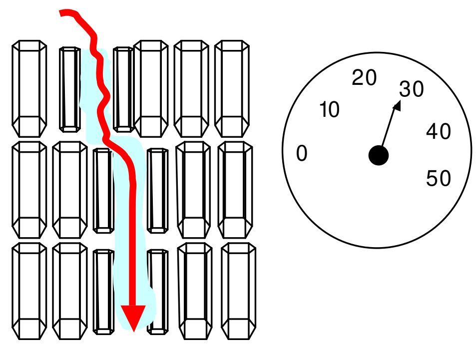
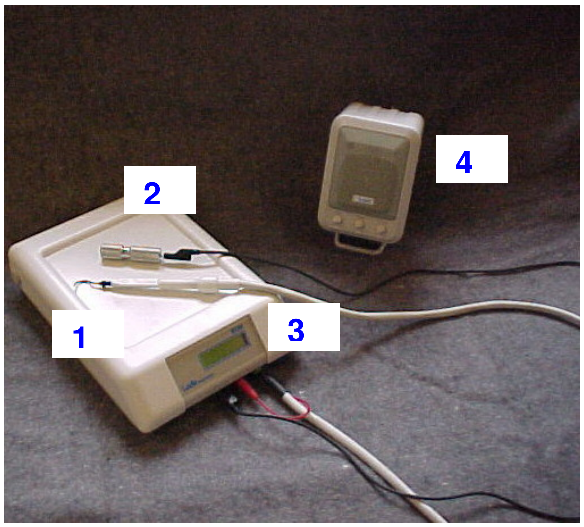
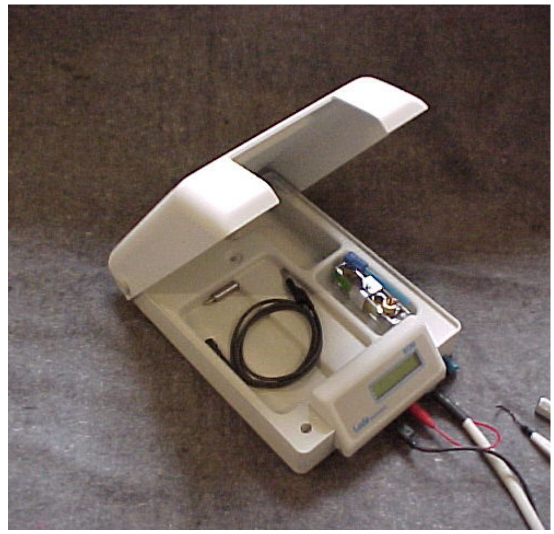


Fig. 15: condutância elétrica aumenta quando o esmalte está desmineralizado

Com base nesses conhecimentos, alguns aparelhos foram desenvolvidos e comercializados e atualmente o aparelho Electronic Caries Monitor ou ECM® (LODE, Groningen, the Netherlands) é o mais conhecido para essa finalidade. O aparelho ECM® opera com uma bateria que produz uma corrente alternada menor que 3mA e freqüência de aproximadamente 21,3 Hz. As medições são realizadas por meio de uma ponteira especial (eletrodo de medição) que apresenta, acoplada ao seu longo eixo, um tubo por onde passa um fluxo de ar contínuo e controlado (cerca de 7,5 l/min), quando se aciona o pedal do equipo odontológico (ao qual está acoplado o aparelho). O aparelho também apresenta o eletrodo de referência, o qual o paciente deve manter em mãos para que o circuito elétrico seja “fechado” e assim as medições possam ser realizadas. Um display localizado na parte frontal do aparelho indica os valores medidos numa escala que pode ir de 0 a 13 ou de 0 a 99, dependendo do modelo utilizado (figuras 16 e 17). Quanto maior o valor medido, maior o grau de desmineralização do tecido dentário. Os dados de alguns estudos indicam que este aparelho é mais sensível que os métodos tradicionais de diagnóstico para a detecção e quantificação da profundidade das lesões cariosas (Ie & Verdonschot, 1994; Lussi et al., 1995; Ricketts et al., 1996, 1997; Ekstrand et al., 1997; Huysmans et al., 1998,1998 a; Pereira et al., 2001).

Figuras 16 e 17: aparelho ECM® (LODE, Groningen, the Netherlands)





Figs. 16 e 17: aparelho ECM® - 1) eletrodo de medição, 2) eletrodo de referência, 3) painel numérico, 4) indicador sonoro

A fim de melhorar-se o desempenho do exame visual para o diagnóstico das lesões cariosas em superfícies oclusais, alguns autores (Ekstrand et al., 1997, 1998, Nyvad et al., 1999) formularam critérios, baseados nas características clínicas das lesões, a partir do exame visual, de forma a melhorar a quantificação das profundidades de desmineralização delas. Um dos métodos mais amplamente aceito para se classificar as lesões cariosas em superfícies oclusais, por meio do exame visual, é o proposto por Ekstrand et al. (1997; 1998) o qual apresenta uma escala ordinal de classificação da profundidade da lesão (Quadro 1) e que tem sido extensamente validado (Ekstrand et al., 1997,1998; Côrtes et al, 2003). Os resultados de alguns estudos demonstraram resultados promissores em relação à utilização dessa escala clínica de classificação, evidenciando valores de sensibilidade e de especificidade próximos aos encontrados nos métodos de diagnóstico supracitados (Ekstrand et al., 1997, 1998; Côrtes et al, 2003).

Quadro 1: Escala de critérios clínicos desenvolvida por Ekstrand et al., 1997,1998.

Quadro – Correlação entre os critérios clínicos e os achados do exame histológico (Ekstrand et al., 1997, 1998)	
Critério Clínico	**Profundidade de penetração (histológico)**
0 – Nenhuma ou leve mudança na translucidez no esmalte após secagem prolongada (>5 seg)	**0 –** Nenhuma desmineralização ou pequena zona superficial apresentando opacidade
1- Opacidade (mancha branca) dificilmente visível em superfície úmida, mas visível após secagem **1a –** Opacidade (sulco pigmentado) dificilmente visualizado em superfície úmida, mas visível após secagem	**1-** Desmineralização do esmalte limitado a 50% da metade externa da camada de esmalte
2- Opacidade (mancha branca) perceptível sem a necessidade de secagem **2a** – Opacidade (pigmentação) perceptível pelo exame visual sem a necessidade de secagem	**2-** Desmineralização envolvendo cerca de 50% da camada de esmalte e 1/3 da dentina
3- Fratura localizada do esmalte (microcavidade) e presença de mancha branca ou pigmentação e/ou descoloração acinzentada na dentina subjacente	**3-** Desmineralização envolvendo o terço médio da camada de dentina
4- Cavitação em esmalte opaco ou descolorido com exposição da dentina subjacente	**4-** Desmineralização envolvendo o terço da camada interna de dentina

De forma a avaliar-se o desempenho de tecnologias emergentes para a detecção e a quantificação da profundidade da lesão cariosa em superfícies oclusais, em relação aos métodos tradicionais de diagnóstico, foi realizado o estudo intitulado "**Comparison of conventional methods and emerging technologies used for occlusal caries detection in permanent teeth**" (Capítulo 2).

Quando se está testando o desempenho de um método de diagnóstico, os resultados dos exames devem ser comparados com os resultados de um teste que confirme o estado verdadeiro da doença. Para alcançar-se este objetivo, os pesquisadores utilizam os denominados "métodos de validação", também denominados de métodos “Gold Standard” (Pine & tem Bosch 1996; Wenzel & Hintze, 1999; Hintze & Wenzel, 2002, 2003).

Para um método de validação ser considerado fidedigno, ele deve preencher três critérios fundamentais (Wenzel & Hintze, 1999, Hintze & Wenzel, 2003):

- o método deve ser preciso (reprodutível);
- o diagnóstico deve ser estabelecido por meio da utilização de um critério o qual defina a doença (preferivelmente, um critério anatomopatológico);
- o método de validação deve ser independente do método de exame que está sendo avaliado.

Para que o primeiro critério seja satisfeito, fazem-se necessários o treinamento e a calibração dos examinadores, que atuarão como validadores, e também a utilização de poucos e bem definidos escores. O segundo critério deve ser alcançado por meio da visualização de áreas de desmineralização nos sulcos e nas fissuras. Observa-se, entretanto, que este critério não é alcançado em estudos “in vivo”, pois o diagnóstico da presença ou da ausência da lesão cariosa só pode ser alcançado por algum tipo de intervenção como cirurgia e preparo cavitário (Wenzel & Hintze, 1999; Hintze & Wenzel, 2002, 2003).

O terceiro critério é facilmente alcançado, porém, em estudos “in vivo” muitas vezes não é observado. Em estudos “in vitro” geralmente, o segundo e terceiro critérios são alcançados, utilizando-se o método histológico como validação (Wenzel & Hintze, 1999; Hintze & Wenzel, 2002, 2003).

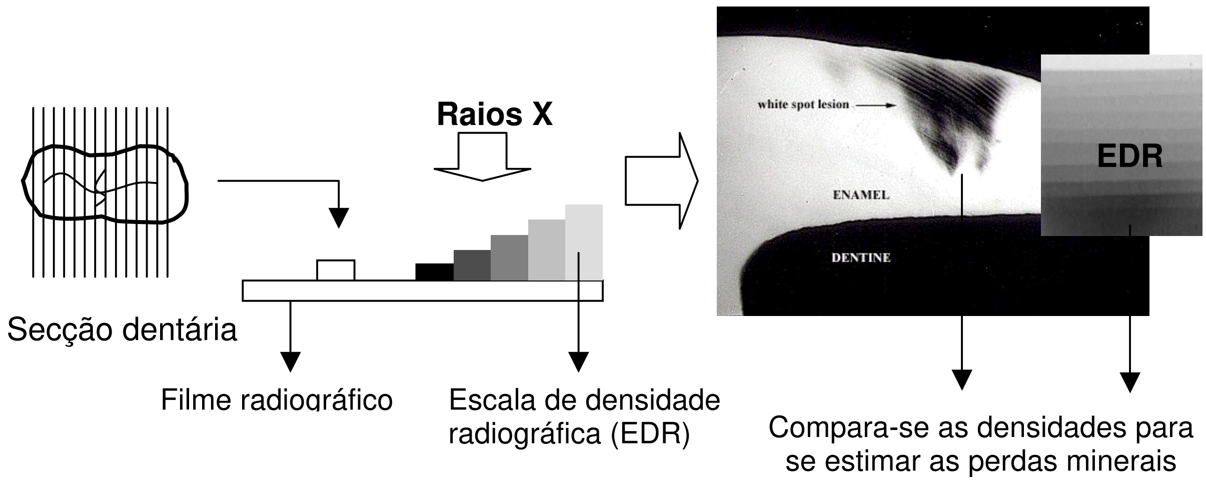
Classicamente, o exame histológico tem sido empregado para se validar o resultado de métodos de diagnósticos em estudos “in vitro” e “in situ” e também nos estudos “in vivo” quando o dente está indicado para ser extraído por razões ortodônticas e/ou cirúrgicas (de Josselin de Jong et al., 1995; Lussi et al., 1995; Ekstrand et al., 1998; Rocha et al., 2003). Entretanto, quanto mais acurado os métodos de diagnóstico se tornam, mais acurado deve ser o método de validação (Ricketts et al. 1998).

O exame de validação histológico realizado por meio de estereomicroscópio é um dos mais utilizados nos estudos para a avaliação “in vitro” dos métodos de diagnóstico (Wenzel, 1994; Ricketts et al., 1996; 1997). Esse tipo de equipamento, também denominado de microscópio estereo-binocular ou microscópio de luz refletida, é geralmente utilizado para a visualização de material opaco sem grandes graus de aumento. Na maioria dos casos, a fonte de luz é externa e refletida sobre o objeto a ser examinado ao invés de ser transmitida através dele, como ocorre em outros tipos de microscópio.

Outro tipo de exame que pode ser utilizado para se avaliar o grau de desmineralização tecidual é a microrradiografia transversa, também denominada de microrradiografia de contato (Arends & ten Bosch, 1992). Segundo essa técnica, a amostra seccionada deve ser plano-paralela e ter espessura bem fina (entre 80mm e 200μm), de forma a que o feixe de raios X a atravesse em ângulo perpendicular a sua superfície (Hintze et al., 1995).

Para se avaliar a profundidade e se estimar as perdas minerais da amostra, é preciso posicioná-la sobre um filme radiográfico que apresente alta resolução, juntamente com uma escala de densidade radiográfica, sendo ambos irradiados por um feixe de raios X. A microradiografia é digitalizada por uma câmera de vídeo ou por um fotomultiplicador (aparelho que transforma energia luminosa em elétrica). A quantidade de minerais presentes na amostra pode ser automaticamente calculada por meio da avaliação dos diferentes níveis de cinza das imagens das secções em relação à escala de densidade radiográfica. Os parâmetros de interesse mais freqüentemente avaliados são o conteúdo de mineral perdido na área da lesão e a profundidade da mesma em relação à superfície.

O esquema 1 mostra como é realizado o exame de microradiografia.



Esquema 1: Realização do exame de microradiografia.

O sistema de aquisição da imagem é conectado a um computador que apresenta um software especial destinado a avaliar as perdas minerais da amostra (Ten Bosch & Angmar-Mansson, 1991; Arends & ten Bosch, 1992).

Atualmente a microscopia confocal de varredura a laser tem sido considerada uma ferramenta valiosa na obtenção de imagens de alta resolução e na reconstrução tridimensional de uma variedade de amostras biológicas, sendo utilizada principalmente em biologia celular (Duschner et al., 1995, 1996). É uma técnica não destrutiva de tomografia microscópica (Engelhardt & Knebel, 1993). Num microscópio convencional a amostra inteira recebe a radiação luminosa de uma fonte de luz de mercúrio ou de xenônio e a imagem pode ser visualizada diretamente através da ocular do microscópio ou captada por um filme fotográfico. Em contraste, na microscopia confocal de varredura a laser, um feixe de luz laser (geralmente de gás He/Ar, operando a 488 nm), de aproximadamente 1mm de diâmetro realiza um movimento de varredura, linha por linha, controlado por espelhos defletores e, apenas a luz refletida ou emitida do exato ponto em foco da amostra é captada por meio de sensores que a transformam em sinais elétricos e os envia para um microcomputador que reconstrói a imagem obtida e a apresenta na tela do computador (figura 18). Alterando passo-a-passo o plano de foco, obtém-se uma pilha de imagens que pode ser reconstruída eletronicamente, formando uma imagem 3D (Duschner et al., 1995, 1996).

Figura 18: exame por meio de microscopia confocal de varredura a laser (MCVL)

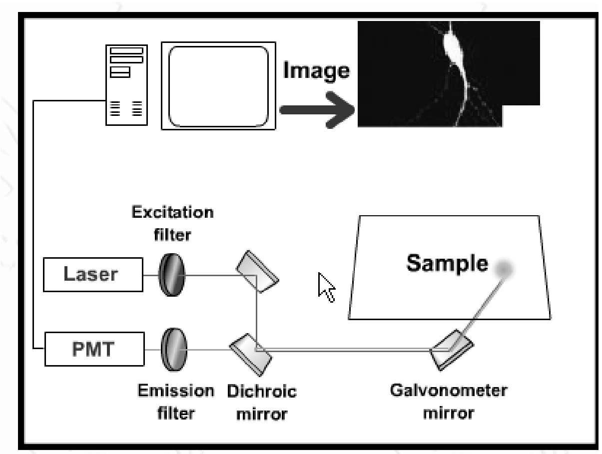


Fig. 18: escaneamento da amostra por meio de MCVL

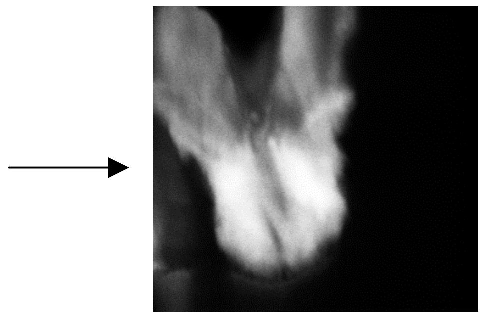


Fig.19: fissura cariada

Quando uma secção histológica é examinada por meio desse método, a luz laser penetra alguns micrômetros abaixo da sua superfície, permitindo a visualização detalhada da estrutura do esmalte subsuperficial (Watson, 1991; Duscher et al., 1996).

Considerando-se que a avaliação do desempenho diagnóstico de um método depende do resultado obtido a partir do método de validação utilizado no estudo e, considerando-se que há poucos trabalhos desenvolvidos nessa área, foi realizado o estudo "**Comparison of validation methods used in dental caries detection**" (Capítulo 3).

2. Proposição

O presente trabalho é composto por 3 estudos tendo como objetivo geral avaliar "in vitro" a eficácia e a reprodutibilidade de diferentes métodos de diagnóstico para a detecção e quantificação da profundidade das lesões cariosas em superfícies oclusais e os seguintes objetivos específicos:

1. Avaliar "in vitro" o desempenho de dois sistemas CCD (Charge Couple Device) de radiografia digital, ou seja, o sistema CDR (Schick Technologies, EUA) e o sistema Sidexis (Sirona, Bensheim, Alemanha) em relação ao método radiográfico convencional para a detecção de lesões cariosas em superfícies oclusais, utilizando o exame histológico como método de validação;

2. Comparar "in vitro" o desempenho de 5 métodos de diagnóstico, ou seja, o exame clínico-visual, o radiográfico convencional, o aparelho QLF (Quantitative Light-induced Fluorescence - Inspektor Research System BV, Amsterdam, Holanda), o aparelho ECM (Electronic Caries Meter - LODE, Groningen, Holanda), e o aparelho DIAGNOdent (Kavo, Biberach, Alemanha) para a detecção de lesões cariosas em supefícies oclusais;

3. Comparar o desempenho de vários métodos de diagnóstico em detectar e estimar a profundidade das lesões cariosas em superfícies oclusais por meio dos resultados obtidos a partir de três métodos de validação, ou seja, exame estereomicroscópio, microrradiografia e microscopia confocal de varredura a laser, analisando também, a correlação entre estes métodos.

3. Estudos

3.1. Estudo 1

EVALUATION OF THREE RADIOGRAPHIC METHODS PERFORMANCE IN THE DETECTION OF OCCLUSAL CARIES LESIONS

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Antonio Carlos Pereira - DDS, MPH, Dr PhD *
Fábio Luiz Mialhe - DDS, MSc *
Hafsteinn Eggertsson, DDS, MSc, PhD**
Analoui Moustafa, DDS, MSc, PhD**
Domenick T. Zero, DDS, MSc **

* University of Campinas, São Paulo, Brazil
** Indiana University, Indianapolis, USA

Corresponding author:
Prof. Dr. Antonio Carlos Pereira
Faculdade de Odontologia de Piracicaba – FOP/UNICAMP
Departamento de Odontologia Social
Av. Limeira 901 – Areião CEP:13414-903 Piracicaba-SP
Tel: 19-3412 5209 FAX: 19-3412-5218 e-mail: apereira@fop.unicamp.br

ABSTRACT

The aim of this study was to evaluate, in vitro, the performance of three radiographic methods for the detection of occlusal caries at D1 diagnostic threshold in permanent teeth. Ninety-six molar teeth with no apparent occlusal cavitation were selected and photographed. One site was recorded in the photographs so they could be located in all examinations. The teeth were then radiographed under standardized conditions using a conventional E-plus films (Kodak, USA) and two CCD-based sensor systems that is, CDR (Schick Technologies, USA) and Sidexis (Sirona, Bensheim, Germany). The films were developed and viewed on a view-box and the digital images were captured using the representative software and viewed on a computer screen. Two examiners viewed all films and images, recording the presence and lesion depth. One quarter of the teeth were re-examined for intra-examiner repeatability. The teeth were subsequently hemisectioned through the selected investigation site using a diamond saw and examined under a stereomicroscope. Diagnostic accuracy of each method were evaluated by calculating the sensitivity, specificity and the area under ROC curve. Out of 96 occlusal surfaces, 41 were sound, 31 had lesions in enamel, and 24 had dentin lesions. Inter-examiner repeatability varied from substantial for the almost perfect and was better for the digital images than the conventional films. The area under the ROC curve for enamel and dentine caries (at D1diagnostic threshold) was 0.61 for films, 0.68 for CDR and 0.54 for Sirona. There was no significant difference in diagnostic accuracy between conventional Kodak Ektaspeed- Plus film and the two CCD-based sensor systems.

UNITERMS: Digital radiography, dental caries, ROC curve, diagnostic test

1- INTRODUCTION

Caries prevalence and disease pattern has changed in the past three decades in most industrial nations. The caries reduction has been site dependent and the oclusal surfaces of teeth are now the sites most frequently affected by dental caries [11,16] .

The data from several studies have shown that bitewing radiographs provide more information compared to visual inspection alone [4,19] . Thus, there have been attempts to improve the reliability of conventional radiographs since the number of deep oclusal lesions (known as “hidden caries”), which are not easily detected clinically, is increasing due to use of topical fluorides[26]. Recent year’s developments have made it possible to use computer technologies to acquire and display digital radiographic images[29]. Advances in radiographic detection methods include the development of several digital radiography systems for dental use. Nowadays there are two types of receptors for direct digital image acquisition in intraoral radiography: the CCD-based image detector and the storage phosphor systems. In CCD systems, a wire connects the sensor to the computer, and image is displayed almost immediately on a computer monitor after exposure of the sensor. In the other system, a storage phosphor plate is exposed to x-radiation and a latent image is created. The information contained in the plate is released by exposure to a laser scanner[29]. These systems have shown a number of advantages compared with conventional film radiography e.g. the numbers of retakes could be reduced and wet processing errors be avoided[29].

Several digital radiographic systems are disposal for the clinicians, who are replacing conventional radiography. However, minimal data is available on the diagnostic differences between intra-oral digital systems and conventional film radiographs for the detection of occlusal caries lesions[10]. In addition, there are few studies evaluating the diagnostic efficacy of CCD-based sensor systems for that purpose. Hence, there is a need for continual evaluation of the performance of these methods regarding their clinical performances in order to generate informations that could help the dentists to select the best system for their clinical purposes.

In view of these considerations, the aim of this study was to compare the accuracy of conventional film radiographs and two CCD-based sensor systems for the detection of caries lesions in occlusal surfaces.

2- MATERIAL AND METHODS

Ninety six extracted permanent molars without fillings which had been stored in 10% buffered formalin were selected for this study. All teeth were without macroscopic oclusal cavity formation, while varying degrees of fissure discoloration could be present. None of the teeth had clinically visible approximal caries. The teeth were first cleaned with a prophylaxis brush and a pumice slurry, rinsed with a 3-in-1 syringe and mounted in plaster blocks in sets of 3 teeth simulating anatomic positions. The occlusal surfaces were photographed (x 4), and one site was recorded in the photographs. The teeth were then radiographed under standardized conditions and the exposures were made using a General Electric GE 1000 Intra-oral X-ray machine operating at 70 kVp and 10 mA. The blocks of teeth were placed in a holder specially designed to provide standardized projection geometry during exposure. The focus-film distance was 21 cm and a 15-mm thick soft tissue equivalent material was placed between the cone end and the teeth blocks.

The radiographs were taken using a conventional dental film (Ektaspeed Plus-Eastman Kodak Co, Rochester, NY, USA) and two CCD-based sensor systems, that is, Sidexis (Sirona, Bensheim, Germany) and CDR (Schick Technologies, NY, USA) were used.

The films were developed in a Dürr automatic processor (1330, AC 245 L, Bietighein-Bissingen, Germany). The processed radiographs were mounted in frames and examined using a viewing box and a dental x-ray viewer (magnification x 4).

The digital images were displayed on a SVA 17-inch monitor screen.

The conventional radiographs and the digital images were examined by two independent observers.

Examination Methods

The selected recorded sites in the photographs were used to locate the precise investigation site on the radiograph in a mesial-distal plane. Oclusal caries depth in the radiographs were assessed on the rank scale by the criteria[28]: 0 = no caries, 1 = radiolucency extending to the ½ outer of the enamel, 2 = radiolucency extending to the ½ inner of the enamel, 3 = radiolucency extending to ½ the outer of the dentin, 4 = radiolucency extending to the ½ inner of the dentin. One week elapsed between individual sets of recordings.

After one week, twenty-four teeth were re-examined for intra-examiner repeatability.

The diagnostic performance of each observer with the three recording systems was compared with the histological diagnosis.

Examiners training

Prior the experimental fase, a training was performed in a two-hours of theory and four-hours of practice to calibrate the examiners. After that, a pilot test was accomplished using nine teeth.

Histological preparation and examination

For the validation of the true presence or absence of caries, the teeth were sectioned in a bucco-lingual direction through the selected investigation site using a Silverstone Taylor microtome (Scifab, Lafayete, Colo, USA). The histologic examination was done with a stereomicroscope (Model BH2, Olympus Optical Co., Ltd., Tokyo, Japan) with at x 40 magnification and performed by two different observers (joint decisions). Both sides of each tooth section were examined. Caries were defined to be present when a demineralization seen as white or discolored (yellow/brown) area was observed. The following classification criteria was applied: 0 = no caries, 1 = demineralization extending to the ½ outer of the enamel, 2 = demineralization extending to the ½ inner of the enamel, 3 = demineralization extending to the 1/2 outer of the dentin, 4 = demineralization extending to the outer ½ of the dentin.

Statistical analyses

To assess the intra-examiner agreement, 25 teeth were re-examined. Intra-examiner agreement and inter-examiner agreement for the x-ray methods were assessed using weighted kappa statistics[14]. Diagnostic performance for occlusal in occlusal enamel or deeper (cut-off ≥ 1) was evaluated using the parameters sensitivity and specificity, and area under ROC curve (Az), with the histological ratings as a gold standard (at D1 threshold: caries in enamel in histological sections was considered "positive") using generalized estimating equation methods (GEE) applied to logistic regression models. The use of GEE methods are necessary because of correlations between observations due to multiple examiners and multiple methods for each specimen. Pairwise comparison between the methods were made using the Sidak method to adjust for multiple comparisons: adjusted p-value = 1–(1-p). Comparisons were considered to be statistically significant if the adjusted p-value was less than 0.05 (or if the unadjusted p-value was less than 0.0034). Area under the ROC curve was computed using the c-statistic from logistic regression models for each examiner for each method.

3- RESULTS

According to the histological examination, 41 teeth were sound, 31 had caries confined to the enamel, and 24 had caries in dentin.

Intra-examiner repeatability was substantial for conventional film (EF) (Kappa = 0.65-0.70), and for the CCD digital systems (Kappa = 0.68). Inter-examiner agreement was on the other hand better for the digital images (0.71-0.80) than the conventional film (0.36).

The results of the diagnostic parameters are summarised in table 1. In general, CDR had the highest values of sensitivity, specificity and diagnostic accuracy whereas the Sidexis had the lowest values. However, there were no statistically significant differences among the radiographic methods (p>0.05)

Table 1: Diagnostic performance for enamel and dentinal caries diagnosis of the three radiographic systems and two examiners expressed in sensitivity, specificity and accuracy.

	Sensitivity			Specificity			Diagnostic Accuracy		
Method	Exam.1	Exam.2	mean	Exam.1	Exam.2	mean	Exam.1	Exam.2	mean
EF	0.44	0.69	0.56a	0.63	0.68	0.65a	0.52	0.69	0.60a
CDR	0.56	0.60	0.58a	0.73	0.76	0.74a	0.63	0.60	0.61a
Sidexis	0.34	0.45	0.39a	0.63	0.71	0.67a	0.47	0.50	0.49a

Different superscript letters show statistical differences between diagnostic methods (p<0.05)

The area under the ROC curves for the two observer's assessments for the occlusal surfaces in relation to depth are shown in Table 2.

Table 2: ROC curve area for caries detection by the two observers.

Method	Examiner 1	Examiner 2	Average
EF	0.54	0.69	0.61a,b
CDR	0.67	0.69	0.68a
Sidexis	0.50	0.58	0.54b

Different superscript letters show statistical differences between diagnostic methods (p<0.05)

The average ROC curve areas ranged from 0.54 (Sidexis) to 0.68 (CDR). The average scores of the two observers were used to calculate ROC curves for the three radiographic systems. CDR tended to be the most accurate system, followed by conventional film and Sidexis conventional film and Sidexis. No statistically significant differences were found among conventional radiography and the two digital radiographic systems ($p>0.05$). Only the differences between CDR and Sidexis were statistically significant ($p<0.05$).

4- DISCUSSION

The literature is still sparse in studies evaluating the accuracy of Sidexis and CDR dental digital imaging systems for the detection of occlusal caries lesions[10] and until the moment, there are none study evaluating the diagnostic accuracy of CDR Schick and just one10 of Sidexis for that purpose. Studies in this field of the knowledge were mainly carried out using a storage phosphor digital system [2,8,27] (Digora – Sorendex, Helsinki, Finland). The majority of the studies comparing these two CCD systems evaluated the performance of methods for the detection of approximal caries lesions [10,13,22,23,30].

In relation to the digital radiographic systems, there was a significant difference in accuracy between Schick and Sidexis ($p=0.03$). In spite of there not being studies in the literature comparing these two methods for the detection of occlusal caries lesions, the data obtained by other studies have demonstrated that there were also significantly differences in the diagnostic accuracy among CCD digital radiographic systems. The mean area under ROC curve for Sidexis system (0.54) in the present study was smaller than that obtained by Hintze et al. (0.8-0.92). However, in that study the authors considered that the cutoff for the presence of disease was caries in dentine which naturally increase the diagnostic performance of the radiographic methods. The data of some studies demonstrate that Sidexis presents a poorer contrast of its images of dental structures, hindering the differentiation of relevant details [17,21]. To achieve optimal image clarity of the areas that were diagnosed, the authors modified the images by adjusting the contrast and brightness, in which it could have influenced the performance of the method, fact this it was not carried out in the present study. In addition, the data of other study[13] shows that Sidexis system underestimated in 51% the depth of approximal caries lesions compared to the gold standard examination. According to Pfeiffer et al.[17] it would happen due to the fact that automatic optimizing of Sidexis system, during which the 12-bit digitized image, is reduced to 8-bit displayed image.

In relation to CDR, a new version of this equipament was introduced in 1998[3]. It has a sensor with a smaller pixel size and uses so-called Active Pixel Sensor (APS) and Complementary Metal-Oxide Semiconductor (CMOS) technologies which improved the physical performance of this direct digital radiographic sensor when compared with the earlier generation[3]. Analoui[1] suggest that based in their inherent characteristics, the new technologies employed in the CCD sensors have potential for surpassing film in virtually all of relevant aspects. In spite of there not being studies relating the performance of this new model of CDR in detecting carious lesions, these technical aspects should be reassessed in other studies in order to assess if this system is so or more efficient than the conventional radiographic film for this purpose.

The poor performance of the radiographic methods evaluated in the present study may be attributed to several reasons. It is well-known that radiograph could not detect initially demineralised occlusal enamel and dentine lesions, resulting in low sensitive values. In the present study, of all teeth presenting caries lesions, 56.3% had caries in enamel and 36.3 presented carious lesions extending to the half outer of the dentin and just 7.4% of the lesions was located in the half inner of the dentin, which can underestimate the performance of the methods. As such, there are two aspects of the study design which restrict the interpretation of the results. First, as none metal strip was fixed by tape on the pre-selected site before exposure in order to secure its precise identification, the localization of clinical site on radiographic examination was not precise. This fact could allow that the examiners evaluated different sites from those selected at the individual photographs. Second, the histological sections were made in a bucco-lingual direction through just the selected investigation. In spite the study of Ekstrand et al.[5] used the same methodology, the majority of the studies evaluating the performance of radiographic methods in detecting occlusal caries lesions sectioned serially the teeth in various sections into buccolingual direction [2,8,9,10,12]. It is obvious that in these studies, the probability of a carious lesions be found is larger in and the diagnostic performance of the method be better in that studies. However, many times in the daily clinical practice, the dentist wants to know the radiographic extension of a clinical detectable lesion in a single site in the occlusal surface. It is suggested in this way, that new studies be carried evaluating the site-specific performance of radiographic methods.

In spite of the examiners didn't enhanced the images by manipulating brightness and contrast, it looks, from the data of other investigations, that there are not significant differences in accuracy between enhanced and unenhanced images [23,25,27]. In addition, it looks that dentists in their clinical practices use the enhancement facilities of digital radiographic systems (manipulation of brightness and contrast) very differently and enhancements not properly used may actually reduce diagnostic accuracy[7,23]. However, others studies should be carried to evaluate if the management of this variables can influence the accuracy of the tested methods.

The present study demonstrates the diagnostic equivalence of digital systems with conventional film using similar viewing conditions. These findings agree with the data presented by others studies wich verified that digital intra-oral radiographic systems seem to be as accurate as current conventionl dental films for the detection of occlusal caries lesions [22,23,30].

In conclusion, there was no significant difference in diagnostic accuracy between conventional Kodak Ektaspeed-Plus film and the two CCD systems evaluated. The CDR Shick system was significantly inferior to the Sidexis system.

Resumo

O objetivo deste estudo ”in vitro” foi avaliar três diferentes métodos radiográficos para a detecção de lesões cariosas em superfícies oclusais. Noventa e seis dentes molares extraídos foram radiografados sob condições padrão utilizando filmes convencionais Ektaspeed Plus (Kodak, USA) e dois sistemas de sensores digitais CCD: o sistema CDR Schick (Schick, Technologies, USA) e o sistema Sidexis (Sirona, Bensheim, Alemanha). Os filmes convencionais foram processados e visualizados em um negatoscópio enquanto que as imagens digitais foram capturadas utilizando sistemas próprios e visualizadas no monitor. Dois examinadores avaliaram a presença e profundidade da lesão. Um quarto dos dentes foi re-examinado para o cálculo da reprodutibilidade de intra-examinador. Os dentes foram subseqüentemente seccionados e examinados por meio de um estereomicroscópio. Das 96 superfícies oclusais examinadas, 41 estavam hígidas, 31 apresentavam lesões em esmalte e 24 lesões de dentina. A reprodutibilidade interexaminador foi considerada melhor para as radiografias digitais do que para o filme convencional. A área calculada abaixo da curva ROC foi de 0,61 para o filme convencional, 0,68 para o sistema CDR e 0,54 para o sistema Sirona. A única diferença estatisticamente significante encontrada ($p<0,05$)) foi entre os sistemas digitalizados CDR e Sidexis. Concluindo-se, não houve diferenças entre o desempenho do filme radiográfico convencional Ektaspeed-Plus e os dois sistemas digitais CCD para a detecção “in vitro” de lesões cariosas em superfície oclusais de molares.

UNITERMOS: Radiografia digital, cárie dentária, curva ROC, teste de diagnóstico.

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3.2. Artigo 2

Comparison of conventional methods and emerging technologies used for occlusal caries detection in permanent teeth

Manuscrito enviado ao periódico Caries Research

Antonio Carlos Pereira - DDS, MPH, Dr PH *
Fábio Luiz Mialhe - DDS, MSc *
Hafsteinn Eggertsson, DDS, MSc, PhD**
E. Angeles-Martinez Mier, DDS, MSc, PhD **
Carlos Gonzéles-Cabezas, DDS, PhD**
Domenick T. Zero, DDS, MSc**

* University of Campinas, Piracicaba, São Paulo State, Brazil
** Indiana University, Indianápolis, USA

Corresponding author:
Prof. Dr. Antonio Carlos Pereira
Faculdade de Odontologia de Piracicaba – FOP/UNICAMP
Departamento de Odontologia Social
Av. Limeira 901 - Areião
CEP:13414-903
Piracicaba-SP
Tel: 19-430 5209 FAX: 19-430-5218
e-mail: apereira@fop.unicamp.br

Abstract

Nowadays, the premature diagnosis of carious lesions on occlusal surfaces has been assuming increasing importance due to the changes in the prevalence and pattern of dental caries. Several new methods have emerged in recent years to improve the diagnosis of occlusal caries. The purpose of the present study was to compare the reproducibility and accuracy of some of these diagnostic methods. Ninety-six extracted permanent molars with macroscopically intact occlusal surfaces were selected. Three examiners were trained to perform the examinations by using visual inspection (VI), bitewing radiography (RX), electrical conductance measurement (ECM), quantitative laser/light-induced fluorescence (QLF) and laser fluorescent examination (DIAGNOdent). Twenty-five percent of the teeth were re-examined for repeatability. Stereomicroscopic examination was used as a gold standard. Intra- and interexaminer agreement were assessed by using weighted kappa statistics, except for ECM and DIAGNOdent (intraclass correlation). All methods were compared considering differences in accuracy, sensitivity, and specificity using generalized estimating equation methods applied to logistic regression models. Intra- and interexaminer agreement ranged from substantial to almost perfect. Areas under the ROC curves were 0.82, 0.60, 0.79, 0.84 and 0.88, respectively for VI, RX, ECM, QLF and DIAGNOdent examinations and none statistically significant differences were observed among these methods, except for RX. Although appealing, emerging technologies did not improve significantly the detection of occlusal caries when compared to traditional methods. Further studies using different classification criteria should focus on the applicability and effectiveness of traditional methods or on the combination of the conventional methods with emerging technologies.

Key words: occlusal caries, visual inspection, bitewing radiography, DIAGNODent, Electronic caries meter, QLF

Introduction

Changes in the epidemiological patterns of dental caries, mainly a reduction on prevalence and severity, have been observed around the world during the past three decades (Beltran-Aguilar, 1999; Hugoson, 2000). The caries reduction has been considered as site dependent and the occlusal surfaces of teeth are now the sites most frequently affected by the dental caries (Mejàre et al., 1998; Pereira et al., 1999; Hugoson et al., 2000). A reduction in the rate of the lesion progression resulted in changes in the morphology and pattern of the lesions, increasing the difficult to detect and assess the extent of the lesion (Weerheijm et al., 1989; Pitts, 1997; Shi et al., 2000).

The detection of carious lesions in earliest stages is very important to prevent the progression of disease before the occurrence of cavitation (Ismail, 1997). Unfortunately, traditional diagnostic systems used for the detection of caries lesions, such as visual inspection and radiography are inaccurate and insensitive when diagnosing occlusal caries prior to cavitation (Ricketts et al., 1995; Atrrill & Ashey, 2001; Bader et al., 2002). New non-invasive, instrument based, techniques for the detection and quantification of demineralization have been developed and tested in order to improve the accuracy of diagnosis (Ashely et al., 1998; Ferreira-Zandona et al., 1998; Lussi et al., 1999; Shi et al., 2000; Angmar-Månsson et al. 2001; Pereira et al., 2001; Shi et al., 2001; Bader et al., 2002; Côrtes et al., 2003; Côrtes & Ellwood, 2004). These techniques include electrical conductance measurement (ECM), light scattering and quantitative laser/light-induced fluorescence (QLF).

Electrical conductance measurement is based on the detection of the increase of the electric conductivity of teeth resulting from the reduction of mineral amount in carious lesion in enamel. The high electrical resistance of sound dental tissue decreases when demineralization creates pores, which are filled by water and soluble electrolytes. Studies comparing visual, radiographic and electrical conductance measurements showed the ECM was the most accurate and reliable method for detecting occlusal caries in posterior teeth (Lussi et al., 1995; Ismail, 1997; Ashley, 1998; Huysmans et al., 1998b).

In 1998, a portable laser-based diagnostic system called Kavo DIAGNOdent (Biberach, Germany) was introduced in the market to aid clinicians to detect occlusal caries. This device emits a red laser light (l=655 nm) which is absorbed by both inorganic and organic components inside tooth structure. Part of the light can be re-emitted as infrared fluorescent light. The fluorescent radiation is registered and evaluated by the device presenting to the operator a digital read-out. The fluorescent light emission is proportional to the severity of carious lesions. DIAGNOdent has shown better accuracy and reproducibility than conventional methods (Lussi et al., 1999; Attrill et al., 2001; Shi et al., 2000; Shi et al., 2001).

Other method is called quantitative light-induced fluorescence (QLF) and it is based on the auto-fluorescence properties of the teeth. The surface of interest is exposed to the violet-blue light (l=290-450 nm) at 13 mW/cm2 emitted from the camera handpiece. The tooth structure emits light in green shades, which are acquired by a miniature CCD camera in the handpiece through a 520 nm wave-length high-pass filter. The images produced are stored, processed, and analyzed by the proprietary software (QLF, Inspektor Research Systems BV, Amsterdam, The Netherlands). The potential of QLF to detect occlusal caries were previously investigated by a small number of studies (Angmar-Månsson et al., 1996; Ando et al., 2000).

The possibility to observe changes in the lesion over the time is one of the advantages of quantitative diagnosis methods (Huysmans et al., 1998). The aim of this study was to compare, in vitro, traditional examination techniques and emerging technologies used to detect occlusal caries in permanent molars without apparent cavitations.

Materials and methods

Sample Material

Ninety-six extracted permanent molars exhibiting macroscopically intact occlusal surfaces and complete root formation were selected from a pool. Teeth with dental fluorosis, tetracycline stain, hypoplasia, clinically visible proximal caries or dental restorations were excluded. Different degrees of discoloration in the fissures were acceptable.

All teeth were first cleaned by prophylaxis through brush and pumice slurry, rinsed by water-jet of 3-in-1 syringe and all teeth were mounted in plaster blocks in sets of three teeth. The occlusal surfaces were photographed (magnification x 4) and one site in each picture received a mark to allow localization. All teeth were stored under refrigeration in thymol-saturated saline in individual plastic containers.

Three examiners were submitted to calibration by training (2h of theory and 4h of practice). After this procedure, they performed a pilot test using nine supplementary teeth prepared as previously described, which were submitted to the following five diagnostic methods.

1-Visual Inspection (VI)

Each site previously chosen was examined by conventional clinical examination, one week after sample selection. Visual inspection was carried out by using dental mirror and operating light. Probing was not allowed.

Each surface was ranked through the following criteria (Ekstrand et al., 1997): 0 = no or slight change in enamel translucency after prolonged air-drying (>5s); 1 = opacity or discoloration hardly visible on the wet surface, but distinctly visible after air-drying; 2 = opacity or discoloration distinctly visible without air drying; 3 = localized enamel breakdown in opaque or discolored enamel and/or grayish discolored from the underlying dentine; 4 = cavitation in opaque or discolored enamel with dentine exposition.

2-Bitewing Radiography (RX)

Each block was placed in a holder specially designed to provide standardization during exposure. The focus distance was 21 cm and a 15 mm-thick plastic compound equivalent to the soft tissues was placed in front of blocks.

A conventional film (Ektaspeed Kodak) was used to take the radiography (70 kVp and 10 mA) of each block. The evaluation was carried out using a light box and a magnification glass (x 4). The presence and extent of lesions were classified following the codes: 0 = no caries; 1 = radiolucency extending to the ½ outer of the enamel; 2 = radiolucency extending between ½ inner of the enamel; 3 = radiolucency extending to the ½ outer of the dentin; 4 = radiolucency extending to the ½ inner of the dentin.

3-Electrical Conductance Measurement (ECM):

Electrical conductance method was performed by using the Electronic Caries Monitor III (ECM III, LODE, Groningen, The Netherlands) in the fissure surfaces at the selected investigation site.

Each block of teeth was placed in a small vial with the teeth roots suspended in physiological saline, which was in contact with the reference electrode. Before each measurement, the occlusal surface of the tooth was moistened with physiological saline and a toothpaste gel was applied in the fissure after gently air-drying.

Measurements were obtained in the previously identified sites by touching the probe of the ECM on the fissure enamel until stable readings were obtained right after the co-axial air-flow (7.5 l/min) were applied. ECM readings ranged from 0.00 (lowest conductance) to 99.99 (highest conductance). The average of three examinations on each site was considered as the definitive score.

4-Laser Fluorescent (LF) Examination

LF measurements were performed by using the DIAGNOdent device (Kavo, Biberach, Germany) previously calibrated through a porcelain standard provided by the manufacturer.

The probe A was individually adjusted by holding the tip against a sound-smooth enamel surface and pressing the ring button until complete calibration. The conical probe was then positioned perpendicularly and rotated around its long axis on each previous selected site. The average of three readings (0-99 range) on each site was considered as the definitive score.

5-Quantitative Light-induced Fluorescence (QLF)

Images of occlusal surfaces were captured by using the portable intra-oral camera (Panasonic WV-KS 152) connected to a computer. Each occlusal surface was illuminated with 13 mW/cm2 of the violet-blue light. The images were captured, stored, processed and analyzed by the proprietary software (QLF, Inspektor Research Systems BV, Amsterdam, The Netherlands).

The following scoring criteria were used: 0 = no change in enamel fluorescence; 1 = slightly changes in enamel fluorescence; 2 = loss of fluorescence distinctly visible without enamel broken; 3 = loss of fluorescence distinctly visible with enamel broken; 4 = loss of fluorescence distinctly visible with cavitation.

Histological Validation

After all assessments were completed, the teeth were removed from blocks and serially sectioned by a diamond-coated disc in a water-cooled microtome (Silverstone Taylor, Scifab, Lafayete, Glues, USA). The sections, which had approximately 200 µm thickness, were obtained perpendicularly to the occlusal surfaces in a buccolingual direction through the previously chosen site.

The histological examination was performed in a stereomicroscope (magnification x 25 to 40) using reflected light. Both sides of each tooth section were examined.

The histological criteria used to classify the caries lesion depth were: 0 = no caries; 1 = demineralization extending to the ½ outer of the enamel; 2 = demineralization extending to the ½ inner of the enamel; 3 = demineralization extending to the ½ outer of the dentin; 4 = demineralization extending to the ½ outer of the dentin.

Statistical Analysis

Twenty-five teeth were re-examined to obtain the intraexaminer and interexaminer agreement. Since the scores obtained for intra- and interexaminer reproducibility considering VI, RX and QLF were ordinal, weighted kappa statistic was used (Landis & Kock, 1977). Intra- and interexaminer reproducibility for ECM and DIAGNOdent were calculated through intraclass correlation coefficient (ICCs) (Shrout, 1979).

The differences in sensitivity, specificity and percentage of correct diagnostics of all methods were compared by generalized estimating equation (GEE) applied to logistic regression models (Zegher, 1992), considering the histological ratings as gold standard (at D1 threshold: enamel caries in histological sections was considered "positive"). Sensitivities and specificities were calculated considering the following cutoffs: > 0 ratings for VI; ≥ 2 for QLF and ≥ 15 for ECM. Considering DIAGNOdent method, the cutoff was ≥ 5 (Lussi et al., 1999).

The use of GEE methods was necessary because correlations between observations performed for multiple examiners and multiple methods for each specimen were used.

Pairwise comparison among the methods was performed by using the Sidak method adjusted for multiple comparisons (adjusted p-value = 1–(1-p)). The comparisons were considered statistically significant when the adjusted p-value was less than 0.05. The area under the ROC curve was computed using the c-statistic from logistic regression models for each examiner considering each method at D1 diagnostic threshold. This parameter was considered an indicator of overall diagnostic performance. The relationship between each ranked scoring system and the histological scoring system were assessed using Spearman correlation test.

Results

The histological examination revealed that 41 sites (42.7%) were sound; 31 (32.3%) had enamel demineralization and 24 (25.0%) had demineralization extended to dentine.

Table 1 shows weighted kappa values for intra- and interexaminer reproducibility at D1 diagnostic threshold considering VI, RX and QLF. Intraexaminer reliability was considered substantial to perfect for the three examiners and the interexaminer reliability was fair to moderate among the examiners.

The ICCs for intra- and interexaminer reproducibility considering DIAGNOdent and ECM are presented in Table 2. Intraexaminer agreement was substantial for both methods, except for examiner 1 using ECM. Interexaminer agreement was generally fair to moderate. Considering the DIAGNOdent, the scores of examiner 3 were significantly lower than the scores of the other examiners.

Sensitivity, specificity, accuracy and the average of the area under the ROC curve (Az) considering each method at D1 diagnostic threshold assessed by all examiners are shown in Figure 1. DIAGNOdent showed the best results considering all parameters, while bitewing radiography showed the worst results. Considering the Az parameter, none statistically significant differences were found among methods, except for bitewing radiography.

Table 3 shows the unadjusted p-values for sensitivity, specificity, accuracy and Az considering the methods at D1 diagnostic threshold for occlusal caries using histological validation. The performance of bitewing radiography was statistically significant lower in comparison to the other methods studied considering all parameters. Considering the area under the ROC curve, it was not observed statistically significant differences among all methods, except for bitewing radiography.

Spearman correlation coefficients values considering examiners and methods are shown in Table 4. The DIAGNOdent showed the higher positive correlation with histological examination.

Discussion

Considering the recent knowledge about the dynamics of the carious process, the preventive methods can be useful to treat initial lesions can be treated more efficiently than operative procedures (Ismail, 1997). Diagnostic methods able to accurately detect dental caries at very early initial stage remain a challenge for dental research.

The typical visual diagnosis of occlusal caries has a very low sensitivity and high specificity (Bader, 2002). However, in the present study, substantial sensitivity (0.81) and specificity (0.73) were found considering this method. Other previous studies (Ekstrand et al., 1997; Pereira et al., 2001; El-Housseiny et al., 2001; Cortês et al.; Rocha et al., 2003) showed similar sensitivity (0.80-0.98) and specificity (0.38-0.98) at D1 and D2 diagnostic threshold using visual scores first related by Ekstrand et al. (1997), which showed higher sensitivity than the ones commonly reported.

In the present study, the better performance of the visual inspection compared with other methods could be also attributed to shallow or wide fissures in the majority of used teeth. Previous studies showed a decreased of 30% in the overall sensitivity, specificity and the percentage of teeth correctly diagnosed in narrow fissures in comparison to wide fissures (Lussi, 1996; Housseiny et al., 2001). Thus, the visual detection of caries in occlusal surfaces could be also dependent on the fissure morphology.

Similarly to Côrtes et al. (2003), a moderate relationship between histological lesions depth and visual criteria (0.54-0.65) was found among examiners. However, other authors (Ekstrand et al., 1997 and 1998) obtained smaller indexes, maybe due to the absence of differentiation between histological demineralization limited to enamel or extended to the dentine.

The poor performance and weak correlation with histological lesion depth of the bitewing radiography verified at the present study could be attributed to some reasons. First, it is well established that radiography could not detect initial demineralization on occlusal enamel and dentine lesions, resulting in low sensitive values (Pitts, 1996). At the present study, considering all teeth presenting caries, 56.3% had caries in enamel; in 36.3 % carious lesions extending to the half outer of the dentin were verified and just 7.4% of the lesions were located in the half inner of the dentin, which could underestimate the performance of bitewing radiography.

Two aspects of the present study design could be responsible for restricted interpretation of the results. First, a metal strip was not fixed on the pre-selected site before exposure in order to guarantee the precise localization of clinical site on radiographic examination. For this reason, the examiners could have evaluated different sites from those really selected at the individual photographs.

Second, in the present study, the histological sections were performed perpendicularly to the occlusal surfaces in a buccolingual direction through each selected investigation site. Although the study of Ekstrand et al. (1997) has used the same methodology, other studies observed various sections (buccolingual direction) in serially sectioned teeth (Hintze et al., 1995; Ashley et al., 1998; Huysmans et al., 1998b), obviously increasing the probability to find carious lesions and improving the diagnostic performance.

Few studies have evaluated the ability of QLF to detect and to quantify caries lesions on smooth surfaces (Angmar-Månsson et al., 2001; Shi et al., 2001; Pretty et al., 2002) and occlusal surfaces (Ando et al., 2000). In the present study, QLF had strong positive correlation with histological examination (0.66-0.71) and it had good performance to detect lesions at D1 diagnostic threshold in occlusal surfaces, which was evident by the calculated Az (0.84).

The sensitivity and specificity for ECM found in the present study were smaller when compared to the ones from other studies (Lussi et al., 1995; Ekstrand et al., 1997; Pereira et al., 2001; Côrtes et al., 2003). However, the estimated Az for occlusal caries were very similar to the data presented by other authors (Ricketts, 1997a,b; Huysmans, 1998a; Côrtes et al., 2003).

A moderate but direct relationship with lesion depth was found, which is in agreement with Ricketts et al. (1996 and 1997), but lower than data presented by Ekstrand et al. (1997 and 1998). These differences could be attributed to distinct histological scores, which could influence the correlation, or to the variation between the electrical current flow along the tooth and the histological section site. It is well-know that the electrical current goes through a complex tree-dimensional and tortuous course, which is unlikely to follow the single histological section made at the investigation site in this study. This aspect of study design may have underestimated the relationship between ECM scores with depth of lesions.

DIAGNOdent showed the high specificity at D1 diagnostic threshold, which is similar to results from previous studies (Lussi et al., 1999; Shi et al., 2000; Baseren et al., 2003). According to Baseren & Gokalp (2003), good specificity for detection of enamel caries would have significant influence on the overall use of prevention techniques, such as control of plaque and diet, use of sealants and fluorides.

Analyzing the Spearman's rank correlation coefficient between the histological examination and DIAGNOdent was possible to observe a strong positive correlation between both methods (rs=0.71-0.74). Previous studies showed higher values (Shi et al., 2000; Bamzahim et al., 2002; Côrtes et al., 2003). Probably, the recommended cutoffs points were the source of variation, which explains the differences (Sheehy et al., 2001).

Although QLF, ECM and DIAGNOdent have provide an appealing high-tech approach in the dental chair, the present study could not show significant better results when they were compared to a visual score performed by a well-trained eye, which showed high correlation with histological examination. Although QLF, ECM and DIAGNOdent provided quantitative information, allowing the observation of the progression or inhibition of the carious lesions along the time, the effectiveness of these emerging technologies in comparison to the traditional methods is questionable. However, the high specificity found by using these new technologies indicated more accuracy than traditional methods to detect sound surfaces.

Considering the actual and drastically decrease of caries prevalence in many populations around the world, these new methods could be used to reduce the probability of overtreatment. Côrtes et al. (2003) observed similar diagnostic performance comparing the combined use of FOTI/visual examination, DIAGNOdent and ECM. Despite many advantages, QLF and ECM are expensive. Less expensive devices, like DIAGNOdent, would probably find better acceptance among clinicians. Further studies should focus on the applicability and effectiveness of traditional methods using different classification criteria and the combination of conventional and emerging technologies.

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Table 1. Kappa values for Intra- and interexaminer reproducibility considering visual examination, radiography and QLF for examiners.

Method	Intraexaminer			Interexaminer		
	exam 1	exam 2	exam 3	exam 1-2	exam 1-3	exam 2-3
Visual	0.78	0.84	0.89	0.64	0.49	0.43
Radiography	0.69	0.90	1.00	0.71	0.36	0.40
QLF	0.79	0.87	0.77	0.86	0.54	0.50

Table 2. Intraclass correlation coefficients for intra- and interexaminer reproducibility for ECM and DIAGNOdent diagnostic methods.

Method	Intraexaminer			Interexaminer
	exam 1	exam 2	exam 3	all examiners
ECM	0.40	0.81	0.95	0.70
DIAGNOdent	0.66	0.69	0.93	0.87

Table 3. Unadjusted p-values for sensitivity, specificity, accuracy and the area under the ROC curve considering the methods at D1 diagnostic threshold for occlusal caries using histological validation.

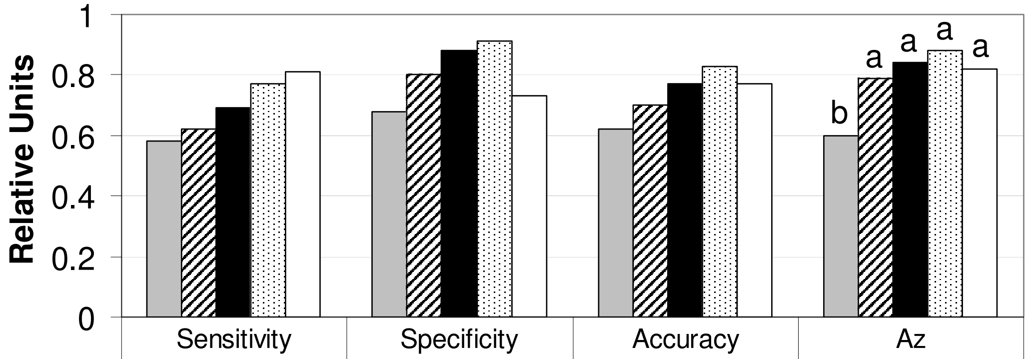
Methods	Sensitivity	Specificity	Accuracy	Az
Visual X Radiographic	**0.000**	**0.002**	**0.000**	**0.002**
Visual X ECM	**0.000**	0.606	0.054	0.606
Visual X DIAGNOdent	0.115	0.217	0.324	0.217
Visual x QLF	**0.017**	0.674	**0.020**	0.674
Radiographic x ECM	0.056	**0.009**	**0.013**	**0.009**
Radiographic x DIAGNOdent	**0.000**	**0.000**	**0.000**	**0.000**
Radiographic x QLF	**0.000**	**0.000**	**0.002**	**0.000**
ECM x QLF	0.084	0.364	0.778	0.364
ECM x DIAGNOdent	**0.028**	0.075	**0.013**	0.075
QLF x DIAGNOdent	0.318	0.366	**0.000**	0.366

Values in bold indicate significance ($p<0.05$).

Table 4. Spearman correlation coefficients considering the relationship between each scoring system and the histological score for each examiner.

	Examiner		
Methods	**1**	**2**	**3**
Visual scores	0.65	0.54	0.64
Radiography	0.44	0.35	0.10
QLF	0.66	0.65	0.71
ECM	0.52	0.57	0.62
DIAGNOdent	0.74	0.72	0.71

Figure 1. Sensitivity, specificity, accuracy and the average of the area under the ROC curve (Az) considering each method at D1 diagnostic threshold.



	Sensitivity	Specificity	Accuracy	Az
Radiography	0.58	0.68	0.62	0.6
ECM	0.62	0.8	0.7	0.79
QLF	0.69	0.88	0.77	0.84
DIAGNOdent	0.77	0.91	0.83	0.88
Visual inspection	0.81	0.73	0.77	0.82

Different superscript letters means statistically significant differences among methods ($p<0.05$)

3. 3. Estudo 3

COMPARISON OF VALIDATION METHODS USED FOR OCCLUSAL CARIES DETECTION

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Antonio Carlos Pereira - DDS, MPH, Dr PH *

Fábio Luiz Mialhe - DDS, MSc *

Carlos Gonzalez –Cabezas- DDS, PhD**

Hafsteinn Eggertsson, DDS, MSc, PhD**

Domenick T. Zero – DDS, MSc **

* University of Campinas, São Paulo, Brazil

** Indiana University, USA

Corresponding author:
Prof. Dr. Antonio Carlos Pereira
Faculdade de Odontologia de Piracicaba – FOP/UNICAMP
Departamento de Odontologia Social
Av. Limeira 901 - Areião
CEP:13414-903
Piracicaba-SP
Tel: 19-3430 5209
FAX: 19-3412-5218
e-mail: apereira@fop.unicamp.br

Abstract

The aim of this study was to assess the performance of visual inspection (VI), QLF, ECM and DIAGNOdent diagnostic systems to detect carious lesions in comparison to different validation methods and to analyze the correlation among them. The occlusal surfaces of 88 extracted molars were assessed by all diagnostic methods by three examiners. After the examinations, the teeth were sectioned and analyzed for occlusal caries by the following gold standard methods: stereomicroscopy (ST), transversal microradiograph (TMR) and confocal laser scanning microscopy (CLSM). The performance of the diagnostic methods was calculated by comparison with gold standard histological methods. None statistically significant differences were observed among the area under ROC curve (Az) for the diagnostic methods, independently of the validation methods used. Strong positive correlations among the validation methods were demonstrated through Spearman correlation coefficients. We concluded that the performance of diagnostic methods used to detect occlusal caries was not influenced by validation methods and all methods presented the same trustworthy to detect occlusal caries.

Key words: Occlusal caries, validation, stereomicroscope, transverse microradiography, confocal laser scanning microscopy, QLF, ECM, DIAGNOdent

Introduction

Caries prevalence has been decreased in the past three decades in most of developed countries. The reduction has been site dependent and an increased proportion of total caries is now found in fissures (Beltran-Aguilar et al., 1999; Hugoson 2000).

The slow progression of the carious lesions has caused morphological changes affecting the accuracy of traditional diagnostic methods. An increased interest in alternative approaches to classifying the presence and/or extent of occlusal lesions has been verified (Ekstrand et al., 1997, 1998; Bader et al., 2002).

The more accurate a diagnostic technique becomes the more accurate must be the validation method. The validity of a caries diagnostic system is achieved by comparing its outcome to the true state of the lesions, often called as "the gold standard diagnosis" (Pine & ten Bosh 1996; Hintze et al., 1995; Wenzel & Hintze, 1999; Hintze & Wenzel, 2003).

The choice of gold standards depends basically on the study design. Considering *in vitro* studies, the histological examination is the widely used validation method (Lussi e Francescult, 2003; Côrtes et al., 2004). Other validation methods, such as radiographic examination (Wenzel et al., 1994; Ricketts et al., 1998), transverse microradiograph (Hintze et al., 1995; Fontana et al., 1996; Ando et al., 1997; Shi et al., 2001) and confocal scanning laser microscopy (Fontana et al., 1996; Ando et al., 1997) also have been used. These last two methods are well-established for the estimation of mineral content in sections of carious lesions (ten Bosch & Angmar-Månsson, 1991).

In vivo studies usually utilize histological examination of teeth scheduled for extraction due to surgical or orthodontic indications (Ekstrand et al., 1998). The histological examination, in this case is more reliable than cavity preparation, visual or radiographic examination (Pine & ten Bosch, 1996; Hintze & Wenzel, 2003).

Since the validation method can influence the accuracy of the tests under evaluation (Wenzel & Hintze, 1999; Hintze & Wenzel, 2003), the aim of this study was to compare some diagnostic methods with three validation methods in order to detect occlusal lesions.

Materials and methods

Sample Material

Ninety-six extracted permanent molars exhibiting macroscopically intact occlusal surfaces and complete root formation were selected from a pool. Teeth with dental fluorosis, tetracycline stain, hypoplasia, clinically visible proximal caries or dental restorations were excluded. Different degrees of discoloration in the fissures were acceptable.

All teeth were first cleaned by prophylaxis through brush and pumice slurry, rinsed by water-jet of 3-in-1 syringe. The occlusal surfaces were photographed (magnification x 4) and one site in each picture received a mark to allow localization. All teeth were stored under refrigeration in thymol-saturated saline in individual plastic containers.

Visual Inspection (VI)

Each site previously chosen was examined by conventional clinical examination, one week after sample selection. After gently air-dry during 15 s, visual inspection was carried out by using dental mirror and operating light. Probing was not allowed.

Each surface was ranked through the following criteria: 0 = no caries; 1 = caries in enamel; 2= caries in dentine.

Quantitative Light-induced Fluorescence (QLF)

Images of occlusal surfaces were captured by using the portable intra-oral camera (Panasonic WV-KS 152) connected to a computer. Each occlusal surface was illuminated with 13 mW/cm^2 of the violet-blue light (wave length: 290-450 nm). The images were captured (yellow light at 520nm in a high-pass filter), stored, processed and analyzed by the proprietary software (QLF, Inspektor Research Systems BV, Amsterdam, The Netherlands).

The following scoring criteria were used: 0 = no change in enamel fluorescence; 1 = slightly changes in enamel fluorescence; 2 = loss of fluorescence distinctly visible without enamel broken; 3 = loss of fluorescence distinctly visible with enamel broken; 4 = loss of fluorescence distinctly visible with cavitation.

Electrical Conductance Measurement (ECM):

Electrical conductance method was performed by using the Electronic Caries Monitor III (ECM III, LODE, Groningen, The Netherlands) in the fissure surfaces at the selected investigation site.

Each tooth was placed in a small vial with the roots suspended in physiological saline, which was in contact with the reference electrode. Before each measurement, the occlusal surface of the tooth was moistened with physiological saline and a toothpaste gel was applied in the fissure after gently air-drying.

Measurements were obtained in the previously identified sites by touching the probe of the ECM on the fissure enamel until stable readings were obtained right after the co-axial air-flow (7.5 L/min) were applied. ECM readings ranged from 0.00 (lowest conductance) to 99.99 (highest conductance). The average of three examinations on each site was considered as the definitive score.

Laser Fluorescent (LF) Examination

LF measurements were performed by using the DIAGNOdent device (Kavo, Biberach, Germany) previously calibrated through a porcelain standard provided by the manufacturer.

The probe A was individually adjusted by holding the tip against a sound-smooth enamel surface and pressing the ring button until complete calibration. The conical probe was then positioned perpendicularly and rotated around its long axis on each previous selected site. The average of three readings (0-99 range) on each site was considered as the definitive score.

VALIDATION METHODS

Stereomicroscope (ST)

After all assessments were completed, the teeth were removed from blocks and serially sectioned by a diamond-coated disc in a water-cooled microtome (Silverstone Taylor, Scifab, Lafayete, Glues, USA). The sections, which had approximately 200 µm thickness, were obtained perpendicularly to the occlusal surfaces in a buccolingual direction through the previously chosen site.

The histological examination was performed in a stereomicroscope (magnification x 25 to 40) using reflected light. Both sides of each tooth section were examined.

The histological aspect was considered as caries lesion when a white or discolored (yellow/brown) demineralization area was observed.

Confocal Laser Scanning Microscopy Examination (CLSM)

One week after the histological examination, each tooth section was stained overnight with a freshly prepared 0.01 M Rhodamine B solution (Aldrich Chem. Co., Milwaukee, Wisc., USA). The samples were dried in the air and the lesion depth was analyzed (magnification x 100) by using CLSM (Odyssey, Noran Instruments, Midleton, Wisc. USA). The sections were exposed to a 488-nm argon laser light. The confocal slit was set at 15 µm. A high pass barrier filter (515 nm) was used. Focus was achieved using x4 and x10 magnification objectives. The images were captured and analyzed through software (IMAGE-1 version 4.03C, Universal Images, West Cheater, PA, USA).

Transverse Microradiograph Examination (TMR)

One week after CLSM examination, the same specimens were analyzed by transverse image analysis microradiograph (TMR). The 200 µm thickness tooth sections were manually polished to 150 µm thickness. Eight samples were lost due to fracture during the section polish.

All sections were fixed through an aluminum step wedge on a glass plate coated with X-ray sensitive emulsion (Imtec type 1A, Imtec Products Inc., Sunnyvale, Calif., USA). After exposition to Ni-filtered Cu (Kα) radiation (20 kV, 30 mA and 30 cm of focal distance) during 20 min, the plates were developed according to standard techniques. The images were examined in a microscope (Zeiss UEM), captured by CCD camera and stored by computer software (Inspektor Research Systems B.V., Amsterdam, The Netherlands).

Lesion depth was defined as the depth (in µm) from surface to the area below where the mineral amount was 95% of the healthy enamel.

The data generated by the validation methods were used to classify each specimen using the following five-point scale to stratify the sites according to the histopathological evidence of demineralization:

0 = no caries;

1 = demineralization extending to the ½ outer of the enamel;

2 = demineralization extending to the ½ inner of the enamel;

3 = demineralization extending to the ½ outer of the dentin;

4 = demineralization extending to the ½ outer of the dentin.

All examinations of the sections were performed by two well-experimented examiners.

Statistical analysis

From ninety-six samples, eighty-eight were considered for data analysis due to fracture of eight specimens during TMR.

The methods were compared for differences in sensitivity, specificity, and accuracy and the area under the ROC curve (Az), which was considered as an indicator of overall diagnostic performance of each method. It was computed using the c-statistic from logistic regression models considering each validation method at D1 diagnostic threshold by each one of the observers.

Sensitivities and specificities were calculated considering the following cutoffs: > 0 ratings for VI; ≥ 2 for QLF and ≥ 15 for ECM. Considering DIAGNOdent method, the cutoff was ≥ 5 (Lussi et al., 1999).

The Spearman rank-order correlation coefficient was calculated to analyze the agreement among ST, CLSM and TMR to detect and estimate the depth of carious lesions at enamel and dentin. Coefficients values from 0 to 0.3 were considered as weak relationship, from > 0.3 to 0.7 as moderate relationship and from > 0.7 to 1.0 as strong relationship.

Results

Table 1 shows the comparison among the diagnostic and validation methods observing sensitivity, specificity and accuracy at D1 diagnostic threshold.

Table 1. Sensitivity, specificity, and accuracy of each system considering the validation methods assessed at D1 diagnostic threshold.

		Validation method		
	Examination method	**ST**	**CLS**	**TMR**
SENSITIVITY	***VI***	0.77	0.74	0.74
	QLF	0.68	0.65	0.63
	ECM	0.61	0.62	0.62
	DIAGNOdent	0.77	0.75	0.74
SPECIFICITY	***VI***	0.74	0.77	0.75
	QLF	0.87	0.87	0.86
	ECM	0.80	0.84	0.83
	DIAGNOdent	0.91	0.93	0.89
ACCURACY	***VI***	0.75	0.75	0.75
	QLF	0.76	0.73	0.73
	ECM	0.70	0.70	0.71
	DIAGNOdent	0.83	0.82	0.81

Different superscript letters show statistically significant differences among diagnostic methods ($p<0.05$)

None statistically significant difference in the sensitivity, specificity and accuracy values for diagnostic methods was observed in relation to different histologic examinations used.

The areas under the ROC curve (Az) for all diagnostic and validation methods are shown in Table 2.

Table 2. Average of the area under the ROC curve (Az) considering diagnostic and validation methods.

	Diagnostic Method			
Validation Method	**VI**	**QLF**	**ECM**	**DIAGNODent**
ST	0.78	0.84	0.79	0.88
CLS	0.78	0.80	0.78	0.87
TMR	0.77	0.80	0.78	0.85

The areas under the ROC curve did not showed statistically significant differences among methods (p>0.05).

The Spearman correlation coefficients among the validation methods are presented in Table 3. A strong positive correlation among the methods could be observed. The strongest correlation was found between ST and CLSM.

Table 3. Spearman correlation coefficients observed between the validation methods.

Validation method	TMR	CLSM
ST	0.91	0.95
CLSM	0.94	

Discussion

A careful selection of a trustworthy validation method must be considered in research projects designated to test and to validate new methods for detection of carious lesions.

A validation method should fulfill at least three criteria: (1) it should be reproducible; (2) it must reflect the patho-anatomical appearance of the lesion; and (3) the true diagnosis should be established independently of the diagnostic methods under evaluation (Wenzel & Hintze, 1999, Hintze & Wenzel, 2003). Validation techniques using sections of teeth, combining training and calibration of the validators usually satisfy these criteria (Wenzel et al., 1994).

None of the three criteria are fulfilled when the test under evaluation is also at same time the gold standard method, like in some *in vivo* studies, which represents a serious methodological error (Wenzel & Hintze, 1999).

In the present study, a high accuracy to detect carious lesions by visual inspection independently of the validation method could be observed between the examiners. These results were similar to the ones observed in previous studies (Ekstrand et al., 1997, 1998; Cortês et al., 2004) which showed high sensitivities values (0.70-0.91) and moderate to high values of specificity (0.59-0.85) at D1 diagnostic threshold.

Histological examination of teeth by stereomicroscope is frequently used to validate the results obtained by diagnostic methods, especially in *in vitro* studies. However, this method is unable to quantify mineral loss. The use of TMR or CLSM to analyze tooth sections of carious lesions is relatively recent. The strong correlation between TMR and CLSM found in the present study is similar to the finds of Fontana et al. (1996).

Hintze et al. (1995) compared the efficacy of stereomicroscopy, film radiography, microradiography and naked-eye inspection to detect occlusal caries in unerupted and erupted third molars with an unknown “true state of disease”. They observed that the stereomicroscopy (ST) resulted in a specificity of 1.00. The only method that allowed the correct identification of all unerupted teeth as sound by all observers was the ST. Therefore, they concluded that ST was the most reliable validation method.

In the present study, a stronger correlation was found between ST and both CLSM (r_s=0.95) and TMR (r_s=0.91). Ando et al. (1997) verified that TMR was partially unable to detect absence of mineral loss in specimens not exposed to demineralizing fluid. These data suggest that CLSM is more reliable than TMR as validation method to detect and to quantify the depth of lesion. The present study, however, showed stronger correlation between both CLSM and TMR.

The ability of TMR to measure demineralized areas could be compromised when the sections were not cut in a flat parallel way or were not perpendicularly to the anatomical tooth surface. This parallelism is difficult to obtain in manual polish of the sections to 150-µm thickness, resulting in lack of standardization, which could interfere in the X-rays ability to pass at 90° through sections. This fact could compromise the method precision to detect the presence and the depth of the carious lesions in the sections.

Some disadvantages have been attributed to TMR technique for detection of occlusal caries. Quantification of mineral loss on occlusal surfaces is difficult to observe with TMR, since it requires a smooth surface as the start point for measurements. The deepest aspect of the lesion could be neglect or lost in the grinding debris. The thin sections are readily damage, especially when substantial demineralization is present (Hintze et al., 1995; Ricketts et al., 1998). The fracture of specimens, which occurred in the present study, due to the use of thin sections could be avoided by the use of ST or CLSM.

Usually the diagnostic methods are subjectively evaluated by human observers and, therefore, exposed to variability. Thus, the validation methods must be performed by well-experienced and trained researchers. Unfortunately, literature about training and calibration of the examiners to perform reliable validation methods is rare in the literature. Usually, more experienced researchers prepare young researchers. More handbooks and protocols should be developed to standardize the calibration procedures of the validation methods around the world.

It could be concluded that none diagnostic methods evaluated in the present study for occlusal caries detection were influenced by the validation methods. The three validations methods assessed showed the same reliability to detect occlusal caries lesions.

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4. Considerações Finais

A detecção das lesões cariosas incipientes em esmalte é muito importante para a manutenção da saúde bucal dos pacientes, pois o clínico pode atuar nessas lesões de forma eficiente, por meio de tratamentos não-invasivos, promovendo a manutenção da estrutura dentária. No entanto, mesmo que o processo tenha evoluído para o nível de dentina, é importante que o clínico tenha meios de detectar e de quantificar os sinais da doença o mais precocemente possível, a fim de poder instituir tratamentos operatórios minimamente invasivos, necessitando desgastar apenas uma pequena quantidade da estrutura tecidual e, conseqüentemente, favorecendo a integridade do elemento dentário. Dessa forma, com a evolução do conhecimento científico na área de cariologia, fundamentando novas estratégias para o tratamento da doença cárie, há a necessidade de se aperfeiçoar a capacidade diagnóstica dos métodos já existentes e também de se desenvolver outros para a estimação da presença, da profundidade e da atividade das lesões cariosas.

O exame radiográfico tem sido, há muito tempo, uma ferramenta diagnóstica auxiliar muito importante ao exame clínico, pois, mesmo não apresentando uma boa capacidade de evidenciar lesões cariosas oclusais em nível de esmalte, detecta com razoável exatidão a presença de lesões cariosas em nível de dentina, principalmente aquelas que já atingiram mais de 1/3 da extensão da profundidade da dentina. Com os recentes avanços na área de radiografia digital há um crescente interesse em se saber se estes apresentam melhores características que os sistemas radiográficos convencionais. Entretanto, a partir dos dados do primeiro estudo, ficou claro que o sistema radiográfico convencional apresenta a mesma capacidade diagnóstica que os sistemas digitais para a detecção e quantificação das lesões cariosas em superfícies oclusais.

Dessa forma, parece-nos que a radiografia digital, apesar de suas vantagens, ainda não apresenta uma relação custo-benefício favorável em relação ao método radiográfico convencional para a finalidade a qual foi avaliada pelo presente estudo.

O aperfeiçoamento dos métodos de diagnóstico não tem se restringido apenas àqueles que utilizam a radiação X para estimar o estado do tecido dentário. Outras tecnologias vêm sendo desenvolvidas baseadas em diferentes princípios. Entretanto, apesar de elas exercerem algum tipo de fascínio na imaginação dos clínicos, sustentado por um apelo mercadológico das empresas que os fabricam, os resultados provenientes do segundo estudo demonstraram, a partir da análise da área abaixo da curva ROC, que os aparelhos QLF, ECM e DIAGNOdent apresentaram o mesmo desempenho em detectar e quantificar a extensão das lesões cariosas ($p>0,05$) que o exame clínico realizado a partir de uma escala de critérios bem fundamentadas por estudos científicos. Apesar disso, os aparelhos QLF e DIAGNOdent apresentaram altos valores de especificidade e número de dentes corretamente diagnosticados, podendo portanto, ser importantes adjuntos aos exames convencionais nos casos em que há dúvidas quanto à higidez de uma fissura que não apresenta uma imagem radiolúcida ao exame radiográfico. Portanto, espera-se que a acurácia diagnóstica do examinador seja aumentada quando utiliza dados provenientes de mais de um método. Devido ao alto custo dos aparelhos QLF e ECM, o aparelho DIAGNOdent talvez seja atualmente, o sistema de diagnóstico mais acessível ao clínico, como adjunto ao exame clínico e radiográfico, permitindo ainda, o monitoramento longitudinal das perdas minerais e ganhos minerais das lesões cariosas.

A fim de se analisar o desempenho de um método de diagnóstico para a cárie dentária, comparam-se os resultados encontrados com o verdadeiro estado do tecido dentário, geralmente denominado de método validação ou “gold standard”. Nos estudos “in vitro”, os quais geralmente precedem as avaliações “in vivo”, os resultados dos testes devem ser preferencialmente validados pelo exame histológico em estereomicroscópio, ou por outra técnica, utilizando secções do dente para que o estado do tecido dentário possa ser avaliado, com determinada exatidão. Nesse sentido, os métodos de validação empregados no estudo 3 são bem resguardados, pois são reconhecidos, comprovadamente, como identificadores e determinadores da perda mineral da estrutura dentária. Verificou-se que a performance dos métodos de diagnóstico avaliados seguiram o mesmo padrão de desempenho, independente do método de validação utilizado e os métodos de validação apresentaram uma alta correlação positiva entre eles. Assim, fica claro que a microradiografia ou o exame confocal a laser não subestimam ou sobrestimam a presença e a extensão da lesão cariosa, comparados ao exame por estereomicroscopia e, portanto, determinam, com a mesma fidedignidade, a efetividade dos métodos para o diagnóstico dessas características.

Concluindo-se, os resultados dos estudos realizados e apresentados neste trabalho sugerem que um exame clínico, efetuado a partir de uma escala de critérios fundamentada por estudos científicos, aliado ao exame radiográfico convencional e ao exame por laser fluorescente (DIAGNOdent), pode ser suficiente para detectar e para estimar a extensão da grande maioria das lesões cariosas localizadas nas superfície oclusais. Novos estudos devem ser realizados com a finalidade de aperfeiçoar-se a capacidade diagnóstica dos exames convencionais e de desenvolver novos métodos mais acurados para o diagnóstico do processo carioso em superfícies oclusais, baseados em exames de validação fidedignos.

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ANEXOS

COMITÊ DE ÉTICA EM PESQUISA

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CERTIFICADO

Certificamos que o Projeto de pesquisa "Avaliação in vitro da confiabilidade de diferentes métodos para o diagnóstico da cárie dentária", protocolo CEP nº **027/2004**, dos Pesquisadores ***Fábio Luiz Mialhe*** e ***Antonio Carlos Pereira***, está de acordo com a Resolução 196/96 do Conselho Nacional de Saúde - MS e foi aprovado pelo Comitê de Ética em Pesquisa da Faculdade de Odontologia - UNICAMP.

We certify that the research project "In vitro evaluation of the reability of different dental caries diagnostic methods", register number **027/2004**, of ***Fábio Luiz Mialhe*** and ***Antonio Carlos Pereira***, is in agreement with the recommendations of 196/96 Resolution of the National Health Committee - Brazilian Health Department and was approved by the Research Ethics Committee of the School of Dentistry of Piracicaba - State University of Campinas - UNICAMP.

Piracicaba - SP, Brazil, June 15 2004

Profa. Dra. Cinthia Pereira Machado Tabchoury
Secretaria
CEP/FOP/UNICAMP

Prof. Dr. Jacks Jorge Júnior
Coordenador
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