REVISTA SAÚDE

DIAGNOSIS OF ARTIFICIAL SECONDARY CARIES ON ENAMEL: CORRELATION BETWEEN VISUAL EVALUATION AND SUPERFICIAL MICROHARDNESS

DIAGNÓSTICO DE CÁRIE SECUNDÁRIA EM ESMALTE: CORRELAÇÃO ENTRE AVALIAÇÃO VISUAL E MICRODUREZA SUPERFICIAL

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ABSTRACT: This *in vitro* study evaluated the correlation of artificial secondary caries diagnosis on enamel between visual evaluation and superficial microhardness test. Cavities with standardized diamond burs (1.6mmØ) were prepared on thirty-six enamel blocks obtained from unerupted human third molars and were assigned to 3 groups. Each group was restored with glass-ionomer cement (GI), resin-modified glass-ionomer (RM), or composite resin (CR). Blocks were thermocycled and submitted to a pH challenge to develop artificial caries-like lesions. Lesions were analyzed by visual evaluation using scores and the results were submitted to Kruskal Wallis and Dunn Test. The hardness of the enamel surface surrounding the restored cavity was evaluated using Knoop microhardness test and results were submitted to ANOVA followed by Tukey's post-hoc test. Afterwards, the correlation between visual and microhardness analyses was verified by Spearman's rho nonparametric correlation test. Regarding visual analysis, no significant difference was observed between GI and RM groups, which showed less caries development than CR group. The microhardness evaluation showed significant differences among all groups with the least caries development in GI group, followed by RM and CR, respectively. The Spearman's rho coefficient of correlation demonstrated a significant weak negative correlation between the response variables. The superficial microhardness test was more sensitive to detect artificial secondary caries than visual evaluation.
KEY-WORDS: Dental caries. Composite resin. Glass ionomer cement. Dental enamel. Hardness. Visual evaluation.

RESUMO: *Este estudo in vitro avaliou a correlação entre a inspeção visual e a microdureza superficial no diagnóstico de lesões artificiais de cárie secundária em esmalte. Trinta e seis blocos de esmalte obtidos de terceiros molares humanos inclusos foram utilizados para a confecção de cavidades circulares padronizadas (1,6 mmØ) e distribuídas em 3 sub-grupos. Cada sub-grupo foi restaurado com cimento de ionômero de vidro (GI), ionômero de vidro modificado por resina (RM), ou resina composta (CR). Os fragmentos foram termociclados e submetidos ao desenvolvimento de lesões artificiais de cárie por ciclagem de pH. As lesões foram avaliadas por inspeção visual empregando-se escores e foram avaliadas estatisticamente pelos testes de Kruskal Wallis e Dunn; e por ensaio de microdureza Knoop microhardness, que foi avaliado por ANOVA e teste de Tukey. Em seguida, a correlação entre inspeção visual e o teste de microdureza foi avaliada pelo teste não paramétrico de correlação de Spearman. Os resultados da inspeção visual não apresentaram diferença significante entre os grupos GI e RM, os quais apresentaram menor desenvolvimento de cárie do que o grupo CR. A avaliaçào de microdureza demonstrou diferenças significantes entre todos os grupos, sendo o menor desenvolvimento de lesão no GI seguido por RM e CR, respectivamente. O coeficiente de correlação de Spearman foi significante e demonstrou uma fraca correlação negativa entre as variáveis de resposta. O ensaio de microdureza superficial foi mais sensível para o diagnóstico da cárie secundária do que a inspeção visual.*
PALAVRAS-CHAVE: *Cárie dental. Resina composta. Cimento de ionômero de vidro. Esmalte dental. Dureza. Inspeção visual.*

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INTRODUCTION

The knowledge of the etiology and development of caries disease has allowed a reduction in caries risk and activity by preventing and arresting primary and secondary caries lesions. Secondary caries should be firstly prevented by the reduction in their determinant and modulating factors to revert the patient condition from high to low risk disease status by hygiene procedures such as brushing and flossing[1].

However, the fluorides released from restorative materials may be a viable alternative to prevent secondary caries development in high-risk patients[2,3,4]. The potential cariostatic effect of restorative materials is described in researches showing high cariostatic effect of conventional glass ionomer cements, moderate cariostatic effect of glass ionomer and composite resin hybrid materials, and no cariostatic effect of composite resin materials by different analysis[2,3,5,6].

These analyses may involve less complex and cheaper methods such as visual evaluation and superficial and sub-superficial microhardness, or more difficult evaluation techniques involving expensive equipments, such as microradiography and polarized light microscopy. Since all these analyses are based on different parameters of evaluation, there is a need to verify the correlation among methods. The main objective of the present study was to evaluate the agreement between visual evaluation and superficial microhardness analysis used for the diagnosis of artificial secondary caries development.

METHODS AND MATERIALS

This study was performed using 20 unerupted human third molars. The research protocol was approved in accordance with the resolution CNS# 196/96 of the National Health Committee/Health Departments by the Research Ethics Committee of the Guarulhos University (Brazil). Following extractions, teeth were stored in 0.1% Timol solution (pH 7.0) for no longer than 30 days. Soft-tissues were removed using periodontal curettes (HU-FRIEDY do Brasil, Rio de Janeiro-Brazil) and teeth were cleaned using pumice slurry in a webbed rubber cup applied with a slow-speed handpiece (Kavo do Brasil, Joinville- Brazil). The crowns were longitudinally and transversally sectioned to obtain 36 dental blocks measuring 4x4x3 mm^3 using double-faced diamond discs (#7020, KG Sorensen, São Paulo- Brazil).

Cavity preparation and restoration

The 36 enamel blocks (n=12 per group) were assigned into three subgroups according to the restorative material described in Table 1. The response variables were visual evaluation and surface microhardness expressed in Knoop Hardness Number (KHN).

Table 1- Experimental groups, manufactures and composition.

Group	Restorative material	Ingredients
GI	Glass ionomer cement (Ketac-Fil,3M/ ESPE, Seefeld, Germany)	Powder: glass powder 100% Liquid: water 60-65%, polyethylene, polycarbonic acid 30-40%, tartaric acid 5-10%
RM	Resin modified glass ionomer (Vitremer, 3M/ESPE, St. Paul, MN, USA)	Primer: 2-hydroxyethyl methacrylate 45-55%, ethyl alcohol 35-45%, copolymer of itaconic and acrylic acids 10-15%. Powder: silane treated glass 90–100%, potassium persulfate < 1% Liquid: copolymer of acrylic and itaconic acids 45-50%, water 25-30%, 2-hydroxyethyl methacrylate 15 – 20%. Finish gloss: triethylene glycol dimethacrylate 40-60%, bisphenol a diglycidyl ether dimethacrylate (bisgma) 40 – 60%.
RC	Resin composite (Z250, 3M/ESPE, St. Paul, MN, USA)	Silane treated ceramic 75-85%, bisphenol a polyethylene glycol diether dimethacrylate (bisema6) 5-10%, diurethane dimethacrylate 5-10%, bisphenol a diglycidyl ether dimethacrylate (bisgma) 1-10%, triethylene glycol dimethacrylate (tegdma) <5%, water <2%.

Standardized circular cavities with 1.6 mm in diameter and 1.6 mm deep were prepared in the enamel blocks with diamond burs No. 2292 (KG Sorensen, Barueri, SP, Brazil, 06454-920) at high speed under a constant water spray coolant. Afterwards, the blocks were randomly distributed to the subgroups, and were restored in one increment with each restorative material according to the manufacturers' instructions.

In cavities filled with Ketac-Fil, the Ketac conditioner was applied for 10 s, rinsed off and dried for 10 s. Ketac-Fil was prepared within 20-25 s, inserted into the cavity with a Centrix injector, protected with a Mylar strip (Dentart, Polidental, São Paulo, Brazil) for 5 min, coated with Vitremer Finish Gloss and light-activated for 20 s with an Optilux 501 light curing unit (light tip diameter: 11 mm; irradiance: 700 mW/cm^2; Demetron/Kerr, Danbury, CT, USA). The power density was constantly measured by placing the light tip on the radiometer attached to the light curing unit.

In cavities filled with Vitremer, the Primer was applied for 30 s, dried for 5 s and light-activated for 20 s. Vitremer was prepared within 45 s, inserted into the cavity with a Centrix injector, light-activated for 40 s, coated with Vitremer Finish Gloss and light-activated for 20 s.

In cavities filled with Z-250, the 35% phosphoric acid (Scotch Bond Etchant; 3M ESPE) was applied for 15 s, rinsed off for 10 s and the cavity was air-dried. Two coats of Adper Single Bond (3M ESPE) were applied, air-dried for 5 s and light-activated for 10 s. The composite resin was inserted and light-activated for 20 s.

All restored blocks were stored in 100% humidity for 24 h and were then polished using the Sof-lex (3M ESPE, St. Paul, MN, USA) disks system for 15 s with each disk.

Thermal and acid challenge

The restored blocks were placed into separate bags with 1 mL of deionized water and were thermocycled for 1000 cycles in water with temperature ranging from 5±2ºC to 55±2ºC with a dwell time of 2 min in each bath and 15 s-transfer time between baths.[2] The external enamel surfaces of blocks were covered with wax, leaving a 1.5 mm-wide margin around the restoration free of wax.

The acid challenge was designed to simulate a daily demineralization challenge of 6 h and 18 h repair (remineralization) by saliva as described by Featherstone *et al.* (1986)[6] and Serra & Cury (1992)[7], to simulate a high *in vitro* caries risk and to produce artificial caries like-lesions around the restorations[2,7].

The demineralization stage was based on the use of an acid buffer containing 2 mmol/L Ca, 2 mmol/L PO_4, 0.075 mol/L acetate at pH 4.3. The remineralization solution contained calcium and phosphate at a previously established degree of saturation (1.5 mmol/L Ca, 0.9 mmol/L PO_4), to mimic the remineralizing properties of saliva, and 50 mmol/L KCl, 20 mmol/L tri-hydroxymethylaminomathan buffer at pH 7.0.[6,7] The slabs were immersed separately in 15 mL of demineralization solution for 6 h, were immersed in 15 mL of remineralization solution for 18 h, washed and immersed in demineralization solution, thereby initiating a new cycle. The pH cycles were conducted for 14 days with 10 daily cycles. In the 6th, 7th, 13th, and 14th days of the cycle, the blocks were kept only in the remineralization solution.

At the end of the pH cycles, the wax was eliminated and the blocks were stored at 100% humidity until the moment of visual evaluation and microhardness test.

Visual evaluation

The blocks were air-dried for 15s and standardized images were obtained from each block with a Nikon D70 digital camera with lens #105. Three calibrated examiners (Kappa>0.73) independently and blindly evaluated the images of all images projected in a dark room with approximately 100x magnification. The examiners evaluated the specimens scoring the presence and severity of caries-like lesions according to an ordinal scale ranked from 0 to 3 based on visual examination, as described in previous studies (Figure 1).[2,8] A median score was obtained from scores given by the 3 examiners for each specimen. Differences among medians were analyzed by Kruskal-Wallis and Dunn non-parametric tests.

Figure 1 - Scores used to visual evaluation

Microhardness test

The demineralization of the restored enamel blocks was assessed with a microhardness tester (PanTec, Panambra Ind. e Técnica SA, São Paulo- Brazil) and a Knoop indenter. The indentations were made keeping the long axis of the diamond instrument parallel to the outer-leveled enamel surface, using a 25 g load applied for 5 s, and the highest diagonal length was measured in micrometers and was automatically changed to KHN. Four measurements were made on the enamel surface 100 µm far from the restoration margins in the upper, left, right, and bottom sides (Figure 2). The means of the four indentations represented the block microhardness value. The mean values of each block were analyzed by ANOVA and Tukey's post-hoc test at a pre-set alpha of 0.05.

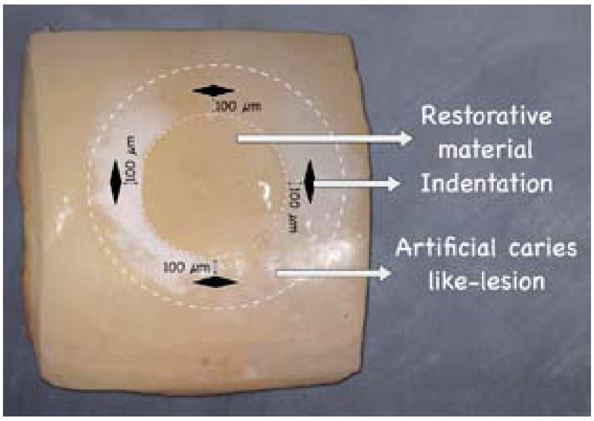

Figure 2 – Location of indentation in the microhardness test.

Correlation between visual evaluation and microhardness test

The correlation between non-parametric visual evaluation and parametric evaluation of microhardness test was evaluated by the Spearman's rho coefficient of correlation, which ranges in value from r=+1.0 for a perfect positive correlation to r=-1.0 for a perfect negative correlation. The midpoint of its range (r=0.0) corresponds to a complete lack of correlation. Values falling between r=0.0 and r=+1.0 represent a range in degrees of positive correlation, while those falling between r=0.0 and r=-1.0 represent a range in degrees of negative correlation[9].

RESULTS

The medians, minimum, and maximum scores of visual evaluation and the means of microhardness values and standard deviations per restorative material are presented in Table 1. The statistical analysis of visual data showed no differences between GI and RM groups, which in turn showed significantly less caries development than CR group ($p<0.01$). The microhardness data showed significant differences among groups with less caries in GI than in RM and CR, which in turn showed the highest incidence of caries ($p<0.05$).

Table 2- Medians, minimum, and maximum of visual evaluation and the means of microhardness values and standard deviations per restorative material; Tukey's and Dunn test results.

	GI- Ketac Fil	RM- Vitremer	CR- Z-250
Visual Evaluation	1 (0-3) A	1 (0-3) A	3 (2-3) B
Microhardness test	235.5 (75.5) a	137.1 (64.1) b	39.3 (26.5) c

Different upper case letters indicate no statistical difference (Dunn test, $p<0.01$);
Different lower case letters indicate no statistical difference (Tukey's test, $p<0.05$);

The Spearman's rho coefficient of correlation between the response variables was statistically significant ($p<0.01$) but the negative correlation was considered weak ($r=-0.51$).

DISCUSSION

This study evaluated the development of artificial caries lesion on enamel around cavities filled with restorative materials with or without fluoride release. A dynamic cyclic model of demineralization and remineralization was applied to simulate acid challenge in patients with high caries risk[6]. The highest development of artificial caries lesions in this study was observed in cavities restored with composite resin. As expected, the composite resin associated to an adhesive system deprived of fluoride in their compositions do not inhibit caries progression.[2] This is consistent with reports from other studies, in which only bioactive composite resins and adhesive systems containing fluorides or antibacterial monomers were capable of showing few cariostatic effect, which was lower than that promoted by glass ionomer cements[4,10].

In agreement with dental literature, the ionomer-based materials showed some cariostatic effect, as they mobilize and release increased amounts of fluoride into the environment during acid challenges, so enamel demineralization is prevented. Then, the presence of fluorides continuously released from ionomers is an important feature for improving enamel remineralization or inhibiting demineralization[11]. This is the reason for less artificial caries lesion development around cavities restored with conventional glass ionomer cement and moderated inhibition of resin modified glass ionomer evaluated by microhardness test. Most studies showed that the smaller fluoride concentration released from resin modified glass ionomer in comparison to that released by conventional glass ionomer cement causes moderate development of artificial caries lesion, which is generally considered less than that observed when glass ionomer cement is used[2,3,7,11]. Therefore, the visual evaluation was not able to detect the difference in caries inhibition between the group using conventional glass ionomer and that using resin modified glass ionomer. It can be supposed that the protection rendered by the glass ionomer cement is extended to some distance from the restoration and is the greatest one in the cavity preparation area[5]. Based on such assumption and on the distance of 100 μm from cavity margins stipulated for the microhardness test, the caries inhibition area provided by conventional glass ionomer could be higher than that created by the resin modified glass ionomer. Therefore, microhardness test may be considered more specific, as the visual evaluation allowed the examiners to check all enamel area free of wax around the restoration. This area was exposed to fluoride released from the glass ionomer material to the solution resulting in a general caries inhibition which was clinically similar to the resin modified glass ionomer.

Thus, it can be considered that for a specific evaluation site, superficial microhardness may be required while a general evaluation of wider surrounding area may be performed by visual evaluation. This difference explains the weak agreement between visual and microhardness evaluation observed in Spearman's correlation test. The Spearman's rho correlation measures how well two variables are connected without making any assumption about the frequency distribution of the variables. The negative coefficient value observed in the present study indicates that the two evaluations are systematically inversely related, as caries lesions visually increase while the superficial microhardness tends to decrease. However, a coefficient value closer to -1.00 could have showed a perfect negative association.

Another aspect that should be considered is that visual evaluation is subjective and this exam depends on the examiner expertise and calibration. The examiners in the present study were calibrated and kappa qualified the agreement from excellent to good. In a similar methodology, Serra induced artificial secondary caries lesion and found a good agreement between visual evaluation and sub-superficial analysis ($r=-0.78$; $p<0.01$).

Visual evaluation has been associated with scores in clinical[12], epidemiological[13] studies to quantify opacities, fluorosis and white spots resulting from enamel demineralization. Also in *in vitro* [2,8,14], and *in situ* studies[15] are well accepted. When compared to other methodologies, this evaluation has some advantages, such as low cost and the possibility of the identification of differences in the cariostatic potential of restorative materials under conditions similar to clinical conditions[2,8]. As showed in the current study, visual evaluation is simple to perform, which facilitates laboratory investigation and allows the conduction of studies in less time and at lower costs[2,8]. In addition, reproducible results have been shown between visual evaluation and microradiography and polarized light microscopy[15].

However, the use of visual evaluation needs to be cautiously inferred by the bias of the macro vision of the secondary artificial caries development by the examiner and the cariostatic effect of restorative materials close to cavity margins could not be totally observed. Then, when specific analysis of a site is required, microhardness profiles are recommended and may be used in association with visual evaluation to provide a micro and a macro response of caries development.

CONCLUSIONS

The superficial microhardness test was more sensitive regarding the diagnosis of artificial secondary caries development than visual evaluation, and specific analysis microhardness profiles may be recommended when a micro-site analysis is required.

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