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Original Article

Reliability analysis of two methods for measuring active enamel demineralization: An *in vitro* study

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ABSTRACT

Objective: The aim was to evaluate the intra- and inter-examiner reliability of two methods, namely, digital caliper [DC] and computerized image analysis software (Image Tool [IT] version 4.1) for measuring the area of an active enamel demineralization lesion. The effect on that measurement of a window that delimits the lesion was also assessed. **Materials and Methods:** Thirty-eight circular artificial enamel demineralization lesions (r = 2 mm) were produced on bovine teeth *in vitro,* which were then measured three times by three examiners, with and without the presence of a window delimiting the area to be measured. The data were analyzed by the intraclass correlation coefficient (ICC) with 95% confidence interval (CI) and by the Altman and Bland analysis. **Results:** The intra-examiner correlation using the IT method showed excellent reproducibility (mean ICC values 0.922-0.970 with the delimiting window and 0.915-0.990 without the window). However, the intra-examiner correlation using the DC method showed comparatively less reproducibility in the measurements (mean ICC values 0.458-0.648 with the delimiting window and 0.378-0.665 without the window). The inter-examiner correlations showed very good reproducibility of the measurements regardless of the presence or absence of the window for both the DC method (0.811-0.846) and IT method (0.953-0.994). However, the latter method showed less variability within the measurements. **Conclusion:** However, statistically no significant difference was found between both methodologies. Nevertheless, computerized image analysis with the Image Tool software demonstrated higher intra- and inter-examiner reliability than the digital caliper method, in estimating the area of the enamel demineralization lesion, regardless of the delimiting window.

Key words: Computerized image analysis, dental caries, image processing, observer variation, reproducibility of results, tooth demineralization

INTRODUCTION

Dental caries is caused by a dynamic imbalance between continuous demineralization and remineralisation processes occurring on a tooth, resulting in a net loss of minerals.[1] These processes begin when the acidogenic bacteria existing in the dental plaque ferment the dietary carbohydrates producing organic acids.[2] These acids dissolve the hydroxyapatite crystals, releasing mineral contents, calcium, and phosphate, resulting in a carious lesion.[1]

Clinically, the initial stage of dental caries in the enamel is seen as an opaque white spot, exhibiting an intact surface, but an affected sub-surface. This lesion area is rendered more porous than the sound enamel, due to loss of minerals. Therefore, it appears opaque and white and the opacity increases with air drying.[3] Such early carious processes, if detected at this incipient stage, can be stopped or even reverted with adequate noninvasive, preventive, and therapeutic measures, thus avoiding invasive cavity preparations and restorative procedures.[4,5]

There is lack of a reliable diagnostic method for detecting, measuring, and monitoring the progress of

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a carious lesion. The use of conventional diagnostic techniques seems to be satisfactory for the diagnosis of cavitated lesions,[6,7] but not enough for the detection of the non-cavitated ones.[8]

The reliability of a diagnostic method is determined by its precision, reproducibility, and exactness.[9] The lower the variations in the results, after repeated use of the method, the greater is its reliability. However, the use of multiple methods and examiners is needed to enhance the validity of the results and confirm their reliability, credibility, and research quality.[10]

Studies have evaluated photographic images as a reliable tool for various measurement purposes.[11] Today, computing technology has made photographic image conversion into digital formats a simple process. These images can be measured and analyzed, as well as stored. This has been advocated by Willmot[12] in a clinical study on white spot lesions, photographed with conventional and digital cameras.

However, there are a few studies identifying the reliability of the methods for the measurement of active enamel demineralization lesions.[12,13] This study aims at assessing intra- and inter-examiner reliability for the measurement (surface area – mm^2) of artificially created active enamel demineralization, with and without a lesion delimiting window, by using manual (digital caliper) and computing (Image Tool version 4.1 software) methods.

MATERIALS AND METHODS

Sample preparation

Twenty sound bovine teeth were selected for the study, none of which presented with grooves, hypoplasia or stains. The teeth were fixed on glass laminas by means of sticky wax and then sectioned into 38 fragments (dimension of 5 × 5 mm) with a double-faced diamond disk mounted on an electric cutter (Isomet Low Speed Buheler, USA). A circular area of approximately 4 mm in diameter was isolated with adhesive tape and the fragment was made completely waterproof with nail varnish.

After drying, the adhesive tape was removed from the enamel with a sharp-tipped instrument and the circular area exhibiting the enamel surface was exposed. The dental fragments were randomly enumerated (1 to 38). To make the sample handling easier, the samples were inserted (six at a time) into devices containing epoxy resin. The fragments were then submersed in 600 ml of demineralizing solution (3 mmol/L calcium, 3 mmol/L phosphate, and 50 ml/L acetic acid, pH 4.5 adjusted with NaOH)[14] for five days at a constant temperature of 37^{o}C, in an incubator, for artificial formation of an active demineralization area, simulating an incipient carious lesion.

After this exposure period, the dental fragments were washed with deionized water for one minute and dried with an air-jet for 15 seconds. Digital images of these dental fragments with active enamel demineralization, delimited by the nail varnish window, were obtained with a digital camera (Nikon Coolpix S4). The camera was fixed in a photographic tripod 10 cm high. The photo was obtained by using the camera flash, and a final focal distance of 10.0 cm was employed. The end result magnification was approximately 4X. As mentioned, both distance and angulation were standardized by means of a photographic apparatus [Figure 1]. Also, a millimeter ruler was placed close to this apparatus, along the same plane of the dental fragments, so that the photographic image could also capture a metric parameter, for further measurements [Figure 2a].

Later the nail varnish was carefully removed by using acetone and cotton, exposing the whole fragment surface. Digital images of these samples without the window were collected [Figure 2b].

Methods of evaluation

In order to measure the surface area (mm^2) of the enamel demineralization, with or without a window, two different methods were employed:

- Digital caliper (Model #500-144B, Mitutoyo Sul

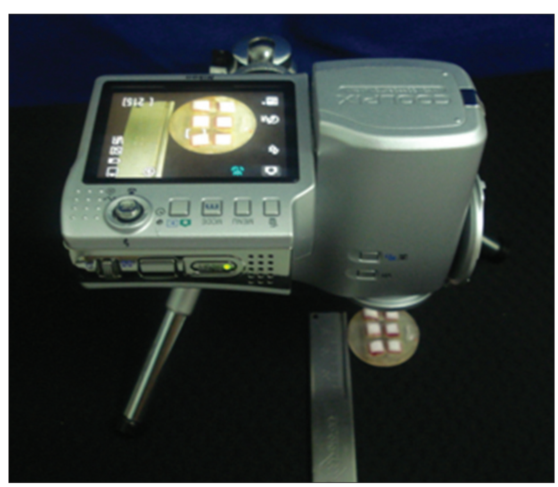

Figure 1: Photographic apparatus used for standardization of distance and angulation

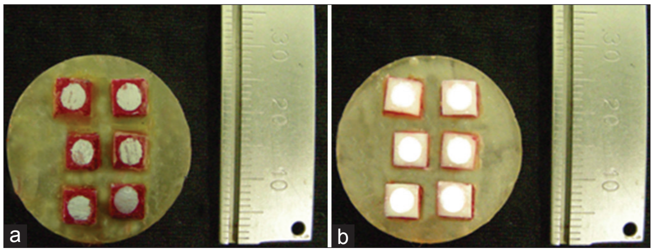

Figure 2: Dental fragments with (a) and without (b) window. Note the millimeter rule used as a metric parameter for further measurements

Americana Ltda., Suzano, Brazil) — precision instrument for measurement with 0.01 mm graduation.

- Image Tool version 4.1 (The University of Texas Health Science Center, San Antonio, TX, USA)—software, for analysis and image processing, including dimensions (distance, angulation, perimeter, area) and grayscale measurements. Precision was graduated in 0.01 mm.

In the first method (digital caliper), after direct measurement of the cut fragment, the enamel demineralization lesion area (mm^2) was calculated by using the Excel software (Microsoft Office Excel 2007) via the formula πr^2 (where r = d/2 and π =3.1416), based on the arithmetic mean of the diameter, measured in duplicate (from two distinct points), of the circle, made by enamel demineralization.

In the second method (Image Tool), the enamel demineralization lesion area was measured using a digital pen (Digitizing tablet, model MousePen 5 × 4 Genius) and then converted into a digital image on the computer screen. This digital pen functioned as a mouse, thus facilitating the delimitation of the enamel demineralization area of each fragment. Initially, the software was calibrated according to the standard distance provided by the millimeter ruler (10 mm).

All enamel demineralization lesions (with and without window) were measured three times by each method, by three pre-trained examiners (3 × 3). The examiners were blind to each other's measurements as well as to their own measurements. A one-hour interval was kept between each examiner. In order to avoid memory bias, each examiner had a one-week interval between measurements.

Statistical analysis

The data were tabulated by using the Excel software (Microsoft Office Excel 2007) and then analyzed with SPSS 16.0 software for Windows (SPSS Inc, Chicago, Illinois, USA). The intra-examiner and inter-examiner concordance was expressed by the intraclass correlation coefficient (ICC), with a confidence interval (CI) of 95%. The Altman and Bland[15] analysis was carried out to evaluate the concordance of the methods in the presence and absence of the window around the lesion.

RESULTS

Intra-examiner concordance

The mean values (of all the examiners and also from all the three readings) for the enamel demineralization lesions area measurement with window, using the digital caliper, ranged from 11.05 ± 0.44 to 11.26 ± 0.47 mm^2. For the Image Tool software, these values ranged from 11.55 ± 0.74 to 11.60 ± 0.77 [Table 1].

The mean values for the measurements without window using the digital caliper ranged from 11.08 ± 0.65 to 11.59 ± 0.71, whereas the Image Tool software had mean values ranging from 12.03 ± 0.93 to 11.90 ± 0.95 [Table 2].

The reproducibility of the Image Tool results for each examiner can be considered excellent, as no influence, either by the presence or absence of a window delimiting the measured area, was observed. On the other hand, intra-examiner concordance regarding the digital caliper ranged from fair to good, regardless of the presence or absence of a window [Table 3].

Inter-examiner concordance

By analyzing the mean values obtained from the

Table 1: Statistical analysis for enamel demineralization lesions areas (mm^2) with presence of window, measured by three examiners, considering the two methods studied

Statistical analysis	Examiner 1		Examiner 2		Examiner 3	
	Digital caliper	Image tool	Digital caliper	Image tool	Digital caliper	Image tool
Mean	11.05	11.55	11.09	11.60	11.26	11.60
Standard deviation	0.44	0.74	0.55	0.77	0.47	0.74
Median	11.01	11.53	11.08	11.75	11.17	11.72
Minimum	10.14	10.31	9.94	10.18	10.39	10.15
Maximum	12.02	13.70	12.36	13.88	12.21	13.98

*Three repeated measurements with regard to each examiner

Table 2: Statistical analysis for white spot areas (mm^2) without window, measured by three examiners, considering the two methods studied

Statistical analysis	Examiner 1		Examiner 2		Examiner 3	
	Digital caliper	Image tool	Digital caliper	Image tool	Digital caliper	Image tool
Mean	11.08	12.03	11.31	12.26	11.59	11.90
Standard deviation	0.65	0.93	0.70	0.96	0.71	0.95
Median	11.07	12.01	11.36	12.24	11.79	11.70
Minimum	9.63	10.23	10.19	10.40	9.97	10.06
Maximum	12.46	14.08	12.78	14.22	13.18	13.98

*Three repeated measurements with regard to each examine

Table 3: Intra-examiner reliability for white spot measurement using image tool software and digital caliper

Methods	With window ICC (95% CI)	Without window ICC (95% CI)
Image tool		
Examiner 1	0.922 (0.870-0.956)	0.915 (0.859-0.951)
Examiner 2	0.977 (0.960-0.987)	0.916 (0.862-0.952)
Examiner 3	0.970 (0.949-0.983)	0.990 (0.983-0.994)
Digital caliper		
Examiner 1	0.478 (0.284-0.658)	0.378 (0.178-0.578)
Examiner 2	0.458 (0.262-0.643)	0.665 (0.506-0.794)
Examiner 3	0.648 (0.483-0.782)	0.491 (0.298-0.668)

*ICC: Intra-class correlation coefficient, **CI: Confidence interval

Table 4: Inter-examiner concordance for measurements of active white spots using digital caliper and image tool software

Inter-examiners	Image tool ICC (95% CI)	Digital caliper ICC (95% CI)
With window	0.988 (0.980-0.994)	0.811 (0.676-0.895)
Without window	0.973 (0.953-0.985)	0.846 (0.737-0.915)

*ICC: Intra-class correlation coefficient, **CI: Confidence interval

three examiners simultaneously, the inter-examiner correlations showed excellent reproducibility of the measurements for both digital caliper and the Image Tool software. However, the latter method was found to have less inter-examiner variability than the digital caliper [Table 4].

Concordance for the presence and absence of frame

Figure 3 shows concordance between the measurements performed with the digital caliper and the Image Tool software regarding the presence and absence of a delimiting window. The former method had a greater difference in measurements than the latter, with the variation ranging from 1.78 to 1.64.

DISCUSSION

Visual examination is one of the most common methods for early detection of caries lesions in which parameters such as color and brightness of the enamel surface are usually assessed.[16] In spite of the advantages of being a non-invasive, simple, quick, and effective method, visual examination does not allow estimation (measurement) of the dimensions of the enamel demineralization lesion.

Combining different methods to diagnose and quantify caries lesions gives better results.[17-20] As not many reliability studies have been reported in the literature on measuring the size of an incipient enamel demineralization carious lesion, this study has been designed to evaluate the image analysis method. For this, bovine teeth were used, which according to the literature had no difference in the average values of hardness[21] and roughness[22] of the enamel from that of humans, suggesting a similar behavior between both structures of teeth. Moreover, they had larger dimensions, which favored research tests on a single element, and therefore, were excellent substitutes for human teeth.[23]

In the study performed by Pierro, *et al*.,[24] using similar methodologies such as digital caliper and Image tool software, for the measurement of alveolar bone height from the bitewing radiographs, it was concluded that both methods were reliable, although the digital caliper was considered better, because it was cheaper and easier to use. This was not corroborated in this study, as the Image Tool method showed better intra- and inter-examiner correlation. Such discordance can be justified by the difference in the image format being measured; Pierro, *et al*.[24] used a linear measure, whereas, we had irregular circumferential areas to measure.

The use of the digital caliper was indeed simpler and easy to perform, however, even calculations were required to obtain the final values. Based on the inter-examiner results, regardless of the presence or absence of a window, the ICC with the digital caliper had a concordance ranging from satisfactory to excellent [Table 4] and with the intra-examiner correlations, the concordance ranged from low to intermediate, resulting in a reliability ranging from fair to moderate. Such variability could have occurred due to the difficulty in establishing a limitation for both the window and external margin of the enamel demineralization lesion, mainly because the area had a few millimeters and the measuring instrument was not able to reproduce very small irregularities. Another disadvantage regarding the use of the digital caliper for measuring an enamel demineralization lesion was that only linear dimensions could be measured accurately, which reduced its use to areas of irregular shapes.

The use of photographic images has the advantage of being less time-consuming. It also allows a more detailed analysis of the lesion. A study by Benson, *et al*.[25] corroborates the same. The demineralized enamel can be quantified not only by determining the size of the enamel demineralization lesion, but also by the amount of mineral loss, according to the change in optical properties, such as luminescence.[26,27] In another study, Willmot, *et al*.[28] also evaluated the reproducibility and quantitative measurement of white spots resulting from enamel demineralization. They used photographic analysis and software SM Camera II to evaluate the mean pixel values in grayscale within the affected area. The authors concluded that grayscale image analysis is a reproducible method for quantifying loss of demineralized enamel.

In the present study, image measurement using Image Tool software was performed by the examiners, who had been previously trained in order to master the technique involved, which was simple and easily executed. Standardized angles and image calibration are crucial when capturing the photographic images. The Image Tool software has been found to be a highly reliable method in terms of intra and inter-examiner reliability [Tables 3 and 4]. This software has functions that allow images to be amplified and irregular contours to be delimited, resulting in a more precise and detailed analysis, which could have contributed to the quality of the results. Besides, the measured area was calculated automatically, thus not requiring any further mathematical intervention. In addition, storage of the photographic images was a great advantage for further analysis.

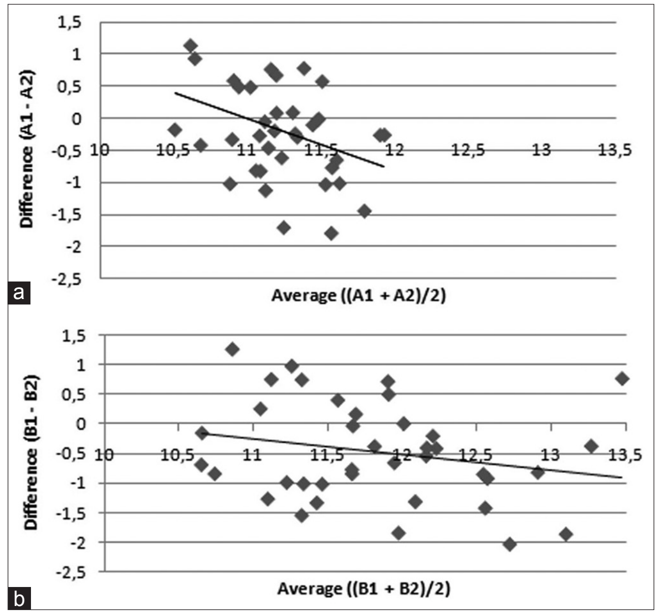

Figure 3: Comparison between digital caliper (a) and image tool software (b), with presence (1) and absence (2) of a window established according to Altman and Bland analysis

In the Altman and Bland analysis [Figure 3], when comparing the presence and absence of the window with the Image Tool and the digital caliper it was observed that the Image Tool had a smaller variation of the measurement differences, showing that this method might be more reliable.

As the measurements using the Image Tool software demonstrated more reliable mean ICC values, this method should be validated for research and clinical use. As this method provided a numerical value of the measured area, comparison of the demineralized and re-mineralized white spot areas seemed to be viable, allowing numerical quantification and image superposition. This would help in assessing the reliability of the therapeutic efficacy.

Drawing a contour of the spot (which could be clinically reproduced with graphite) was not a way to improve the reproducibility of the measurements, as the mean intra-examiner values for the digital caliper showed a weaker correlation compared to that of the Image Tool software.

CONCLUSIONS

In the present study, no statistically significant difference was observed in the measurements performed by the three examiners by using both the methods (digital caliper *versus* Image Tool). However, analysis of the photographic images using the Image Tool software yielded better results, which could be supported by higher intra- and inter-examiner reliability values. The presence or absence of a window delimiting the lesion had no effect on the area measurement of the lesion in both methods.

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