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Evaluation of a Computerized Method for Denture Biofilm Quantification: Inter-Examiner Reproducibility

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Abstract

Purpose: The objective of this work was to evaluate the accuracy and inter-examiner reproducibility of the computerized method Image Tool 2.02 (Windows, version 2.02, University of Texas Health Science Center, San Antonio, TX) in the quantification of complete denture biofilm.

Materials and Methods: Two hundred digital photographs of the internal surface of complete upper dentures with biofilm dyed in 1% neutral red were used. The photographs were burned to a CD and sent to two researchers who received practical training and written instructions on how to use the method; then, in a 20-day period, the stained biofilm on the internal surface was measured, and ten photographs were analyzed daily. Each examiner independently quantified the percentage of stained area on each photograph. The inter-examiner reproducibility (i.e., agreement) was estimated by calculating an intra-class correlation coefficient (ICC) based on an ANOVA mixed model (SPSS software, Chicago, IL).

Results: The ICC showed excellent agreement (ICC = 0.993, $p < 0.001$). The difference in the percentage of the stained area recorded by the two examiners was $\leq$1 point for 187 (93.5%) of the 200 photographs.

Conclusions: The obtained results suggest that the computerized method aided by the Image Tool software (2.0) can be employed to quantify complete denture biofilm, seeing that it demonstrated inter-examiner reproducibility of the results.

Biofilm on the internal surface of complete dentures accumulates fungi and bacteria, causing inflammation and infections, for instance, chronic atrophic Candidiasis, a frequent complication observed for complete denture wearers. Moreover, colonization of oral surfaces, including the fitting surface of dental prostheses, can serve as a reservoir for microorganisms, disseminating infections such as gastrointestinal and pleuropulmonary contaminations.[1] This type of information is important and can be used to motivate and instruct patients with regards to the hygiene of complete dentures.

Dental health education programs and efficient hygiene methods and materials are indispensable for the oral hygiene routine of denture wearers. The material should be easy-to-use, bactericidal, fungicidal, non-toxic to patients, non-deleterious to the material, low-cost, and effective in removing organic and inorganic deposits and stains.[2] The hygiene methods of complete denture cleansing are separated into two main groups: mechanical (brushing and ultrasonic devices) and chemical (hypochlorite, peroxide, neutral peroxide with enzymes, enzymes, acids, unrefined drugs, and denture rinsing).

An important factor in complete denture hygiene, besides the correct use of hygiene methods and materials, is being informed of biofilm quantification methods, an essential procedure when testing the efficacy of the hygiene products.[3,4]

Studies that compare the efficacy of complete denture hygiene methods (chemical or mechanical) employ varied biofilm quantification methods, hence making it difficult to compare the results obtained.[4,5] In some experiments, assessment is clinically performed (in vivo) by using the biofilm evidencing method (with or without photographs), protean assessment, and microbiological quantification; in other studies, laboratory experiments have been developed (in vitro).[1] Independent of being clinical or laboratory, the biofilm quantification method should be viable, simple, precise, reliable, and reproducible, so that it can be used as a parameter for effectivity tests of specific hygiene products in complete dentures. Furthermore, the adequate use of a quantification method is important for evaluating oral hygiene conditions and providing instructions and motivation to edentulous individuals, hence contributing to the planning and implementation of preventive geriatric odontology.[3-6]

In the last 40 years, many clinical indexes have been elaborated to measure the presence and severity of dental pathologies. Some of these indexes have been construed for population epidemiologic studies and others for aiding clinical instruction and individual patient follow-up. These indexes provide numeric values for health and illnesses. Several of these indicators were developed for epidemiological protection of populations and others for clinical assistance in the continued control of individual patients.[4]

Among the quantification methods are score methods Prosthesis Hygiene Index (PHI), Budtz-Jørgensen Index, and Augsburger and Elahi Index, and quantitative methods (planimeter, computerized, point-counting, and paper-weighing).

In some investigations of the quantification method, disclosed biofilm has been used, combined or not with photographs of the analyzed prostheses' surfaces. With regards to denture biofilm evidencing, literature indicates Erythrosin,[7] Methylene Blue,[8] Fluorescein,[9] and Monosulfate Proflavine can be used.[10,11]

The biofilm evidencing method is frequently associated with the indexes (score attribution methods). Its association with quantitative methods is less frequent,[12-14] and few works discuss the reliability of such methods.[1,4,5,7,12,15]

Generally, the biofilm indexes for complete dentures evaluate the internal surfaces of evidenced upper prostheses directly or by photos.[16,17]

The scoring methods should be reliable, effective, and easy to apply, even under unusual conditions. Undoubtedly, the first requirement is difficult to meet, given the inherent subjectivity of the method, which compromises meeting the second requirement.[1]

With relation to the quantitative methods, these have been denominated as "quantitative based on physical analytical parameters,"[18] "photographic methods,"[19] "morphometric methods,"[7] or simply "methods with overlapping quantitative image."[20]

According to Sheen and Harrison,[13] using quantitative methods is more laborious than subjective methods (score attributions). The difficulty of quantitative method application does not only refer to the measuring instruments, but also primarily to the delimitation of the biofilm area.

Many quantification methods can be used for complete dentures. One of these, the computerized method, which is effective in biofilm quantification, presents advantages with relation to the other methods, and correlates with other tested methods.

As this method is still recent and not widely used, the present study assessed the reproducibility of this method by two examiners, due to the fact that this element has not yet been assessed in previous studies, and since it is very important for the feasibility of employing a quantification method in complete dentures.

The objective of this work was to assess the accuracy and inter-examiner reproducibility of the computerized method Image Tool 2.02 (Windows, version 2.02, University of Texas Health Science Center, San Antonio, TX) in biofilm quantification evidenced on the internal surface of upper complete prostheses.

Materials and methods

Two hundred digital photographs of the internal surface of upper complete dentures with biofilm evidenced with 1% neutral red, from a previously conducted work, were employed using a digital camera (Coolpix 950, Nikon, Melville, NY).[3]

For biofilm quantification, a computerized method was used, aided by the software Image Tool 2.02, which enables measuring the areas by numeric data. Two researchers received the CD with the recorded photographs (Data CD-R, TDK, São Paulo, Brazil) with demonstrative and written instructions to use the software. The images were measured by the researchers during a stipulated period (20 days), and each examiner measured ten photographs daily. The biofilm quantification was performed on the same computer during different periods so the researchers would not have contact with each other, in that way not influencing the results. Regarding the areas to be measured (total and stained), the researchers were instructed to measure the total area, using as a basis the external edge line of the prosthesis, and with relation to the stained area, only the stained biofilm. Many prostheses show irregularities that become marked by the evidencing, which are easily recognized from the stained biofilm. Accordingly, the researchers were instructed to identify what was biofilm and what was an irregularity.

Each examiner independently quantified the percentage of stained area on each photograph. The inter-examiner reproducibility (i.e., agreement) was estimated by calculating an intra-class correlation coefficient (ICC) based on an ANOVA mixed model. The difference in the percentage of the stained area recorded by the two examiners was summarized by calculating the mean, median, and standard deviation of the 200 differences. In addition, the proportion of photographs for which the two examiners' measurements were ≤ 1 point, >1 to ≤ 3 points, >3 to ≤ 5 points, and ≥ 5 points were tabulated. All statistical analysis was performed using SPSS (SPSS Inc., Chicago, IL) software.

Results

Figure 1 depicts the percentages of the stained areas on the internal surface of the upper complete denture, for each examiner.

Table 1 shows a descriptive summary of the paired differences between the two examiners (mean, standard deviation, and median) and the magnitude of the differences between the two examiners. The difference in the percentage of the stained area recorded by the two examiners was ≤ 1 point for 187 (93.5%) of the 200 photographs.

The result of the ICC is presented in Table 2.

Discussion

Using the biofilm quantification method in complete dentures is a difficult process due to the scarceness of published studies. Thus, biofilm quantification methods are inadequately applied, or not many criteria are used. The methodology of biofilm quantification in complete dentures should be a customary procedure by surgeon-dentists, with research work conducted for reliable methodologies, hence disseminating the acquired information

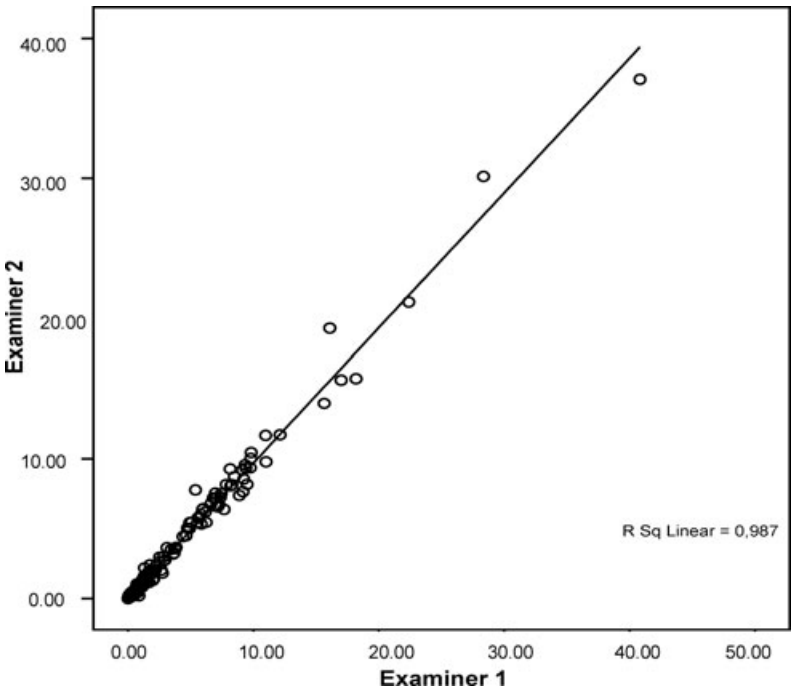

Figure 1 Percentages of the stained areas on the internal surface of the upper complete denture for examiner 1 and examiner 2.

to clinicians. There are many difficulties encountered in the quantification of biofilm in complete dentures. Accuracy, reliability, and validity of the methods must be considered when conducting a clinical or laboratory experiment.[4]

Despite the many clinical experiments relating to the effectiveness of hygiene agents, the results are questionable due to the diversity and lack of standards in biofilm quantification methods.

Shannon et al[21] emphasized that the intricacy in performing valid and reliable comparisons among many experiments, particularly concerning the hygiene of complete dentures, consists mainly in choosing reliable methods to evaluate the presence of biofilm deposits. Ambjørnsen et al[7] called attention to the need for precise methods in the quantification of biofilm distribution in complete dentures. According to the authors, this can be obtained by using a biofilm evidencing solution associated with a morphometric method, emphasizing that for the analysis of a product's biofilm removal, using a precise and systematic methodology could be important to making its use among various examiners viable.

Owing to the need for a precise and reliable method that can be reproduced by different researchers, this work assessed the reproducibility of the Image Tool using two examiners. This computerized method enables quantifying the biofilm in digital photos of complete dentures, by measuring the total area of the prosthesis and the area with the stained biofilm before the photograph was taken.

Image Tool software was employed for biofilm quantification by several authors[4,14,22] to evaluate hygiene products and was used in a comparative study for various methods of biofilm quantification in complete dentures.[4]

The ICC coefficient indicated high correlation between the data of both examiners ($p < 0.001$; $r = 0.993$), suggesting that there is data reproducibility obtained by the computerized method of assessed denture biofilm quantification. The scale of points (Table 1) indicated a difference ≤ 1 point in 93.5% of the 200 photographs measured by the examiners. For the analysis of that variable (biofilm), it is believed that this difference may be considered small.

There are many quantification methods, including scoring methods Prosthesis Hygiene Index (PHI), Budtz-Jørgensen Index, Additive Index, and the Augsburger and Elahi Index, and quantitative methods (planimeter, computerized, point-counting, and paper-weighing). The use of the computerized method is relatively new for complete dentures.[13,23] Paranhos and Silva-Lovato[4] compared the application and reliability of four methods for biofilm quantification (computerized—Image Tool, paper-weighing, point-counting, and planimetric) in

Table 1 Mean (standard deviation), median of the 200 paired differences, and number (%) of the 200 differences ≤ 1 points, >1 to ≤ 3 points, >3 to ≤ 5 points, and ≥ 5 points of each other

Mean (standard deviation)	Median	Differences in points			
0.12 (0.5)	0.12	≤ 1 points 187 (93.5%)	>1 to ≤ 3 points 11 (5.5%)	>3 to ≤ 5 points 2 (1%)	≥ 5 points 0

Table 2 Intra-class correlation coefficient

		95% Confidence interval		
	Intra-class correlation	Lower bound	Upper bound	Significance
Single measures	0.993[b]	0.991[a]	0.995[a]	0.001

Two-way random effects model where both people effects and measures effects are random.

[a]Type A intra-class correlation coefficients using an absolute agreement definition.

[b]The estimator is the same, whether the interaction effect is present or not.

complete dentures, verifying the correlation between them (0.82 to 0.99). The methods of biofilm quantification, although more challenging than scoring methods, offer objective and accurate results. Because these methods do not rely on ability, calibration, or number of examiners, they should be the methods of choice in clinical experiments for the evaluation of complete denture cleansers. The difficulty in differentiating biofilm from food residues and stains in the photographs is considered a limitation of these methods. Although the examiner was trained on the four methods, the time spent to measure the areas of interest was considerably high for all methods; however, the computerized method was clearly faster (average time: 20 minutes), since the measurements were performed directly from the scanned image, and the program measured the selected area. The authors concluded that quantitative methods were efficient and reliable for measuring quantity of biofilm in complete dentures, and may be useful in experimental studies on the efficacy of hygiene products. The computerized method was fast and easy to perform.

McCabe et al[20] called attention to the difficulty encountered in the quantification methods that employed photos. These difficulties involve standardizing prosthesis positioning, lighting conditions, and contrast of the photographic methods. Sheen and Harrison,[13] aiming to standardize such conditions, recommended the use of digitalized images. In the present work, quantification was performed on slides obtained under controlled lighting and processing conditions. To make use of photographs, it is essential to point out the quality of the photos obtained and the procedure's measuring standard of the images, whether by a scanner coupled to the computer, or by means of a digital camera.

McCabe et al[20] underscored the importance of calibration when using different examiners. Some experiments use more than one, citing the participation of two,[24] three,[20,25] four,[19] and even five[26] examiners.

For this method, however, the use of specialized equipment is necessary. In other words, the images have to be scanned before measuring, and a microcomputer is needed. Such a method can be used without a scanner, by using a digital camera directly coupled to a computer.[4,13,22,27] That way, the prostheses are photographed with a digital camera, and the images are transferred and stored in the computer, where the quantification method is later applied.

The results of the present work suggest that the computerized Image Tool method can be reliably employed in clinical experiments, and that it can be the chosen method for clinical experiments to assess the effectivity of different hygiene methods in complete dentures for the effective control of biofilm when precision is very important.[27] However, further work with more data will be developed.

Conclusions

Based on the results of this work, it can be concluded that there was high correlation ($r = 0.993$) between the data obtained by the two examiners, suggesting that the computerized method, aided by Image Tool (2.0) software can be used with reliability in the denture biofilm quantification.

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