Validity and reliability of autofluorescence-based quantification method of dental plaque

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Background: The aim of this study was to evaluate validity and reliability of autofluorescence-based plaque quantification (APQ) method.

Methods: The facial surfaces of 600 sound anterior teeth of 50 subjects were examined. The subjects received dental plaque examination using Turesky modified Quigley Hein plaque index (QHI) and Silness & Løe plaque index (SLI). The autofluorescence images were taken before the plaque examination with Quantitative Light-induced Fluorescence-Digital, and plaque percent index (PPI) was calculated. Correlation between two existing plaque indices and the PPI of the APQ method was evaluated to find which level of plaque redness on tooth (∆R) by the APQ method shows the highest correlation. The area under the ROC curve (AUC) analysis and intra- and inter-examiner reliability tests were performed.

Results: The PPI ∆R0.48 of the APQ method showed a moderate correlation with two existing plaque indices (rho of QHI = 0.48, SLI = 0.51). This methodology fell in the fair category and it had an excellent reliability. The APQ method also showed possibility to detect heavy plaque with fair validity.

Conclusions: The APQ method demonstrated excellent reliability, and fair validity, compared with 2 conventional indices. The plaque quantification described has the potential to be used in clinical evaluation of oral hygiene procedures.

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1. Introduction

Quantification of dental plaque is important for oral health maintenance as dental plaque has been known to be a main etiologic factor for oral diseases [1]. As a quantitative method, dental plaque indices have been used to assess oral hygiene status of individuals and communities.

The dental plaque indices generally focus on the area or thickness of plaque covering the tooth surface. Quigley Hein plaque index (QHI) [2] and its modification by Turesky [3], and O’Leary index [4] consider the surface area of plaque from gingival margin. These indices need to dye tooth surface using a disclosing agent before measuring, so they may cause discomfort and social embarrassment to patients owing to the unwanted staining of oral tissues. Silness & Løe plaque index (SLI) is basically based on the thickness of gingival plaque without disclosing agents. This index was designed to demonstrate the relationship between plaque accumulations and gingivitis [5]. It considers differences in thicknesses of the plaque without paying attention to the coronal plaque extension. Hence, it is likely to be related with plaque maturity and thus potential pathogenicity. Since it is measured by visual examination without disclosing, differences in examination techniques can affect the result. However, there has been no report of dental indices which consider the area and the thickness of plaque concurrently.

Most conventional plaque scoring methods have some limitations for assessing oral hygiene status and treatment effects. Previous indices, such as QHI, SLI, O’Leary index and Rustogi’s modified navy plaque index, are based on categorical or ordinal scales on plaque measurement by dividing demarcation lines on tooth surface [6,7]. These approaches cannot demonstrate slight changes and various types of plaque accumulation as they yield scores based only on the presence of the plaque in one or divided zones [7]. In addition, scores are likely to be influenced by the examiner’s subjective decisions as these indices rely on only visual examination [6]. Calibration between examiners are always needed due to the subjective nature of the indices. This requires a lot of time in clinical studies [8].

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Computerized dental plaque quantification has been attempted to overcome these limitations [9,10]. Computer-based methods focus on quantifying the plaque area which is covering the tooth surface. There are some advantages of this methodology; first, it can detect a slight change in the plaque accumulation due to individual oral hygiene behavior; second, it can objectively detect plaque and consider a spatial localization of the plaque related to the tooth structure [7].

Analysis of the images is used to produce an index called percentage plaque index (PPI) which is an indicative of the extent of plaque coverage on a tooth surface [9,11–13]. Although the procedure of selecting the area of plaque coverage could be considered subjective, the planimetric method has demonstrated its objectivity and reliability in several studies [11,14].

Quantitative Light-induced Fluorescence-Digital (QLF-D Biluminator, Inspektor Research System BV, Amsterdam, The Netherlands) is a newly developed device specifically designed to achieve the red-reversibility and the quantification of plaque [15,16]. It has been proved to detect red fluorescence representing endogenous porphyrins particularly produced by late colonizing oral bacteria [15]. While, most planimetric methods used fluorescent dyes or disclosing agents to distinguish plaque from the tooth, the QLF-D does not need any additional disclosing procedure [17,18].

The QLF-D has many advantages to be used in plaque quantification. It would be able to obtain high-quality images with small focal depth. Moreover, this method can compensate for the defect of the planimetric method considering only the area of the plaque since the red fluorescence intensity is linked closely to plaque depth and maturation [19,20]. The intensity of the plaque fluorescence is given as a delta R (ΔR), increasing examiners’ ability to conduct research on this matter. The ΔR is given that percent of increase in the red per green ratio of plaque with respect to that of tooth surface. In accordance with different ΔR levels, it would be able to evaluate plaque accumulation at various redness levels.

The purpose of the present study was therefore to evaluate the validity and reliability of autofluorescence-based plaque quantification (APQ) method using the QLF-D in the clinical situation.

2. Materials and methods

2.1. Ethical approval

A prospective, non-interventional study received ethical approval from the Yonsei University Dental Hospital, Republic of Korea (IRB No: 2-2012-0045). This study was conducted in accordance with the Helsinki Declaration of 1975, as revised in 2000.

2.2. Participants

Participants included 50 persons aged between 20–60 years old through clinical trial recruitment. The inclusion criteria were that the subjects were in good general health with sound anterior teeth on facial surfaces. Teeth with stains or dental caries were excluded due to a possibility of confounding factors. All participants agreed to refrain from oral hygiene procedures and food intake of minimum 4 h before visiting. They were given information about this study, and informed consent forms were signed. On the same day, plaque measurement was done, and intra-oral photographs were taken.

2.3. Oral examination

Fifty healthy participants included 27 males and 23 females with a mean age 34.6. Twelve anterior teeth from each person were tested in this planimetric method. They received two types of dental plaque examination (SLI and QHI) by two different examiners, respectively. Only facial anterior surfaces of maxillary and mandibular arches were examined (central incisors, lateral incisors and canines). And then full-mouth oral examination was performed by a dentist. If participants had severe dental caries or lesion which demands for urgent dental treatment, they were excluded from this study and received treatment recommendation. All participants had full-mouth scaling and polishing by a dentist or a dental hygienist after examination.

The plaque measurement was performed in the same order in consideration of disclosing procedure; the SLI and then the QHI. The score of the SLI, which considers the thickness of plaque, was calculated without the score of lingual surface. And the score 1 was given if plaque exists when the QHI was assessing because the plaque could be disturbed by using probe [21]. The measurement method of the QHI, which considers the area of plaque, was followed method of Mankodi, et al. [22] which use mean score of three zones per a facial surface by line angle (disto and mesio-line angle) to improve accuracy.

Two examiners participated in this study after calibration training process. The ICC (Intraclass Correlation Coefficient) values of intra-examiner agreement for the examiner’s were monitored throughout the study period and the value was kept more than 0.900, respectively.

2.4. Quantitative light-induced fluorescence-digital examination

The QLF-D images were taken before oral examination for the APQ method. First, the participants swallowed saliva. If there was any debris on the surface of the teeth, it was removed. Then the dental unit was placed by supine position and the participants were taken images on edge to edge bite to obtain overall shape of their anterior teeth. Lip retractors were used to allow a good view of the anterior teeth. The QLF-D was vertically placed to the anterior tooth surface. And then the cone of the device was closely attached to their mouth to take images with a certain depth of focal length (0.32). Focus was adjusted to the maxillary lateral incisor and canine. The fluorescence images were captured with a ‘Live view’ at the following setting: shutter speed of 1/30s, aperture value of 5.6, and ISO speed of 1600. The images were automatically saved by default as a bitmap image (Fig. 1). The cone of the QLF-D was covered with a blackout fabric to block out ambient light.

2.5. Planimetric analysis and plaque percent index

The APQ method was based on the planimetric method. Planimetric analysis is a technique based on images of the teeth which
uses plaque percent index [12,13]. The score was calculated by following figures: the number of pixels of red fluorescence and the number of pixels of tooth. Plaque patch of the proprietary software (QA v.1.23, Inspektor Research Systems BV, Amsterdam, The Netherlands) was used to obtain the pixel numbers of plaque and tooth in every level of red fluorescence intensity (ΔR). The ΔR means that percent of increase in red and green ratio of object with respect to that of sound tooth. This figure is related to the presence of porphyrins. Through the analysis program, total 13 levels of ΔR (from ΔR0 to ΔR120) and its pixel numbers were obtained (Fig. 2). For example, ΔR30 means that the redness difference between the tooth and the plaque is at least 30%. The ΔR30 contains the number of the pixels at higher ΔR levels. The data were transferred to an Excel spreadsheet. The PPI (plaque area) was calculated as a ratio of the pixel number of plaque with respect to that of tooth in every levels of ΔR. And it was used to determine an optimal ΔR level which shows the highest correlation with conventional indices for further analysis. This analysis was performed by one single examiner.

2.6. Data analysis

Relationship between two dental plaque indices and the APQ method was evaluated using a Spearman’s rank correlation analysis (PASW statistics ver.18.0, SPSS, Chicago, IL, USA) (α = 0.05). The level of ΔR showing the highest correlation with QHI and SLI was investigated.

The area under the ROC curve (AUC) was analyzed to verify whether the APQ method is able to detect presence of plaque. Score 1 and more of conventional plaque indices was criterion to divide into two levels (1 and 0) according to existence of plaque for AUC analysis. And then additional analysis was conducted that whether the method is able to perceive difference between young (thin) plaque and heavy plaque. The score of the SLI was criterion to divide into two levels (score 1 and lower = 0, score 2 and more = 1). Sensitivity and specificity with cutoff value on two conventional indices were assessed (MedCalc 12.7.0., Mariakerke, Belgium), respectively.

Among 600 teeth, 10% of teeth were randomly selected by random function of excel to evaluate intra and inter-examiner reliability. The reliability was tested by Pearson’s correlation analysis and ICC. The significance level was 0.05.

3. Results

Total 600 teeth were tested the correlation between the PPI of the APQ method and scores of the conventional plaque indices. Each PPI from ΔR0 to ΔR120 was compared with scores of the two conventional plaque indices by tooth (N = 600) and person (N = 50) as a unit of analysis. The highest correlation coefficient with the QHI was 0.479 by tooth and 0.517 by person as the unit of analysis at PPI_{ΔR20}. With the SLI, the coefficient was 0.506 by the tooth unit at PPI_{ΔR30} however, there was no significantly differences with PPI_{ΔR20}. And 0.582 of coefficient by the person unit was the highest at PPI_{ΔR20} (Table 1). The PPI_{ΔR20} was distributed from 0% to 81.82% (mean = 5.36, s.d. = 11.7).

In the AUC analysis based on the QHI as a standard, sensitivity and specificity were the highest as 72.34–83.33 at the curve of ΔR20, respectively (Table 2). The cut-off value was PPI_{ΔR20} > 0 and AUC was 0.779 (p < 0.0001). Following analysis with the SLI, the sen-

<table>
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<tr>
<th>Table 1</th>
<th>Correlation between PPI of APQ method and scores of two conventional plaque indices.</th>
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<tr>
<td>PPI by ΔR value</td>
<td>Unit: Tooth (n = 600)</td>
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<tr>
<td></td>
<td>QHI</td>
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<tr>
<td>⎯</td>
<td>⎯</td>
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<tr>
<td>ΔR0</td>
<td>0.202*</td>
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<tr>
<td>ΔR10</td>
<td>0.465*</td>
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<tr>
<td>ΔR20</td>
<td>0.479*</td>
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<tr>
<td>ΔR30</td>
<td>0.470*</td>
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<tr>
<td>ΔR40</td>
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<tr>
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<td>0.421*</td>
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<tr>
<td>ΔR90</td>
<td>0.411*</td>
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<tr>
<td>ΔR100</td>
<td>0.401*</td>
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<tr>
<td>ΔR110</td>
<td>0.388*</td>
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<tr>
<td>ΔR120</td>
<td>0.387*</td>
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Spearman’s rank correlation; **p < 0.01, *p < 0.05.

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<th>Table 2</th>
<th>Results of the area of under the ROC curve analysis.</th>
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<tr>
<td>Purpose</td>
<td>Type of index</td>
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<tr>
<td>Presence of plaque</td>
<td>QHI</td>
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<tr>
<td></td>
<td>SLI</td>
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<td>Heavy plaque detection</td>
<td>SLI</td>
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sitivity and specificity were respectively 71.01–70.97% at the curve of the $\Delta R_{20}$ as well (Table 2). The cutoff criteria was $\text{PPV}_{\Delta R_{20}} > 0.34$ and AUC was 0.747 ($p < 0.0001$). In additional analysis, the curve of the $\Delta R_{30}$ presented the highest AUC value (0.752, $p < 0.0001$) as a threshold level for heavy plaque detection (Table 2).

Reliability test of the APQ method showed 0.995 (95% Confidence Interval = 0.994–0.995) for intra-examiner repeatability and 0.980 (95% CI = 0.977–0.983) for inter-examiner reproducibility (Table 3).

### 4. Discussion

The present study assessed that the APQ method using QLF-D for clinical plaque was a reliable and valid method to quantify clinical plaque without disclosing. There have been some reports that QLF planimetric analysis has a potential for plaque quantification [8,19,23]. These previous studies, however, used a different system and also disclosed tooth images using a previous QLF system (Inspektor™ Pro, Inspektor Research System BV, Amsterdam, The Netherlands). The current study used a different hardware and software system and involved the facial surfaces of the anterior teeth. This study was thus conducted to assess sensitivity and validity of plaque quantification using two well-known plaque indices (QHI and SLI).

This new method demonstrated a moderate correlation with two conventional plaque indices (QHI = 0.48, SLI = 0.51, $p < 0.01$) (Table 1). In a previous study, the correlation between the planimetric method using digital camera images and the QHI was higher ($r = 0.92, p < 0.01$) than the results of this present study [11]. The other study also showed a good correlation between the planimetric method and the QHI ($r = 0.77, p < 0.01$) [24]. However, the lower correlation in this study would be carried out because we used undisclosed tooth surface for the APQ method.

The characteristics of the QLF-D detecting red autofluorescence of plaque by porphyrins (i.e., a particular product produced by late colonizing oral bacteria) is more applicable to measurement of undisclosed plaque than the disclosed plaque. According to a previous study, area of the red fluorescent (RF) plaque was obviously smaller than that of the disclosed plaque [8]. This difference was due to red fluorescence in plaque visualized after 3 days of plaque growth in contrast to the disclosed plaque [20]. Therefore, previous QLF studies have used disclosing agents to assess the efficacy of the APQ method [19]. However, the disclosing procedure can cause socially embarrassing and it may over estimate the actual plaque area due to the agents attached not only to plaque, but also to proteins in oral cavity. This study was thus conducted to evaluate whether the quantification of RF plaque area without disclosing is a valid and reliable tool in the clinical environment.

Result from analyzing area under the ROC curves of $\Delta R_{0}$–$\Delta R_{50}$ revealed that the curve of the $\text{PPV}_{\Delta R_{20}}$ was selected by the sensitivity and specificity. The results revealed that the APQ method showed fair predictive power with respect to being able to distinguish between the presence and absence of plaque (Table 2). As results, it has been proved that the APQ method has the possibility to be used as a novel plaque detection method if the plaque has more than 20% of redness difference with respect to that of sound tooth. In other studies [14,24], validity was tested by Pearson's correlation coefficient and covariance analysis which measured plaque accumulation at different times (baseline, 24, 32, 48, and 96 h) between two conventional indices (QHI and Addy plaque area index) and a test index. This present study evaluated the validity using Spearman's rank correlation coefficient and the AUC analysis as comparison with two representative indices. Then it showed this new method had a potential to detect the presence of plaque compared to two different typical dental plaque indices, respectively.

Previous QLF studies mainly have been conducted to compare performance against only the QHI which depend on measuring of plaque covered area. Pretty et al. recommended that the assessment of the threshold for plaque depth should be compared with the SLI to see if the stronger redness relates to an increased depth of plaque [8]. In this study, the correlation coefficient with the SLI was still kept in moderate range as $\Delta R$ level increased ($r = 0.44$ at $\Delta R_{120}, p < 0.01$). The results showed a stronger correlation with the SLI than that with the QHI at all $\Delta R$ levels. It is assumed that the redness intensity can reflect thickness of the plaque. Since the SLI is fundamentally based on the same principle with the Silness & Löe Gingival Index [21], the score of the SLI would match the severity of gingivitis. In the additional analysis, the result thus showed that this method can detect heavy plaque (score 2 and more of SLI) from young (thin) plaque (score 1 and lower of SLI) with fair validity at $\Delta R_{30}$ (AUC = 0.752, sensitivity = 64.00, and specificity = 80.25, Table 2). The sensitivity was decreased but the specificity was increased up to 80.25. Regarding this result, it can be explained that the APQ method is capable of distinguishing plaque levels based on the thickness and its plaque area. It could be the reason why the APQ method showed moderate correlation with conventional indices in Table 3. Above all things, this new plaque quantification method using QLF-D would have possibility as a tool to manage progression of oral diseases.

The good reliability of planimetric methods has been reported by previous researches [8,25–27]. Smith et al. [14] showed that the correlation coefficient on each tooth was in the excellent reliability category of 0.82 to 0.99. Another study conducted to test on lingual surface also reported that the total correlation coefficient among teeth was in the excellent reliability range [24]. In common with these studies, the present study using the QLF-D showed high intra- and inter-examiner agreements as well (Table 3). ICC values for measuring repeatability and reproducibility fell in the 'excellent' range (0.995 and 0.980, respectively).

In this study, the main statistical unit was a tooth due to the possibility that the use of person as a unit could lose information in reliability analysis. Since the result of examined tooth is not influenced by that of next tooth, use of tooth as a unit can be acceptable [28].

The most important point of the image analysis depends on obtaining high-quality images. Among various external factors, the optimum photographing condition should be carefully considered. Several researchers have tried to employ a method using a specially designed frame to standardize illumination [14,19]. It was developed for a high degree of standardization of camera/patient positioning and lightening. An additional space for this frame however must be needed in the dental clinic. If the frame needs to move when taking pictures in a clinical study, it can be a limitation of the study.

To reduce these limitations, it can be recommended that applying the same condition and position of photographing by camera. Söder et al. [11] have used a photographing standard with its horizontal plane and vertical plane on each imaging lesion. In this study, taking fluorescence images using QLF-D was conducted by a single examiner, and the photographing condition and its position was same for all participants. Also, we covered the cone of the QLF-D with a blackout fabric to block out ambient light instead of taking photographs in a dark room. This might be a practicable method.
for use in dental clinics for photographing the anterior or partial sections of the tooth.

However, unlike previous studies which had subjects refrained from food consumption or oral hygiene care over night or for at least 24 h, the present study assessed the plaque accumulated for 4 h and more due to ethical issue [7,14,24]. The heavy plaque would thus be less than that in the previous studies. Nevertheless, the intensity of red fluorescence was differently revealed depending on caries risk of plaque even if the plaque had been accumulated during the same duration [29]. Thus, this study focused on whether the amount of plaque is enough to analyze; individual oral hygiene status and time of plaque accumulation were not serious considerations. Also, the plaque was obtained not giving dental scaling because the oral hygiene restriction may cause ethical issue. All subjects were received dental scaling after plaque measurement. Therefore, red fluorescence of plaque may be associated with calculus as well. However, it was not considered since most calculus was covered with dental plaque by oral hygiene restriction for 4 h.

Despite these limitations, this study demonstrated the utility of the APQ method by QLF-D for use in clinical environment and research. The results highlighted the ability to assess plaque without disclosing as a new plaque measurement method. Detecting heavy plaque could be used for high-risk assessment of dental plaque associated diseases. This method also can reduce various errors appearing in dental clinic, such as disagreement between examiners. However, further studies are needed to assess clinical significance of this methodology based on detecting RF plaque.

5. Conclusion

The APQ method using QLF-D on undisclosed plaque was confirmed to have excellent reliability and fair validity against conventional indices. Also, this method was able to reliably measure tooth coverage by heavy plaque that fluorescence red when imaged by the QLF-D.

Acknowledgment

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