ORIGINAL REPORT

Longitudinal Analyses of Early Lesions by Fluorescence: An Observational Study

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Abstract: *Previous caries experience correlates to future caries risk; thus, early identification of lesions has importance for risk assessment and management. In this study, we aimed to determine if Quantitative Light-induced Fluorescence (QLF) parameters—area (A [mm²]), fluorescence loss (ΔF [%]), and ΔQ [%×mm²]—obtained by image analyses can predict lesion progression. We secured consent from 565 children (from 5-13 years old) and their parents/guardians and examined them at baseline and regular intervals over 48 months according to the International Caries Detection Assessment System (ICDAS), yearly radiographs, and QLF. QLF images from surfaces with ICDAS 0/1/2/3/4 at baseline that progressed (N = 2,191) to cavitation (ICDAS 5/6) or fillings and surfaces that did not progress to cavitation/fillings (N = 4,141) were analyzed independently for A, ΔF, and ΔQ. Linear mixed-effects models were used to compare means and slopes (changes over time) between surfaces that progressed and those that did not. QLF A, ΔF, and ΔQ increased at a faster rate for surfaces that progressed than for surfaces that did not progress* (p = *.0001), regardless of type of surface or baseline ICDAS score. AUC for ICDAS ranged from 0.65 to 0.80, but adding QLF information improved AUC (0.82-0.87,* p *< .0005). We concluded that faster changes in QLF variables can indicate lesion progression toward cavitation and be more clinically relevant than actual QLF values.*

Key Words: dental caries, prospective study, visual examination, early diagnosis, fluorescence imaging.

Introduction

Previous caries experience has been the only risk factor that consistently correlates to future caries risk (Bader *et al.*, 2008). Therefore, the detection of caries lesions at their earliest stage can significantly assist in determining future risk (Zero *et al.*, 2011). However, it is known that not all lesions progress to cavitation (Backer Dirks, 1966; Ferreira Zandoná *et al.*, 2012); thus, identifying which of these early lesions are likely to progress will allow dentists to provide focused early preventive intervention (Zero *et al.,* 2011; Ferreira Zandoná *et al.*, 2012).

There has been considerable interest in developing methodologies that can help dentists to detect caries at early stages. The International Caries Detection and Assessment System (ICDAS), a visual method that assesses the severity of dental caries using 7 scores (0-6), has been shown to be a reliable method to assess early lesions (Ekstrand *et al.*, 2007; Ismail *et al.,* 2008; Varma *et al.,* 2008). Additionally, we have reported that these early lesions (ICDAS 1 or 2) can be monitored by visual examination. When it is observed that they transition to a moderate lesion (ICDAS 3 or 4), the lesions can be used as surrogates of cavitation in a high-risk population (Ferreira Zandoná *et al.*, 2012). Quantitative Light-induced Fluorescence (QLF) is a technology-based method that has been stated to have high sensitivity for the detection of early lesions (Diniz *et al.*, 2012; Jablonski-Momeni *et al.*, 2012; Souza *et al.*, 2012). Because this method is quantitative (Ando *et al.*, 2001; Karlsson *et al.*, 2007; Kühnisch *et al.*, 2007), it allows for longitudinal monitoring of lesion progression (Ferreira Zandoná *et al.*, 2000; Tranaeus *et al.,* 2001; Kambara *et al.*, 2003). The combined use of ICDAS with QLF (Ferreira Zandoná *et al.*, 2010)

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allows for the earlier detection of caries lesions, avoiding the pitfalls that have been associated with QLF: the confounding effects of developmental defects, fluorosis (Pretty *et al*., 2006), stain (Shi *et al*., 2001), swollen gingival margin, presence of plaque, and quality of fluorescence images (Heinrich-Weltzien *et al*., 2005).

The objective of this four-year longitudinal study was to determine if QLF parameters (ΔF - %; Δarea - mm^2; and ΔQ - mm^2 x %) obtained by the analyses of fluorescent images could be used to predict lesion progression toward cavitation.

Study Population & Methods

As previously reported (Ferreira Zandoná *et al.,* 2010, 2012; Fontana *et al*., 2011), children (N = 565) from public schools (kindergarten to 9th grade) in Aguas Buenas, Puerto Rico were recruited as approved by the institutional review board committees from Indiana University (IU-IRB #0608-15) and the University of Puerto Rico (UPR-IRB#A1340107). Parental consent was obtained along with assent from the child for children older than 7 yrs. The sample size was chosen based on preliminary data, to allow for a sufficient number of clinically significant lesions at the end of the study. Inclusion criteria were age (5-13 yrs), no medical problem contraindicating participation, and tolerance for oral examination, including radiographs, digital photographs, and QLF examinations. Both caries-free and caries-active children, with at least one permanent molar and at least one unrestored surface, were recruited.

Examinations—which consisted of an oral soft tissue examination, a visual examination according to the ICDAS criteria, which range from 0 to 6 (Ferreira Zandoná *et al.,* 2010), and an examination with QLF, all completed by a single calibrated examiner—were conducted at baseline, 8, 12, 20, 24, 28, 32, 36, 40, 44, and 48 mos. The occlusal and buccal surfaces of all permanent molars and the lingual surfaces of upper molars were examined, and fluorescence images were acquired with Quantitative Light-induced Fluorescence (QLF Pro, Inspektor Research Systems B.V., Amsterdam, The Netherlands) in a controlled darkened environment, immediately after the ICDAS examination. Bitewing radiographs were obtained yearly, but the results are not included in these analyses. Details of the ICDAS examinations were published previously (Ferreira Zandoná *et al*., 2012). The study complied with the STROBE criteria.

Fluorescence Image Analyses

QLF images from surfaces with ICDAS scores 0/1/2/3/4 at baseline that progressed to cavitation (ICDAS scores 5/6) or fillings (N = 2,191) at any subsequent examination and a random sample of surfaces that did not progress to cavitation/filling (N = 4,141) at the end of the four-year study were analyzed independently for average area (ΔA [mm^2]), fluorescence loss (ΔF [%]), and ΔQ [%×mm^2], with dedicated software (QLF 2.00g, Inspektor Research Systems B.V., Amsterdam, The Netherlands) as previously described (Ando *et al.,* 2001) (Fig. 1). A patch was placed on the last image of the lesion on the visit that preceded cavitation, and that patch was copied on all previous images including the baseline visit images.

Statistical Analyses

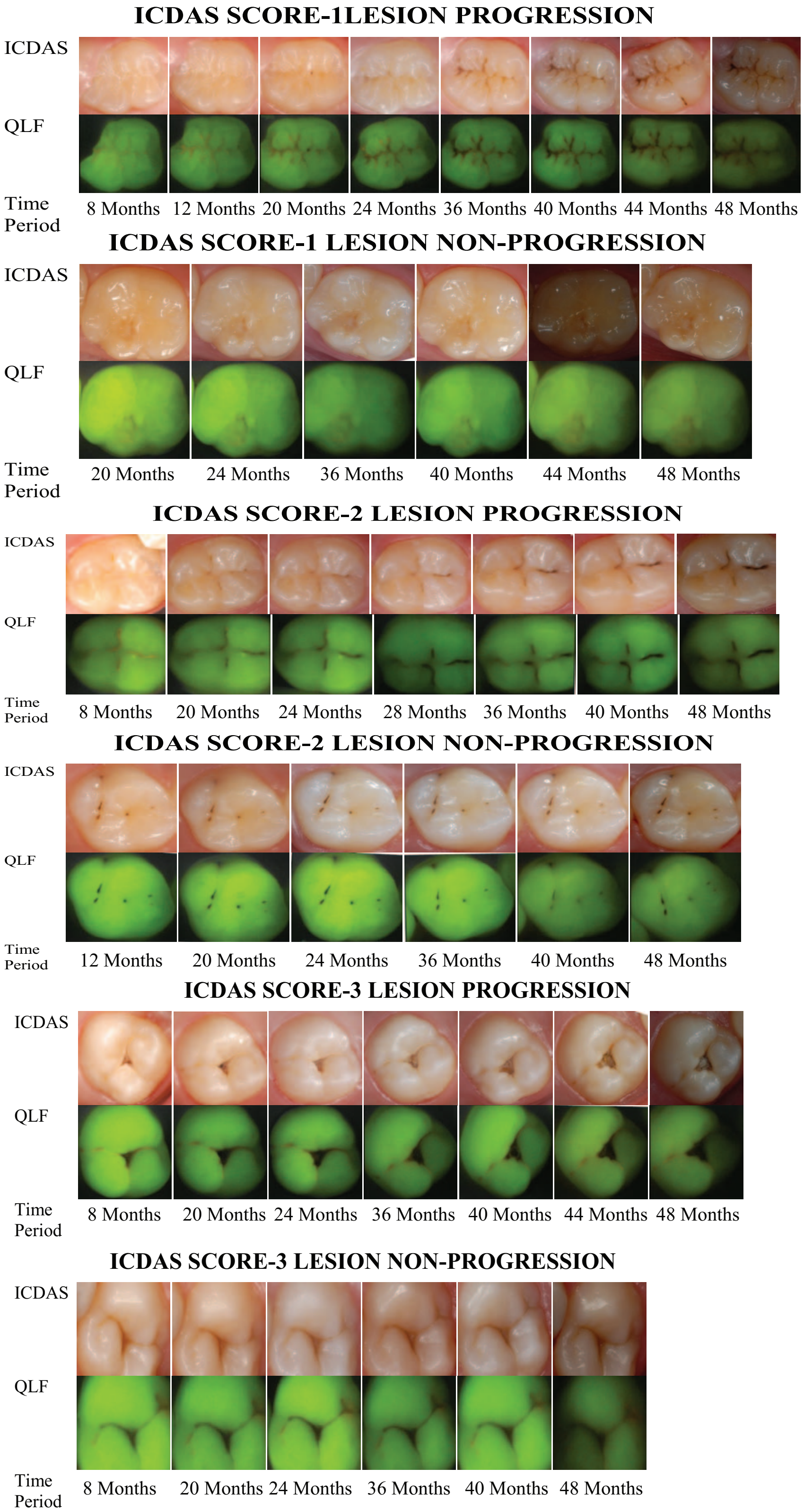
The last visit with QLF image analyses was determined for each tooth surface. Visits were re-numbered as the number of 4-month periods prior to the last visit for the tooth surface. Summary statistics (mean, standard deviation, standard error, range) for QLF ΔA, ΔF, and ΔQ were calculated by visit and whether or not the surface progressed to cavitation or filling by the end of the study period. Linear mixed-effects models were used to compare the means and slopes (changes over time) between surfaces that progressed and those that did not progress. The models included random effects for each participant, participant-by-tooth, and participant-by-tooth-by-surface to model within-participant correlations between observations. Analyses were performed overall (adjusted for surface and baseline ICDAS as well as the demographic characteristics age, gender, race, and ethnicity), by surface (adjusted for baseline ICDAS and demographics), and by baseline ICDAS (adjusted for surface and demographics). These models indicate the average trajectories of the QLF parameters over time for lesions that ultimately progressed and those that did not. Additionally, slopes representing the changes in QLF measurements over time were calculated for each individual surface; one slope was estimated over all time-points for each surface for each participant. The surface type (buccal, lingual, occlusal), individual QLF slopes, baseline ICDAS score, last ICDAS (at final visit if no cavitation, at visit before cavitation if surface cavitated), last QLF values, and the interactions between baseline ICDAS and QLF slopes were included in a logistic regression model to calculate the area under the receiver operating characteristic (ROC) curve (AUC). To evaluate the associations between the ICDAS levels and QLF ΔF with data from all time-points, QLF ΔF values were compared among ICDAS levels in a linear mixed-effects model which included random effects for participant, participant-by-tooth, and participant-by-tooth-by-surface to model within-participant correlations between observations and included surface type and demographics as covariates.

Results

There were 338 children who completed the examinations at the 48-month visit. Details have previously been published (Ferreira Zandoná *et al.,* 2012). The surfaces that cavitated and, thus, had the images analyzed from each of the examination periods are from 359 children at baseline, 317 at visit 1, 272 at visit 2, 281 at visit 3, 244 at visit 4, 220 at visit 5, 168 at visit 6, 136 at visit 7, 116 at visit 8, 71 at visit 9, 46 at visit 10, and 46 at visit 11. On average, there were images of 2.3 to 3.3 surfaces analyzed from each of the visits, ranging from 1 to 16 images *per* child. (Characteristics of the lesions are provided in Appendix Table 1.) Fillings were included as part of the study outcome definition. Progression was not considered after

Figure 1.

Examples of reflectance and fluorescent images of lesions that progressed to cavitation and did not progress to cavitation based on baseline ICDAS scores.



sealants were placed. Caregivers were asked to complete yearly questionnaires, which included an item about fluoride treatments received at the dentist's office. Nearly 90% of the respondents indicated that the child received a fluoride treatment at least once *per* year.

All the QLF parameters (QLF A, ΔF, and ΔQ) increased at an overall faster rate (*i.e.*, larger slope) on average for surfaces that progressed than for surfaces that did not progress (p = .0001) to cavitation, regardless of type of surface or baseline ICDAS score (Appendix Tables 2 and 3). Since the differences were significant for all parameters, only ΔF values are presented.

As can be seen in Fig, 2, independent of surface type (buccal, occlusal, or lingual), surfaces that progressed to cavitation had significantly more fluorescence loss (ΔF) than surfaces that did not progress (p < .0001).The rate of progression (slope difference) of lesions that progressed to cavitation was significantly greater (progressed faster) than that of those that did not progress (p < .0001). QLF ΔF values tended to be higher at baseline for lesions that progressed to cavitation and had a progressive increase prior to cavitation. QLF ΔF values tended to be higher for lesions on occlusal surfaces compared with those on buccal or lingual surfaces.

The results based on the ICDAS scores at baseline can be seen in Fig. 3. Independent of the ICDAS score at baseline, lesions that progressed to cavitation had QLF ΔF values increasing at a faster rate than those of lesions that did not progress to cavitation. This was true for all surfaces and for all QLF parameters.

Because of the differences in progression rates observed between lesions that progressed to cavitation and those that did not, the slopes for the QLF parameters were calculated for each surface (individual slopes for every surface evaluated), and the individual slopes and last QLF measurement were used to predict cavitation. As indicated in Fig. 4, the AUC for ICDAS alone was low for buccal and lingual surfaces and still below 0.80 for occlusal surfaces. When the QLF information was also included in the model, significant

Figure 2.
Fluorescence loss (ΔF) from baseline to last visit before cavitation as determined by ICDAS based on progression status and surface.

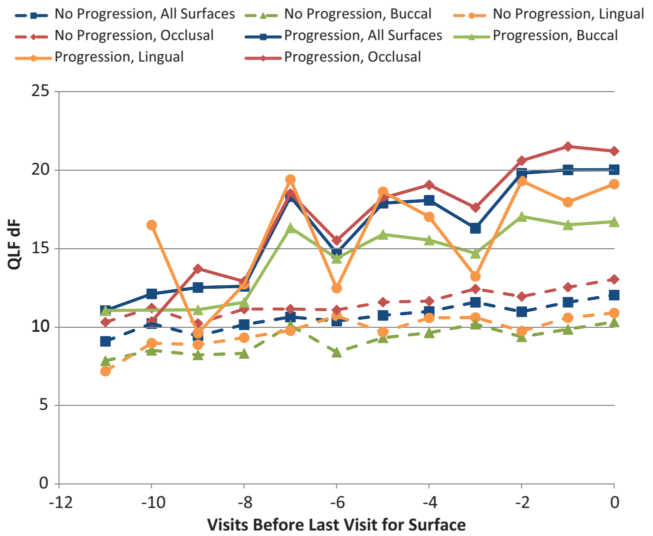
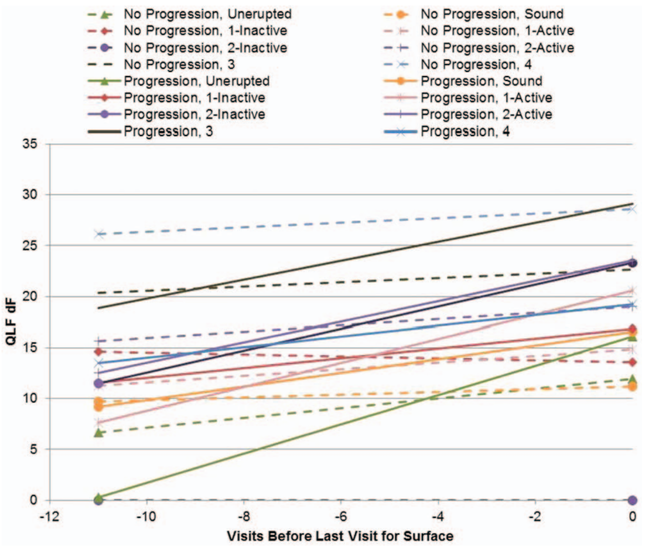


Figure 3.
Regression slopes for QLF ΔF values by ICDAS scores at baseline for lesions that progressed to cavitation and lesions that did not progress to cavitation, based on the number of visits that preceded cavitation.



improvements in AUC were observed for buccal and lingual surfaces. The increases in the AUC with the addition of the QLF parameters were statistically significant (p = .0005 for lingual surfaces, $p \leq .0001$ for all other comparisons).

QLF ΔF values were lower for ICDAS 0 than for all other scores (p = .0298 *vs.* ICDAS 1 active, $p < .0001$ *vs.* all others); lower for ICDAS 1 active than for ICDAS 1 inactive (p = .0031) and for all higher scores ($p < .0001$); lower for ICDAS 1 inactive than for ICDAS 2 inactive (p = .0147), ICDAS 2 active ($p < .0001$), ICDAS 3 ($p < .0001$), and ICDAS 4 ($p < .0001$); and lower for ICDAS 2 inactive and ICDAS 2 active than for ICDAS 3 and ICDAS 4 ($p < .0001$) (Appendix Table 2). There was no difference between ICDAS 2 inactive and ICDAS 2 active. There was no difference among ICDAS 2 active or inactive and ICDAS 3 or 4.

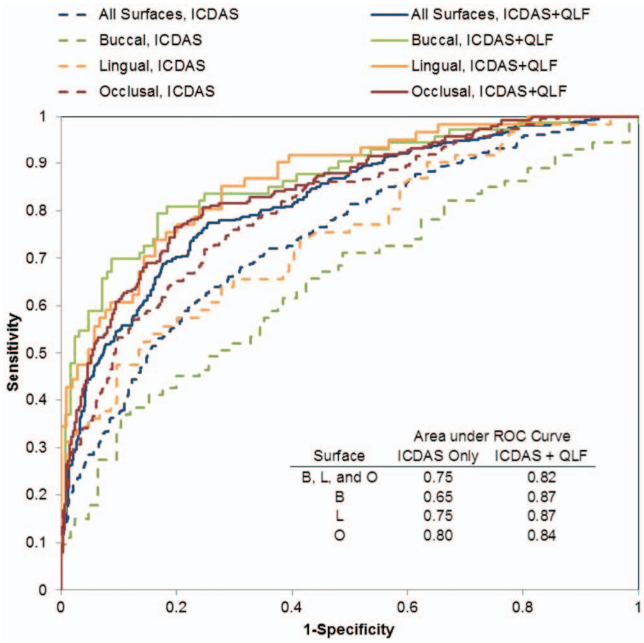
Discussion

Detection of early lesions is the cornerstone for the modern management of dental caries. The ability to determine which of these early lesions will progress to cavitation and how fast they are progressing can provide the dentist with a quantifiable way to assess the efficacy of preventive approaches, essential for clinical decision-making.

Several clinical studies have used QLF (Tranaeus *et al.*, 2001; Kambara *et al.*, 2003; Meller *et al.*, 2006; Karlsson *et al.*, 2007). However, there are few reports of longitudinal studies with QLF that follow caries lesions longer than 6 mos (Ferreira Zandoná *et al.,* 2000, 2003a; Kambara *et al.*, 2003; Karlsson *et al.*, 2007; Mattousch *et al.,* 2007; van der Veen *et al.*, 2007). The interpretation of QLF images without the aid of visual examinations is challenged by confounding effects, as previously reported (Pretty *et al.*, 2006). To avoid this issue, we have proposed to combine the use of QLF with a visual criterion (ICDAS), to benefit from the high sensitivity of the QLF and high specificity of the visual method (Ferreira Zandoná *et al.*, 2010). In this study, we report on the natural progression of dental lesions as monitored by QLF over a 4-year period.

The progression of dental lesions is not a continuous process; neither is there a single pathway that lesions follow. Lesions can progress to cavitation, arrest, or reverse (Backer Dirks, 1966; Ferreira Zandoná *et al.*, 2012). Using the ICDAS criteria to follow the natural progression of dental lesions, we have found that surfaces that transition to moderate-sized lesions (ICDAS 3 or 4) are likely to progress to cavitation (Ferreira Zandoná *et al.*, 2012). The activity of early lesions (ICDAS 1 and 2) was shown to be rather difficult to determine, since some lesions deemed inactive, by clinical signs, did progress to cavitation (Ferreira Zandoná *et al.*, 2012). The results of the longitudinal analyses of the QLF images indicate that this methodology allows for the

Figure 4.
Receiver operating characteristic (ROC) curves to predict progression with and without the inclusion of QLF parameters.



identification of the lesions that are progressing at an earlier stage than was clinically possible. That is, lesions that clinical assessment would indicate as progressing only when a moderate severity (ICDAS scores 3 and 4) is reached can be shown to be progressing by the rapid changes in QLF values at the early stages (ICDAS 1 and 2). In the early stages, lesions can be truly reversing or progressing, and there is also a higher variability on the calls (Nelson *et al.*, 2011); however, by focusing the analyses on true progressions, it could be demonstrated that QLF was able to identify the surfaces that are likely to become cavitated.

Several studies have used QLF *in vivo* to evaluate differences between and among different fluoride products, indicating that the method can separate different treatment groups (Feng *et al.*, 2007; Karlsson *et al.,* 2007) or to follow lesions after removal of fixed orthodontic appliances, indicating the ability of the method to quantitate changes in the lesions (Mattousch *et al.*, 2007; van der Veen *et al.*, 2007). In the present study, the QLF parameters (ΔF and ΔQ) were able to demonstrate the natural progression of dental caries and distinguish between lesions that progressed to cavitation and those that did not progress. Analysis of the data from the questionnaires indicated that approximately 90% of the study participants received a professional fluoride treatment at least once a year, but any potential impact was not included in the analyses. *In vitro*, QLF has been shown to have a good correlation with mineral loss, but this correlation did not hold when there was surface loss in a study of artificial lesions (Meharry *et al.*, 2012). ICDAS score 3 is lesions with microcavitation or surface loss. In this clinical study, QLF was able to monitor the changes in the lesions that were scored as ICDAS 3 at baseline as well as the lesions scored as ICDAS 1 and 2, but with intact surfaces. An *in vivo* study that compared the ability of QLF and other methods to discriminate between and among ICDAS scores found, as in our study, that although the QLF values for each of the scores were statistically significantly different, the absolute value differences were relatively small and not likely to be of clinical significance (Rechmann *et al.*, 2012). Additionally, the actual values provided by various devices can differ, as we have reported (Ferreira Zandoná *et al,* 2003b). This longitudinal off-site study caused considerable wear on the equipment, requiring different devices to be used, which may explain the drops observed in the ΔF values at -3 and -6 visits. Thus, it is more probable that the rapid changes will be more clinically significant in predicting progression than the actual values.

In conclusion, QLF is able to monitor changes in lesion severity and discriminate between lesions that are rapidly progressing and those that are arrested. The value of this technology may lie in its ability to follow lesions through time and determine changes, more than in providing a quantitative value that relates to a clinical score.

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