Clinical Trial for Early Detection of Tooth Caries using Fluorescence Ratio Reference Standard
8.1 INTRODUCTION

Laser-induced fluorescence (LIF) has been increasingly used in recent years as a powerful tool for caries detection. The fluorescence detection technique may be an alternative to the dental probe or radiographic examination. This chapter presents the clinical applicability of a diagnostic algorithms or fluorescence reference standards (FRS) based on LIF spectral ratios to discriminate different stages of caries and to detect early tooth caries. Towards this, LIF emission spectra were recorded in the 400-800 nm spectral range on a miniature fiber-optic spectrometer in 105 patients, with excitation at 404 nm from a diode laser. The spectral results were correlated with visual-tactile and radiographic examinations. The LIF spectra of sound tooth shows a broad emission at 500 nm that is characteristic of natural enamel whereas in carious tooth, additional peaks were seen at 635 and 680 nm, due to emission from porphyrins present in oral bacteria. In order to discriminate different stages of tooth caries, FRS ratio scatter plots of the fluorescence intensity ratios F500/F635 and F500/F680 were developed to differentiate sound from enamel caries, sound from dentinal caries and enamel caries from dentinal caries using spectral data obtained from 65 carious sites and 25 sites of sound tooth in 65 patients. The sensitivity, specificity, PPV and NPV of the FRS to detect tooth caries are calculated and presented. A blind-test was carried out in 40 patients at 15 sound and 40 carious sites to check the accuracy of the developed standard for early detection of tooth caries.

8.2 STUDY MATERIAL, PROTOCOL AND ETHICAL ISSUES

The study was conducted at the Department of Conservative Dentistry and Endodontics of the Government Dental College, Thiruvananthapuram, India. Ethical approval for the study was provided by the Institutional Ethical Committee of the Government Dental College, Thiruvananthapuram (IEC/C/01-A/2008/DCT). All volunteers were provided with patient information sheet and consent forms. After explaining details of the study, consent form was endorsed by each patient prior to the initiation of study.

A total of 105 patients, aged between 20 and 50 years, with clinically suspicious incipient caries or radiographically proven tooth caries participated in the study. An experienced clinician identified the tooth for spectral measurement in each patient and recorded its visual-tactile findings. If clinical observation was positive, bitewing radiographs were taken. Thereafter, LIF measurements were carried out.
After positioning the patients in the dental chair, visual-tactile examination was performed, with the aid of a light reflector, air/water spray and dental mirror. The teeth observed as sound during clinical examination were included as control in the study. Samples studied covered smooth surface as well as occlusal caries. The study population comprised of 40 sound (clear enamel-intact tooth surfaces), 50 enamel (white spot, loss of luster and rough) and 55 dentinal caries lesions (localized enamel breakdown or cavitation in opaque or discolored enamel exposing the dentin). Samples with any kind of staining, hypoplasia and fluorosis were excluded.

Before initiation of measurements, the patients were asked to wash their mouth with saline to reduce the effects of recently consumed food. The oral cavity was then cleaned and the measurement site dried with cotton-swab. The OOI Base32 software was configured to record the spectra, averaged for 40 scans, with boxcar width of 8 nm and an integration time of 50 ms. Due to the diverse nature of the caries lesions, 15 sets of LIF measurements were taken from each of the caries lesions and sound tooth of the same patient for comparison, and the mean spectra of each site is determined for

![Fig. 8.1a](image_url). Mean in vivo LIF spectra from sound and caries lesions belonging to different stages and b) LIF spectra normalized to maximum fluorescence emission intensity. Sound tooth spectra represent the mean of 15 measurements each in 25 samples, whereas enamel caries spectra is of 15 × 30 measurements and the dentinal caries spectra is of 15 × 35 measurements.
further analysis. Depending upon the visual-tactile and radiographic results, each tooth was classified as sound, enamel or dentinal caries by the clinician, who was blinded to the spectral findings. ANOVA was performed to detect whether the average LIF spectral intensity differed between each of these groups.

Fluorescence intensity ratios are then calculated from the recorded mean spectra and correlated with visual-tactile and radiographic findings. To account for the broad nature of the peaks and sample-to-sample variation in peak position due to changes in chemical composition and fluorophore content of the tooth, the mean LIF spectral intensity over an interval of ±10 nm at the emission peak was used to determine the LIF spectral ratios. In-order to discriminate sound from enamel caries, sound from dentinal caries and enamel from dentinal caries, fluorescence reference standard (FRS) scatter plots of the respective intensity ratios (F500/F635 and F500/F680) were developed using the spectral data obtained from 65 carious sites (30 enamel caries and 35 dentinal caries) and 25 sites of sound tooth in 65 patients. A blind-test was carried out in 40 patients and the data obtained from 40 carious sites and 15 sound sites were used to check the accuracy of the developed standard. An independent Student t-test was performed on the FRS ratios to discriminate different stages of caries, and to determine the statistical significance of the developed method for early detection of dental caries.

8.3 RESULTS

8.3.1 LIF spectral features

Fig. 8.1a) shows the mean in vivo LIF spectra recorded from 25 sites of sound tooth, 30 sites of enamel caries and 35 dentinal caries lesions and Fig. 8.1b), the same LIF spectra normalized to the peak emission intensity. The standard deviation is shown for the prominent peaks in the mean LIF spectra of sound and caries tooth belonging to various stages of decay. The overall fluorescence intensity of caries lesions were found lower than that of sound tooth. Nevertheless, the fluorescence spectral intensity in the red wavelength region was greater for caries lesions as compared to sound tooth. The LIF spectrum of sound tooth shows a broad emission around 500 nm with a long tail extending towards the red wavelength, whereas, two additional peaks are seen at 635 and 680 nm in caries tooth. In advanced caries lesion, 500 nm band appears broadened and red-shifted by about 30 nm. The peaks at 635 and 680 nm are very prominent in advanced caries lesions. However, the 680 nm peak appears broadened in incipient caries. The spectra shows a sharp rising
edge in the short wavelength side (400-450 nm), which is due to absorbance of the 420 nm long-wavelength pass filter (Schott UG420) used for blocking the back-scattered laser light from entering the spectrometer.

One way ANOVA test was used to determine the average spectral intensity variation among the 3 groups. Statistically significant differences in mean spectral intensities (p <0.001) were noticed between sound tooth, enamel caries and dentinal caries lesions.

**Table 8.1** Mean LIF spectral ratios determined from in vivo LIF spectral data from sound and caries tooth.

<table>
<thead>
<tr>
<th>Tooth Types</th>
<th>Population (n)</th>
<th>F500/F635</th>
<th>F500/F680</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sound</td>
<td>40</td>
<td>10.94±1.7</td>
<td>27.89±6.8</td>
</tr>
<tr>
<td>Enamel Caries</td>
<td>50</td>
<td>6.48±1.7 (41)</td>
<td>14.59±6.3 (48)</td>
</tr>
<tr>
<td>Dentinal Caries</td>
<td>55</td>
<td>2.59±1.4 (76)</td>
<td>5.16±3.4 (82)</td>
</tr>
</tbody>
</table>

The percentage given in parentheses shows the variation with respect to the sound tooth

**8.3.2 LIF intensity ratios**

Mean LIF spectral intensity ratios (F500/F635 and F500/F680) determined from the sound and caries tooth samples are listed in Table 8.1. It was observed that both the F500/F635 and F500/F680 ratios of caries tooth have values lower than those of sound tooth. The F500/F680 ratio shows a maximum variation of 48% between sound and enamel caries and 82% between sound and dentinal caries, whereas between enamel and dentinal caries lesions, this ratio has a variation of 64%.

**8.3.3 Discrimination using FRS ratio Scatter plots**

A reference standard consisting of fluorescence spectral intensity ratio scatter plot was developed to facilitate discrimination between different types of caries. Fig.8. 2 (a-b) shows the scatter plots of the FRS ratios F500/F635 and F500/F680 in 65 patients, with their lesions diagnosed as enamel and dentinal caries, along with the control data from 25 sites of sound teeth. The fluorescence intensity ratios (Table 8.1) used in this classification has low independent Student t-test values of p<0.001. Cut-off lines were drawn between sound tooth and enamel caries, sound tooth and
dentinal caries, enamel and dentinal caries data points, at values that correspond to the average ratio values of the respective groups. For example, to differentiate the sound tooth from enamel caries, the cut off line was drawn at 8.69 (the mean ratio of
the sound tooth and enamel caries lesion data points). The classification sensitivity, specificity, positive predictive value and negative predictive value for discriminating each category were calculated based on the cut-off values, by validation with visual-tactile and radiographic observation results.

For F500/F635 and F500/F680 ratios, by selecting a cut-off at the mean value of sound tooth and dentinal caries values (6.87 and 16.67) respectively, a sensitivity and specificity of 100% were achieved for discriminating these categories, with a positive and negative predictive value of 1. In Fig. 8.2a, by selecting 8.69 as the cut-off value in the F500/F635 scatter plot to discriminate sound tooth from enamel caries, a sensitivity and specificity of 87% and 100% respectively were obtained, with a positive predictive value of 1 and negative predictive value of 0.86. In this study, only 4 out of the 30 enamel caries lesions were misclassified as sound. For discriminating enamel from dentinal caries, a sensitivity and specificity of 89% and 80% respectively, were achieved for the same ratio by using 4.73 as the cut-off value, with a corresponding positive predictive value of 0.84 and negative predictive value of 0.86. Here, only 4 out of 35 dentinal caries lesions were misclassified as enamel and 6 out of 30 enamel caries lesions were misdiagnosed as dentinal caries. Furthermore, an overall sensitivity and specificity of 85% and 90% respectively, were achieved for discriminating sound tooth from enamel caries, whereas a sensitivity and specificity of 100% was obtained to discriminate sound tooth from dentinal caries. Table 8.2 illustrates independent and overall sensitivity, specificity, PPV and NPV of FRS ratios, F500/F635 and F500/F680 for classifying different stages of tooth caries.

8.4 DISCUSSION

Until now visual inspection alone or its combination with radiography is considered as the gold standard for caries detection. However, x-rays are ionizing and hazardous in nature and cannot detect caries until they are well advanced. Hence development of diagnostic algorithms or reference standards based on LIF spectral ratios would be of great help for the real-time, non-invasive detection of tooth caries in the clinic.

8.4.1 LIF spectral features

The mean LIF spectra from sound and caries tooth shows characteristic features based on the absorption and scattering properties of light by cariogenic substances and fluorophores in the tooth. The reduction in the fluorescence intensity of the caries lesion could also be attributed to the changes in the physical structure and chemical composition during the disintegration of tooth enamel leading to caries or due to the
import of exogenous molecules during the caries process. This clearly supports the progressive rise of fluorescence spectral intensity in the red wavelength region with caries progression, and the consequent decrease in the autofluorescence emission around 500 nm.

Normally, tooth enamel is composed of millions of prisms or rods with waveguide properties that facilitate deep penetration when illuminated with visible light. In the case of dental caries, the prism structure is damaged and the waveguide properties are lost so that the irradiated light cannot penetrate deeply. This leads to a reduction in the fluorescence intensity in caries lesion (Ando et al, 2001).

The broad autofluorescence emission around 500 nm in sound tooth is due to the emission from natural enamel and dentin (Konig et al, 1998) and emissions observed at 635 and 680 nm are due to endogenous porphyrins and metalloporphyrins, in particular protoporphyrin IX (PpIX), meso-porphyrin and copro-porphyrin synthesised by bacteria (Konig et al, 1998; Hibst et al, 2001). PpIX concentration is reported to be higher in Gram-negative oral bacteria and its level increases as the dental biofilm becomes more mature, which is responsible for the red fluorescence in teeth (Walsh and Shakibaie, 2007). Pretty et al (2005) reported that the fluorescence due to porphyrins in certain oral plaque species, particularly Gram-negative anaerobes, is more abundant in late than early plaque (Marsh and Martin, 1999). Similarly, Buchalla (2005) reported that caries lesions fluoresce at 624, 650 and 690 nm, as due to porphyrins, more efficiently when excited in the wavelength range between 400 and 420 nm.

Konig et al (1993) observed that the autofluorescence emission in the red spectral region of carious lesions with 407 nm krypton ion laser excitation, is caused by the oral microorganisms such as *Prevotella intermedia*, *Actinomyces odontolytics*, *Corynebacterium species* and *Candida albicans*, which are able to synthesise high levels of endogenous metal-free fluorescent porphyrins. They also found that lactic acid bacteria, such as lactobacilli and streptococci, did not show typical porphyrin fluorescence in the red wavelength region. Thus the maturity of dental plaque, rather than the presence of cariogenic streptococci, is the basis for the red fluorescence when excited with near-UV light (Coulthwaite et al, 2006).

### 8.4.2 LIF intensity ratios

As compared to the mean fluorescence spectral intensity ratios of sound tooth given in Table 8.1, the caries tooth belonging to different categories has lower ratios.
Both LIF ratios showed 100% sensitivity and specificity for detecting dentinal caries (p<0.001). Nevertheless, by selecting suitable cut-off value, both FRS ratios could detect enamel caries with an average sensitivity of 85% and specificity of 90%.

### 8.4.3 Validation of FRS ratio

The best standard for validating detection accuracy in a clinical setting should be one that is derived from actual patient data. Blind-test for validation of FRS used data from 40 patients with the results correlated by clinical and radiographic findings (Fig.8.2a-c). The corresponding sensitivity, specificity, PPV and NPV for differentiating different stages of tooth caries are given in Table 8.2. It is found that both FRS ratios discriminates sound tooth from enamel and dentinal caries with 100% sensitivity and specificity. An overall sensitivity of 100% and specificity of 93% was achieved in discriminating enamel from dentinal caries lesions with a positive predictive value of 0.95 and negative predictive value of 1.00.

**Table 8.2** Independent and overall sensitivity, specificity, PPV and NPV of FRS ratios, F500/F635 and F500/F680 for classifying different stages of tooth caries.

<table>
<thead>
<tr>
<th>Type</th>
<th>LIF ratios</th>
<th>Sound vs Enamel caries</th>
<th>Sound vs Dentinal caries</th>
<th>Enamel vs Dentinal caries</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Sensitivity (%)</td>
<td>Specificity (%)</td>
<td>PPV (%)</td>
</tr>
<tr>
<td>FRS results</td>
<td>F500/F635</td>
<td>87</td>
<td>100</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>F500/F680</td>
<td>83</td>
<td>80</td>
<td>0.83</td>
</tr>
<tr>
<td>Overall</td>
<td></td>
<td>85</td>
<td>90</td>
<td>0.92</td>
</tr>
<tr>
<td>Blind-test</td>
<td>results</td>
<td>F500/F635</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>F500/F680</td>
<td>100</td>
<td>100</td>
<td>1.00</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>100</td>
<td>100</td>
<td>1.00</td>
</tr>
</tbody>
</table>

LIF: Laser-Induced fluorescence, FRS: Fluorescence reference standard; PPV: Positive predictive value; NPV: Negative predictive value

Independent t-Test: P<0.01

Validation of the diagnostic method usually takes place under clinical conditions, if follow-up treatment is planned. Nevertheless, if no operative intervention is intended, validation of the results is difficult for want of proper gold standard. Visual inspection
appears to have very low specificity and high sensitivity for detecting enamel caries and the opposite is true for dentinal caries of primary and permanent teeth. This shows that changes in enamel are easily identified by visual examination, while several dentinal caries might be covered up by mineralized enamel (Rodrigues et al, 2008; Valera et al, 2008; Ie and Verdonschot, 1994). Instead, visual inspection combined with radiography classified 82% of caries correctly (Bader et al, 2004). Recently in an in vitro study, Valera et al (2008) compared visual inspection, radiographic examination and use of DIAGNOdent device as well as their combinations for occlusal caries detection and found that visual and radiographic examination resulted in a specificity of 99%, whereas visual inspection alone resulted in 100% specificity. Therefore, we used a combination of visual-tactile and radiographic examinations as the gold standard for caries diagnosis in this study.

In a recent study, Alkurt et al (2008) reported LIF to be more reliable to assess actual lesion depth than visual inspection or bitewing radiography. Similarly, in a clinical study with permanent and primary molar teeth, Costa et al (2007) observed that laser fluorescence measurements yielded results similar to visual examination. In another study, Huth et al (2008) reported that LIF shows good discrimination between sound and carious tooth with area under the curve (AUC) of 0.92 and 0.78 for discriminating between enamel and dentin caries. Reis et al (2006) reported that no significant difference was observed between LIF measurements and visual inspection under both in vitro and in vivo conditions.

Attrill and Ashley (2001) compared LIF, visual examination and radiography and found that LIF was the most precise method (sensitivity of 77-80%) for detection of occlusal caries extending into dentine in extracted primary molars. In another study, Lussi, et al (2001) reported a sensitivity and specificity of 92% and 86%, respectively for detecting occlusal dentin caries using DIAGNOdent device as compared to visual inspection and bite-wing radiography. When carious enamel was used as threshold, sensitivity was about 96%. Anttonen, et al (2003) observed a sensitivity of 92% and specificity of 82% by selecting a cut-off point of 30 for occlusal caries detection in children. Similarly, the study conducted by Heinrich-Weltzien, et al (2005) revealed sensitivity values of 93%; however the specificity was lower (63%) when compared to the other reports. In another study, Olmez et al (2006) reported that LIF has higher sensitivity and specificity as compared to visual inspection and bitewing radiography for occlusal caries detection. However, Burin et al (2005) assessed the efficiency of LIF, visual examination and bitewing radiography and found that visual inspection was as valid as LIF, which should be considered a better adjunct than bitewing
radiography for caries diagnosis.

In comparison, the results of this clinical study showed improvements in discrimination between sound and carious tooth and between different stages of caries. It may be noted that both FRS ratios gave 100% sensitivity and specificity for discriminating sound tooth from enamel (incipient) caries and dentinal (advanced) caries. As regards differentiation of enamel caries from dentinal caries, the F500/F635 ratio showed 100% sensitivity and 90% specificity. Furthermore, the sensitivity and specificity shown in Table 8.2 for blind tests are also higher than the earlier reports (Lussi et al, 2001; Anttonen et al, 2003; Rodrigues et al, 2008; Huth et al, 2008; Angnes et al, 2005).

The investigation shows that diagnostic algorithms based on fluorescence intensity ratio of the emission peaks can localize and discriminate caries lesions from sound tooth. With the help of FRS threshold, the F500/F635 and F500/F680 ratio algorithms classified caries lesions from sound tooth with an average sensitivity and specificity of more than 90% with a positive predictive value of 0.96 and a negative predictive value of 0.92. This study relies on the assumption that the gold standard (a combination of visual-tactile and radiographic examination) provides diagnosis with 100% of sensitivity and specificity (i.e., 0% false-positives and false-negatives).

8.5 CONCLUSIONS

The blind-test results of this clinical study illustrate that information provided by non-invasive LIF spectroscopy has excellent potential to detect dental caries in its early stage. The FRS ratio diagnostic algorithm based on tissue autofluorescence was found to be sensitive and specific in discriminating different stages of tooth caries and in detecting early changes in tooth enamel that lead to caries formation. Therefore, LIF spectroscopy could function as a tool to dentists for early detection of tooth caries, in particular those not visible to the eye, hidden under restorations and beneath the exposed enamel surfaces in a fast and sensitive manner. Both FRS ratios (F500/F635 and F500/F680) were found suitable to understand caries progression from sound to enamel and dentinal caries with 100% sensitivity and specificity with PPV of and NPV of 1.00. Our results confirm that classification of tooth caries from fluorescence signatures, with 404 nm diode laser excitation allows precise visualisation and quantification of both the intrinsic green fluorescence of dental hard tissues as well as the red fluorescence of bacterial origin. The potential of LIF spectroscopy to detect secondary caries has not been tested and would be interesting.