Endoscopic Fluorescence Detection of High-Grade Dysplasia in Barrett’s Esophagus

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See editorial on page 247.

Background & Aims: Early detection and treatment of esophageal cancer in Barrett’s esophagus may improve patient survival if dysplasia is effectively detected at endoscopy. Typically, four-quadrant pinch biopsy specimens are taken at 2-cm intervals. This study was conducted to determine whether laser-induced fluorescence spectroscopy could be used to detect high-grade dysplasia in patients with Barrett’s esophagus. Methods: Four hundred ten–nanometer laser light was used to induce autofluorescence of Barrett’s mucosa in 36 patients. The spectra were analyzed using the differential normalized fluorescence (DNF) index technique to differentiate high-grade dysplasia from either low-grade or nondysplastic mucosa. Each spectrum was classified as either premalignant or benign using two different DNF indices. Results: Analysis of the fluorescence spectra from all patients collectively using the DNF intensity at 480 nm (DNF480) index showed that 96% of nondysplastic Barrett’s esophagus samples were classified as benign, all low-grade dysplasia samples as benign, 90% of high-grade dysplasia samples as premalignant, and 28% of low-grade with focal high-grade dysplasia samples as premalignant. Using the two DNF indices concurrently, all patients with any high-grade dysplasia were classified correctly. Conclusions: Laser-induced fluorescence spectroscopy has great potential to detect high-grade dysplasia in Barrett’s esophagus when using the DNF technique.

Laser-induced fluorescence (LIF) spectroscopy has been used in vitro and in vivo to distinguish normal from malignant tissue in different organs, such as the breast, lung, colon, and esophagus.1–6 These studies clearly show the potential application of LIF spectroscopy as a noninvasive diagnostic technique. However, most of these lesions are visible or palpable and are readily diagnosed by the clinician. In gastroenterology, the importance of LIF spectroscopy would be in diagnosing premalignant lesions unrecognizable by the physician during endoscopy.

Barrett’s esophagus is a condition in which the squamous lining of the esophagus is replaced by specialized columnar epithelium.7,8 The prevalence of adenocarcinoma in Barrett’s esophagus is approximately 10%.9,10 The estimated incidence of adenocarcinoma in Barrett’s esophagus ranges from 1 in 52 to 1 in 441 patient years, reflecting an increased risk of 30–125-fold.9,11–14 Development of cancer is preceded by dysplastic transformation of Barrett’s mucosa,15 thereby providing a clinical setting that allows identification of patients at high risk for developing esophageal adenocarcinoma.

Although Barrett’s mucosa is easily detected during endoscopy, the dysplasia within the Barrett’s mucosa is not visually distinguishable from nondysplastic areas. Extensive pinch biopsy specimens are required to detect dysplasia in such patients, typically four-quadrant biopsy specimens at 1–2-cm intervals.16

An alternative technique to improve the detection of dysplasia in patients with Barrett’s esophagus is desirable. This report describes the use of endoscopic LIF spectroscopy to detect high-grade dysplasia during endoscopy in patients with Barrett’s esophagus.

Materials and Methods

Patient Population

A total of 36 patients were studied, including 26 men and 10 women ranging from 35 to 87 years of age. This study was approved by the Institutional Review Board of the Thompson Cancer Survival Center. All patients signed an informed consent form before being included in the study.

LIF Measurement System

The LIF system has been described previously.6 Figure 1 shows a schematic diagram of the overall system. In sum-
mary, a nitrogen-pumped dye-laser (model LC300C; Laser Photonics, Inc., Orlando, FL) was used to deliver 5-nanosecond pulses of excitation light. The dye laser was tuned to generate pulses of 410-nm light for excitation of tissue proven to be effective for detection of esophageal cancer. The pulse energy was highly stable with a typical energy of 20.0 ± 0.3 µJ. This pulsed laser is suitable for such studies because it provides a sufficient amount of energy per pulse that can be coupled easily to a fiber or fiber bundle. The laser was activated externally by an optical multichannel analyzer to allow synchronization with a gated intensified diode array. Such a system allows fluorescence measurements without concern for background light interference.

The laser beam was coupled via a quartz lens to the excitation fibers of a sheathed fluorescence probe (C Technologies, Verona, NJ). The probe was constructed of seven 200-µm fibers for delivery of the excitation light and 12 200-µm fibers surrounding the excitation fibers for collection of tissue fluorescence emission. The distal end of the probe (the end that is passed through the scope and comes in contact with the tissue) was flat and was encased in a 4-mm-long hypodermic tube with an outside diameter of 1.7 mm. The output end of the collection fibers (the end that is attached to the spectrograph) was arranged in a linear array of 1 × 12 fibers to match the entrance slit of the spectrograph. The length of this fiber assembly was 6 m. The overall outside diameter of the probe, including the protective sheath, was 2.4 mm.

The linear array of collection fibers formed the input to the entrance slit of a f/3.8, 0.28-m triple-grating spectrograph (model 1235; EG&G Princeton Applied Research, Princeton, NJ). A 150-grooves/mm grating was selected to allow wide-band analysis of the fluorescence spectra. The center wavelength of the spectrograph was set at 733 nm to shift the excitation component of the collected light 20 nm off the detector.

The spectrally dispersed emission spectrum was imaged on a gated intensified 1024-diode array detector (model 1456B-990-HQ) controlled by an optical multichannel analyzer (OMA III; EG&G Princeton Applied Research). The diode array allowed simultaneous analysis of a wide range of wavelengths within seconds (1023 different wavelengths, corresponding to the number of active diodes). Synchronization of laser pulses and gating of the intensified diode array was achieved by programming a 1336-nanosecond delay between laser trigger and detector activation. The intensifier was gated for 100 nanoseconds, during which a 5-nanosecond laser pulse was delivered to the tissue. The diode array was spectrally calibrated using a mercury/argon spectral calibration lamp (model 6035; Oriel Corp., Stratford, CT).

A personal computer (model 486DX2, 50 MHz; Gateway 2000, North Sioux City, SD) was used to control the entire system. OMA-Vision-PDA spectroscopy software (EG&G Princeton Applied Research) was used to conduct the measurements. This software allowed automatic storage of spectra into the hard disk with a file name coded using a combination of numbers representing the current date and a three-digit file number. Each measurement was conducted and stored on the hard disk by depressing a foot switch attached to the keyboard to allow remote activation of the measurements by the endoscopist. Each measurement required 1–2 seconds for processing and storage.

The software was operated in accumulate/subtract mode. In this mode, the following three operations form a measurement cycle: the optical multichannel analyzer system sends a trigger signal to the excitation laser; after a programmed delay of 1336 nanoseconds, the optical multichannel analyzer system also sends a signal to the detector to be activated; and the optical multichannel analyzer system performs background measurement, subtracts the background from the signal, and stores/adds the result in memory. In this study, 10 emission scans from tissue were accumulated per measurement, and the result was stored in the hard disk.

Overall System Response

To assure optimum system performance, the fluorescence of a standard dye solution (kiton red) and the laser pulse energy were measured among all patients. In this manner, any variation in fluorescence signal caused by laser energy, detector gain, or probe damage could be detected and corrected immediately. It should be emphasized that fluorescence line shape is independent of fluorescence emission intensity. Therefore, laser pulse energy and standard dye fluorescence intensity were not needed for calculation of fluorescence line shape.

Endoscopic Fluorescence Measurement

Fluorescence measurements (often called optical biopsy specimens) were performed during routine upper endoscopy procedures. The endoscopist passed the flexible fiber-optic fluorescence probe through the biopsy channel of the endoscope and touched it lightly to the tissue. Measurements were initiated by pressing the foot switch. The collected spectra were automatically saved in separate coded data files. In a timed
study, 6–7 optical biopsies could be performed during the same time required to perform a single pinch biopsy.

Fluorescence measurements were conducted in 36 patients with Barrett’s esophagus. The probe was gently pressed against the tissue to distort the surface superficially and to imbed the tip of the probe onto the tissue fold. Because of the small diameter of the probe tip, good contact was made easily with the tissue. The proper placement of the probe against the tissue was verified on the endoscopy monitor by an independent observer and noted during each measurement. If verification was not possible, the measured fluorescence was discarded. It must be noted that, although fluorescence intensity is strongly affected by probe placement against tissue, the line shape (normalized fluorescence spectrum) is not affected. Multiple measurements were obtained, typically in four quadrants every 2 cm of the columnar-lined mucosa (occasionally every 1.0 cm). The location of each measurement was determined by measurement of the endoscope from the dental margin. Each measurement was analyzed as a separate biopsy specimen, and the data collected from the same distance were not averaged.

Four-quadrant pinch biopsy specimens were then obtained from where fluorescence measurements were taken. The majority of the biopsies were performed using jumbo forceps. The pinch biopsy results were used for tabulation of the fluorescence spectra.

**Differential Normalized Fluorescence Spectral Analysis**

A mathematical model based on the differential normalized fluorescence (DNF) index has been developed for the endoscopic LIF diagnosis of esophageal cancer. A brief description of the development of the model is given here.

The total integrated light (sum of all photons detected by the selected diodes within the array) from 430 nm to 716 nm was determined for each measured fluorescence spectrum. All fluorescence spectra were normalized with respect to the total integrated light. A set of normalized fluorescence spectra from 15 patients with normal esophagus (squamous epithelium) were collected and used to determine an average normalized fluorescence spectrum to be used as a baseline value. Although these spectra were not superimposable, the variation was negligible. DNF spectrum for each new fluorescence spectrum was determined by subtracting the baseline spectrum from the new normalized spectrum. DNF indices DNF_{480} and DNF_{660} were defined as the magnitude of the DNF spectrum at wavelengths of 480 nm and 660 nm. Four hundred eighty nanometers and 660 nm were selected because the normalized fluorescence spectra from normal and malignant esophageal tissue had significant spectral differences around these wavelengths.

Development of the models using DNF_{480} and DNF_{660} indices to differentiate normal from malignant tissue were similar but only DNF_{480} will be described as an example. A set of DNF_{480} index values for normal and cancerous tissue were selected and graphed in a scatter plot. Two separate populations of data points were generated representing normal and malignant DNF_{480} indices.

The goal was to determine effectively a threshold value that separated normal DNF_{480} indices from cancerous DNF_{480} indices using statistical concepts. The mean and 1SD was determined for each group of data. The DNF_{480} index threshold value was determined by calculating the midpoint between two DNF_{480} index values, DNF_{480(mean + SD)} and DNF_{480(mean – SD)}, where DNF_{480(mean + SD)} is the mean + 1SD of DNF_{480} index values for the cancerous set and where DNF_{480(mean – SD)} is the mean – 1SD of the DNF_{480} index values for the normal set. Using this technique, the threshold value for the DNF_{480} index was $−7.5 \times 10^{-4}$. DNF_{480} index values less than this threshold were classified as malignant. DNF_{480} index values greater than this threshold value were classified as normal.

Similarly, the threshold value for the DNF_{660} index was calculated. The threshold was $+4.3 \times 10^{-4}$. The DNF_{660} index values greater than this threshold value were classified as malignant. The DNF_{660} index values less than the threshold value were classified as normal tissue.

**Testing the DNF Models for Detection of Dysplasia in Barrett’s Mucosa**

The fluorescence spectra from Barrett’s mucosa were analyzed using the DNF models to determine whether high-grade dysplasia could be diagnosed in patients with Barrett’s esophagus. In this study, the DNF indices classified each spectrum as either premalignant or benign, noting that all spectra were taken from Barrett’s mucosa with and without dysplasia and without any cancer. Each spectrum was classified using two different DNF indices, DNF_{480} and DNF_{660}, where DNF_{480} and DNF_{660} were defined previously.

In summary, all collected fluorescence spectra were analyzed as follows: each spectrum was first normalized, DNF_{480} and DNF_{660} indices were obtained for each normalized spectrum, the DNF_{480} and DNF_{660} indices classified each spectrum as either benign or premalignant based on the model described above, and the classifications results were then verified/compared with histological results.

**Data Tabulation Methods**

Measurements from 36 patients were analyzed and entered into this study using two different tabulation methods: collective data analysis and individual patient data analysis.

**Collective data analysis.** In this technique, the fluorescence spectra from all patients were analyzed collectively. DNF_{480} and DNF_{660} were used independent of each other to classify each spectrum as either benign or premalignant tissue.

A total of 216 spectra were obtained from nondysplastic Barrett’s mucosa. Thirty-six spectra were obtained from mucosa with low-grade dysplasia, 10 spectra were obtained from high-grade dysplasia, and 46 spectra were obtained from mucosa with low-grade dysplasia with focal high-grade dysplasia.

**Individual patient data analysis.** In the second technique, DNF_{480} and DNF_{660} were used concurrently by forming the Logical OR Operation of the classification of each spectrum (benign or premalignant) in each patient. Logical OR operation may be considered as a black box with two inputs...
Figure 2. (A) A typical normalized fluorescence spectrum from a nondysplastic Barrett’s mucosa. (B) A typical normalized fluorescence spectrum of Barrett’s mucosa with high-grade dysplasia.

Histological Categories

The biopsy specimens were blinded. The histological diagnoses as determined by one of the investigators (R.C.H.) were as follows: Barrett’s mucosa without dysplasia, referred to as nondysplastic Barrett’s mucosa; Barrett’s mucosa with low-grade dysplasia; Barrett’s mucosa with high-grade dysplasia; and Barrett’s mucosa with low-grade dysplasia with focal high-grade dysplasia. Biopsy specimens from benign esophageal erosions and ulcerations were excluded from this study. The criteria used for the diagnosis and grading of dysplasia have been published previously.

In this study, it is assumed that nondysplastic Barrett’s mucosa and low-grade dysplasia are conditions requiring no immediate clinical intervention (except for endoscopic surveillance) and, therefore, were assigned as benign for the purpose of specificity analysis. On the other hand, any high-grade dysplasia (including focal high-grade dysplasia) was assigned as premalignant to facilitate sensitivity analysis.

Results

The DNF spectrum was calculated for each spectrum. Figure 2A shows a typical normalized fluorescence spectrum of nondysplastic Barrett’s mucosa. Figure 2B shows the normalized fluorescence spectrum of a Barrett’s mucosa with high-grade dysplasia. Figure 3A shows the corresponding DNF spectrum for the above nondysplastic Barrett’s mucosa. Figure 3B shows the corresponding DNF spectrum for the high-grade dysplasia sample in Figure 2B.

The specificity for detecting benign tissue in nondysplastic Barrett’s mucosa. Figure 3B shows the corresponding DNF spectrum for tissues with low-grade dysplasia was similar to that of nondysplastic Barrett’s mucosa samples.

Using Figure 2A and B, small spectral difference can be observed. However, the spectral differences are highlighted effectively in the corresponding DNF spectra shown in Figure 3A and B. The DNF spectrum for high-grade dysplasia shows two prominent features at 480 nm and 660 nm, indicating that DNF_{480} and DNF_{660} indices may be used to differentiate nondysplastic Barrett’s from high-grade dysplasia in Barrett’s mucosa.

Each spectrum was classified as either benign or premalignant using the two different DNF indices at 480 nm and 660 nm. The data (histological diagnosis vs. DNF index classification) were grouped using two different tabulation techniques: collective data analysis and individual patient data analysis.

Collective Data Analysis

Table 1 shows the results using the collective data tabulation. Using this technique, DNF_{480} and DNF_{660} indices were used independently, and the fluorescence spectra from all patients were analyzed collectively. Using the DNF_{480} index, 96% of nondysplastic Barrett’s mucosa samples (208 of 216) and all low-grade dysplasia samples (36 of 36) were classified as benign. Ninety percent of high-grade dysplasia samples (9 of 10) were classified as premalignant tissue. In contrast, 28% of biopsy specimens with low-grade with focal high-grade dysplasia (13 of 46) were classified as premalignant tissue, a result anticipated because of the focal nature of high-grade dysplasia in patients with mixed low-grade and focal high-grade dysplasia.

The specificity for detecting benign tissue in nondys-
fluorescence detection of Barrett's dysplasia.

plastic Barrett's mucosa was 96% (208 of 216), noting the definitions of benign and premalignant tissues previously described. The specificity for detecting benign tissue in low-grade dysplasia samples was 100% (36 of 36). The sensitivity for detecting premalignant tissue was 28% (13 of 46) and 90% (9 of 10) for samples with low-grade with focal high-grade dysplasia and for samples with high-grade dysplasia, respectively.

When the DNF660 index was used to classify the spectra, the results were similar except that 95% of nondysplastic Barrett’s mucosa samples (206 of 216) were classified as benign tissue compared with 96% when using the DNF480 index.

**Individual Patient Data Analysis**

Table 2 shows the individual patient data analysis. This technique classified each individual patient as having either benign or premalignant tissue. Sixteen of 23 patients with histologically nondysplastic Barrett’s mucosa and all 6 patients with low-grade dysplasia were classified as having benign Barrett’s mucosa. Seven patients with nondysplastic Barrett’s mucosa were classified as having premalignant sites. All patients with high-grade dysplasia, including those with low-grade with focal high-grade dysplasia, were classified as having premalignant lesions.

Using the individual patient data analysis, the specificity for detecting benign tissue in patients with nondysplastic Barrett’s mucosa and those with low-grade dysplasia was 70% (16 of 23) and 100% (6 of 6), respectively. The sensitivity for detecting premalignant tissue in patients with high-grade dysplasia was 100% (7 of 7).

In the 7 patients with nondysplastic Barrett’s mucosa that were classified as having premalignant lesions (false

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**Table 1. Collective Data Analysis Comparing Spectroscopic and Histological Results in Nondysplastic and Dysplastic Tissue in Barrett’s Esophagus**

<table>
<thead>
<tr>
<th>Spectroscopy</th>
<th>Histology</th>
<th>low-grade dysplasia</th>
<th>High-grade dysplasia</th>
<th>High-grade dysplasia</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNF480</td>
<td>Benign</td>
<td>208</td>
<td>36</td>
<td>33</td>
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<tr>
<td></td>
<td>Premalignant</td>
<td>8</td>
<td>0</td>
<td>13</td>
</tr>
<tr>
<td>DNF660</td>
<td>Benign</td>
<td>206</td>
<td>36</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td>Premalignant</td>
<td>10</td>
<td>0</td>
<td>13</td>
</tr>
</tbody>
</table>

**Table 2. Individual Patient Data Analysis Comparing Spectroscopic and Histological Results in Nondysplastic and Dysplastic Tissues in Barrett’s Esophagus**

<table>
<thead>
<tr>
<th>Spectroscopy</th>
<th>Histology</th>
<th>low-grade dysplasia</th>
<th>High-grade dysplasia</th>
<th>High-grade dysplasia</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNF480,660</td>
<td>Benign</td>
<td>16</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Premalignant</td>
<td>7</td>
<td>0</td>
<td>7</td>
</tr>
</tbody>
</table>

DNF480,660: Logical OR of DNF480 index and DNF660 index results.

* Number of patients classified as having a benign lesion using the DNF480,660 index.

* Number of patients classified as having a premalignant lesion using the DNF480,660 index.

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positive), only a small fraction of the spectra from each patient (except P311) was classified as premalignant. Table 3 shows a list of these patients.

### Discussion

LIF spectroscopy is a noninvasive technique that has been used to differentiate normal from malignant tissue. Several reports have suggested endogenous fluorescence differences between normal and malignant tissue. Alfano et al.² used different laser lines from an argon laser to excite in vitro fluorescence of normal and malignant tissues from lung and breast tissue. Using two pairs of malignant and normal breast and lung tissue, they showed marked differences between spectra from normal and cancerous samples. Additional data from this study have been published by Tang et al.¹.

Fluorescence imaging has been used to detect dysplasia and carcinoma in situ in lung. Lam et al.²⁰ described a fluorescence bronchoscope used to complement white-light bronchoscopy. They stated that the sensitivity of fluorescence bronchoscopy was 50% greater than that of white-light bronchoscopy in detecting dysplasia and carcinoma in situ. They also indicated that moderate/severe dysplasia could not be differentiated from carcinoma in situ because of significant overlap of data.

LIF has been used for detection of premalignant lesions of the gastrointestinal tract. In an in vitro study, Kapadia et al.³ used a helium-cadmium laser for differentiation of adenomatous polyps from normal mucosa and hyperplastic polyps. Using stepwise multivariate linear regression analysis, they developed an algorithm that classified correctly all 34 normal mucosal specimens and all 16 adenomatous polyps. Fifteen of 16 hyperplastic polyps were classified as normal tissue.

Schomacker et al.⁴ used a nitrogen laser to excite in vivo and in vitro fluorescence of colonic tissue. Using a multivariate linear regression analysis, they distinguished neoplastic tissue from nonneoplastic tissue with sensitivity and specificity of 80% and 92%, respectively. Sensitivity and specificity of 86% and 77% were obtained when multivariate linear regression analysis was used to differentiate neoplastic polyps from nonneoplastic polyps. In a similar study, Schomacker et al.²¹ studied the changes in tissue fluorescence occurring post mortem. They indicated that in vivo and in vitro fluorescence emissions were significantly different in adenomatous polyps and concluded that LIF should be acquired in vivo.

In an in vitro study, Richards-Kortum et al.²² used fluorescence excitation-emission matrices to identify the optimal excitation wavelength for obtaining fluorescence emission spectra that could be used to differentiate normal and pathological colonic tissue. They indicated that excitation at 330, 370, and 430 ± 10 nm was optimal for this purpose.

Cothren et al.⁵ used a 370-nm excitation light to examine in vivo fluorescence of colonic tissue. Using a two-dimensional scatter plot of fluorescence intensities at 460 nm vs. 680 nm, they defined a straight line representing a decision surface that minimized the misclassified samples. Using this technique, adenomas could be distinguished from nonadenomatous tissue in approximately 97% of cases.

Panjehpour et al.⁶ used LIF with an excitation wavelength of 410 nm to diagnose esophageal cancer during routine endoscopy using a new classification algorithm called linear discriminate analysis. Using this technique, normal esophagus could be distinguished from malignant esophageal tissue with a sensitivity of 100% and a specificity of 98%. Vo-Dinh et al.¹⁷ described a new spectral analysis technique, DNF index, to distinguish normal from malignant tissues in the esophagus during routine endoscopy. Using DNF indices at 480 nm and 660 nm, esophageal cancer was distinguished from normal tissue with a high degree of reliability.

Many of these studies report detecting malignancies that are either visible or are palpable by the clinician. In contrast, spectroscopy offers the potential to diagnose accurately premalignant changes, such as dysplasia, before development of a visible lesion. In Barrett’s esophagus, the squamous lining of the esophagus is replaced by specialized columnar mucosa.⁷,⁸ Cancer is preceded by dysplastic transformation of Barrett’s mucosa⁹ that is not detectable through white-light endoscopy. Dysplasia can only be detected histologically from biopsy specimens. Because of the focal nature of dysplasia, extensive biopsy specimens are required for reliable diagnosis.

Barrett’s esophagus is found in 10% – 12% of patients with symptomatic gastroesophageal reflux disease who undergo endoscopy,²³,²⁴ but its frequency may be 20

### Table 3. Patients with Nondysplastic Barrett’s Mucosa With a False-Positive LIF Diagnosis Using Individual Patient Data Analysis Technique

<table>
<thead>
<tr>
<th>Patient</th>
<th>Total no. of data points from the patient</th>
<th>No. of data points classified as premalignant (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P271</td>
<td>16</td>
<td>1 (6)</td>
</tr>
<tr>
<td>P276</td>
<td>30</td>
<td>4 (13)</td>
</tr>
<tr>
<td>P283</td>
<td>36</td>
<td>2 (5)</td>
</tr>
<tr>
<td>P289</td>
<td>24</td>
<td>4 (16)</td>
</tr>
<tr>
<td>P290</td>
<td>18</td>
<td>2 (11)</td>
</tr>
<tr>
<td>P300</td>
<td>16</td>
<td>2 (12)</td>
</tr>
<tr>
<td>P311</td>
<td>6</td>
<td>3 (50)</td>
</tr>
</tbody>
</table>
times greater. In patients with Barrett’s esophagus with no apparent adenocarcinoma, the prevalence of positive dysplasia is 5%–10%. Dysplasia may be found in different levels of severity, including low-grade or high-grade dysplasia, with occasional mixed low-grade and focal high-grade dysplasia. Altorki et al. reported detecting carcinoma in 45% of the surgically resected specimens from symptomatic patients with Barrett’s esophagus who underwent surgery for high-grade dysplasia.

An established methodology that provides extensive sampling for detecting dysplasia involves four-quadrant biopsy specimens taken at 2-cm intervals within the Barrett’s mucosa. However, this method is far from ideal. First, the focal nature of dysplasia creates the possibility of sampling error even when performing four-quadrant biopsies at 2-cm intervals. Second, performing endoscopic biopsies in patients with long segments of Barrett’s mucosa results in a prolonged procedure. Third, bleeding during and after endoscopic biopsies interferes with the accuracy of determining the precise location of biopsies. In addition, bleeding may limit the number of biopsies that may be taken. Furthermore, there is always a potential risk of greater bleeding after biopsy is performed in patients with a coagulation disorder. Fourth, variability exists in the histological interpretation of pinch biopsy samples by different laboratories, creating concerns and problems for clinicians.

Compared with pinch biopsies, optical biopsy offers certain advantages. First, because of the rapidity of the fluorescence measurement, the endoscopic LIF technique allows examination of a practically unlimited number of sites, thereby reducing the sampling error, while reducing the length of the procedure significantly at the same time. Typically, 6–7 optical biopsies can be performed in the time it takes to perform one pinch biopsy. In addition, the noninvasive nature of the measurements allows for multiple optical biopsies to be taken without any bleeding.

Furthermore, this technique may potentially reduce the human variability in histological interpretation of biopsy samples by establishing a standard mathematical/statistical technique for interpretation of the data. Last, the results of the optical biopsy can be available to the endoscopist within minutes, perhaps seconds, rather than days. This may allow the endoscopist to decide on further diagnostic studies or on a choice of treatment, possibly with implementation during the same procedure.

The DNF index technique was applied in this study to determine if high-grade dysplasia associated with Barrett’s mucosa can be distinguished from nondysplastic Barrett’s mucosa. Analyzing all data collectively using the DNF$_{480}$, 96% of nondysplastic Barrett’s and all low-grade dysplasia samples were classified as benign. Ninety percent of tissues with high-grade dysplasia were classified as premalignant. Only 28% of the samples that were histologically diagnosed as low grade with focal high-grade dysplasia were classified as premalignant. This is to be expected when the data are collectively analyzed. Because multiple four-quadrant biopsy samples from the same level are placed in the same histology vial for interpretation, a mixture of low-grade and high-grade samples might be included from the same level. However, when fluorescence measurements are conducted, each spectrum is analyzed individually as either benign or premalignant, perhaps explaining why only 28% of the samples containing low-grade with focal high-grade dysplasia were classified as premalignant.

The overall results of the collective data analysis using the DNF$_{660}$ index were similar but not identical to those obtained using the DNF$_{480}$ index. This suggests that each DNF index may be used independently or the two DNF indices may be used concurrently to improve the likelihood of detecting high-grade dysplasia.

An alternative tabulation technique was therefore used; the logical OR of the two DNF classifications was used to reduce the chance of misclassifying patients with high-grade dysplasia. If any of the two (or both) DNF indices classified a spectrum as premalignant, the final diagnosis was premalignant. A minimum of one premalignant spectrum in a patient was sufficient to place that patient in the premalignant category. Using this technique, 100% of patients with any high-grade dysplasia were classified as having premalignant lesions. However, 7 of 23 patients with nondysplastic Barrett’s mucosa (30%) were classified as having premalignant lesions using this technique. The high rate of false positives may be explained in several ways. First, this may simply be caused by the limitation of the technique. Another possible explanation may arise from the high probability of sampling error when performing pinch biopsies. It is quite possible that the optical biopsy sampled tiny islands of high-grade dysplasia that the pinch biopsy missed. Therefore, it is possible that the 30% false-positive result may not reflect a true false-positive result. Although a high rate of false-positive results was obtained using this technique, only a small fraction of spectra from each patient was classified as premalignant. However, in patients with high-grade dysplasia, a high percentage of spectra in each patient was classified as premalignant, ranging from 50% to 100%. Using the two DNF indices concurrently, all patients with high-grade dysplasia were classified as having premalignant lesions, indicating that optical biopsy may be used as a reliable screening technique for detecting high-grade dysplasia in patients with Barrett’s esophagus.
In a preliminary data analysis, it was determined that the line shape of nondysplastic Barrett’s mucosa was similar to that of normal esophagus in the majority of the cases. Occasional line shape variations were small, classifying the spectra within the benign category. This is clearly evident in the accuracy of classifying nondysplastic Barrett’s mucosa sample as benign tissue in this study (specificity of 96%).

This study showed that the fluorescence line shape of high-grade dysplasia has similar characteristics as that of esophageal cancer. Therefore, this model could not differentiate between high-grade dysplasia and carcinoma, similar to results reported by Lam et al. However, high-grade dysplasia could be distinguished from nondysplastic Barrett’s mucosa and low-grade dysplasia with relatively high sensitivity and moderate specificity.

Pathologists often have difficulty classifying a lesion as dysplastic in the presence of active inflammation (reactive atypia). If biopsy specimens are not obtained and if the endoscopist depends entirely on spectroscopy, it may be necessary to prove that spectra obtained from inflamed mucosa with reactive atypia can be differentiated from those with high-grade dysplasia. Such studies are in progress at our institution. Preliminary data indicate that benign erosive esophagitis may misclassify as premalignant. Therefore, until further data is available for analysis, we caution against making a spectral diagnosis of premalignant based on data obtained from esophageal erosions and ulcerations.

Thus, endoscopic LIF spectroscopy provides the clinician with a rapid, accurate, and less invasive technique for detecting high-grade dysplasia in patients with Barrett’s esophagus compared with standard methods. If optical biopsy specimens are abnormal, the physician can proceed to pinch biopsy for histological diagnosis. If endoscopic LIF optical biopsy specimens are normal, the patient would not necessarily need to undergo an extensive sampling protocol with pinch biopsies and could be scheduled for routine follow-up.

In summary, LIF spectroscopy has a great potential to detect high-grade dysplasia in Barrett’s esophagus during routine endoscopy. Further studies are needed to determine whether this technique can correctly classify tissue with active inflammation. We believe this technique may eventually serve as the preferred endoscopic screening procedure for patients with Barrett’s esophagus.

References

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