Detection of early laryngeal cancer and its precursor lesions by a real-time autofluorescence imaging system

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Abstract
The prognosis of laryngeal carcinoma patients can be improved by early diagnosis. The autofluorescence endoscopy has been developed to gain more information about the biologic character of the precancerous or cancerous lesions. The aim of the present study was to evaluate the diagnostic potential and limitations of this imaging technique applied during indirect laryngoscopy and comparing with white light and microlaryngoscopy with biopsy. In a prospective study, 56 patients with suspected precancerous or cancerous lesions were investigated preoperatively by white light and fluorescence endoscopy during indirect laryngoscopy. The procedure was followed by microlaryngoscopy and biopsy. Results were compared to histopathological findings. Normal laryngeal mucosa displayed a typical green fluorescence, moderate to high epithelial dysplasia, in situ carcinoma and cancer showed a diminished green fluorescence. In 47 of 56 (84%) cases, we found concordant results. The experience from this study shows that autofluorescence by indirect laryngoscopy may be a useful complementary method for detecting laryngeal malignancies. Its advantages are non-invasiveness, high sensitivity and repeatability.

Keywords: autofluorescence endoscopy, early diagnosis, precursor lesions of larynx, laryngeal cancer.

Introduction
Carcinomas of the upper aerodigestive tract represent a major problem in modern health care. Laryngeal cancer constitutes 1–2% of all malignancies diagnosed worldwide [1].

A recent U.S. study has pointed out that survival rates are significantly higher for early stage carcinomas [1]. Therefore, it is essential to concentrate on the initial steps in tumor development in order to facilitate early detection and timely implementation of suitable therapy [1].

Complete diagnosis of laryngeal pathology includes the detection of their presence, precise determination of their size and boundaries, and providing biopsy from adequate tissue to establish exact histopathological diagnosis [2].

Nowadays, the microlaryngoscopy (MLS) introduced in the sixties by Kleinsasser [3] is a worldwide standard diagnostic procedure for the detection and description of laryngeal pathology, as well as for many endoscopic microsurgical procedures involving the larynx.

Up until today, indirect and direct white light laryngoscopy was considered the standard imaging technique in the assessment of laryngeal cancer and its precursor lesions [4].

To improve the recognition of precancerous lesions and cancer, supravitral staining of mucosa with Toluidine Blue [5], selective demonstration of tumor tissue using Lugol’s solution [6] and many other methods have been developed. However, none of these methods improved diagnostics during MLS [7].

Recently, autofluorescence endoscopy has been developed to enhance endoscopic information of mucosal lesions and has been successfully applied in different medical specialties, e.g., urology, pulmonology and gastroenterology [8–11].

Autofluorescence is a natural capacity of tissue to fluoresce when exposed to a certain light wavelength. This feature is a consequence of the presence of some substances in the tissue that fluoresce when exposed to a narrow wavelength range [12].

Autofluorescence endoscopy is based on the excitation of tissue-specific fluorescence of the mucosa by short-wave blue light of the visible spectrum (375–440 nm). The causes for changes of the fluorescence characteristics are complex. Changes in metabolism, reactive changes of the adjacent stroma and the frequent thickening of the epithelium are a few possible reasons [13]. Also, atypical and larger nuclei increase the loss of autofluorescence.

In addition, autofluorescence is also markedly reduced when elastic fibers in the lamina propria are diminished.

The appearance and degree of autofluorescence depend especially on tissue structure’s fluorophores content. Fluorophores are predominantly proteins, e.g., elastin and keratin, as well as nicotinamide adenine dinucleotide (NADH) [14, 15]. In cancer cells, NADH is reduced, and the non-fluorescent oxidative form NAD prevails.

We describe the value and limitation of the method in the diagnosis of precancerous and cancerous lesions of the larynx applied during indirect laryngoscopy and the comparison with white light endoscopy and microlaryngoscopy with biopsy.

Patients and Methods
In this prospective pilot study, 56 patients (40 males, 16 female) were examined between December 2013 and July 2014. The mean age was 58 years (35–78 years). All of them suffered from one or more laryngeal symptoms (e.g., hoarseness, dysphagia, foreign body sensation, cough, etc.).

In 40 (71.42%) of them, malignancy was suspected...
after mirror laryngoscopy (Table 1). Benign pathology (Reinke’s edema, papilloma’s, polyps, cysts, nodules) was included for the basic evaluation of the system. Informed consent for the present study was obtained for each patient.

Table 1 – Diagnoses and malignancy suspected on mirror laryngoscopy

<table>
<thead>
<tr>
<th>Histological diagnosis</th>
<th>Malignancy suspected on mirror laryngoscopy</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chronic laryngitis (simplex hyperplasia)</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td>Nodules, polyps, cysts, Reinke’s edema, papilloma (without dysplasia)</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>Precancerous lesions, keratosis, atypical hyperplasia, etc.</td>
<td>0</td>
<td>12</td>
</tr>
<tr>
<td>Laryngeal tumors</td>
<td>0</td>
<td>25</td>
</tr>
<tr>
<td>Total</td>
<td>16</td>
<td>40</td>
</tr>
</tbody>
</table>

All patients were evaluated preoperatively by white light and autofluorescence endoscopy during indirect laryngoscopy. The procedure was followed by micro-laryngoscopy with biopsy.

For white light and autofluorescence endoscopy, we used a 70° rigid hypopharyngoscope (Karl Storz, Germany) and the D-LIGHT C/AF System (Karl Storz, Germany). In this system, the excitation blue light source is provided by a Xenon arc lamp with an excitation wavelength ranging from 375 to 440 nm. The remitted excitation light up to 440 nm was blocked out by an integrated optic filter. This system allows real-time white light endoscopy and switches over to autofluorescence endoscopy by pressing a button. We used a high-resolution color charge coupled device (CCD-camera system, Endovision TRICAM SL-PDD and Endovision TELECAM SL-PDD, Karl Storz, Germany) camera system. All findings were recorded on a digital video system for documentation and analysis. Intraoperatively obtained tissue samples were taking according to the size of the pathology by excisional biopsy, e.g., CO₂ laser decortication of the vocal fold, CO₂ laser cordectomy, or a biopsy in case of larger tumors. Results were compared then to histopathological findings.

Results

Histopathological findings of 56 cases revealed: 10 (17.85%) cases of hyperplasia, 16 (28.56%) cases of mild dysplasia, eight (14.28%) cases of moderate dysplasia, six (10.71%) cases of severe dysplasia/in situ carcinoma and 16 (28.56%) cases of laryngeal cancer.

Normal mucosa of the larynx with no visible alterations in either indirect white light or in autofluorescence laryngoscopy shows the typical homogeneous, pale green fluorescence. Transitional areas with different fluorescent properties between the healthy squamous epithelium of the vocal cords and the respiratory epithelium of the supra- and subglottic region cannot be identified. Vessels were better contrasted to the surrounding tissue due to light absorption by the hemoglobin. Areas of edematous swelling (e.g., Reinke’s edema) display normal fluorescence intensities.

In normal light, hyperplastic epithelium with or without mild dysplasia (non-cancerous) presented a milky-white aspect. Keratosis or leukoplakia appeared as a small white warty, sharply circumscribed lesion. According to its high autofluorescence intensity, keratosis appeared as a more-or-less bright green area.

In precancerous or cancerous alterations of the mucosa, the autofluorescence signal changed from green fluorescence to a reddish-violet color (Figures 1–4).

In some cases of severe dysplasia or in situ carcinoma, as well as infiltrating cancer, the reddish-violet autofluorescence signal was more intense when compared to moderate dysplastic changes. However, we were not able to differentiate between various higher grades of epithelial dysplasia. Moreover, we could not distinguish in situ carcinoma from invasive cancer. In some cases of laryngeal cancer, we were able to detect an orange fluorescence caused by bacterial overgrowth.

The diagnostic accuracy by white light laryngoscopy was 71% (n=40) and that of autofluorescence 84% (n=47).

The sensitivity by white light laryngoscopy for detection of precancerous as well as cancerous lesions was 85%, and specificity was 75%. The sensitivity by autofluorescence laryngoscopy was 92% and specificity to 87%.

Figure 1 – Normal mucosa of the larynx with no visible alterations in either white light (a) or in autofluorescence laryngoscopy (b) displays the bright-green fluorescence.
Figure 2 – Laryngeal cyst: White light endoscopy (a); Autofluorescence endoscopy (b) shows a homogeneous intensity and distribution.

Figure 3 – Invasive carcinoma (pT1a): White light endoscopy (a) demonstrates a bulging tumor of the left vocal fold; Autofluorescence endoscopy (b) shows a marked loss of autofluorescence, whereas induced fluorescence endoscopy.

Figure 4 – Moderate differentiated squamous cell carcinoma of the right vocal fold with lesions of carcinoma in situ (pT1a): White light endoscopy (a); Autofluorescence endoscopy (b) shows a marked loss of autofluorescence, whereas induced fluorescence endoscopy.

Discussion

Optimization of early diagnosis of laryngeal carcinomas represents an objective of ENT specialists. It is not only the key to reducing the mortality rate of oncological patients, but also the condition for improving the modalities of minimally invasive treatment. Early detection and preoperative assessment are important to a curative and function-preserving therapy, because the
The AFE method exploits the fact that cancer cells have a different metabolism from that of normal cells. A specific molecular mechanism in the tumor leads to the accumulation of fluorescent substances in the tumor; the defective cells will fluoresce and show up in the image. Demarcation of precursor lesions and dysplastic margins of carcinomas are intensified by the color contrast. In such a manner, AFE can contribute to guided biopsy and surgical resection as well as oncological follow-up of precancerous and cancerous lesions.

The AF system can only detect changes on laryngeal epithelium, but not in deeper structures. For these reason, laryngeal nodule, polyp, Reinke’s edema, papilloma and other lesions covered by normal epithelium will not result in any disturbance in the autofluorescent signal. If a reduction of autofluorescent intensity appears, it is necessary to consider this area as a suspicious, and to take a biopsy.

In 1924, Policard [16] observed the ability of tissue to fluoresce under certain conditions. Alfano et al., in 1984 [17], reported the possibility of differentiating between healthy and malignant tissue by means of their fluorescent characteristics. In 1933, Sutro and Burmann described the phenomenon of the different fluorescences between healthy and malignant tissue by means of their fluorescent characteristics. In 1995, Harries et al. [18] in a pilot study of eight cases with carcinoma of the vocal folds concluded that the technique can increase the accuracy of staging of cancer of the larynx and allows earlier diagnosis of tumors and their recurrence.

In 2002, Malzahn et al. [19] in a report of a case series of 127 patients, of which 111 were suspected of having precancerous and cancerous lesions, concluded that: normal laryngeal mucosa displays a typical green fluorescence signal, moderate and severe epithelial dysplasia, CIS and invasive carcinoma shows a diminished green fluorescence. They reported two false-negative cases and eight false-positive cases, and a sensitivity of 97%, while specificity was 83%.

In 2006, Arens et al. [20] in a prospective study of 116 cases with suspected precancerous or cancerous lesions of the larynx with “Indirect Autofluorescence Laryngoscopy” and comparing the images with direct autofluorescence reported concordant results in 103 cases. It was therefore concluded that the technique can increase the accuracy of staging of cancer of the larynx and allows earlier diagnosis of tumors and their recurrence.

In this study, we found that the AF imaging system was a useful complementary procedure for detecting laryngeal malignancies. However, when used alone it did not significantly improve the diagnostic evaluation in comparison with microlaryngoscopy. From our study, we can say that AFE and WLE do improve diagnostic findings when used in combination with MLS.

Conclusions

Indirect autofluorescence laryngoscopy is a quick, reproducible, non-invasive imaging technique, without drug application, which facilitates the detection and delineation of precancerous lesions, in situ carcinoma, and cancer of the larynx more accurately than white light examination alone. AFE could be an important additional diagnostic method, which coupled with the standard MLS could significantly improve the diagnostic efficacy of laryngeal pathology.

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