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Research Article

The importance of magnification chromoendoscopy with methylene blue in detecting specialized intestinal metaplasia in short segment Barrett's esophagus

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Abstract. Background:S pecialized intestinal metaplasia (SIM) is not identifiable in Barrett esophagus in standard white light endoscopy. In this study we assessed the utility of magnification chromoendoscopy with methylene blue in detecting SIM in patients with short Barrett's esophagus. Material and method: The study included 50 patients with suspected short Barrett's esophagus: 26 followed standard endoscopy with random biopsies and 24 underwent magnification chromoendoscopy with methylene blue with targeted biopsies. In the magnification chromoendoscopy group, magnified images were analyzed and specific patterns were classified using Endo's classification. Results: In the standard endoscopy group, 17 of 26 patients were detected as having SIM and in the magnification chromoendoscopy group 20 of 24 patients were SIM positive. Magnification chromoendoscopy increases the probability of detecting SIM up to 2,4 times (OR=2.39, p=0.028). Sensitivity and specificity of MB staining in diagnosis of SIM was 78.7% and 55.5%. SIM was diagnosed in areas with tubular and villous patterns, but had a significant correlation only with villous pattern (p=0.017).Conclusion: Magnification chromoendoscopy with methylene blue improves SIM detection in patients with short Barrett's esophagus.

Key Words: Barrett's esophagus, specialized intestinal metaplasia, magnification chromoendoscopy, methylene blue

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Background

Barrett esophagus is an acquired, premalignant condition caused by chronic gastroesophageal refflux, in which the normal squamous epithelium in the distal esophagus is replaced by columnar epithelium. Barrett's esophagus is considered to follow a multistep process progression from intestinal metaplasia to low-grade dysplasia (LGD) to high-grade dysplasia (HGD) and finally to esophageal invasive adenocarcinoma in a subset of patients (Hameeteman *et al* 1989; Miros *et al* 1991). In epidemiologic studies, BE is associated with an increased risk of esophageal adenocarcinoma (EAC), at least 40-fold higher than the general population (Drewitz *et al* 1997; Spechler *et al* 1984). The risk of developing EAC among patients with BE is estimated between 0.5% and 1% per patient per year (Falk 2002). The diagnosis of BE relies initially on the endoscopic recognition of the columnar lined distal esophagus and is confirmed by histological examination. The metaplastic Barrett epithelium is a mosaic of different types of columnar epithelium which frequently coexist in the same patient (Paull *et al* 1976): cardial, fundic and specialized intestinal metaplasia (SIM) with goblet cells. Due to the identification of histologically confirmed SIM as a precursor lesion for dysplasia and malignancy, obtaining biopsies from the columnar lined distal esophagus is mandatory (Chalasani *et al* 1997; Schnell *et al* 1992).

Even if columnar metaplasia in the distal esophagus can be easily recognized as a displacement of scuamocolumnar junction over the gastroesophageal junction, it is difficult to identify SIM in standard endoscopy. The most widely accepted method to identify the presence of SIM in standard endoscopy is the use of four-quadrant random biopsy at 1-2 cm intervals over the entire length of the specialized columnar epithelium. This protocol, referred to as the Seattle protocol, has certain disadvantages and limitations: use of multiple biopsies, sampling errors, long time procedure because of the number of biopsies and high cost (Sampliner 1998; Falk *et al* 2000).

Because of the reminded limitations of the Seattle protocol, several techniques that could improve recognition of SIM and provide a more accurate way to guide biopsies have been tried.

Chromoendoscopy and magnification endoscopy is a technique that can provide high resolution mucosal details and improve detection of SIM. One type of chromoendoscopy uses methylene blue (MB) sprayed over the surface of the distal esophagus. MB stains actively absorbing tissues such as intestinal epithelium and intestinal metaplasia, but it will not stain nonabsorptive squamous mucosa or gastric mucosa.

Magnification endoscopy and chromoendoscopy with methylene blue can identify several mucosal surface patterns, which were classified and described by Endo et al in 2002: small/ round pattern, straight, long oval, tubular and villous pattern. Round and straight pattern corresponded to gastric epithelium, whereas area showing tubular and villous pattern contained intestinal-type epithelium.

The goal of the present study was to use magnification endoscopy in combination with methylene blue chromoendoscopy to help identify intestinal metaplasia in short Barrett's esophagus.

Materials and Methods

Our study group consisted of patients with suspected short BE after a standard endoscopic examination of the upper intestinal tract in the Gastroenterology Clinic of Tg. Mures County Hospital. They gave written informed consent and were enrolled in this prospective study, in which every second patient underwent magnification chromoendoscopy with targeted biopsies. The patients were divided into 2 groups: one group (24 patients) underwent magnification chromoendoscopy with directed biopsies and the other group (26 patients) followed standard endoscopy with random biopsies. Collected data included age, gender, symptoms of GERD (heartburn, acid reflux , regurgitation), endoscopic appearance of scuamocolumnar junction, the presence of a hiatal hernia, methylene blue staining pattern, magnification endoscopy pattern and presence of SIM on histopathology were collected .

Scuamocolumnar junction was identified as the abrupt change of colour from the pink pale esophageal squamous epithelium to the red salmon-colored columnar epithelium, and the esophagogastric junction as the most proximal margin of gastric folds. A hiatus hernia was considered present when there was a distance of at least 2 cm between the diaphragmatic hiatus and the gastroesophageal junction (GEJ).

The length of BE was measured as the distance between the proximally displaced SCJ and the GEJ. Short or ultra short Barrett esophagus was identified as an irregular, proximally located, Z-line relative to GEJ, and/or the appearance of islands or short tongues of columnar-type mucosa that extend into the distal esophagus less than 3 cm in length above GEJ.

We excluded from the study patients with severe cardio-pulmonary disease and with increased risk of bronchial inhalation, patients with severe coagulation disorders or with anticoagulant treatment .

The patients in the standard endoscopy group had four quadrant random biopsies taken every 1 (in columnar mucosa <2cm) or 2 cm in case of circular columnar lined-esophagus. Biopsies were taken from all reddish tongue-like or island-like columnar appearing mucosa extended proximal to the GEJ.

Patients in the magnification endoscopy group had a detailed examination of columnar mucosa in esophagus using optical magnification up to 115 times (Olympus GIF Q160Z).Mucus was removed by the 10% solution of acetylcysteine instillation. Then MB 0,5% was flushed from the upper to the lower portion of the distal oesophagus followed after 2-3minutes by a water rinse to remove excess dye. The magnified images were analyzed and the different mucosal pit patterns were carefully observed under magnification and were classified according to Endo's scale. After a mucosal pattern was observed, biopsies samples for histopathologic examination were taken with standard forceps from the regions colored with MB, from the regions with patterns described by Takao Endo classification, especially from the patterns most often related to SIM according to Endo's study (tubular and villous) .

All biopsy specimens were fixed in formalin and submitted for histopathological examination. All biopsy specimens were then embedded in paraffin, stained with hematoxylin-eosin and analyzed by the same pathologist, who was blinded to the magnification endoscopy results and patterns. Histological data required presence of goblet cells as criteria for SIM.

Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS, ver. 19, Chicago, IL, USA). Continuous variables were tested for normality of distribution using the Kolmogorov-Smirnov test. Descriptive analysis included frequencies for ordinal variables and the mean for continuous variables with normal distribution. Differences of the mean between groups were analyzed using the T test. The correlation between continuous variables was assessed using Pearson correlation. Chi-square test was used to compare quantitative variables. A binary logistic regression was used to assess the influence of certain parameters on the probability of detection of SIM. A p value of less than 0.05 was considered to be statistically significant.

Results

Our study population was composed of 50 patients, 24 of them in the magnification chromoendoscopy group and 26 in the standard endoscopy with random biopsy. Table 1 shows the characteristics of the patients in both groups. We analyzed mean age between in the two groups using T test for independent variables and found no differences (p=0.589). We used chi- square test to analyze sex distribution (p=0.139) , symptoms of GERD, esophagitis(p=0.964) and hiatal hernia (p=0.161) and found no statistical difference between two groups.

Table 1. Demographic, clinical and endoscopic chartacteristics

Variables	Magnification chromoendoscopy group N=24	Standard endoscopy group N=26
Age (year. mean)	58.73 (29-80)	56.54 (32-84)
Sex F/M	7/17	14/12
Body mass index	23.2	24.03
Symptoms of GERD	13 (54.1%)	12 (46.1%)
Esophagitis	5 (20.8%)	7 (26.9%)
Hiatal hernia	14 (58.3%)	18 (69.2%)
Ultra short BE	8 (33.3%)	9 (34.6%)

N - number of patients

In the magnification chromoendoscopy group 20 of 24 patients (83.3%) had SIM on the histological exam. In this group we performed 51 biopsies (with an average of 2.1 biopsies per patient); in 34 of these biopsies SIM was positive in histological examination. In the standard endoscopy group 17 of 26 (65.3%) patients had SIM; we took 71 biopsies (an average of 2.7 biopsies per patient) and in 27 biopsies histology detected SIM (table 2).The number of biopsies in the magnification chromoendoscopy group was reduced compared to standard endoscopy group(p=0.016).

Table 2. Detection of specialized intestinal metaplasia

	Number of patients	Patients with SIM	Number of biopsies	Biopsies with SIM
Magnification chromoendoscopy group	24	20 (83.3%)	51	34 (66.6%)
Standard endoscopy group	26	17 (65.3%)	71	27 (38%)

A complex multivariate analysis showed that magnification chromoendoscopy increases the probability of detecting SIM up to 2.4 times (OR=2.39, p=0.028) and every biopsy taken increases the probability of detecting SIM up to 3.8 times (OR=3.87, p=0.042).

In the magnification chromoendoscopy group we took 33 biopsies from the methylene blue stained sites and in 26 biopsies histology detected SIM. Histological examination proved SIM in 8 out of 18 biopsies (44.4%) from MB unstained areas. Sensitivity and specificity of MB staining in the diagnosis of SIM was 78.7% and 55.5%. We found no significant correlation between MB stained areas and the diagnosis of SIM in histology (p=0.442) (table 3).

All types of mucosal pit patterns in Endo's classification were identified after using methylene blue and magnification endoscopy: small round, straight, long oval, tubular and villous. (table 4) Of the 51 biopsies in the chromoendoscopy group, 15 were performed in patients with ultrashort segment Barrett's esophagus with islands or small tongues of columnar mucosa less than 1 cm and histology proved SIM in 9 biopsies (60%). In patients with short segment Barrett's esophagus we took 36 biopsies and SIM was found in 25 samples (69.4%). No sample with small round, straight or oval patterns according to Endo's classification had SIM or dysplasia; those patterns corresponded to gastric or cardiad metaplasia. SIM was diagnosed in sites covered with tubular and villous patterns, but had a significant correlation only with the villous pattern (p=0.017) . Among of the 36 studied biopsies in SSBE patients, low grade dysplasia was found in one biopsy with tubular pattern.

Discussion

Barrett's esophagus is a premalignant condition with an increased risk of developing dysplasia and esophageal adenocarcinoma. Detection of early cancers or precancerous lesions in the esophagus may lead to better prognosis and survival, but unfortunately, these early lesions are often macroscopically normal or with very subtle mucosal changes which can be missed in a standard endoscopy examination. Malignant transformation in Barrett's metaplasia occurs most often in the presence of specialized intestinal metaplasia, but the distribution of SIM in columnar lined distal esophagus is patchy and not identifiable by white-light endoscopy.The accuracy in detection SIM using standard biopsy is low and some endoscopic techniques like magnification chromoendoscopy with methylene blue can help target areas with suspected specialized intestinal metaplasia and may improve the diagnosis of Barrett's esophagus.

In this study, cromoendoscopy with methylene blue have a moderate sensitivity of 78.7% and a specificity of 55.5% in detecting SIM in patients with short Barrett's esophagus, but the simultaneous use of chromoendoscopy and magnification increased the probability of detecting SIM in histology up to 2.4 times. The moderate sensitivity of methylene blue chromoendoscopy could be explained by difficulties in staining in esophagus, which is often patchy especially in short or ultrashort Barrett's esophagus. Other studies showed controversial results regarding the use of chromoendoscopy with methylene blue.

Previous studies have used chromoendoscopy with methylene blue to evaluate the utility in detecting SIM or dysplasia in patients with BE. In a study on 26 patients, Canto et al (Canto *et al* 1996) showed that chromoendoscopy with methylene blue is a highly accurate method of diagnosing specialized columnar epithelium in Barrett's esophagus with a sensitivity of 95% and a specificity of 97%. Sharma et al (Sharma *et al* 2001) studied a group of 75 patients with endoscopic appearance of short segment Barrett and methylene blue directed biopsies and compared with a control group of 83 patients with randomly obtained biopsies. Specialized intestinal metaplasia was detected in 61% of cases in methylene blue directed biopsies and 42% of cases in random biopsies. Kiesslich et al (Kiesslich *et al* 2001), in a study performed in 51 patients with Barrett's osophagus and 21 control subjects, showed that targeted biopsy of stained areas provided histological proof of specialized columnar epithelium with a sensitivity of 98% and a specificity of 61% and confirmed the ability of methylene blue staining to highlight areas of specialized intestinal metaplasia.

In a more recent study (Wasielica-Berger *et al* 2011), MB staining had a very low specificity (40.6%) and a moderate sensitivity (71.4%) Other studies have not demonstrated a benefit of methylene blue staining in the identification of intestinal metaplasia or dysplasia. In a prospective randomised crossover trial, Wo and coworkers (Wo *et al* 2001) compared the diagnostic yield of methylene blue directed biopsies with that of four quadrant 2 cm interval biopsies and found no additional benefit. Sensitivity and specificity for specialised intestinal metaplasia were 53% and 51%, respectively. Relative frequencies for specialised intestinal metaplasia were 20% and 18% from methylene blue directed and conventional biopsies, respectively. Some other studies (Saporiti *et al* 2003; Horwhat *et al* 2008) also concluded that methylene blue chromoendoscopy offered no advantage over the conventional method of random biopsies in the diagnosis of Barrett's esophagus

The combination of methylene blue chromoendoscopy with magnification endoscopy has been used in previous studies to identify specific mucosal patterns which are more frequent associated with SIM in patients with Barrett's esophagus. Endo and colleagues used magnification endoscopy and methylene blue staining in 30 Barrett's esophagus patients.

Table 3. Detection of specialized intestinal metaplasia and dysplasia in sites stained or not stained with methylene blue

	Number of biopsies	Specialized intestinal metaplasia	Patients with SSBE		Patients with USSBE	
			Number of biopsies	Specialized intestinal metaplasia	Number of biopsies	Specialized intestinal metaplasia
Methylene blue stained sites	33	26 (78.7%)	25	19 (76%)	8	7 (87.5%)
Methylene blue not stained sites	18	8 (44.4%)	11	6 (54.5%)	7	2 (28.5%)
Total	51	34 (66.6%)	36	25 (69.4%)	15	9 (60%)

Table 4. Patterns identified during magnification chromoendoscopy with MB and frequency of specialized intestinal metaplasia and dysplasia in short segment Barrett's esophagus (SSBE) and ultra-short segment Barrett's esophagus (USSBE)

Patterns	Patients with SSBE			Patients with USSBE		
	Number of biopsies	SIM	Low grade dysplasia	Number of biopsies	SIM	Low grade dysplasia
Small round I	0	0	0	3	0	0
Straight II	2	0	0	1	0	0
Long oval III	2	0	0	0	0	0
Tubular IV	11	8	0	9	7	0
Villous V	21	14	1	2	1	0
Total	36	22	0	15	8	0

They identified five different mucosal staining pit patterns and found that specialized intestinal metaplasia was detected in 100% of biopsy specimens who exhibited tubular and villous patterns. Round pits and straight lines corresponded to gastric fundic type epithelium. In addition, the tubular and villous areas showed absorption of methylene blue, whereas this was lacking in areas with small round pits and/or straight lines. This study identified two specific patterns (tubular and villous) observed under magnification, which might be an useful tool in detecting intestinal metaplasia. W –Berger and colleagues (Wasielica-Berger *et al* 2011) reported the presence of SIM in areas with the same patterns as in Endo's original study, but less frequently: 13,6% from biopsies with tubular pattern and in 29% from biopsies with villous pattern. The sensitivity and specificity of tubular and villous pit patterns to detect SIM according to Endo's classification were respectively, 85.7% and 21.1%.

In our study, we identified all five pit patterns, but SIM was diagnosed only in sites covered with tubular and villous patterns. Histological examination found SIM in 75% from biopsies with tubular patterns and in 65.2% from biopsies in villous patterns. In one biopsy with villous patterns low grade dysplasia was identified.

In summary, SIM was a common finding in tubular and villous patterns according to Endo's classification and simultaneous use of magnification and chromoendoscopy with methylene blue improve SIM detection in patients with short Barrett's esophagus.

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