The utility of cytokeratins 7 (CK7) and 20 (CK20) immunohistochemistry in the distinction of short-segment Barrett esophagus from gastric intestinal metaplasia: Is it reliable?

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Abstract

Background: The purpose of the present correlative immunohistochemical study was to assess the utility of cytokeratin (CK7 and CK20) expression in the diagnosis of short-segment Barrett esophagus, particularly its efficacy in differentiating Barrett mucosa from intestinal metaplasia of the gastric cardia and corpus.

Methods: Two groups of endoscopic biopsy specimens were examined, including 20 endoscopic biopsy specimens of short-segment Barrett esophagus (Group A) and equal number exhibiting *Helicobacter pylori* associated intestinal metaplasia of the gastric cardia and corpus (Group B). All were investigated by immunohistochemistry using the standard ABC method for CK7 and CK20 expression.

Results: The anticipated pattern of reactivity in Barrett mucosa (CK 7 – strong diffuse positivity in superficial and deep glands; CK 20 – positivity in surface epithelium and superficial glands) was seen in 2 cases of Group A specimens. The expected gastric pattern (CK 7 – patchy immunostaining with variable involvement of deep glands; CK20- patchy immunostaining of superficial and deep glands in incomplete intestinal metaplasia / absence of CK7 immunoreactivity with strong CK20 staining in superficial and deep glands in complete intestinal metaplasia) was seen in 8 cases of Group B specimens. The respective sensitivity values of CK7/20 staining for Barrett pattern and Gastric pattern in Group A and Group B were as follows: 10% and 40%, respectively.

Conclusions: We concluded that these hypothesized and recently applied diagnostic criteria involving CK7 and CK20 immunoreactivity are not reliable in distinguishing short-segment Barrett esophagus from intestinal metaplasia as seen in gastric cardia and corpus.
Background

Barrett esophagus (BE) is associated with an increased risk of esophageal adenocarcinoma, a malignancy that has had a rapid rising incidence recently [1,2]. The etiology of BE is unknown, but genetic predisposition with ulcerative changes from gastro-esophageal reflux disease (GERD) might be responsible [3].

BE should be suspected at the endoscopy when the normal, whitish-appearing squamous epithelium is replaced by a red, velvety-appearing mucosa in the distal esophagus. The reliable diagnosis of BE is difficult related with sampling errors due to determination of the exact location of the gastro-esophageal junction such as in hiatal hernia or normal occurrence of gastric type epithelium in the distal esophagus. Diagnostic difficulties arise when the endoscopist deals with the short-segment BE. Junctional, fundic and specialized (intestinal) types of glandular epithelium were described for the definition of BE by Paull et al. [4]. But it was recently reported that the detection of the latter is a reliable criterion for the diagnosis of BE [5,6]. Helicobacter pylori (H.pylori) might be associated with carditis and intestinal metaplasia of the cardia [7]. Therefore, intestinal metaplasia involving the cardia related to Helicobacter gastritis can be histologically indistinguishable from intestinal metaplasia of the distal esophagus.

It has been suggested that cytokeratin subsets 7 and 20 (CK7 and CK20) might be useful to distinguish both long- and short-segment of BE from intestinal metaplasia of the proximal stomach [8-11]. In accordance with the study by Ormsby et al. [8], the Barrett (CK7/20) pattern was defined as staining of the superficial epithelium with CK20 and staining of both superficial and deep metaplastic epithelium with CK7. The designated (CK7/20) pattern was grouped into two patterns regarding the type of the intestinal metaplasia (complete/incomplete) by the same authors. According to their suggestion, patchy CK7 expression with variable involvement of deep glands was seen in incomplete gastric intestinal metaplasia, whereas strong CK20 expression in superficial and deep glands with the absence of CK7 expression was seen in complete gastric intestinal metaplasia.

The purpose of this study was to evaluate the hypothesized Barrett (CK7/CK20) pattern. The hypothesized gastric CK7/CK20 pattern was also evaluated.
Methods

Endoscopy specimens of the patients referred from Marmara University Institute of Gastroenterology were reviewed from the files of the Department of Pathology at the same university between 1998 and 2002. All the cases which were diagnosed histopathologically as short-segment BE were included to the study. Short-segment BE was defined endoscopically as tongues less than 3cm in length above the esophagogastric junction (EGJ) with its resemblance to small intestine having well-formed microvilli and intestinal metaplasia either with hematoxylin and eosin (H&E) (Figure 1) or Alcian blue pH2.5 positive goblet cells (Figure 2) on microscopic examination. Biopsy specimens that were obtained within 5mm distance below the EGJ were considered as cardiac mucosa. Esophageal and gastric biopsies were taken antegrade or retrograde with retroflexion of the endoscope when it was necessary. The study group consisted of 20 patients with short-segment BE (Group A) and the equal number of patients with gastric intestinal metaplasia (cardia and corpus) accompanied by *H. pylori* gastritis (Group B).

Specimens were fixed in 10% buffered formalin and embedded in paraffin. Serial sections were cut from each specimen. All sections were stained with routine H&E stain. Periodic acid Schiff (PAS), Alcian blue pH 2.5 and Alcian blue pH 0.5 were used to identify neutral mucin, sialomucin and sulphomucin, respectively. Giemsa stain was used to reveal *H. pylori*. We excluded the cases without detected goblet cell metaplasia by Alcian blue stain for both Group A and Group B. *H. pylori* infection was confirmed with Giemsa stain for the cases in Group B.

Endoscopic biopsies were grouped as complete (type I) or incomplete (type II) intestinal metaplasia according to the previously defined criteria [12]. The proposed Barrett CK7/CK20 pattern and gastric CK7/CK20 pattern were evaluated for all cases. Six control biopsies were constituted retrospectively from patients whose biopsies showed normal squamo-columnar junction (SCJ) (n=3) and normal cardia (n=3) microscopically.

**Group A**

Clinical data including age, sex, gastro-esophageal reflux symptoms, and endoscopy findings, short- or long-segment Barrett mucosa were obtained for each case. The patients with BE within the last 3
cm of distal esophagus were included to the Group A. Patients with positive *H. pylori* were excluded from the study.

**Group B**

Clinical data including age, sex, gastro-esophageal reflux symptoms, endoscopy findings, and *H. pylori* status were obtained for each case. Patients with positive *H. pylori* were included to the study. Twenty endoscopic biopsies (cardia, n=9 and corpus, n=11) with histologic evidence of intestinal metaplasia were studied.

**Immunohistochemical studies**

One tissue block was selected for each case. The blocks were cut at 5µm sections. Slides were deparaffinized and rehydrated through graded alcohols. Antigen retrieval was performed by microwave for CK20. They incubated in 3% hydrogen peroxide/methanol for 20 min to block endogenous peroxidase. Using the standard streptavidin-biotin peroxidase complex (ABC) method, CK7 (clone OV-TL 12/30, Neomarkers, Fremont, CA, USA) and CK20 (clone K20.4, Neomarkers, Fremont, CA, USA) were performed on all slides. After PBS rinsing, the slides were incubated for 30 minutes in secondary antibody solution. The slides were counterstained with hematoxylin. Appropriate positive and negative controls were used to evaluate the CK7 and CK20 staining.

**Results**

In total 40 patients were included to the study. The median age of the patients was 46 (range, 28-77) years in Group A and 50 (range, 25-73) years in Group B. The male to female ratio was 4 to 1 in Group A, whereas it was 2 to 3 in Group B.

**Group A**

Reflux symptoms were found in 14 cases (70%) with BE. Three cases showed low-grade dysplasia out of total. Thirteen cases revealed incomplete intestinal metaplasia and 7 cases were complete intestinal metaplasia by using Alcian blue stain. The anticipated Barrett CK7/20 pattern was identified only 2 of the 20 cases (**Figure 3**). The reminder had variable staining patterns, which did not fit in with
the anticipated Barrett CK7/20 pattern (Figure 4). The sensitivity of CK7/20 staining for Barrett pattern was 10%.

**Group B**

Eleven cases showed complete intestinal metaplasia whereas 9 cases showed incomplete intestinal metaplasia by using Alcian blue stain. Eight cases expressed the gastric CK7/20 pattern (cardia, n=5 and corpus, n=3). Six of them showed complete and 2 incomplete gastric CK7/20 pattern. Barrett CK7/20 pattern was not observed in any patient with gastric intestinal metaplasia. The sensitivity of CK7/20 staining for Gastric pattern was 40%.

**Discussion**

Intestinal metaplasia may develop in the cardia in patients with short-segment BE or with carditis, especially infection with *H. pylori*. Distinction between the two entities is important since the etiology and risk of developing adenocarcinoma are different. BE is believed to be caused by GERD and associated with an increased risk of esophageal adenocarcinoma. Microscopically BE can be defined as replacement of the esophageal squamous epithelium by metaplastic specialized (intestinalized) columnar epithelium. Other epithelial types are junctional type and fundal type of epithelia. High iron diamine-alcian blue has been shown to be superior to H&E stained sections in determination of intestinal metaplasia. However, the related condition still remains to be defined. Couvelard *et al.* [10] suggested that cytokeratin subsets of intestinal metaplasia indicate its etiology. Because of no absolute histological criteria for diagnosing Barrett mucosa has yet been established, recent studies [8-11,14-16] mainly depend on patterns of some cytokeratin subsets especially CK7/20 pattern of Barrett esophagus and gastric intestinal metaplasia.

Cytokeratins are highly conserved polypeptides and represent a group of cytoskeletal structural proteins present in all epithelia. There are at least 20 distinct forms of cytokeratins in epithelial cells and variable patterns of expression depending on the type, location, and differentiation of the epithelium [13]. CK7, essentially, is not expressed in normal epithelium of the gastrointestinal tract whereas CK20 is expressed in intestinal epithelium, gastric foveolar epithelium, and endocrine cells in the upper portions of the pyloric glands. Ormsby *et al.* [8] identified two unique CK7/20 patterns in
cases with long-segment BE, and gastric intestinal metaplasia. According to their study, Barrett CK7/20 pattern was highly sensitive and specific when compared to cases with gastric intestinal metaplasia. *H. pylori* status of the cases was not mentioned in their study. A study by Jovanovic *et al.* [14] confirmed Ormsby's findings in 94% of their cases with long-segment BE. However, studies by some other researchers have not been able to support Ormsby's findings; the proposed Barrett CK7/20 pattern was found in 54% of patients with long-segment BE by Mohammed *et al.* [15] and only 39% of patients with long-segment BE by El-Zimaity *et al.* [16].

Ormsby *et al.* [11] assessed the utility of CK7/20 patterns in short-segment BE in another study and found that “diagnostic” Barrett CK7/20 pattern was present in 82% of patients with short-segment BE. Although Mohammed *et al.* [15] found almost the same percentage (81%) for the short-segment BE cases with Ormsby *et al.* [11], they found the same pattern in 30.7% of patients with intestinal metaplasia in cardia and more interestingly in 55% of biopsies which had either normal or inflamed gastric mucosa without intestinal metaplasia.

To suspect the diagnosis of BE requires identifying the exact level of SCJ, proximal aspect of the gastric folds, linearly oriented mucosal vessels in the distal esophagus, and EGJ [17]. SCJ normally corresponds to the proximal margin of the linear gastric folds which means EGJ. The small vessels oriented parallel to the long axis of the esophagus disappear at the normally located SCJ. However, detection of abnormal extension of these vessels below the SCJ and above the proximal margin of gastric folds is an evidence of the presence of columnar epithelium in the distal esophagus [18].

In the present study, 10% of patients with short-segment BE showed the anticipated CK7/20 pattern and 40% of patients with gastric intestinal metaplasia showed the anticipated gastric CK7/20 pattern with appropriate complete or incomplete intestinal metaplasia. Although Barrett CK7/20 pattern was not observed in any patient with gastric intestinal metaplasia in our study, this particular pattern could not reliably identify short-segment BE cases.

In the Couvelard’s study [10] 31% of patients and in the Ormsby’s study 15% of patients with short-segment BE associated with Barrett CK7/20 pattern had *H. pylori* infection. Although the percentage of
the positive *H.pylori* cases in patients with short-segment BE associated with a Barrett CK7/20 pattern was not mentioned in the Mohammed’s study [15], 6% of patients had *H.pylori* infection with short-segment BE. Since intestinal metaplasia may be seen both in *H.pylori* infection and BE, combination of these entities should not be of the interpreted for evaluating the utility of the cytokeratin subsets. We excluded the *H.pylori* positive cases even if it was diagnosed as BE. Therefore, the differences between our study and previously reported results may have been due to this strict distinction of *H.pylori* positive BE cases in the present study.

**Conclusions**

On the basis of our results, we are less confident of the use of the proposed CK7/20 for differentiating short-segment BE from gastric intestinal metaplasia. We suggest that the definition of BE should be based on the clinic, endoscopic and histological findings rather than the pattern of CK7/20 immunostaining.
**List of abbreviations**

BE: Barrett Esophagus

GERD: Gastro-Esophageal Reflux Disease

*H.pylori*: Helicobacter pylori

CK7: Cytokeratin 7

CK20: Cytokeratin 20

EGJ: Esophago-Gastric Junction

H&E: Hematoxylin and Eosin

PAS: Periodic Acid Schiff

SCJ: Squamo-Columnar Junction

ABC: Standard Streptavidin-Biotin Peroxidase Complex

**Competing interests**

None declared.

**Authors’ contributions**

OK-Y conceived of the study and drafted the manuscript. RG participated in the endoscopic procedures and the redaction of the article. EA and NT participated in the endoscopic procedures. AS participated in the histopathologic evaluations. NB performed the statistical analysis. All authors read and approved the final manuscript.
References


Figure Legends

Figure 1. Section from gastro-esophageal junction showing BE with goblet cells, H&E, X40

Figure 2. Intense blue staining of goblet cells with Alcian blue pH 2.5/PAS, X40

Figure 3. a. Diffuse moderate CK7 immunostaining of superficial and deep glands in BE, X40, b. Band-like CK20 immunostaining of surface epithelium and superficial glands in BE, X40

Figure 4. a. Absent CK7 immunostaining in BE, X100, b. Patchy CK20 immunostaining of superficial and deep glands in BE, X100