COLUMNAR METAPLASIA AND BARRETT'S ESOPHAGUS: MORPHOLOGICAL HETEROGENEITY AND IMMUNOHISTOCHEMICAL PHENOTYPE

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Barrett's esophagus (BE) is a pathologically confirmed intestinal metaplasia (CM) of the distal esophagus. BE is recognized as a potential complication of gastroesophageal reflux disease (GERD) and a premalignant condition with a high risk of neoplastic progression. The aim of this study was to compare the morphology of biopsied BE segments and CM segments extending < 1 cm and > 1 cm above the gastroesophageal junction (GEJ), as well as to perform the immunohistochemical analysis of biopsies with BE and CM > 1 cm above GEJ with or without dysplasia. The study recruited 92 patients with GERD: 42 patients with BE, 24 patients with CM > 1 cm above GEJ (C0M1.5–C13M14) and 26 patients with CM < 1 cm above GEJ (C0M0.3–0.8). Comparative analysis of tissue morphology revealed an association between the reactive changes in the epithelium and the severity of esophagitis in all groups. Reactive changes were detected significantly more often in BE segments than in CM segments > 1 cm (Mann-Whitney U, $p < 0.05$). Eight patients with BE (19.05%) were found to have low-grade dysplasia. One patient with CM > 1 cm above GEJ (4.2%) had high-grade dysplasia with cardiac-type metaplasia and immunohistochemical features of submorphological enteralization. Immunohistochemical testing for the intestinal and gastric markers of cell differentiation revealed the signs of submorphological enteralisation in all esophageal specimens with cardiac and fundic type metaplasia and in the specimens with BE in the areas lacking goblet cells.

Keywords: columnar metaplasia, Barrett's esophagus, low-grade dysplasia, high-grade dysplasia, carcinogenesis

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ЦИЛИНДРОКЛЕТОЧНАЯ МЕТАПЛАЗИЯ И ПИЩЕВОД БАРРЕТТА: МОРФОЛОГИЧЕСКАЯ НЕОДНОРОДНОСТЬ И ИММУНОГИСТОХИМИЧЕСКИЙ ФЕНОТИП

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Пищевод Барретта (ПБ), или доказанная морфологически кишечная метаплазия слизистой оболочки дистального отдела пищевода, является облигатным предраковым заболеванием, которое развивается как осложнение гастроэзофагеальной рефлюксной болезни (ГЭРБ). Цель исследования — выполнить сравнительный морфологический анализ биопсированных фрагментов ПБ, ЦМ на расстоянии < 1 см и > 1 см от гастроэзофагеального перехода (ГЭП), а также провести иммуногистохимическое исследование фрагментов с ПБ и ЦМ > 1 см от ГЭП при наличии и отсутствии дисплазии. В исследование вошли 92 пациента с ГЭРБ: 42 пациента с ПБ, 24 пациента с ЦМ > 1 см от ГЭП (C0M1,5 до C13M14) и 26 пациентов с ЦМ < 1 см от ГЭП (C0M0,3–0,8). При сравнительном морфологическом анализе наличие реактивных изменений эпителия было связано с тяжестью эзофагита во всех группах. Реактивные изменения эпителия выявляли достоверно чаще при ПБ по сравнению с ЦМ > 1 см от ГЭП ($p < 0,05$ при использовании критерия Манна–Уитни). При ПБ в восьми наблюдениях (19,05%) выявлена low-grade дисплазия. В одном наблюдении ЦМ > 1 см от ГЭП (4,2%) выявлена high-grade дисплазия на фоне кардиальной метаплазии с иммуногистохимическими признаками субморфологической энтерализации. При иммуногистохимическом исследовании с маркерами желудочной и кишечной дифференцировки признаки субморфологической энтерализации выявлены во всех фрагментах пищевода с кардиальной метаплазией и у пациентов с ПБ в зонах с отсутствием бокаловидных клеток.

Ключевые слова: цилиндроклеточная метаплазия пищевода, пищевод Барретта, low-grade и high-grade дисплазия, канцерогенез

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Соблюдение этических стандартов: все пациенты подписали добровольное информированное согласие.

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Barrett's esophagus (BE) refers to intestinal metaplasia (CM) of the distal esophagus confirmed by endoscopy and histological examination. BE is recognized as a potential complication of gastroesophageal reflux disease (GERD) and a premalignant condition with a high risk of neoplastic progression. At present, there is no universally accepted definition of BE because the affected tissue is very heterogeneous and can present as a mosaic of various metaplastic phenotypes within one biopsied fragment of the distal esophageal mucosa. The American Gastroenterological Association maintains that BE should be diagnosed only in the presence of intestinal metaplasia in the biopsies collected from the sites of the columnar-lined esophagus [1]. The British Society of Gastroenterology (BSG) defines BE as any type of columnar metaplasia (CM) extending ≥1 cm above the gastroesophageal junction (GEJ) [2]. The International Consensus BOB CAT group (2015) recognizes BE as any type of CM of the distal esophagus but emphasizes that the type of metaplasia should be clearly specified in the pathology report [3].

Embraced by many clinicians, the definition formulated by BSG has become fairly popular. But of course, it did not make the underlying biology of the disease any different. Therefore, for the sake of diagnostic accuracy, it is essential to meticulously compare endoscopy and pathology findings, especially for biopsies collected <1 cm above GEJ [4].

In the distal esophagus, 3 types of metaplasia can be distinguished, including cardiac, fundic and intestinal. The first is often seen in short-segment BE and is the earliest metaplastic transformation of the esophagus in patients with GERD. In cardiac-type metaplasia, squamous epithelium is replaced with foveolar cells. Fundic-type metaplasia is characterized by the presence of chief and parietal cells as normally in the gastric corpus mucosa. Intestinal metaplasia usually presents as a patchwork of goblet and foveolar cells and sometimes Paneth cells [5].

Biopsies of one and the same patient can contain different types of columnar-lined epithelium. As a rule, intestinal metaplasia affects the proximal side of columnar-lined esophagus, whereas cardiac and fundic types usually occur more distally, close to GEJ. Intestinal metaplasia is reported to be twice as prevalent in the biopsies from the proximal side of columnar-lined esophagus than in the fragments close to GEJ [6].

Today, the pathogenesis of esophageal CM is linked to the transdifferentiation of the stratified squamous epithelium or to the transcommitment of progenitor cells involved in the repair of reflux-induced damage [7].

The prevalence of goblet cells is associated with the pH gradient along the BE segment: the lower the pH value (i.e. the closer to GEJ), the fewer and more scattered the goblet cells. This phenomenon might be tied to the solubility gradient of bile acids determined by pH levels: at more acidic pH (closer to GEJ) the solubility of bile acids is the lowest; at neutral pH typical of the proximal BE, the solubility of bile acids increases [8]. A study conducted in mice has shown the role of bile acids in the pathogenesis of BE with intestinal metaplasia and CDX2/MUC2 expression [9].

Another study has demonstrated that cardiac CM can undergo early enteralization, i.e. retain morphological features of cardiac-type cells but start expressing markers of intestinal differentiation, such as Villin and CDX2 [10].

There is ongoing debate about whether goblet cells play an exclusive role in carcinogenesis in the distal esophagus or other CM types can also contribute to dysplasia and development of esophageal adenocarcinoma. In one of epidemiological studies, the incidence of progression to malignancy in patients with intestinal metaplasia was higher (0.38% per year) than in patients without this condition (0.07% per year) [11]. However, this trend was not corroborated by another long-term observation (8–20 years) [12]. In over 70% of patients with small (<2 cm) esophageal adenocarcinomas, cancer was preceded by columnar metaplasia of the esophagus, but the metaplastic transformation of mucosa adjacent to the tumor was not intestinal but cardiac [13]. These findings were confirmed by other researchers [14] who reported expression of gastric markers (MUC5A and MUC6) in minute Barrett's tumors (< 5 mm). Non-goblet cardiac-type CM epithelium without CDX2 expression can also undergo malignant transformation [15]. At the same time, high density of goblet cells can reduce the risk of adenocarcinoma [16, 17]. Perhaps, there are two independent carcinogenesis pathways in the distal esophagus: foveolar and intestinal, i.e. involving both gastric-type and specialized intestinal metaplasia [18–20]. The implicated type of carcinogenesis can be identified by immunohistochemistry from the expression of gastric (MUC1, MUC5A, MUC6) and intestinal (MUC2, CD10, CDX2, Villin, etc.) markers.

According to the definition proposed by BSG, BE should be diagnosed if the metaplastic epithelium extends ≥1 cm above GEJ. Although this criterion is increasingly applied, it is still arbitrary. So far, research into the contribution of ultra-short BE (<1 cm) to esophageal carcinogenesis has produced conflicting evidence.

The aim of this study was to compare the morphology of columnar-lined esophagus segments extending < 1 cm above GEJ (ultra-short BE), classic goblet BE and CM extending > 1 cm above GEJ, to identify the relative prevalence of different metaplasia types, the frequency of reactive changes in the epithelium (indefinite for metaplasia) and dysplasia of the metaplastic esophageal epithelium, as well as to carry out the immunohistochemical analysis of BE specimens with different types of metaplastic transformations with or without dysplasia.

METHODS

The study was carried out at the City Clinical Hospital № 31 between January 1, 2018 and September 1, 2019. The study recruited patients with GERD who were undergoing medical examination at the Hospital at that time. The following inclusion criterion was applied: the presence of columnar-lined esophageal segments of any length detected on endoscopy and subsequently confirmed by histological examination. Mucosal biopsies were taken from the distal esophagus of 92 patients with GERD during upper endoscopy (EGD) and then examined by the pathologists. Of all the examined patients, 42 had BE, 24 had CM extending > 1 cm above GEJ (C0M1.5 to C13M14) and 26 patients had endoscopic features of CM < 1 cm above GEJ (C0M0.3–0.8). Exclusion criteria were as follows: the absence of columnar metaplasia in the biopsy histological examination; the absence of esophageal mucosa components in the biopsied specimens (stratified squamous epithelium, glands of the esophageal lamina propria and their ducts), especially in ultra-short BE, which prevented us from drawing a firm conclusion that the biopsies had been collected from the esophagus but not from the stomach. In the group of patients with CM < 1 cm above GEJ, the mean age was 55.50 ± 1.10 years (range 22–82 years); of them 11 were men (mean age 50.09 ± 18.03) and 15 women (mean age 59.47 ± 14.57); the male to female ratio was 1 : 1.36. The group of patients with CM segment length > 1 cm consisted of 24 individuals aged 19–94 years (mean age 52.21 ± 18.00); of them 7 were male (mean age 47 ± 20.05) and 17 were female (mean age 53.5 ± 17.47); the male to

female ratio was 1 : 2.4. The group of patients with BE was represented by 42 patients aged 19–93 years (mean age 61.80 ± 16.33); of them 29 were men (mean age 54.47 ± 21.79) and 13 women (69.23 ± 13.57); the male to female ratio was 2.23 : 1. The patients with CM length < 1 cm and > 1 cm tended to be younger than those with BE, but the difference between the groups was insignificant. In the group with CM < 1 cm and CM > 1 cm above GEJ, as well as in the BE group, the mean age of male participants was lower than that of women, because the male sex is one of the risk factors for GERD.

Pre-existing conditions predisposing our patients to the CM of the distal esophagus and BE included sliding hiatal hernia and cardiac sphincter incompetence. Endoscopy was suggestive of sliding hiatal hernia in 14 of 26 (53.8%) patients who had endoscopic evidence of CM < 1 cm; another 3 (11.5%) patients with CM < 1 cm had signs of cardiac sphincter incompetence in the absence of hiatal hernia. In the group with CM extending > 1 cm above GEJ, hiatal hernia was detected by EGD in 11 (45.83%) patients and cardiac sphincter incompetence, in 3 (12.5%) individuals. Of all study participants with BE, endoscopy was suggestive of hiatal hernia in 18 (42.86%) patients and of cardiac sphincter incompetence, in 6 (14.29%) participants.

Biopsied esophageal specimens were processed following the standard protocol. The slides were stained with hematoxylin-eosin and Schiff reagent-Alcian blue. The latter helps to identify goblet cells and discriminate between goblet and dystrophic pseudogoblet cells. Pseudogoblet cells were detected in 88.04% of all examined specimens.

Biopsies with intestinal metaplasia were evaluated for the density of goblet cells in the glands. If goblet cells made less than 5% of all epithelial cells in the glands, the sample was categorized as containing few goblet cells; if these cells amounted to 5-50% of all epithelial cells, the sample was categorized as containing low-density goblet cells; if the proportion of goblet cells exceeded 50%, the sample was regarded as containing high-density goblet cells.

All biopsies collected from the patients with CM < 1 cm were analyzed for the presence of esophageal mucosa components, including stratified squamous epithelium (20 patients, 76.92%), esophageal glands (19 patients, 73.07%) and their excretory ducts (5 patients, 19.23%). This helped us to determine whether the studied specimens had been collected from the distal esophagus vs the stomach. The fragments that lacked the aforementioned components of the esophageal mucosa were excluded from the final analysis.

In 24 cases of BE and CM > 1 cm above GEJ, immunohistochemistry was performed using the following antibodies: anti-MUC1 (1 : 100, Ventana; Roche), anti-MUC2 (1 : 125, Ventana; Roche), anti-MUC5A (1 : 250, Ventana; Roche), anti-MUC6 (ready to use, Ventana; Roche), anti-CDX2 (1 : 125, Ventana; Roche), and anti-Villin (ready to use, Leica).

RESULTS

Three metaplasia types were identified in the patients with CM < 1 cm above GEJ: cardiac, fundic and intestinal. In the absence of other metaplastic changes, cardiac-type metaplasia was present in 7 (26.92%) patients and fundic-type, in 4 patients (15.38%) in the group with CM < 1 cm (Fig.1). Intestinal metaplasia with different relative goblet cell density was observed in 15 patients (57.69%) (Fig. 2). In 5 patients (33.33%), goblet cells were few. Low-density goblet cells (10 to 49%) were observed in 8 patients (53.33%); high-density goblet cells (50 and 70%), in 2 patients (13.33%). Biopsies of 6 patients (23.07%) contained a mosaic of 3 metaplasia types. Esophagitis was a pre-existing condition in all study participants. Inflammatory cell infiltration was moderate in 69.23% of specimens and pronounced in 30.77% of cases. Erosions of esophageal mucosa were observed in 8 patients (30.77%); ulceration was detected in 1 patient. Biopsies of 9 patients (34.62%) were described as indefinite for dysplasia (reactive changes of the epithelium characterized by the irregular arrangement and crowding of the glands, their angulated shape, slightly enlarged and hyperchromic nuclei, and single mitotic cells). In 2 specimens, reactive changes of the epithelium were observed in the presence of cardiac-type metaplasia; in 6 cases, in the presence of intestinal metaplasia with low density of goblet cells; in 1 case, in the presence of intestinal metaplasia with high density of goblet cells. Low-grade or high-grade dysplasia was not detected in any of the esophageal specimens with CM < 1 cm.

In the group with CM > 1 cm above GEJ, cardiac-type metaplasia was detected in 14 of 24 patients (58.33%), whereas fundic, in 10 individuals (41.67%). The group of patients with BE included 42 individuals with intestinal metaplasia. If we merge these two groups into one, the ratio of metaplasia types will be as follows (Fig. 3): cardiac type, 14 of 66 patients (21.21%), fundic type, 10 (15.15%), intestinal type, 42 (63.64%). The mosaic of all 3 types was observed in 8 patients with BE (12.12%). In the BE group (42 patients with intestinal metaplasia; Fig. 4), few goblet cells were detected in 8 participants (18.18%); low-density goblet cells, in 15 individuals (43.09%); high-density goblet cells, in 21 patients (47.73%).

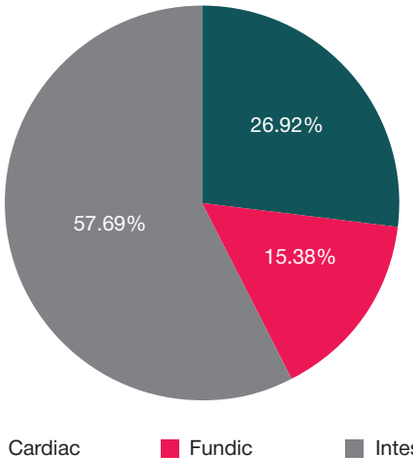

Fig. 1. Prevalence of different metaplasia types in CM segments extending < 1 cm above GEJ

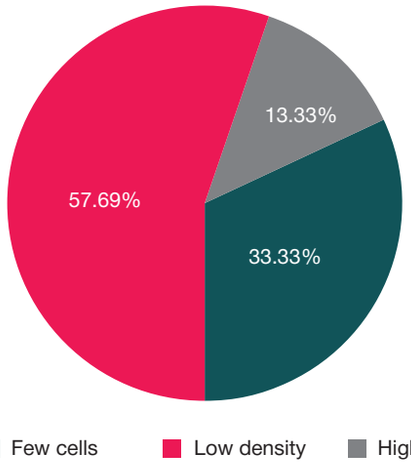

Fig. 2. The relative number of goblet cells in CM segments extending < 1 cm above GEJ

Thus, intestinal metaplasia was more prevalent in the biopsies of patients with CM > 1 cm above GEJ and BE than in the group with CM < 1 cm above GEJ (63.64 and 57.69% respectively; Fig. 5). High-density goblet cells occurred in the patients with BE 3.5 times more often than in those with CM < 1 cm (Fig. 6).

Signs of esophagitis with moderate inflammatory lymphocyte/plasma cell infiltration were detected in 16 patients with CM > 1 cm above GEJ (66.67%), whereas signs of pronounced inflammation, in 8 patients (33.33%). Erosions of the metaplastic mucosa were present in 11 patients (45.83%); of them 1 patient had areas of ulceration. Reactive changes of the epithelium were observed in 5 patients with columnar metaplasia (20.83%); of them 3 had cardiac type and 2, fundic type. One patient was found to have high-grade dysplasia of the metaplastic segment in the presence of cardiac-type metaplasia (4.2%).

Signs of esophagitis with moderate inflammatory lymphocyte/plasma cell infiltration were detected in 18 patients with BE (42.86%), whereas signs of pronounced inflammation, in 26 patients (57.14%); 27 patients had erosions of metaplastic esophageal segments (64.29%), of them 4 individuals had areas of ulceration. Erosions were detected 2.1 times more often in the patients with BE than in those with CM < 1 cm above GEJ and 1.4 times more often than in the patients with CM > 1 cm above GEJ. Reactive changes of the epithelium were observed in 15 patients (35.71%). Thus, reactive changes of the epithelium occurred with the same frequency in the patients with BE and CM < 1 cm above GEJ and were 1.7 more rare in the patients with CM > 1 cm without intestinal metaplasia. Reactive changes of the epithelium were significantly more frequent in the patients with BE than in those with CM > 1 cm above GEJ (Mann–Whitney U test; $p < 0.05$). Such changes were equally prevalent in the samples differing in the density of goblet cells (few goblet cells, low density of goblet cells and high density of goblet). In all cases, the changes were associated with the severity of inflammation (Mann–Whitney U, $p < 0.05$).

Low- or high-grade dysplasia was absent in the patients with CM < 1 cm. Low-grade dysplasia (Fig. 7A) was diagnosed in 8 patients with BE (19.05%); of them 6 patients had high-density goblet cells and 2, single goblet cells. One patient with CM > 1 cm was found to have high-grade dysplasia (4.2%; Fig. 7B).

Eight specimens of CM > 1 cm and 16 specimens of BE were tested for the markers of intestinal (MUC2, CDX2, Villin) and gastric (MUC1, MUC5A, MUC6) differentiation using immunohistochemistry assays. Samples with cardiac and fundic-type metaplasia were characterized by strong diffuse expression of MUC5A in the cytoplasm of the surface epithelial layer, weak diffuse expression of MUC1 in the cytoplasm of the surface epithelial layer, diffuse expression of MUC1, MUC5A and MUC6 in the cytoplasm of glandular epithelium. MUC2 was not expressed in the samples with cardiac or fundic-type metaplasia. CDX2 was expressed in 5 patients with cardiac and fundic-type metaplasia (in up to 30% of the cells in the biopsied fragment); Villin expression was noted in 8 cases (in 15–20 to 80% of glandular epithelial cells). Expression of intestinal markers (CDX2 and Villin) in the samples with cardiac and fundic-type metaplasia is a sign of submorphological enteralization. In our study, intestinal markers (MUC2, CDX2, Villin) were expressed in all 16 patients with BE and intestinal metaplasia. Their expression depended on the density of goblet cells: cytoplasmic expression of MUC2 was observed in 10 to 50% of the cells; nuclear expression of CDX2 was discovered in 10 to 90% of epithelial cells, including cells of the columnar glandular epithelium with the cardiac phenotype; cytoplasmic expression of Villin was detected in 70–100% of epithelial cells. Because goblet cells in the metaplastic BE epithelium are interspersed with foveolar cells, intestinal metaplasia is also characterized by the expression of gastric markers: MUC1, MUC5A and MUC6, which seems to be weaker than in cardiac-type metaplasia and not so spread out.

The immunohistochemical analysis of BE fragments with low-grade dysplasia revealed pronounced expression of MUC2, CDX2 and Villlin and weak expression of MUC1, MUC5A and MUC6. MUC1, MUC5A, MUC6, and Villin were also expressed in one sample with high-grade dysplasia and cardiac-type metaplasia negative for MUC2 and CDX2 expression.

DISCUSSION

This study compares morphological phenotypes of columnar-lined epithelium segments extending < 1 cm above GEJ, > 1 cm above GEJ, and BE segments. Interestingly, our groups of patients with CM > 1 cm and BE were almost mirror-symmetrical in terms of male to female ratio: for the patients with CM > 1 cm, the male to female ratio was 1 : 2.4, whereas for the patients with BE, it was 2.23 : 1. In all groups, the mean age of male participants was a bit lower than the mean age of women because the male sex is one of the risk factors for GERD. It is believed that cardiac-type metaplasia is the earliest type of CM in patients with GERD. In our study, in the

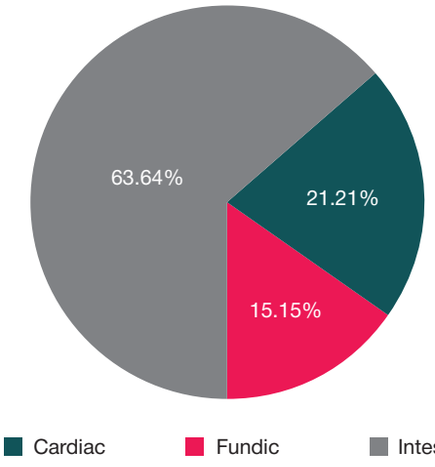

Fig. 3. Prevalence of different types of columnar metaplasia in different parts of the biopsied segment > 1 cm above GEJ, including BE

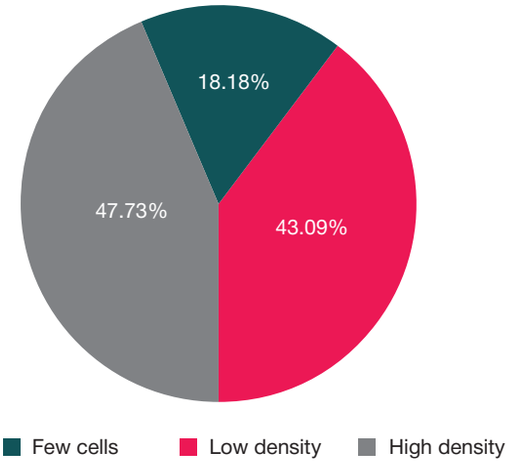

Fig. 4. The relative number of goblet cells in BE segments.

absence of other metaplastic changes cardiac-type metaplasia was detected more frequently in the patients with CM < 1 cm (26.92%) than in C0M1.5–C13M14 biopsies (21.21%). Goblet cells occurred more frequently in the metaplastic segments > 1 cm in length; high-density goblet cells were seen 3.5 times more often in the patients with CM < 1 cm than in those with CM < 1 cm. According to the literature, the frequency of goblet cell occurrence increases with the length of the metaplastic esophageal segment [6]. In our next study, we will be attempting to establish a correlation between the length of the BE segment, the frequency of goblet cell occurrence and their density.

Reactive changes of the epithelium were observed significantly more often in the group of patients with BE vs the group with CM > 1 cm above GEJ and were associated with the severity of inflammation (Mann-Whitney U, p < 0.05). In the patients with BE, dysplasia was detected more often (19.05%) than in the patients with CM > 1 cm (4.2%). Biopsies characterized by low-grade dysplasia in the presence of BE showed pronounced expression of intestinal (MUC2, CDX2, Villin) and weaker expression of gastric (MUC1, MUC5A, MUC6) markers, which is consistent with the mixed metaplastic phenotype dominated by intestinal metaplasia [13, 14, 18–20]. The only case of high-grade dysplasia with CM > 1 cm was characterized by bright expression of MUC1, MUC5A, MUC6, and Villin and was negative for MUC2 and CDX2 expression, suggesting the cardiac phenotype. Thus, we observed both intestinal (BE) and foveolar (CM >1 cm above GEJ) carcinogenesis pathways, but the intestinal one prevailed in our sample, in spite of the miniature size of the dysplastic focus (≥ 2 mm).

It is hypothesized that high density of goblet cells can be protective against dysplasia [16, 17]. In our sample, low-grade dysplasia was detected in 6 of 8 biopsies (75%) characterized by high density of goblet cells in the adjacent esophageal mucosa. This can be explained by a small number of low-grade dysplasia cases in our patient sample. Further research is needed to provide a more objective morphological profile for patients with BE in the early stage of dysplasia and to identify the group at risk for this condition.

CONCLUSIONS

The accuracy of ultra-short BE diagnosis largely depends on precise targeting during biopsy sampling. This diagnosis can be established only if the biopsied specimen contains esophageal components (stratified squamous cell epithelium, excretory ducts and glands of the mucosa) and shows distinct metaplastic changes in the esophageal epithelium. Our comparative analysis of biopsy samples revealed that reactive changes in the epithelium were more frequent in the BE segments than in the specimens with CM > 1 cm above GEJ (Mann-Whitney U, $p < 0.05$); in the group with CM < 1 cm, these

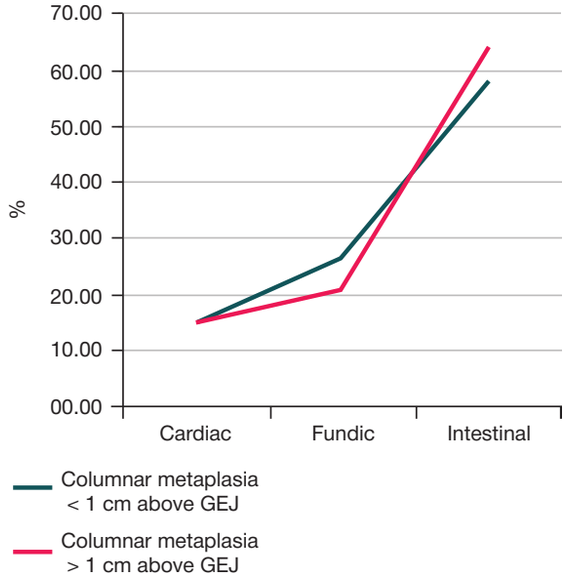

Fig. 5. The relative number of different metaplasia types in CM segments < 1 cm and > 1 cm above GEJ

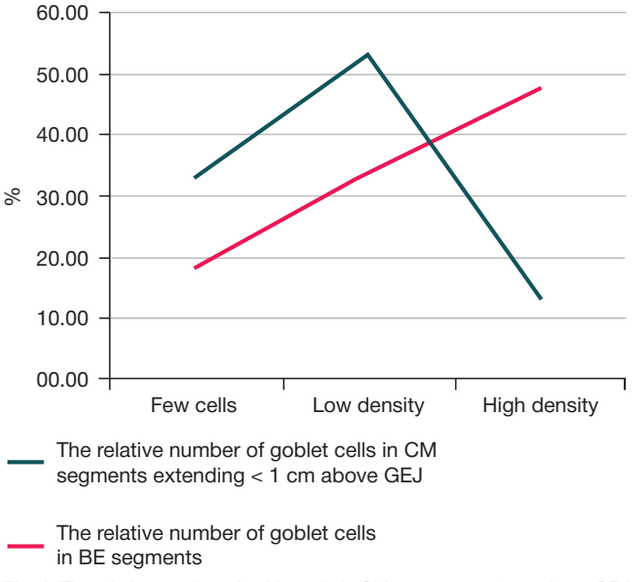

Fig. 6. The relative number of goblet cells in CM segments < 1 cm above GEJ and in BE segments

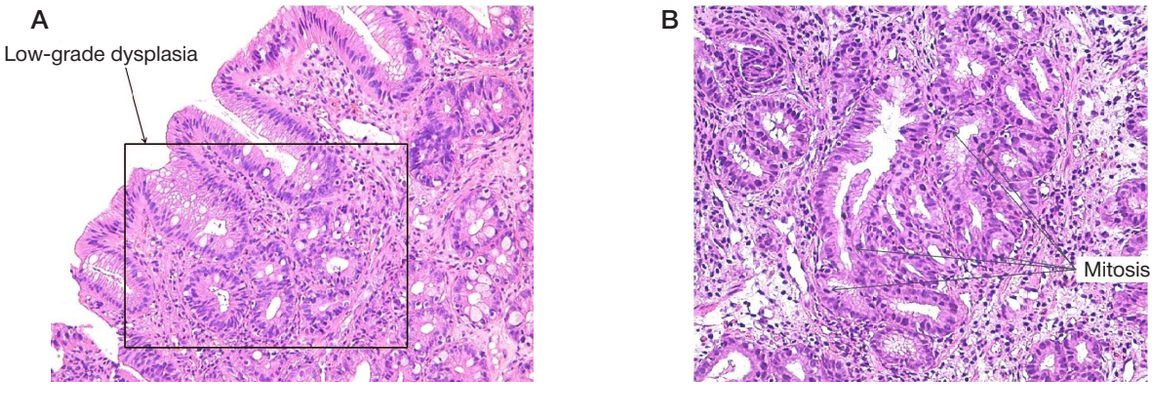

Fig. 7. A. Low-grade esophageal dysplasia in patients with BE. **B**. High-grade esophageal dysplasia in patients with CM > 1 cm above GEJ. Staining: hematoxylin-eosin; magnification ×200

changes were observed more often in the presence of goblet cells. Reactive changes in the epithelium were associated with the severity of esophagitis. Genuine dysplasia was detected in 8 patients with BE (low-grade dysplasia, 19.05%) and in 1 patient with CM > 1 cm above GEJ (high-grade dysplasia, 4.2%). Immunohistochemical testing for the intestinal and gastric markers of cell differentiation revealed the signs of submorphological enteralization in all esophageal specimens with cardiac and fundic type metaplasia and in the specimens with BE in the areas lacking goblet cells.

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