PŮVODNÍ PRÁCE

Histopathologic Changes in Gastroesophageal Reflux Disease. A Study of 126 Bioptic and Autoptic Cases

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Summary

The histologic diagnosis of reflux esophagitis is still complicated by the lack of a consensus opinion on what is the normal mucosa in the area of the gastroesophageal junction (GEJ). Most authors consider GEJ as the junction between the squamous and the cardiac epithelium. The cardiac mucosa is composed of mucinous or mixed mucinous-oxyntic glands. These glands are in fact indistinguishable from metaplastic mucosa that arises in the distal esophagus in consequence of gastroesophageal reflux (GER). The cardiac mucosa shows invariably chronic inflammatory changes referred to as "carditis". The cause of "carditis" is GER and/or *Helicobacter pylori* (HP) infection.
In our series of 120 endoscopic biopsies of the GEJ and distal esophagus the cardia type mucosa (CM) was always present. In 15 cases, it was accompanied by oxyntocardiac mucosa. Both mucosa types showed chronic inflammation that is after exclusion of HP infection regarded as a strong diagnostic sign of the gastroesophageal reflux disease (GERD). In two cases with clinical symptoms of GERD, a few HP were found on the CM. Therefore we diagnosed them as GERD with secondary HP infection. In 17 cases, CM displayed intestinal metaplasia (IM) predominantly of incomplete type and no dysplasia. This IM expressed MUC6 in the glandular zone of the mucosa like it did in the neighboring glands, whereas in the surface and foveolar epithelium the MUC6 was negative or only slightly and focally positive. On the other hand, IM in the surface and foveolar epithelium was reactive for MUC5AC. The positivity and distribution of CK7 and CK20 was very similar in the Barrett's mucosa, cardiac mucosa and antral mucosa.
In one specimen of esophagus resected for adenocarcinoma, CM with incomplete IM was found in the vicinity of the tumor. Squamous metaplastic epithelium was often seen near the orifices of submucosal esophageal glands in these areas, indicating the metaplastic nature of the glandular mucosa in the distal esophagus. In the GEJ of 5 autopsy cases of children with spastic quadriplegia (age range 7-10 years) CM in a short segment (0.5-3 mm in length), probably of metaplastic origin was identified, showing chronic inactive inflammation.

Key words: gastroesophageal junction – gastroesophageal reflux disease – gastric cardia – carditis – metaplasia of the esophagus – intestinal metaplasia

Souhrn

Histopatologické změny u gastroezofageální refluxní nemoci. Studie souboru 126 bioptických a autoptických případů

Histologickou diagnózu refluxní ezofagitidy (RE) komplikuje nejednotný názor na typy sliznic v oblasti gastroezofageální junkce (GEJ). V současné době převládá názor, že v oblasti GEJ přechází dlaždicový epitel jícnu do žlazové sliznice kardie žaludku. Sliznice kardie je tvořena hlenovými nebo smíšenými hlenovými/oxyntickými žlázkami, které jsou neodlišitelné od metaplastické sliznice, vznikající v distálním jícnu při gastroezofageálním refluxu (GER). Ve žlazové sliznici se pravidelně nacházejí chronické zánětlivé změny označované jako „carditis". Jejich příčinou je GER a/nebo infekce *Helicobacter pylori* (Hp). V naší sestavě 120 biopsií z oblasti GEJ a distálního jícnu byla ve všech zachycena sliznice typu kardie (CM), v 15 případech současně s oxyntokardiální sliznicí. V obou typech sliznic byl chronický neaktivní zánět, který se po vyloučení infekce Hp všeobecně považuje za diagnostický pro RE. U dvou nemocných se symptomatologií GER byla na povrchu CM řídká kolonizace Hp. Nález jsme hodnotili jako RE se superponovanou infekcí Hp. V 17 případech byla v CM intestinální metaplazie (IM) převážně nekompletního typu bez dysplazie. Intestinální metaplastický epitel exprimoval ve žlázové zoně – podobně jako okolní žlazky – mucin MUC6, zatímco v povrchovém a foveolárním epitelu byl MUC6

negativní nebo vykazoval jen slabou ložiskovou pozitivitu. V povrchovém a foveolárním epitelu byl intestinální epitel naopak pozitivní v barvení MUC5AC. Imunohistochemické vyšetření prokázalo v CM, Barrettově jícnu a v antrální sliznici shodnou lokalizaci cytokeratinů CK7/CK20. V resekovaném jícnu pro adenokarcinom byla v okolí nádoru CM s nekompletní IM. Pod sliznicí se nacházely submukózní žlazky jícnu a v okolí jejich ústí překrývaly žlazovou sliznici ostrůvky dlaždicového epitelu. Přítomnost submukózních žlazek svědčí pro žlázovou metaplazii sliznice v distálním jícnu. V nekroptických jícnech 5 dětí ve věku 7–10 let se spastickou kvadruplegií byla u všech v GEJ zánětlivě změněná CM v délce 0,5–3mm, pravděpodobně metaplastického původu.

Klíčová slova: gastroezofageální junkce – refluxní ezofagitida – kardie žaludku – carditis – metaplazie sliznice jícnu – intestinální metaplazie

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The histologic features of the gastroesophageal junction (GEJ) are still controversial. In "normal" individuals the GEJ corresponds histologically to the squamocolumnar junction or Z line, i. e. the transition between the esophageal squamous epithelium and the gastric cardia. The length of the cardiac mucosa with pure mucous glands or with a mixture of mucous/oxyntic glands is variable, ranging from 1.0 to 4.0 mm (8, 12-14, 23). In addition, the type and length of the epithelium within the true gastric cardia may vary in different portions of the circumference of the cardia within individual patients (12, 20, 22, 23, 27). The presence of the true gastric cardia (mean length of 1.0 mm) was also documented both in autopsy (8, 20) and biopsy specimens of pediatric patients (12). Until recently, it was believed that the distal 1- 2 cm of esophagus is normally lined by mucinous columnar epithelium similar in appearance to the cardiac mucosa serving from the functional point of view as a "buffer zone" between the esophagus and the stomach (12, 13, 27). However, recent data strongly suggest that mucinous columnar epithelium above the anatomic GEJ is abnormal, namely, metaplastic in origin (1-3, 6, 22, 27). As it is difficult to distinguish the true gastric cardia from the metaplastic columnar epithelium in the distal esophagus, there are problems in the diagnosis of gastroesophageal reflux disease (GERD) and Barrett's esophagus (BE).

In this study, we present the features of GEJ in a series of 120 endoscopic biopsies from GEJ, one resectate of the esophagus, and 5 autopsy specimens of the distal esophagus of children.

Materials and Methods

We examined 120 endoscopic biopsies from the GEJ and distal esophagus. Two to 4 specimens were obtained in every case. 86 patients were males and 34 were females (age range 43-57 years, average 52 years) with clinical symptoms of GERD (102 patients, i.e. 85%) and/or hiatal hernia (18 patients, i.e. 15%). In addition, we examine one resectate of the esophagus with adenocarcinoma in a 58-year-old male, and 5 autoptic specimens of the esophagus from 7- to 10-year-old boys with serious perinatal hypoxic-anoxic injury of the brain resulting in spastic quadriplegia.

In the esophageal resectate and in all autoptic specimens, the whole circumference of the distal part of the esophagus in the length of 2 cm together with the adjacent gastric wall was cut longitudinally and processed in 5 to 6 blocks. All specimens were fixed in 10% formalin and embedded in paraffin. The sections were stained with HE, PAS, Alcian blue (pH 2.5) and silver-impregnated according to Warthin-Starry. Ten bioptic specimens of cardia type mucosa (CM), 5 samples of BE and 10 specimens of antral mucosa were studied immunohistochemically using antibodies to the following antigens: MUC 5AC (CLH2, MW, 1:400, Novocastra), MUC6 (CLH5, MW, 1:400, Novocastra), CK7 (OV-TL12/30, MW, 1:200, DakoCytomation), CK 20 (KS20.8, MW,1:100, DakoCytomation). The primary antibodies were visualised using streptavidin-biotin-peroxidase complex (DakoCytomation).

Results

In all biopsy specimens, both squamous cell and glandular mucosa were seen. These mucosa types were found either apart in isolated fragments, or together in a single tissue fragment in which a transition between the mucosa types could be identified. The squamous epithelium of the esophagus showed non-specific reactive changes (basal cell hyperplasia and an extension of the connective tissue papillae of the lamina propria more than two-thirds of the distance to the surface) and a mild lymphoplasmacytic infiltration in the lamina propria (less than 12 lymphocytes/plasmacytes per HPF). The glandular mucosa contained mucous glands consistent with CM. In 15 cases, CM was accompanied by oxyntocardiac mucosa in which typical parietal cells were found within otherwise mucous glands. In all cases, a chronic inactive inflammation was seen in both

of these mucosa types, i.e. there was no case without inflammation. In addition, all CM specimens showed foveolar hyperplasia.

Helicobacter pylori (HP) was negative in all but two specimens. In these 2 cases, the colonization of CM was mild and HP positive antral gastritis was diagnosed. In 17 cases of BE, IM predominantly of incomplete type without dysplasia was found in the superficial, foveolar and, to a lesser extent, in the glandular epithelium (**figure 1**). In the esophageal resectate, tubular adenocarcinoma infiltrated as deep as into the muscularis propria. In the mucosa adjacent to the tumor, there was CM with mild chronic inflammation and advanced incomplete IM in the foveolae and glands. The submucosa below contained mucinous and mixed seromucinous glands (**figure 2**). The glandular ducts were lined with a double layered cuboidal epithelium ending on the mucosal surface. Several islands of squamous epithelium were found in the superficial epithelium adjacent to the orifices of these submucosal glands. This squamous epithelium overlaid CM glands with IM (**figure 3**).

In 5 autoptic pediatric cases, CM was seen in a 0.5-3 mm-long segment between the squamous esophageal epithelium and the oxyntic gastric mucosa in the entire circumference, always accompanied by chronic inactive inflammation (**figure 4**). No esophageal submucosal glands were found in these short areas of CM. The oxyntic mucosa adjacent to CM lacked any inflammation.

The immunohistochemical stains for MUC5AC and MUC6 showed a similar positivity pattern in all specimens of CM, BE and antral mucosa. MUC5AC was positive in the foveolar epithelium and focally in the glands. MUC6 reacted in the glands of all mucosa types. It was also positive in incomplete IM in the glands but was negative or at most slightly and focally positive in the surface and foveolar epithelium. On the other hand, in the surface and foveolar epithelium IM expressed MUC5AC (**figure 5**).

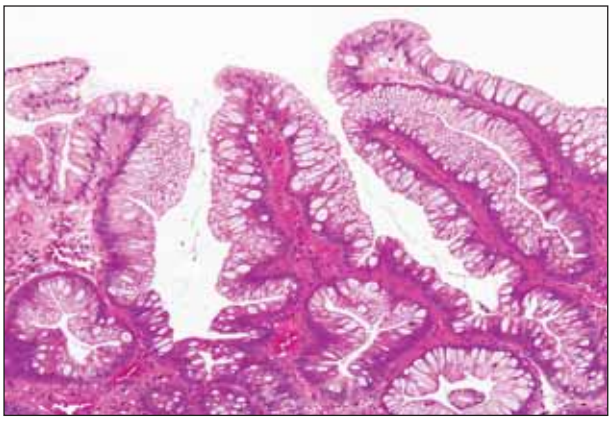

Fig. 1. Incomplete IM of the superficial and foveolar epithelium is seen in the glandular cardia type mucosa. HE, x500

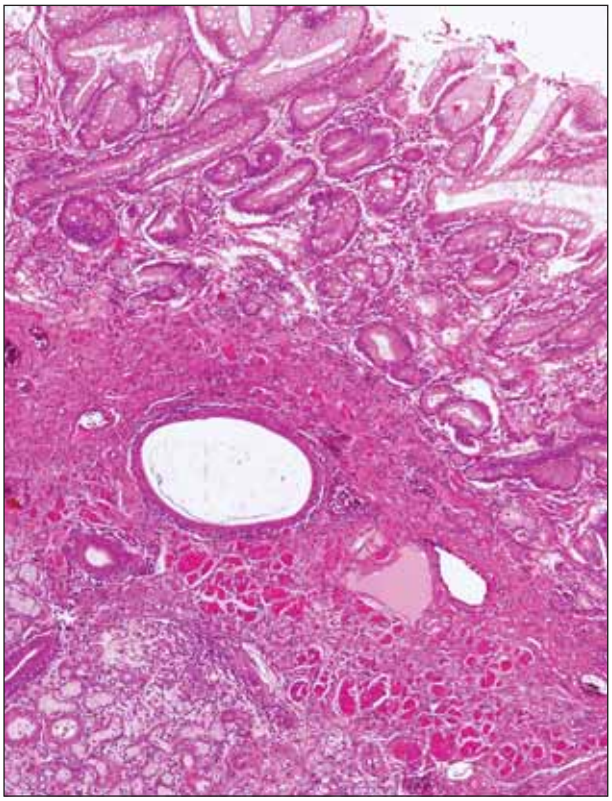

Fig. 2. A finding in the esophageal resectate. Cardia type mucosa with incomplete IM and mixed seromucinous gland (lower left) are shown. HE, x250

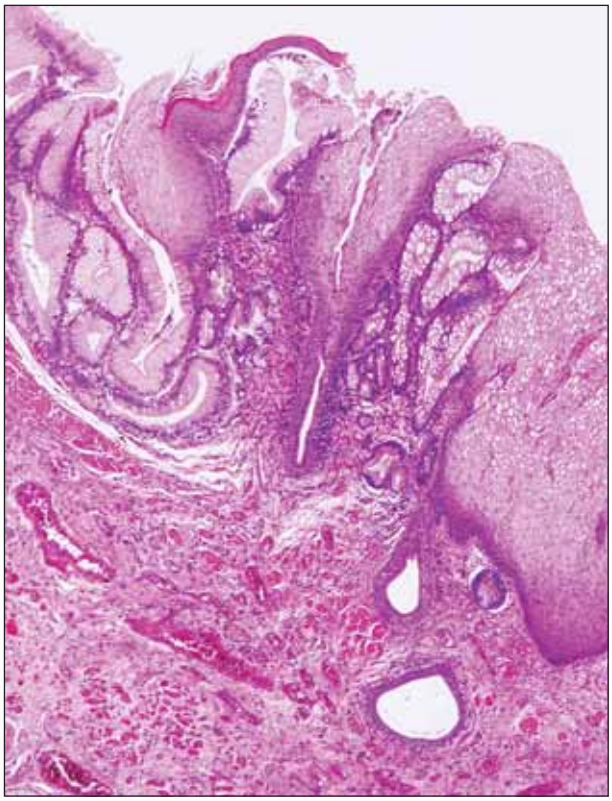

Fig. 3. Squamous metaplasia in the superficial epithelium near the orifices of submucosal esophageal glands in the resected esophagus. HE, x250

CK7 and CK20 were positive in all mucosa types in the foveolae, and focally also in the epithelium of the glands.

Discussion

There are abundant histologic and histochemical data to support the view that columnar epithelium proximal to the anatomic GEJ represents metaplastic epithelium developed as a result of GERD (2, 12, 16, 22, 24, 27). This aquired metaplastic esophageal portion of the columnar mucosa is identical to the physiologic gastric cardia (11-13, 24, 27). As it is not possible to distinguish between these two types of glandular mucosa, there are problems in the evaluation of biopsies from the GEJ area. All biopsies with CM show similar histologic abnormalities indicative of inflammation (3, 8, 12, 13) and reactive changes that include foveolar hyperplasia and smooth muscle proliferation in the lamina propria (3). This condition referred to as "carditis" is considered a result of either GERD or HP infection, or both (6, 7, 12, 13, 25, 32). For this reason, HP negative chronic inflammation in CM indicates GERD in the surgical pathology practice, not regarding the origin of this CM. However, none of these studies explains the cause of the chronic inflammation of the cardia; if CM was a normal part of the gastric mucosa, it should not be damaged by reflux. In addition, the oxyntic mucosa immediately adjacent to CM shows no inflammation (like in our autoptic cases) (5-7). In contrast to previous studies presuming the existence of the gastric cardia, Chandrasoma et al. detected pure mucous glands or mixed mucous/oxyntic glands in the anatomic GEJ in only 44% of cases in a study of 90 adult autopsies (1). Their and other studies documented that presence and extent of pure mucous glands increased progressively with higher patient age and often showed

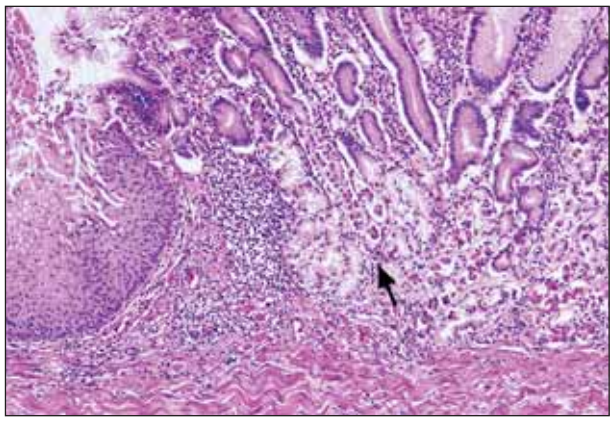

Fig. 4. Cardia type glands with inactive chronic inflammation (arrow) are seen between the squamous esophageal epithelium and the oxyntic gastric mucosa in autoptic pediatric case. HE, x200

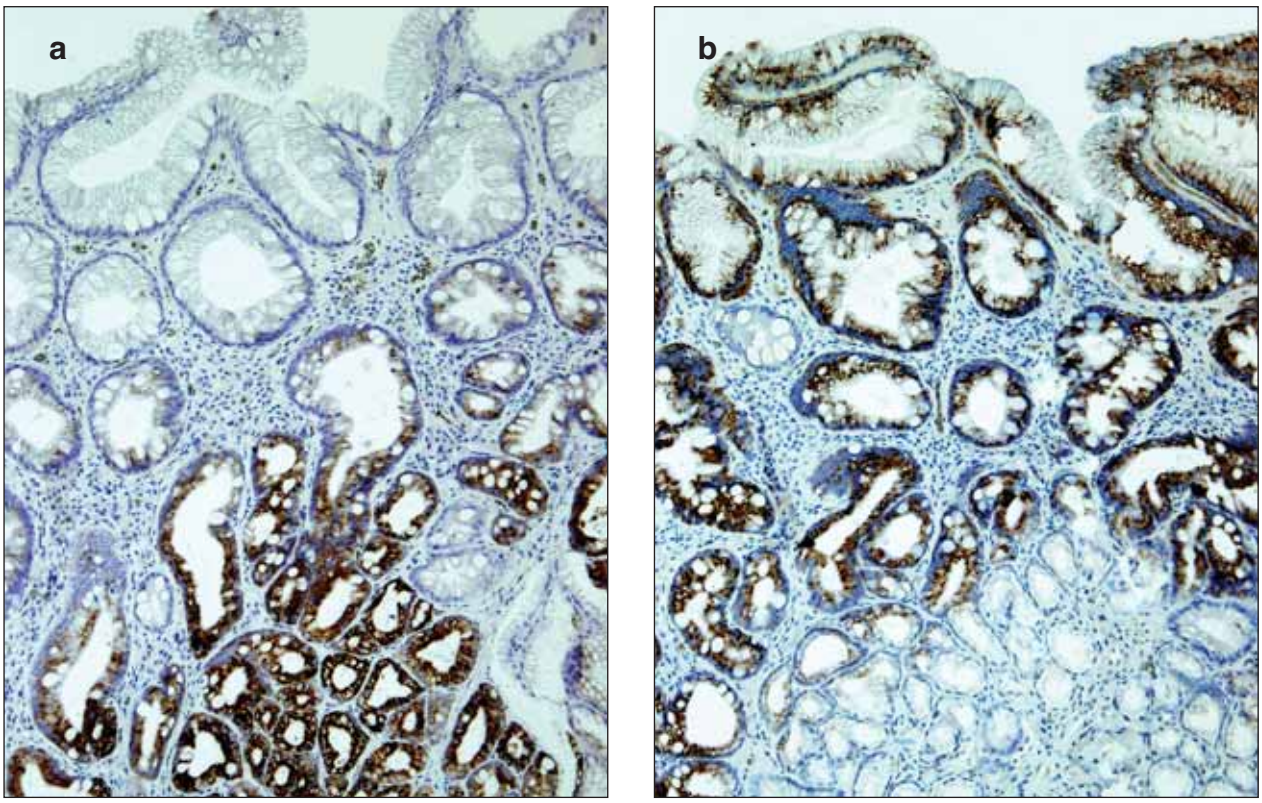

Fig. 5. Immunohistochemical findings. The incomplete IM express MUC6 in the glandular zone of the mucosa, whereas in the surface and foveolar epithelium the reaction is negative or only focally positive (a). In the surface and foveolar epithelium, the incomplete IM is reactive for MUC5AC (b). ABC technique, x250.

an increased amount of chronic inflammation as well (4, 22, 27). Histologic examinations of GEJ performed simultaneously with measurements of pH in the distal esophagus unquestionably proved that histologic changes depend on the gravity of the GER. Twenty-four hour pH monitoring showed that reflux of gastric contents into the lower esophagus occurs almost in everyone (1). Although chronic exposure of the lower esophagus to a lesser degree of reflux is commonly asymptomatic, such exposure is likely to damage the squamous epithelium. This fact can explain the very common presence of a short segment of CM in the population (2, 7, 22). Gastroesophageal reflux is also often present in infants. Although considered normal, "physiologic", and spontaneously resolving by the second year (9), it could cause metaplastic change in the distal esophagus, thus explaining the occurrence of CM in the pediatric population (8). Gastroesophageal reflux occurring in children older than 1.5-2 years is pathologic (21). Severe GERD during childhood has a few well-known risk factors that include neurological disorders, such as spastic quadriplegia. In accordance with this, all our autoptic cases of children with perinatal hypoxic/anoxic encephalopathy had CM in the distal esophagus in the segment measuring 0.5-3 mm in length. In all of these cases, CM showed chronic inflammation whereas adjacent oxyntic mucosa was normal. Therefore, we suppose that this inflamed (i.e. chronically irritated) CM represents metaplasia and not true cardiac mucosa of the stomach. This view is also supported by Park et al. (26) as well as by our previous study (17) in which CM was never found in stillborn mature infants.

In 2000 Chandrasoma et al. (2) characterized three epithelial types interposed between the squamous epithelium and gastric oxyntic mucosa: (1) CM composed of pure mucous glands that involves into (2) oxyntocardiac mucosa (OCM) containing a mixture of mucous cells and parietal cells (16), (3) IM with goblet cells. IM occurs only in CM (11, 12, 16, 30) and its prevalence rises with the increasing length of CM approaching 100% with more than 3 cm CM (4, 22, 29).

In our series, all 120 biopsies from the GEJ area contained CM. Of these, 15 specimens contained oxyntocardiac mucosa as well. All cases showed mild inactive chronic inflammation in both CM and OCM, and foveolar hyperplasia in CM. This finding is considered diagnostic for GERD, irrespective of the theories on the cardia origin. Only two cases with clinical features of GERD were positive for HP. In both cases antral HP positive inactive gastritis was found. Regarding the clinical symptoms, we interpreted this finding as GERD with superimposed HP infection. In 15 cases CM contained incomplete IM (28, 31) without dysplasia.

The immunohistochemical expression of MUC5AC and MUC6 was very similar in all specimens with CM, BE and antral mucosa. MUC5AC was always positive in the foveolae and focally also in the epithelium of the glands. MUC6 was produced by the normal epithelium as well as by the metaplastic epithelium (IM) in the glands. On the other hand, IM in the surface and foveolar epithelium was negative or only slightly focally reactive for MUC6, but showed positivity for MUC5AC. Thus the metaplastic epithelium tends to retain production of the mucin type typical for the respective location (surface, foveolae, glands). This finding of ours is in contrast with the results of Glickman et al. who found MUC6 expression in IM without relationship of this IM to the location in the mucosa. Therefore, these authors consider MUC6 positivity diagnostic for BE (11). Incomplete IM is characterized by the presence of secretory-absorptive cells with the capacity for a wide range of differentiation and mucin production (28). In our cases, this was proved by the simultaneous expression of MUC2 and neutral mucins MUC5AC and MUC6. Gulman et al. (15) observed the same mucin type expression as well as a similar pattern of CK7 and CK20 in BE and in "cardiac" IM. They suggest that both mucosa types represent the same entity and that the malignant potential of BE relates more to its length than to the exact location of IM around the GEJ.

The precise localization of metaplastic epithelial changes above the GEJ is crucial for their classification as GERD. The value of cytokeratin 7 and 20 immunostaining in distinguishing metaplastic columnar epithelium in the esophagus from the true gastric cardia is controversial (11, 23).

Accordingly, our findings confirmed the same distribution of CK7/CK20 in all cases of CM, BE, and antral mucosa. Both CK types were positive in the foveolae and also focally in the glandular epithelium.

Submucosal glands are believed to occur in the esophagus and not in the stomach. Thus, the presence of submucosal glands under CM and OCM indicates that the site is esophagus (6, 18, 27, 30). We found such glands under CM and IM in our esophageal resectate as well. In our autoptic cases of children similar interpretation was not possible because the segment with CM was too short (usually 1mm and less). Similarly to previous studies (18, 27, 30), we found foci of squamous epithelium closely related to esophageal gland ducts in the resected esophagus. Their presence in the columnar epithelium represents an additional proof for the esophageal localisation because the squamous cell foci do not occur in the stomach in GERD.

Although the view that cardia is a normal part of the stomach seems to be still prevailing, our histologic findings in the esophageal resectate and endoscopic biopsies, together with the results of others, support a new alternative opinion that CM and OCM represent metaplastic changes in the

distal esophagus (1-7, 27). For this reason, we have interpreted inflammed CM or/and OCM as GERD, and incomplete IM in CM as BE. Our criteria, however, do not change the diagnosis of GERD, because even pathologists regarding cardia a physiologic part of the stomach interpret "carditis" as a sign of GERD. We consider the inflammed CM in our pediatric patients metaplastic as a result of GERD caused by spastic quadriplegia. The extent of metaplastic changes was similar as described by Derdoy et al. (8) and Glickman et al. (12). As the opinions on origin of the cardiac mucosa are still controversial, we prefer the descriptive term "the cardiac type mucosa".

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