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Morphogenesis of Metaplastic Changes in Gastroesophageal Reflux Disease

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Abstract

The aim of research is studying of morphogenesis of metaplastic changes in esophageal mucous and defining diagnostic and prognostic criteria in Barrett Esophagus (BE) disease.

Materials for histological and immunnohistochemical research were mucosal biopsies of distal esophagus received from 79 patients with clinical and endoscopic indicators of BE. Immunohistochemical research with using markers CK7 and CK20 is characterized by high sensitivity, specificity and accuracy allowing defining a phenotype of various types of metaplastic processes at endoscopically defined Barrett's Esophagus.

Moderate CK 20 expression CK20, high level of Ki67 expression and P53 in mucous epithelium of cardial type testifies about transformation of cylindrical gastric epithelium in intestinal that confirms existence of a transition form of metaplasia in intestinal and can be a precursory diagnostic symptom of Barret's Esophagus development.

Markers Ki67 and P53 are predictors of dysplastic and malignant cell's regeneration due to gradual increase in its expression with the maximal values in biopsies of adenocarcinoma.

Keywords: Morphology; Diagnostics; Esophagus

Introduction

Gastroezophageal reflux disease is an important medical and social-economic problem of the modern society [1,2]. The relevance of studying etiopatogenetic and diagnostic aspects of GERD is defined of its abundance, clinical significance, further strong complications and difficulty of their early diagnostics [3,4]. Special significance gastroezophageal reflux disease got in the last years. The attention of clinicisits is paid on development of heavy form of disease, Barrett's Esophagus (BE) which is considered as before - cancer stage which can bring to adenocarcinoma of esophagus [5-9]. Despite the significant amount of publication for the last 20 years, a question about origin of cylindrical epithelium in mucous of esophagus remains debatable still. Existence of "Flame's tongues» at endoscopic research estimates by clinical doctors as "Barrett's Esophagus", which has morphological manifestation - specialized intestinal metaplasia in mucous of esophagus. But in the majority of researches patomorphologist does not confirm this diagnosis and defines other types of gastric metaplasia. The questions are "whether are they self-contained forms or stages of development of specialized intestinal metaplasias" and "In which metaplasia takes place the risk of development of neoplastic processes". The main purpose of research is searching of rational treatment's protocol and scientifically data about immunohistochemical (IGC) definition of metaplasia's phenotype for application in the daily practice.

Research objective: searching of morphogenesis of metaplastic changes in mucous of esophagus and defining of diagnostic and prognostic criteria at Barrett's esophagus.

Materials and Methods

Material for morphological researches was biopsy of mucous (distal parts of esophagus) of 79 patients with clinical signs of BE.

Biopsy was carried out during esophagogastroduodenoscopy manipulation with using of endoscopic station Olympus Evis Exera 2, series 180, which supports modes of high optical resolution and mode of narrow spectral visualization. Endoscopic research of struck esophagus included border definition between esophagus and stomach (Z-line). The aiming biopsy was received only from the suspicious centers in the bottom third of esophagus, located more proximally than the Z-line and separated from a stomach with the strip of normal epithelium not less than 1, 5 cm wide. In addition to standard histological research with using of coloring (hematoksilin and eosine), we carried out immunohistochemical reactions with cytokeratins 7 and 20 (CK7 and CK 20), which allow to define phenotypic characteristics of a cylindrical epithelium and to estimate its role in development of metaplastic changes which can reveal early stages of intestinal metaplasia.

The CK 7(marker of differentiation of stomach cell's epithelium) is in norm. The CK 20 carries to the intermediate filaments, makes structure basis of an epithelial cells [5] and also is the marker of differentiation of intestinal cells epithelium. In norm it meets in epithelium of glands in thickly intestine. Studying of proliferate activity of epitheliocytes carried out by means of Ki67 marker, which is found in all phases of mitotic cycle, except G0. P53 marker – protein regulator of apoptosis and cell's cycle was used for definition of dysplastic processes degree and forecast of development of malignant process in all groups of our research [9].

Intensity of expression for each marker was estimated with semi quantitative method: – -negative, + - weak, ++ - moderate, +++ - expressed. Viewing and photography carried out with OLYMPUS CX-41 microscope.

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Results and Discussion

The analysis of biopsies (mucous bottom third) of patients with clinic -endoscopic signs of BE showed an in homogeneity of received results.

In all cases in remained mucous of esophagus it was able to see some manifestations of chronic esophagitis corresponding to morphological criteria of reflux esophagitis and being shown by lymphocytic, histiocytic infiltration with impurity of plasmocytes in plate of mucous. Activity of inflammation was characterized by leukocytic infiltration both in stroma, and in interepithelial zone of the multilayer flat epithelium (MFE). It should be noted existence of a small amount of eosinocytes. Besides, moderately expressed signs of a circulatory disturbance in the form of plethora of vessels of different caliber, perivascular petekhial hemorrhages and stroma hypostasis were defined. Pays attention an acanthosis with lengthening of nipples MPE exceeding 60% of thickness of epithelium and basal cell's hyperplasia of exceeding 20% of thickness of epithelium. In some cases erosion and sharp ulcers are found.

A variety of metaplastic changes attracts attention. In 21% of cases of metaplasia researches of gastric type are found, it is morphologically reminding mucous the stomach, covered by the high cylindrical epithelium. Round or oval cores of epithelium cells occupy basal part of a cell; around cores cytoplasm is eosinophilic (Figure 1a and 1b).

There are defined gastric poles in which ostiums of branching tubular glands open. It should be noted existence of erosion in different stages of adhesions. So, in the regions of some of them signs of the foveolyar hyperplasia are defined which is showing in the form of lengthening poles, existence high, sometimes the branchy rollers which are looking like nipples, covered with the flattened epithelium that can be associate to regenerator reaction to mucous inflammation. In sites of the healed erosion the surface and poles epithelium is flattened, basophilic. Looking alike histological structure of fundal part of stomach is shown by available main parietal and mucous cells, simple hyperplasic Pannet's cells in glands making main components of a gastric juice – Pepsin and Acidum hydrochloric.

At immunohistochemical research (with CK7 and CK20) of mucous parts with fundal gastric metaplasia in all cases negative reaction or focal weak surface expression of cytokeratins is noted. The internuclear expression of Ki67 is found in 5.1 ± 0.01% mainly in poles epithelium and is regarded as norm, in epithelia of deep glands 1.2 ± 0.04 of %. P53 expression in the surface cylindrical epithelium structure is 6.1 ± 0.02%, and in parietal and main cells –1.5 ± 0.01%. The greatest number of biopsies (34%) was characterized by presence of a metaplasia of gastric type reminding cardial part of stomach (Figure 2a and 2b).

The surface mucous has the papillary outgrowths covered with a high cylindrical epithelium with eosinophilic cytoplasm, raised mukoidization with formation of chance goblet cells. There are deep gastric poles with seldom located parietal cells, which are the centers of a foveolyar hyperplasia. Besides the inflammatory changes it is important to note emergence of simple lymphoid follicles with expanded terminative centers. In 23% of cases at selective coloring of CK7 in parts of mucous with a gastric metaplasia on cardial type in the surface cylindrical epithelium the cytoplasmatic expression is noted. In an epithelium of deep glands of the metaplastic mucous of cardial type the expression of CK7 is regarded as weak (+), and the expression of CK20 is negative.

To our opinion these manifestations testify about changes of phenotype of cardial type metaplasia in specialized intestinal type, and its focally and in homogeneity are signs of initial transformation of one type in another owing to violation of epithelium differentiation. Positive reaction of Ki67 in the metaplastic epithelia of cardial type is

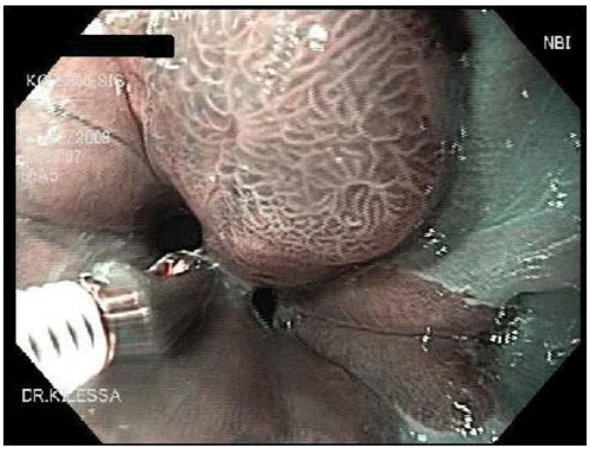

Figure 1a: Gastric type of a metaplasia at Barrett's Esophagus disease. Videogastroscopy. NBI

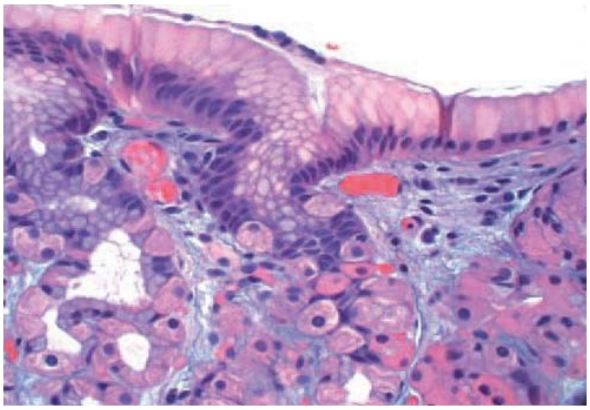

Figure 1b: Gastric metaplasia of esophagus (fundal type). Foveolyar hyperplasia, existence of parietal, main cells, Pannet's cells, mucous cells. Coloring of hematoxylin and eosine. Inc. 20x.

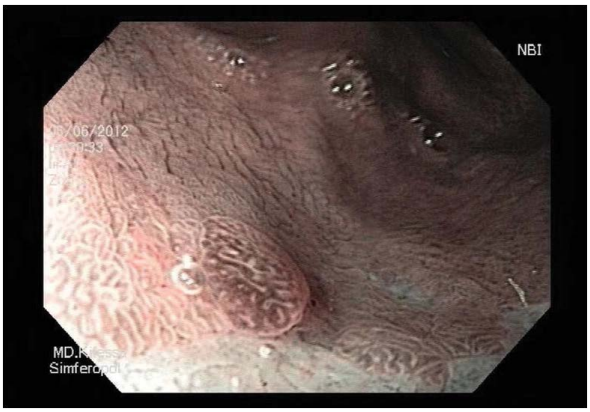

Figure 2a: Cardial type of a metaplasia in Barrett's esophagus with local hyperplasia. Videogastroskopy. NBI.

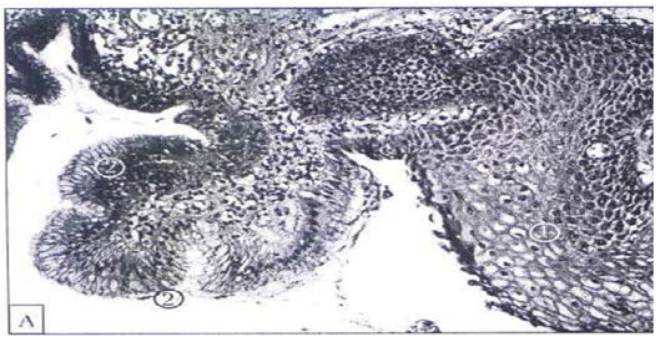

Figure 2b: Gastric metaplasia of esophagus (cardial type). MFE-1. Mucous zone of metaplasia – high cylindrical epithelium with increased mukoidization - 2. Coloring of hematoxylin and eosine. Inc. 20x.

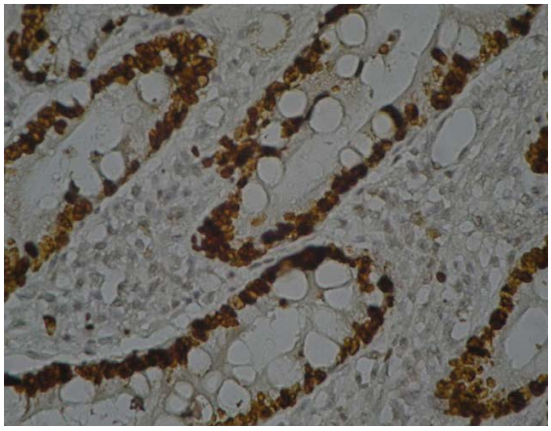

Figure 2c: Cardial metaplasia. Ki-67 expression in a gland epithelium in cardial type of metaplasia. IHC. x400.

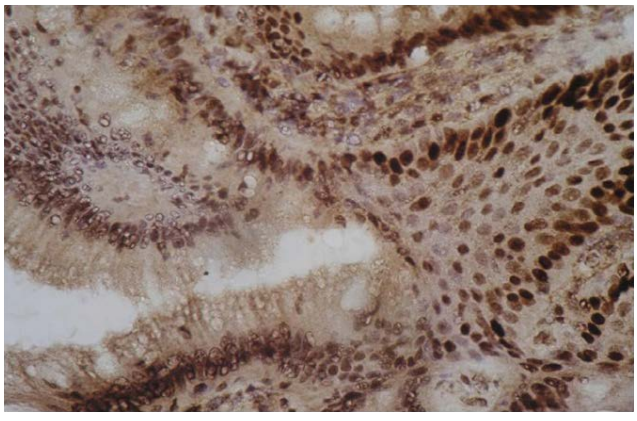

Figure 2d: Cardial metaplasia. P53 expression in a gland epithelium in cardial type of metaplasia. IHC. x400.

defined in 11.7 ± 0.03% of cases in the surface and 37.4 ± 0.03% in deep gland's epithelium and is the manifestation of moderate proliferate activity that in our opinion testifies about violation of epitheliocytes differentiations. Besides, important prognostic criteria is reliable (in comparison with fundal type of a metaplasia) strengthening of expression P53 as in the surface (11.4 ± 0.02%), and in deep glands (12.3 ± 0.02%). In 11% with histological diagnosed metaplasia of cardial type the moderate expression of CK20 (++) in the surface cylindrical epithelium, mainly in apical parts (Figure 5) is found. CK7 gives a weak focal expression or is absent. Besides, authentically significant strengthening of an intranuclear expression of proliferate activity index to 18.1 ± 0,02% in the surface epithelium and 55.3 ± 0.07% in an epithelium of glands is noted. Increase of indexes is a consequence of active cell's proliferation owing to transformation of cylindrical epithelium that confirms existence of transition form of metaplasia in intestinal and can be a precursory diagnostic symptom of Barrett's esophagus.

Important difference of biomaterial with cardial type of gastric metaplasia and a high level of expression of CK20 is authentically comparable level of expressionP53 with established specialized intestinal metaplasia. 17.5 ± 0.03% of positively painted kernels and 41.7 ± 0.03 in an epithelium of deep glands are defined in the surface epithelium, that testifies to gradual increase of potential malignant regeneration. At research of 24% of patients with clinico-endoscopic signs of BE the specialized type of intestinal metaplasia was revealed, which is characterized by signs both thin, and a colic metaplasia that is shown of existence of goblet cells in the surface and deep glands. In the surface cylindrical epithelium the reinforced slim- producing is defined by increase in volume of cell with existence in eosinophilic cytoplasm of multiple shallow vacuoles. However the structure of the surface epithelium has papillary outgrowths of surface mucous, but legibly created fibers (reference for an enteric metaplasia) are absent (Figure 3a and 3b).

The analysis of IGC researches of biomaterials with signs of a specialized intestinal metaplasia shows intensive coloring of CK20 in the surface cylindrical epithelium with legible visualization of goblet cells and moderate coloring in epithelium of deep glands. The expression of CK7 is characterized by poorly expressed focal coloring of simple glands of t surface epithelium or lack of positively painted cells in general (Figure 4). By comparison of results of histological and immunohistochemical research, the absence of legibly created fibers reference for enteric metaplasia was noted. Presence of goblet cells at the surface and deep epithelium of glands, reference for thin and for colic metaplasia is regarded as a specialized intestinal metaplasia (Figure 5). Proliferate activity of epitheliocytes is 21,4 ± 0,03% in the surface epithelium, and in deep glands – 57.6 ± 0.01%. The expression P53 was 18.3 ± 0.02% and 48.7 ± 0.05% in the surface and deep epithelium of

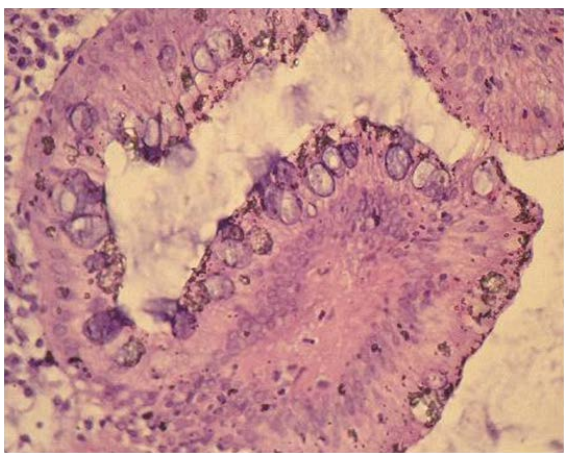

Figure 3a: The specialized intestinal metaplasia of esophagus. Coloring of hematoxylin and eosin. Inc. 20x.

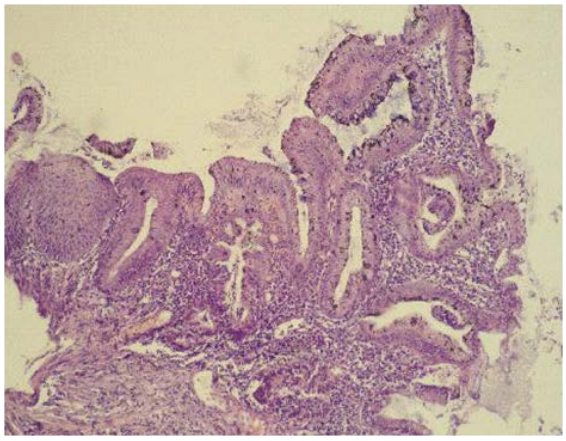

Figure 3b: The specialized intestinal metaplasia of esophagus. Goblet cells. Coloring of hematoxylin and eosine. Inc. 20x.

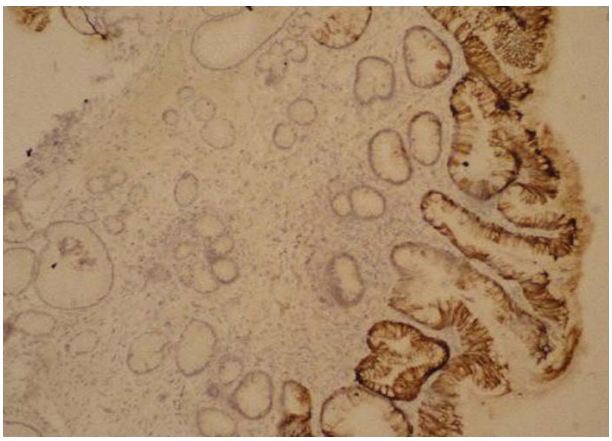

Figure 4: Cardial type of mucous. Moderate expression of the CK 20 in the surface epithelium (++). IGC. Inc. 20x.

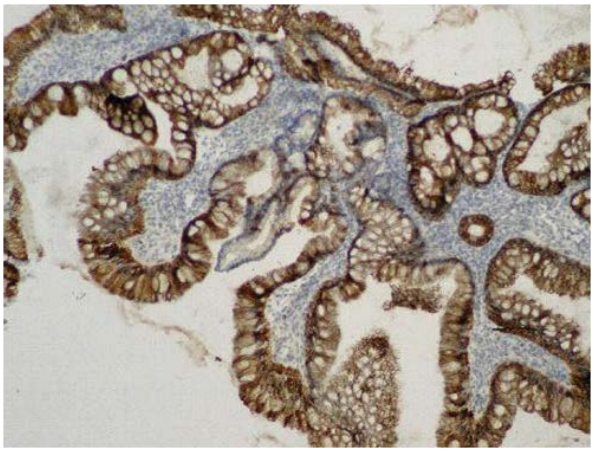

Figure 5: Specialized intestinal metaplasia. The CK20 expression (+++) with visualization of goblet cells.IGC. Inc. 20X.

glands respectively. In 9% of biomaterials with histological diagnosed signs of specialized intestinal metaplasia, 5% decide on dysplasia of gland's epithelium of low degree and 4% – on dysplasia of high degree. At dysplasia of gland's epithelium (low degree), the surface mucous took a form of papillary outgrowths, poles were extended.

There was a proliferation of cambial cells with atypia development in the form of strengthening of nuclear hyperchromatosis and increase of nuclear-cytoplasmatic ratios. As a whole the histological structure is not broken, the multilane of an epithelium met seldom. Proliferate activity increases to 59.8 ± 0.01% in deep and 42.5 ± 0.03% the surface glands respectively. At a dysplasia of low degree the P53 level in epithelium of glands is 36.6 ± 0.01% and 53.5 ± 0.04%. At dysplasia of high degree lack of goblet cells was noted due to expense of expressed proliferation of epithelium and violation of its differentiation. In the majority of cases, signs of cell's atypia were expressed: anisocariosis, nuclear hyper chromatosis , sharp increase of nuclear-cytoplasmatic ratios. Widespread pseudo-stratification of gland's epithelium, stroma reduction, increase of gland's amount and their compact arrangement took place. (Figure 6a and 6b)

The expression Ki67 is 72.3 ± 0.05% and 65.5 ± 0.03% in deep and surface glands respectively. Indexes of P53 increase (55.7 ± 0.03% and 65.4 ± 0.03%) that is an adverse prognostic factor concerning risk of development of adenocarcinoma, level of an expression in which is 93.7 ± 0.05% (Figure 7a, 7b). Application of modern research techniques allows endoscopically and immunohistochemically to define phonotypical features of a cylindrical epithelium and risk of

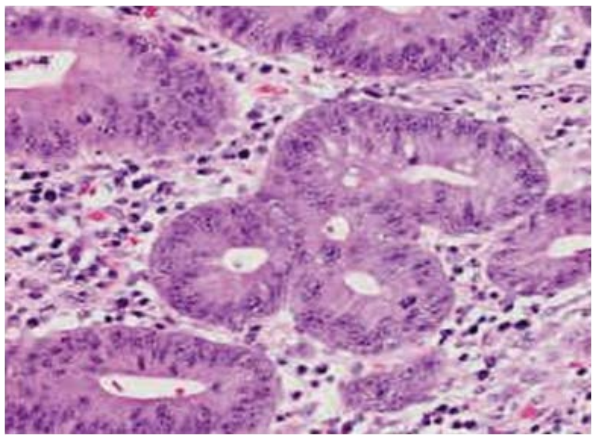

Figure 6a: Dysplasia of low degree. Coloring of hematoxylin and eosine. Inc. 20x.

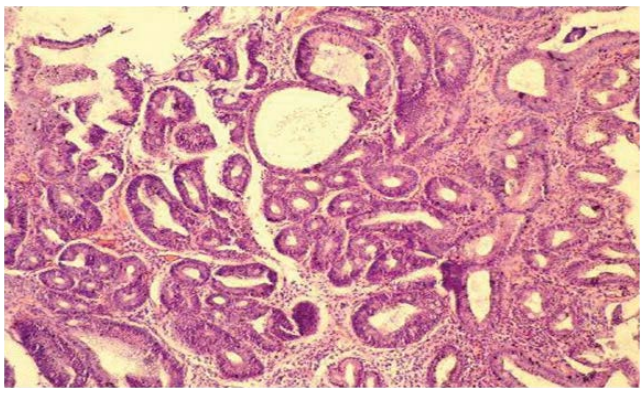

Figure 6b: Dysplasia of low and focal high degrees in specialized intestinal metaplasia of esophagus . Coloring of hematoxylin and eosine. Inc. 20x.

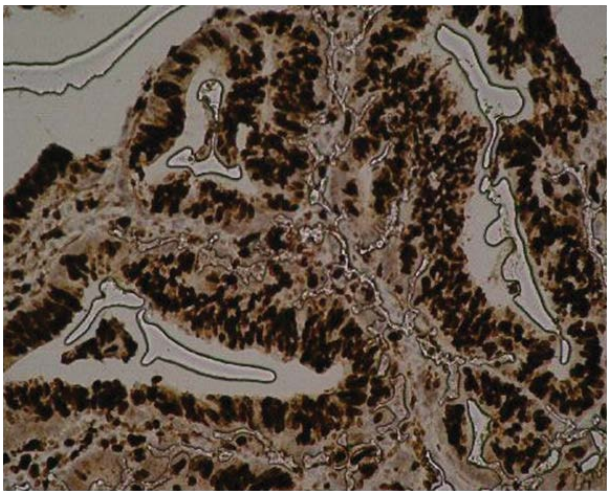

Figure 7a: Dysplasia of high degree. Coloring of hematoxylin and eosine. Inc. 20x.

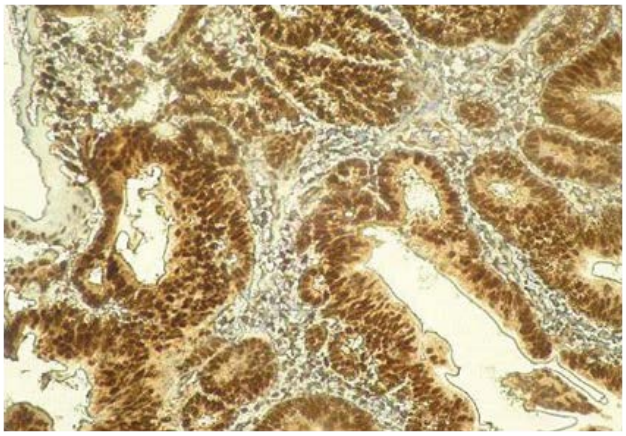

Figure 7b: Dysplasia of high degree in specialized intestinal metaplasia of esophagus. Coloring of hematoxylin and eosine. Inc. 20x.

development of dysplasia and a malignancy, depending on particular type of metaplastic changes – a specialized intestinal metaplasia.

Conclusions

1. Immunohistochemical research with using markers CK7 and CK20 is characterized by high sensitivity, specificity and accuracy allowing to define a phenotype of various types of metaplastic processes at endoscopically defined Barrett's Esophagus.

2. Moderate CK20 expression, high level of Ki67 expression and P53 in mucous epithelium of cardial type testifies about transformation of cylindrical gastric epithelium in intestinal that confirms existence of a transition form of metaplasia in intestinal and can be a precursory diagnostic symptom of Barret's Esophagus development.

3. Markers Ki67 and P53 are predictors of dysplastic and malignant cell's regeneration due to gradual increase in its expression with the maximal values in biopsies of adenocarcinoma.

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