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ENGLISH VERSION: COMPLEX DIAGNOSTICS OF ULCEROINFILTRATIVE GASTRIC CANCER*

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Complex diagnostics, made by the histological method, specifying the mitotic regimen, immunohistochemical Ki-67 marker (MIB-1clone) and ISSR-PCR marker of genetic typing showed lesions in gastric mucosa epithelium, specific to gastric mucosa epithelium neoplasia in patients with ulceroinfiltrative gastric cancer. Expansion of ISSR-PCR marker of genetic typing indicates its high sensitivity and informativeness. There is a strong correlation between the indices of histological, immunohistochemical and indices of genetic typing, detected by the ISSR-PCR method. Between the indices of mitotic regimen, proliferative activity of gastric mucosa epithelium of Ki-67 marker and indices of genetic typing of gastric mucosa epithelium, the Pearson's correlation coefficient, r_{xy}, constitutes 0,197, 0,607 and 0,881, respectively, corresponding to the existence of moderate and considerable relationship between indices. The overall result shows statistically significant dependence with probability of 0,99.

Key words: mitotic regimen, Ki-67 marker, genetic typing.

Introduction

The main cause of death in developed countries, along with mortality from cardiovascular processes and their complications, is mortality from malignant tumors. Every year gastric cancer affects 750 – 870 thousand people in the world; it accounts for 10% from fatal outcomes, caused by neoplastic pathology. In 2009 gastric cancer in Ukraine took the 3rd (9.0%) place in the structure of male oncopathology and the 6th (5.6%) place in the female one; in the structure of oncomortality it takes the 2nd (11.8 and 9.3%) place in both groups [5, 10].

Lesions in mucosa, surrounding gastric ulcer, e.g., chronic gastritis, which are followed by dysregenerative processes with the development of epithelial dysplasia [1, 3, 6] are strongly believed to be predictors of gastric cancer development. The latter, especially the severe ones, are often identified as the marker of cancer, growing nearby [11].

Ulceroinfiltrative gastric cancers are complicated for diagnostics. At early stages this form of cancer may stimulate typical ulcer, making it difficult to make differentiated diagnostic with the latter [4, 6]. Ulcerated gastric cancer, as well as typical ulcer, may be healed. But, again, the process of healing is replaced by ulceration and such cycles may occur repeatedly. Due to the fact that gastric ulcer develops comparatively slowly, such cycles may occur repeatedly [6, 12].

The purpose of the research is complex diagnostics of pretumor changes in gastric mucosa and diagnosis of gastric cancer by histological, immunohistochemical and molecular- biological methods.

Materials and Methods

The paper considers findings of 50 examinations of ulceroinfiltrative gastric cancer. Surgically extracted stomachs have been examined with the purpose to detect morphological features of gastric mucosa condition.

The obtained samples of gastric mucosa have been studied by conventional histological method according to standard regimen, including fixation of tissue in neutral formalin. The material was processed in paraffin coating. Sections were colored in hematoxylin-eosin and picrorosein according to Van-Gison.

Immunohistochemical detection of proliferation of gastric mucosa epithelium has been performed by Ki-67 marker on deparaffined sections of 4-5 mm thick with prior antigen damasking in citrate buffer (pH 6,0) in microwave oven during 10 min. Monoclonal antibodies have been applied to Ki-67 (MIB-1clone) as primary ones. In-

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cubation with primary antibodies has been performed during 18 hours. Identification of reaction has been performed by the chromogen 3,3'= diaminobenzidine tetrachloride (DAB, Dako Cytomation). Sections were contrasted by hematoxylin-eosin.

At the same time the analysis of gastric mucosa has been carried out by the polymerase chain reaction. The ISSR-PCR method has been applied, using the S2 ISSR-primer with the following structure: $(AGC)_6G$[7, 8, 9, 13].

Amplification has been made in 25 mcl of reaction mixture. DNAs were added, numbered in 10 – 20 ng per reaction. The temperature of primer's shooting was 57ºC, synthesis of the DNA fragments ran in 30 cycles of amplification [8].

Electrophoretic amplification-products separation has been made in 2%-horizontal agarose gel (Vagofor, Latvia).

Visualization of electrophoregrams have been done on transilluminator by ultraviolet light with wave length of 365 nm with further photographing.

Amplicones were measured by the marker of molecular weight of 1000 bp DNA-Ledder, pUC 19 DNA/ Msp I («Fermentas», Lithuania)[8, 13].

Mitotic regimen has been conventionally defined to estimate the manifestation of mitosis disorders. Mitoses have been calculated under immersion microscope magnification of 100 visual fields. Mitotic index (MI), i.e., number of mitoses per 1000 cells measured in per mille (‰), number of mitoses in metaphase, measured in percent (%) and number of pathological mitoses, measured in percent, has been defined [2].

Findings of immunohistochemical reactions have been evaluated by calculation of ratio of positive cells with various intensity, estimated visually. 800–1000 epithelial cells have been analyzed on a case-by-case basis. Proliferative potential (index of proliferation) has been defined while calculating the number of cells, expressing the Ki-67 marker. In MI (marker index), where Ki-67<10,0%, proliferative activity is low, and in IM, where Ki-67≥30,0%, proliferative activity is high.

Reliability of difference of mean comparison indices has been estimated by the Student's t-criteria. The difference between comparison values was considered to be significant, if allowable error (p) was less than 0,05.

Quantitative assessment of correlation has been estimated by the value of correlation coefficients within the limits from -1 to +1. Coefficients' negative values indicate the back relation, and positive values indicate the direct relation. Zero value may indicate the absence of relation. Intensity of relation (weak – moderate – considerable – strong relation) has been estimated by the absolute value of correlation coefficients.

Results of the research

No significant difference of mitotic index (MI) has been found in patients with ulceroinfiltrative gastric cancer, developed in gastric mucosa around tumor against the background of manifested forms of chronic atrophic and atrophic- hyperplastic gastritis, in the pyloric part and lesser curvature of stomach (Fig. 1).

The rate of mitotic index in the body(B) of stomach (15,5±4,2‰) was significantly lower (p<0,05) than in the area around the tumor(AT) (33,1±11,8‰), pyloric(P) part (27,5±5,8‰) and lesser curvature (LC)(25,5±3,9‰) (Fig. 1).

No significant difference (p>0,05) has been found between the rates of number of mitoses at metaphase in pyloric part (51,5±3,3%), lesser curvature (51,9±2,2%) and around the tumor (56,9±5,8%).

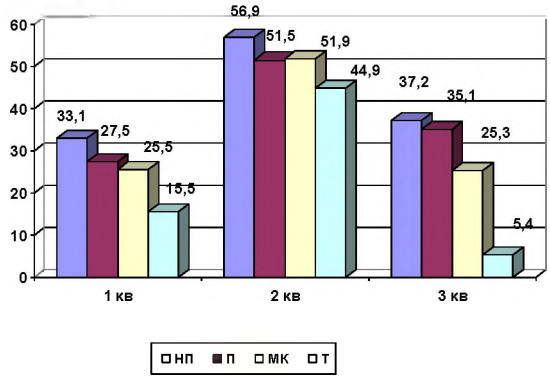

Fig. 1. Mitotic regimen of gastric mucosa epithelium of patients with ulceroinfiltrative gastric cancer. 1кв – mitotic index (‰). 2кв – number of mitoses at metaphase (%). 3кв – number of pathological mitoses кількість (%).

Number of mitoses at metaphase in the body of stomach (44,9±2,8%) was significantly lower (p<0,01) than in the area around the tumor (56,9±5,8%) and other areas of gastric mucosa.

Examined pathological mitoses (Fig.1) were characterized by significant lowering (p<0,001) in the body of stomach (5,4±1,1%) relative to the area around the tumor (37,2±3,5%), pyloric part (35,1±2,7%) and lesser curvature (25,3±3,3%); significant difference between the pyloric part (35,1±2,7%) and lesser curvature of stomach (25,3±3,3%) (p<0,05) has been detected.

High proliferative activity of gastric mucosa epithelium with marker index of >30,0% has been found by the Ki-67 marker (MIB-1clone) (Fig.2).

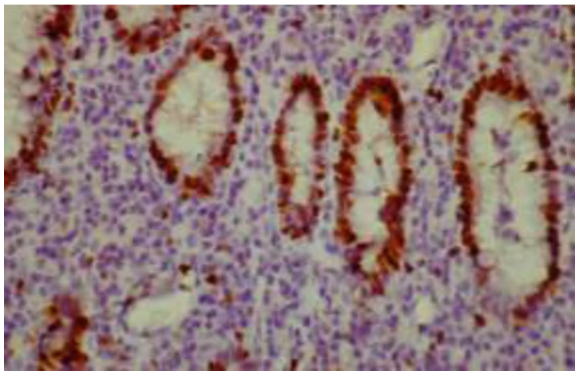

Fig.2. High proliferative activity in recesses epithelium with dysplasia against the background of chronic gastritis. Ki-67marker (MIB-1clone).

Dependence between the rates of number of pathological mitoses, mitoses at metaphase, mitotic index with manifested proliferative activity of mucosa epithelium has been identified in various topographic-anatomical parts of stomach.

In the body of stomach, where indices of mitotic regimen are lower than in other parts of stomach, manifested proliferative activity of epithelium with gastric mucosa dysplasia is significantly rarely identified (p<0,001).

Increase in number of pathological mitoses, mitotic index and mitoses at metaphase in pyloric part, lesser curvature and around the tumor correlates with the increase of proliferative activity of gastric mucosa epithelium with marker index of ≥30,0%.

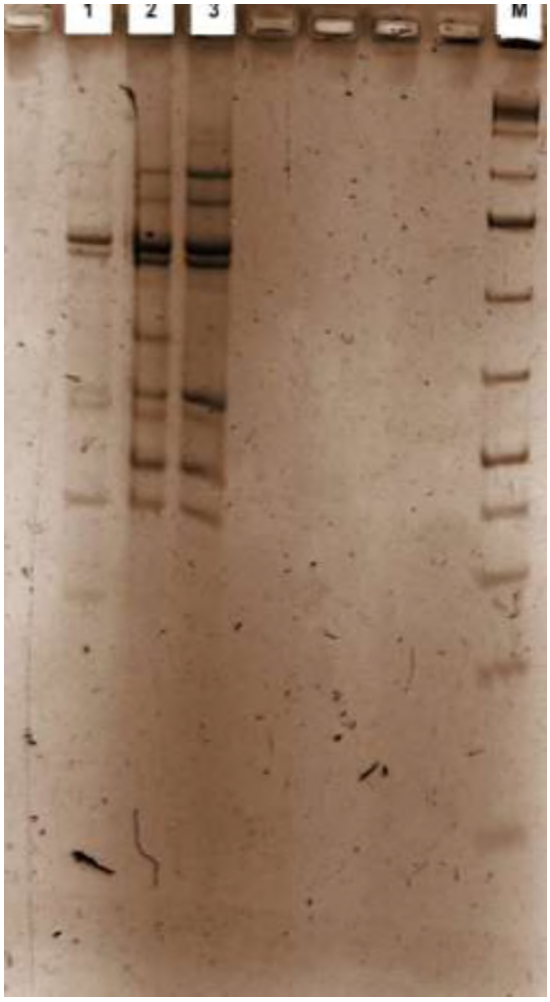

Fig. 3. Electrophoregrams of amplificate products of DNA of gastric mucosa of patients with ulceroinfiltrative gastric cancer: 1,2,3 – DNA-profiles correspond to tumor marker; M – marker of DNA fragments size.

Genetic typing of gastric mucosa epithelium of patients with ulceroinfiltrative gastric cancer detected rather stable DNA-profiles, presented by the expansion of fragments measured 520 and 620 p.n. (pairs of nucleotides) long, in all observations (Fig.3) and were totally different from the profile of normal marker.

The more rates of mitotic regimen are, the grater proliferative activity of integumentary-recessed epithelium and glands epithelium is.

In the body of stomach, where indices of mitotic regimen are the lowest in comparison with other parts of stomach, proliferative activity of epithelium is significantly rarely identified ($p<0,001$) and is defined by the marker index of <10,0%.

The results of genetic typing concluded that, since the DNA-profiles were obtained with evident expansion of fragments measured from 520 to 620 p.n. in all examinations, they are to be considered as the marker of tumor existence.

Between the indices of mitotic regimen of gastric mucosa, manifestation of proliferative activity of dysplasia of gastric mucosa epithelium according to Ki-67 marker of patients with ulceroinfiltrative gastric cancer and indices of genetic typing of mucosa epithelium, the Pearson's correlation coefficient, r_{xy}, constituted 0,197, 0,607 and 0,881, respectively, corresponding to weak, There is considerable and strong relationship. Coefficient of determination, $D=r_{xy}\hat{}2$, was equal to 0,039, 0,369 and 0,776, respectively. Critical value of correlation coefficient with probability of 0,95 is equal to 0,2732. Critical value of correlation coefficient with probability of 0,99 is equal to 0,3511. The comparison of correlation coefficient, r_{xy}, with critical value, r_{cr}, worth of 0,95, corresponded to $r_{xy}<r_{cr}$ and $r_{xy}>r_{cr}$, respectively. The comparison of correlation coefficient, r_{xy}, with critical value, r_{cr}, worth of 0,99, corresponded to $r_{xy}<r_{cr}$ and $r_{xy}>r_{cr}$, respectively. Covariation coefficient constituted 0,389, 3,442 and 0,859, respectively. Statistically significant dependence with probability of 0,99 has been detected between the indices of mitotic regimen, proliferative activity of epithelium dysplasia and indices of genetic typing of gastric mucosa.

Conclusions

Histological method has established that high indices of mitotic regimen of mucosa epithelium have been found in ulceroinfiltrative gastric cancer.

Indices of mitotic regimen in stomach parts tend to be increased in the following direction: B→ LC→ P →AT.

In ulceroinfiltrative gastric cancer genetic typing of gastric mucosa stable DNA-profiles are found, presented by the expansion of amplicones measured 520 and 620 p.n. (pairs of nucleotides) in all examinations. It indicates about their genetic uniformity, malignancy and possibility to use them as malignancy marker.

Application of the ISSR-PCR method provides with the possibility of early diagnostics of gastric mucosa while examining the mucosa material.

Indices of immunohistochemical method correlate with histological method and indices of genetic typing according to ISSR-PCR reaction. The Pearson's correlation coefficient, r_{xy}, between the indices of mitotic regimen, proliferative activity of gastric mucosa epithelium according to Ki-67 marker and indices of genetic typing of gastric mucosa epithelium, is equal to 0,197, 0,607 and 0,881, respectively, corresponding to the existence of moderate and considerable relationship between the indices.

Morphological diagnostics of cancer should be based on complex estimation of symptoms, differentiating its epithelium dysplasia and other lesions, which stimulate tumor, comparing the findings of pathohistological immunohistochemical studies and genetic typing.

Perspectives of further research

It is planned to carry out practical complex studies to diagnose neoplastic lesions of gastric mucosa epithelium in patients with chronic stomach diseases.

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