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Some Morphometric and Microbiological Aspects of Erosive-Ulcerous Lesions in Upper Part of Digestive Tract in Patients with Hepatocirrhosis and Portal Hypertension

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ABSTRACT: The present study is directed to the most principal and debatable questions of combined affection of the upper part of digestive tract and liver. Mucosa state of upper part of digestive tract was evaluated on morphometric and microbiological study of digestive tract and liver. Pathogenesis features of the development of erosive-ulcerous process against the background of hepatocyrrhosis (HC) progression were characterized. Autopsy with stomach examination was held in 52 patients, which died from HC. Microbiological study was conducted in 40 patients with HC and portal hypertension in admitting to the hospital and after their treatment. As a material for microbiological study we used gastric juice, biopsy materials from mucosa of the esophagus and cardia. Serological study included detection of antibodies (IgG) to Helicobacter pylori (Hp). So-called "hepatogenous ulcers" are characterized by specific morphology of atrophic and disseminated with Hp gastric mucosa. HC considerably exacerbates the course of combined erosive-ulcerous affection of esophagus and stomach, which in turn accelerates development of hepatocellular insufficiency. Study shows that so-called "hepatogenous ulcers" have not only specific localization and quantitative evaluation but specific morphologic features that differ them from classic "peptic ulcers".

Author Keywords: Hepatocyrrhosis, Hepatogenous Ulcers, Helicobacter Pylori

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INTRODUCTION

Erosive-ulcerous process spreading to the mucosa of cardioesophageal zone, where esophageal and gastric varicose dilated veins (EGVDV) can be located, is one of the detonators that may provoke profuse, often fatal, esophagogastric bleeding. Erosive-ulcerous process in pyloroantral section of the stomach is more often observed in patients with hepatocirrhosis (HC) and portal hypertension (PH); for description of such cases many authors use the term "hepatogenic ulcers" [1].

Since Helicobacter pylori (Hp) discovery and admitting its etiological role in the development of stomach (SU) and duodenal ulcer (DU), new data on the influence of this microorganism on pathologic processes in other organs of abdominal cavity, including stomach, have appeared [2, 3]. Recent studies Waluga (2015), have provided evidence that H. pylori is also involved in the pathogenesis of some liver diseases. Many observations have proved that Hp infection is important in the development of insulin resistance, non-alcoholic fatty liver disease, non-alcoholic steatohepatitis, liver fibrosis and cirrhosis. [4]. Hp DNA was detected in the livers of primary biliary cirrhosis patients [5].

The prevalence of ulcers of the stomach and/or duodenum caused by Hp is higher in patients suffering from hepatic cirrhosis [6, 7]. A recent meta-analysis suggests that there is also a significantly high prevalence of Hp infection among patients with cirrhosis [8]. Eradication therapy may be beneficial for cirrhotic patients because it diminishes the risk of recurrent peptic ulcers and bleeding [9]. However, Stalke et al. [10] demonstrated a positive correlation between the degree of gastric colonization by this bacterium and parenchymatous liver damage in a group of hospitalized patients without liver cirrhosis.

We have not seen serious investigations on pathogenetic mechanisms of Hp influence on the development and course of erosive-ulcerous lesions in upper part of the digestive tract of patients with HC and complications of PH. Taking into account an important role of erosive processes in the development of bleeding from EGVDV, research in this direction is of great importance.

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So our study was directed to the main and debatable questions on combined affection of upper part of digestive tract and liver included:

1. Influence of diffuse liver affection on stomach wall;
2. A role of Hp in pathogenesis of gastrointestinal hemorrhages in patients with HC.

MATERIAL AND METHODS

Morphologic research

For studying features of stomach mucosa structure in HC, we examined 52 stomachs of dead patients with HC. Material was fixed in buffered formalin (pH=7,4) and in 2,5% solution of glutaric aldehyde. We used light and scanning microscopy. Morphometry was conducted by projecting-metric method with scale of micrometers (μm).

Microbiological methods

Microbiological study was carried out in admission of patients and after treatment. For microbiological study we used gastric juice, biopsy from mucosa of esophagus and stomach cardia. Gastric juice was received in the morning, on an empty stomach in gastric intubation, biopsy material was taken in esophagogastrofibroscopy. We identified micro flora in gastric juice (Hp, fungi of the genus Candida and other microorganisms), microflora and urease activity were studied in biopsy materials. Isolated microorganisms were identified by generally accepted methods; susceptibility to antimicrobial drugs was tested by disco-diffuse method.

Serological methods

IgG antibodies to Hp was detected by ELISA, test-system ("Hexagon H.Pylori", "Human", Germany). "Hexagon H. pylori" is a qualitative one-step test for determining Hp antibodies in serum or whole blood.

RESULTS AND DISCUSSION

Typical feature of stomach wall in patients with HC is decrease of its thickness in all parts; enlarged sizes of stomach were observed in most cases. In macroscopic examination of structural and functional organization of GM decreases of gastric folds height and their flatness were observed as well as high quantity of viscid mucus. Petechial hemorrhages and erosions were often found in the thickness of mucosa, especially in cardial part. Study of mucosa by scanning electronic microscopy revealed disorders in rhythm conformation as well as significant polymorphism of gastric fossae. Microerosions of different size were a constant sign (Figure 1). Large zones of GM with desquamation of integmentary fossa epithelium. Large quantity of microorganisms were found by this method on the surface of mucosa of gastric fossae, marginal parts of erosions and ulcerations (Figure 2).

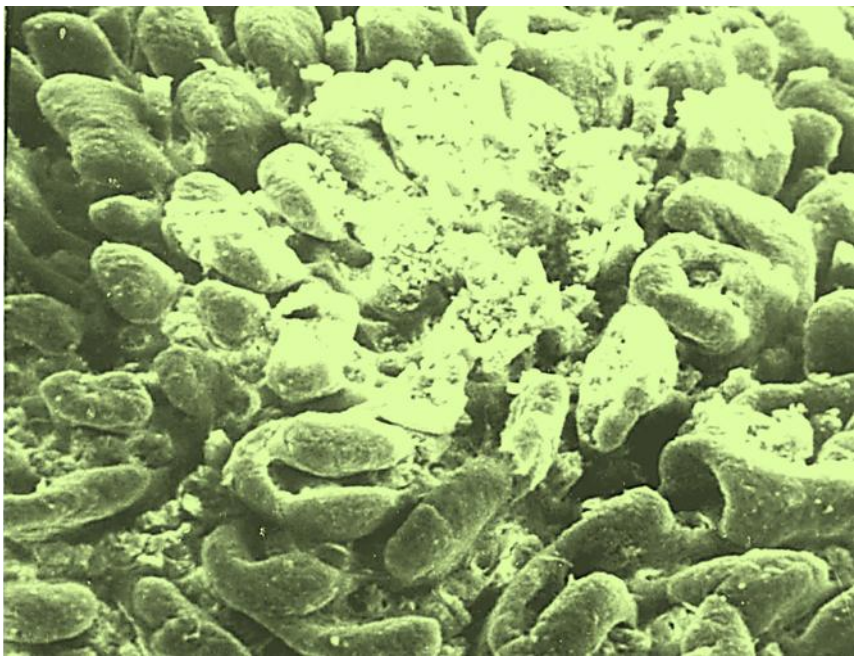

Figure 1. Microerosions of different size were a constant sign SEM x 2000.

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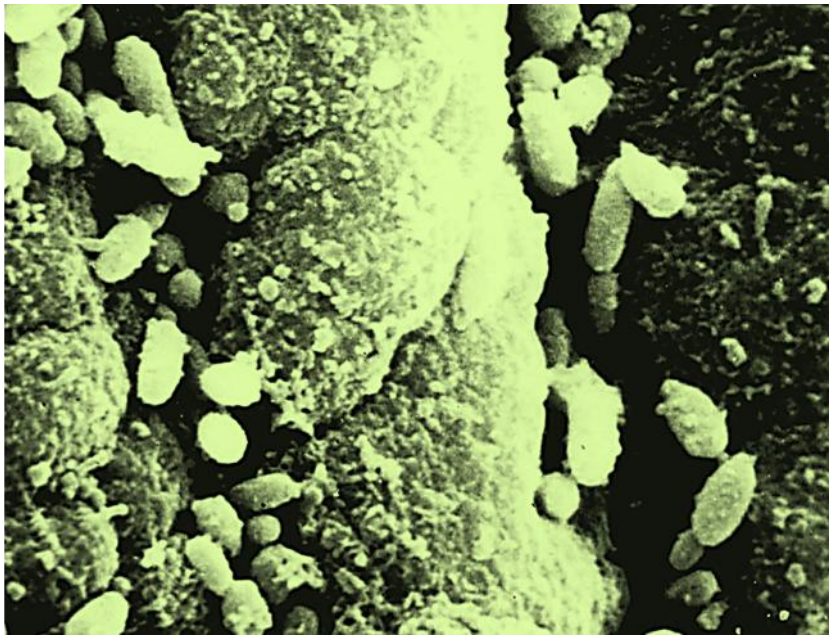

Figure 2. Large quantity of microorganisms were found by this method on the surface of mucosa of gastric fossae, marginal parts of erosions and ulcerations SEM x 10000.

Morphometric study of mucosa showed decreasing of its thickness, most expressed in cardia and minimally in pyloric part. In microscopic study of GM in patients with HC widening and deepening of gastric fossae were observed. The fossae often occupy about 1/3 of GM height, rarely achieve till its center. Surface epithelium is transformed from high prismatic into cubic one. As it was mentioned above its desquamation and denudation of spaces between fossae also take place.

There is a decrease of glands quantity per length unit. The present process is observed both in cardial and fundal parts, its minimal expression can be observed in pyloric part. In all parts interfossal spaces are enlarged at the expense of edema, small thin-walled vessels of vein type are found in quantity as well as considerably enlarged lymphatic fissures. Practically in all cases moderately expressed lymphoid cellular infiltration with increase of intensity in basal parts of GM was observed.

Cardial glands become shorter and straighter; quantity of mucocytes in secretory parts decreases. Parietal cells are not detected in HC. Highly-specialized fundal glands are pathologically changed to the most degree. Quantity of these glands compared to the control samples decreases down to 40, 9 - 3, 2 (70, 3 – 4, 1 in health). Atrophic and dystrophic processes in fundal glands are localized mostly in main and parietal cells, their quantity decrease in comparison to the control samples by 21 and 14,9% respectively. These highly-specialized cells in pathology under investigation are substituted by mucocytes and “pylorisation” of fundal glands takes place. In ultrasound study of cardial glands decrease of size and quantity of mucocytes is observed as well as reduction of amount of mitochondria and granular endoplasmic reticulum in them, diminution of the number of secret granules in apical part of cytoplasm is also detected.

There is also reduction of mitochondria’s number in parietal cells which become small, their intracellular channels are reduced, amount of tubulovesicles is decreased. Nuclei are polymorphic, mainly small, with decreasing of chromatin electronic density. Significant quantity of myelin structures occur in the cytoplasm.

The main cells are of small size, their cytoplasm contains a minute quantity of small mitochondria, content of granular endoplasmic reticulum profiles is decreased, reticulum is cistern–like. Quantity of secretory granules is low; they are often of mixed character (mucoidisation). Content of pathologic inclusions and vacuoles is increased with characters of myelin degeneration in mitochondria. Nuclei are of small size, chromatin is significantly clarified. Structures of the lamellar complex are distended by content with low electronic density. Quite a number of parietal and main cells in fundal glands are replacing by mucocytes.

Glands of the pyloric part are affected by atrophy to a less extent, sometimes their moderate proliferation can be observed. Thin-walled vessels of venous type, lymphatic fissure, moderately expressed edema and fibrosis occur in interglandular strome. Submucous layer of stomach is dilated in patients with HC, complicated by PH, due to expressed edema, various focal or diffuse lymphoid-cellular infiltrates as well as different number of

venous type vessels. These vessels are of various diameter, large veins are often located close to mucosa. Dilatation of lymphatic fissures close to mucosa also can be observed in some cases.

It should be noticed that changes in submucous layer parameters in HC, complicated by PH, are maximally occurred in cardial part and minimal in pyloric part. Venous type vessels of the largest diameter are usually found in cardial part and more rarely in pyloric part. It is also of great importance that changes in submucous layer, described above, depend on a level of vascular system development in the zone of cardioesophageal transition. According to our data muscular layer of stomach in HC is thinned, almost by a factor of two in all cases. This process is more expressed in cardial part and more rarely in pyloric one. The leading sign of decrease of muscular layer thickness is atrophy of smooth muscle cells and their substitution by elements of connective tissue. In this case muscular layer is exposed to fasciculation and fibrosis. Venous type vessels of various diameter and lymphoid-cellular infiltration of various intensity are also found there.

Degree of muscular layer fibrosis directly depends on HC duration and level of PH.

Subserous layer of the organ is also affected by edema and respectively increase in thickness. Fiber structure of the connective tissue is disturbed; a lot of vessels of venous type and different diameter, mostly of small diameter are located there.

Erosion were observed in 22 (42%) of section material, ulcerous lesions of stomach were found in 7 (13,4%).

Thus, our study shows that so-called "hepatogenous ulcers" have not only specific localization and quantitative evaluation but specific morphologic features that differ them from classic "peptic ulcers":

1. Basic number of ulcers were localized in pyloroantral part;
2. In half of the cases ulcers were multiple (2-4);
3. Ulcers were mainly flat, with small depth and flat edges (Figure 3);
4. Fundus of the ulcers was covered with a thin layer of fibrinoid necrosis with a very weak development of granulations (Figure 4);
5. There is a large quantity of venous vessels of various diameters in fibrous layer, often located at a small depth;
6. Signs of ulcer epithelization were absent in almost all the cases;
7. Inflammatory infiltration was present in all the cases.

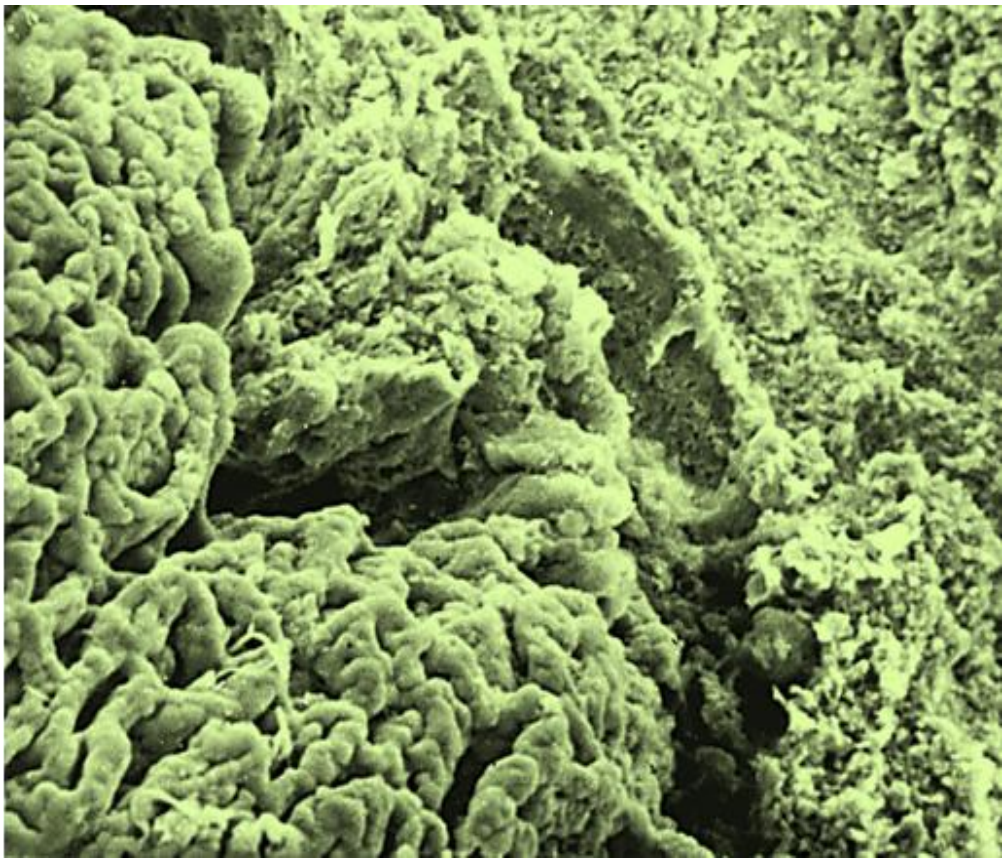

Figure 3. Ulcers were mainly flat, with small depth and flat edges. SEMx60.

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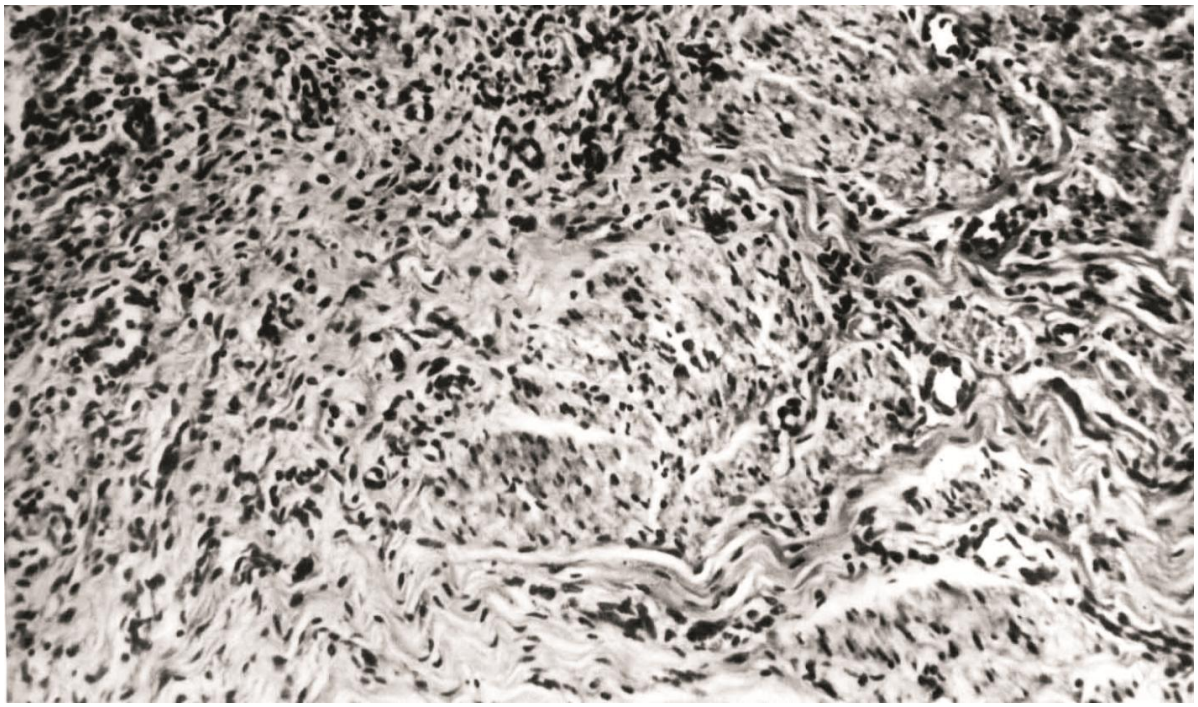

Figure 4. Fundus of the ulcers was covered with a thin layer of fibrinoid necrosis with a very weak development of granulations. LM 10 x 40

Results of our microbiological study are presented in Table 1. The table demonstrates, that Hp in gastric juices persisted in 23 (57,5%) of patients, fungi from Candida genus—in 17 (42,5%) of patients, mold fungi in 3 (7.5%), other microorganisms—in 10 (25,0%) of patients. Hp were more often isolated from gastric juice 23 (57.5 %) of patients, then from biopsy material from cardia 9 (45.0%) of patients and esophagus 5 (25.0%) of patients. IgG antibodies to Hp were detected in 10 (66.6%) of patients and it can be considered as evidence of the fact that persistence of Hp is not indifferent to macroorganism and causes appropriate response.

Along with Hp Candida were often found in examined patients, indicating to a significant decrease of barrier functions of the stomach and organism as a whole. Study of gastric juices acidity showed that only 9 (22,5%) of patients had normal secretion (BAP - basal acid production5-10 mmol/hour), decrease of gastric secretion was detected in other 31 (77.5%) of patients (BAP < 5 mmol/hour). Isolation of Hp depending on gastric juice acidity level was of special interest. Analysis (table 2) shows a tendency of Hh isolation as acidity increases and a reverse tendency was detected for Candida. This tendency was especially clear in analysis content of Hp and Candida content in gastric juice.

One fact also attracts our attention: in all 4 examined patients with bleeding from EVVGD and stomach, who came in emergent order, high intensity of Hp infection (10^4-10^5 CFU/ml) and expressed serologic response (antibodies against Hp), were observed. However we don't have enough data to make concrete conclusions.

Table 1. The results of our microbiological examination of patients with HC and PH

№	Material under study	H.pylori	Candida	Mold fungi	Other microorganisms
1	Gastric juice n=40.	23 (57.5%)	17 (42.5%)	3 (7.5%)	10 (25.0%)
2	Biopsy from gastric cardia.n=20.	9 (45.0%)	13 (65.0%)	0	13(65.0%)
3	Biopsy from esophagus. n=20.	5 (25.0%)	10 (50.0%)	0	10 (50.0%)
4	Blood serum (antibodies to Hp).n=15.	10 (66.6%)	x	x	x

Table 2. Influence of gastric juice acidity on microflora in patients with HC and PH n=40

Acidity level of gastric juice	Number of patients, %	H.pylori		Candida	
		Frequency, %	Contamination CFU/ml M±m	Frequency, %	Contamination CFU/mlM±m
Normal (BAP = 5-10)	9 (22.5%)	6 (66.6%)	$3.9x10^6±2.1x10^5$	4 (44.4%)	$5.0x10^3±1.9x10^2$
Reduced (BAP<5)	31 (77.5%)	16 (51.6%)	$3.0x10^4±1.3x10^3$	21 (67.7%)	$2.0x10^4±1.1x10^3$
P			<0.001		<0.001
Increased (BAP>10)	0	-	-	-	-

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Our data testifies that hypochlorhydria of gastric juice is prevalent in the examined patients, that results in migration of Hp from pyloroantral part of stomach, where acidity is reduced due atrophic processes in mucosa, to upper part of stomach (corpus and cardial part) where they find more appropriate conditions for their vital activity. At the same time reduced gastric acidity favors to Candida development that worsens disbiotic processes in the digestive tract.

DISCUSSION

The relevance of problem erosive-ulcerous lesions in upper part of digestive tract in patients with hepatocirrhosis and portal hypertension in clinical practice is determined by a large number of publications over many years.

Stomach function and secretions are altered significantly in patients with cirrhosis, both with or without portal hypertension motivation by the abnormalities of gastric acid and pepsin secretion, and gastrin release. Histological and endoscopic changes, and the impaired cytoprotection associated with cirrhosis, are discussed in the context of abnormal gastric secretion. In addition, the symptomatology and association of Hp, and treatment of duodenal ulceration in cirrhosis are discussed [11].

The result of many early studies is the conclusion that additional studies are needed to further understand of erosive-ulcerous lesions in upper part of digestive tract in patients with hepatocirrhosis and portal hypertension. Early H. pylori eradication is associated with a lower risk of recurrent peptic ulcers in cirrhotic patients. H. pylori eradication is the mainstay for treating cirrhotic patients who have contracted peptic ulcers [12].

So high frequency of helicobacteriosis in patients with HC and PH may be caused by various general and local factors: total decrease of resistance to infection in patients with HC, disorders in blood circulation in portal system, contributing to local decrease of resistance in mucosa of gastroduodenal zone. In turn Hp invasion worsens all the processes described above, causing progressive mucosa atrophy and development of hypo- and achlorhydria. It is also necessary to remember that various strains of Hp differ by their virulence, i.e. ability for adhesion on GM and, probably, esophagus, and some other pathogenic features that allow Hp to affect tissues of macroorganism up to development of bleeding and other complications. This is confirmed by the studies of Wen et al. [13] H. pylori infection impairs the expressions and functional activities of duodenal mucosal bicarbonate transport proteins, CFTR and SLC26A6, which contributes to the development of duodenal ulcer [13].

Due to unique mobility and some other factors, Hp and products of their metabolism may penetrate into esophagus and manifest pathogenic properties, negatively influencing changed mucosa over EVVGD.

It would be wrong to ignore a question about biological properties of isolated strains of Hp as a possible etiological agent of pathologic processes in mucosa of the gastroduodenal zone and esophagus in patient with HC and PH, however such data were presented in study in our center. The results of the study indicated to the presence of eubiotic drugs characterizing by significant antagonistic activity against Hp cultures, isolated from patients with HC and PH. The author described a group of specific preparations, which can be used in the complex treatment of such patients. Besides correction of intestinal dysbiosis, these preparations exert antagonistic action, direct and indirect, on Hp, persisting in an organism.

Walton [14] in one of the recent issues learn about the virulence factors that have made Helicobacter pylori such a successful pathogen in hepatocirrhosis, when it focuses on in vitro findings that may shed light on epithelial-mesenchymal transition that occurs during the process of fibrosis [14].

Thus, Hp are frequently found in patients with HC and PH and worsen the course of the main disease, contributing to the development of hemorrhages from EVVGD. Pathologic process in these patients lasts for a long time, and they admitted to hospital with advanced cases with considerable atrophy of mucosa and respectively essentially reduced secretory activity; but at the initial phases of the process these symptoms are manifested weaker. Hp infection is much intensive, frequency of the presence of antibodies to Hp is higher than isolation of Hp from stomach, i.e. due to the development of some negative for Hp conditions, microorganisms are eliminated to some extent, but antibodies against Hp continue to circulate in patients' blood.

CONCLUSIONS

1. Distinctive features of so-called "hepatogenous ulcers" are not only their specific localization and quantitative presentation but also some definite morphologic properties: Multiple flat ulcers in pyloroantral part covered with a thin layer of fibrinoid necrosis with a weak development of granulations and number of venous vessels of various diameter in fibrous layer; 2. "Hepatogenous" erosive-ulcerous process in stomach of patients

with HC and PH lasts for a long time with a significant atrophy of mucosa and, respectively, low secretory activity, against background of HP colonization of high intensity, worsening the course of the main disease and contributing to the development of esophageal and gastric hemorrhages.

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Competing interests

The authors declare that they have no competing interests.

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