

ORIGINAL PAPER

The gastric mucosa in portal hypertension: structural and ultrastructural observations

ALINA DRĂGHIA¹⁾, FL. DRĂGHIA²⁾, L. COMAN³⁾

¹⁾*Department of Cellular and Molecular Medicine*

²⁾*Department of Anatomy*

³⁾*Department of Physiology*

"Carol Davila" University of Medicine and Pharmacy, Bucharest

Abstract

The gastric mucosal lesions represent a frequent cause of hemorrhage in the portal hypertension (PHG) and in the hepatic cirrhosis. This study was undertaken to assess the structural and ultra structural modifications in the intimal lamina of the stomach in this pathology. The cells of mucosa show graded alternative transformations. In the gastric mucosa, some of the chief (enzymatic) cells present a quasi-normal histological organization; others increased alterations such as irregular and heterochromatic nuclei, fewer cytoplasm organelles, numerous clear vesicles and heterogeneous lysosomes. The parietal (oxyntic) cells show in their apical cytoplasm wide dilatations of the intracellular canaliculi, vesicles, a reduced number of organelles and irregular nuclei. The enteroendocrine cells (APUD) present an increased number of granules and organelles in the cytoplasm. The connective inter-glandular tissue contains active fibroblasts, micro inflammatory zones, blood vessels with hypertrophied endothelium, irregular and dilated lumen. Subsequent to these alterations in the structures of the gastric mucosa in portal hypertension, the function of synthesis/excretion of the pepsinogen, lipase, hydrochloric acid, intrinsic factor, serotonin, etc. are affected.

Keywords: gastric mucosa, portal hypertension, hepatic cirrhosis, electron microscopy.

Introduction

Gastric mucosal lesions are common in individuals with portal hypertension worldwide [1] with frequent esophageal varices and dangerous sequels. Gastric mucosal lesions can be an important cause of blood loss, which is generally slow and insidious, but can be massive and occasionally fatal [2].

The portal hypertensive gastropathy (PHG), remains the second cause of digestive hemorrhage after those of esophageal varices (70% of patients) [3].

Histophysiological [4] and histochemical [5] alterations of gastric mucosa with hyper-gastric acidity and hypo-pepsin in patients with portal hypertension-gastropathy are frequently signaled. Large clinical observations [6], routine endoscopy [7], magnifying endoscopy [8], and endoscopy associated with screening electron-microscopy [9], represent modalities of examining gastric mucosa lesions, including the portal hypertension. The new technique developed in the last year's "New Band Imaging" (NBI), enhances the diagnosis power of endoscopes, by using narrow band filters, which modify the spectral characteristics of the illuminating light, yielding very clear the images of microvessels on the gastric mucosal surface [10].

The clinical/pathological endoscopical correlations between PHG and the gravity of the hepatic cirrhosis and between the magnifying endoscopic findings with the histopathological aspects represent a problem yet disputed and with some aspects not clearly determined. To have more histopathological precision, the purpose of this presentation is to investigate the structural and

ultrastructural modifications of the gastric mucosa in the portal hypertensive gastropathy.

Material and Methods

Biopsies obtained from 29 patients aged between 30–81 years, suffering of portal hypertension gastropathy, in the Gastroenterology Center, "Fundeni" Hospital, Bucharest, were examined by routine optical microscopy and by electronic microscopy (TEM) Philips, with standard methodology.

Electron microscopy

In the case of the compound examined by electron microscopy, we used a double fixation, initially with a buffer solution of glutaraldehyde, subsequently with a buffer solution of osmium tetroxide (with the function of preserving and coloring the lipids and proteins). We used the inclusion in synthetic resins because this prevents the tissue atrophy caused by the high temperatures needed for the inclusion, and the resulting compounds were of higher quality. Very resistant blocks were obtained, which were sectioned with knives having glass (diamond) blades. The extreme thin sections (40/90 nm) were transferred onto small metal grids, in order to be examined by microscope. Images with very high resolution (0.1 nm) were obtained, which allowed a magnifying up to $\times 20\,000$. The image capture is the result of a balance between the electrons that hit the fluorescent screen and those retained in the microscope tube. Most of the electrons reaching the objective generate a magnified image which, when projected through other lenses, will be magnified additionally.

Optical microscopy

We obtained the optical microscopy compounds by using an isotonic buffer solution of formaldehyde 4%. Subsequently we included the compounds in paraffin. Previously we carried out dehydration and clarification to the initial purpose of extracting the water from the tissues by successively introducing the compounds in seriated solutions of ethanol and water with an ethanol concentration of 70–100%. Subsequently we replaced the ethanol with xylene (which is a miscible solvent with inclusion medium, namely paraffin). After the solvent penetrated the tissues, these were clarified and became transparent. After the initial impregnation with ethanol and subsequently with xylene, we placed the tissue (embedded in melted paraffin) into the oven at a temperature of 50–60°C. The role of this phase is to cause evaporation and to allow the paraffin to penetrate the tissue spaces. After the extraction from the oven, the tissue and paraffin solidify. We obtained solid block that we introduced in the microtome and produced, by using a steel blade, sections with a thickness between 1–10 µm. We colored the compound obtained in this way with Hematoxylin–Eosin, thus highlighting the basophil components (the cells' nuclei, the ARN rich cytoplasm regions) and the acidophil components (mitochondria, secretor granules). The image capture is realized by the interaction between the light fascicle and the tissue components. We used a high resolution and quality lenses in order to obtain a magnified image. To enhance the power of the microscope, we used a photo/video camera, which allowed obtaining digital images that were stored on the computer.

Results

In PHG, the gastric (stomachal) mucous shows the most significant changes, with cellular alteration in the surface epithelium and in the chorion structures. The epithelium appears segmentarily interrupted, with cellular regions of normal aspect interchanging with acellular areas (Figure 1). The cellular lesions that occur on the surface are as frequent as the sanguine cellular

elements from the main stem, existing in the basal part of the epithelium and its basal membrane. There were cases where luminal hemorrhages (Figures 2 and 3) also were seen. The subjacent chorion presents gastric glands with enlarged lumen, with sinuously layout and with losses of secretory cells towards the epithelium surface. Enclosing the gland and inside it there can be observed vascular blood stems with sanguineous stasis, edema, microhemorrhages and inflammatory infiltrate of different degrees (Figure 4). Superficial epithelium shows erosions, and microhemorrhage in some area with inflammatory infiltrate, and fibrin purulent exudates into the lumen, in the acute, active gastritis.

The chief cells (zymogene cells) present a round-oval nucleus with clusters of heterochromatin attached to the inner face of the nucleolemma. The cytoplasm contains abundant synthesizing and exporting organelles. Between the quasi-normal cells, appear others, with modified and altered ultrastructural organization. In these cells, the irregular nucleus and a cytoplasm are charged with great, clear, vesicles, and tertiary lysosomes appear (Figures 5 and 6).

The parietal (oxyntic) cells show irregular nuclei containing equally euchromatin and heterochromatin. A relative number of mitochondria and structures of endoplasmic reticulum can be seen in the cytoplasm. The most striking feature is the presence of very large, irregular intracellular canalicles in the apical region of the cells, below the plasmalemma, and numerous microvilli at the luminal surface (Figures 7 and 8).

The enteroendocrine cells have round nuclei; the nucleolemma presents few short invaginations, the euchromatin is predominant. Cytoplasm is rich in synthesizing organelles (mitochondria, endoplasmic reticulum, Golgi complex), and in a great number of electron-light and electron-dense secretor granules. Short microvilli are present at the cellular membrane surface (Figure 9).

The inter-glandular connective tissue presents microzones of hemorrhage, active fibroblasts, scattered neutrophils, frequent lymphocytes and macrophages (Figure 10).

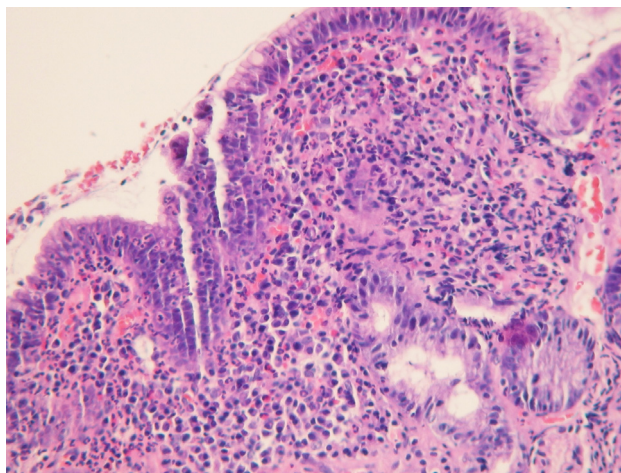


Figure 1 – PHG. Gastric mucosa: optic microscopy. Gastric mucosa present erosion of the surface epithelium, microhemorrhage and inflammatory infiltrate in the chorion (×132).

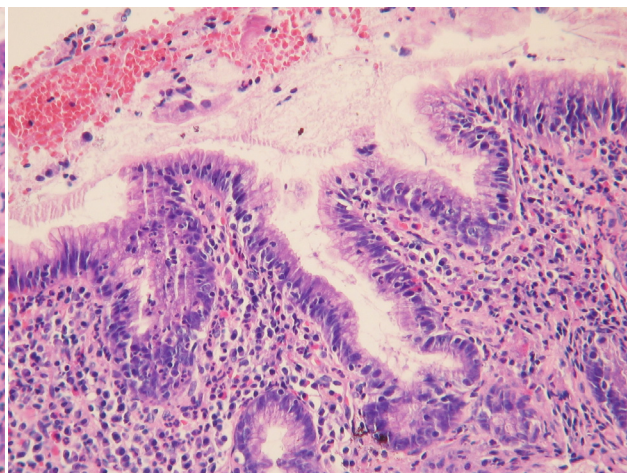


Figure 2 – PHG. Gastric mucosa: optic microscopy. Gastric mucosa with abundant inflammatory infiltrate. Hemorrhage in the gastric lumen (×132).

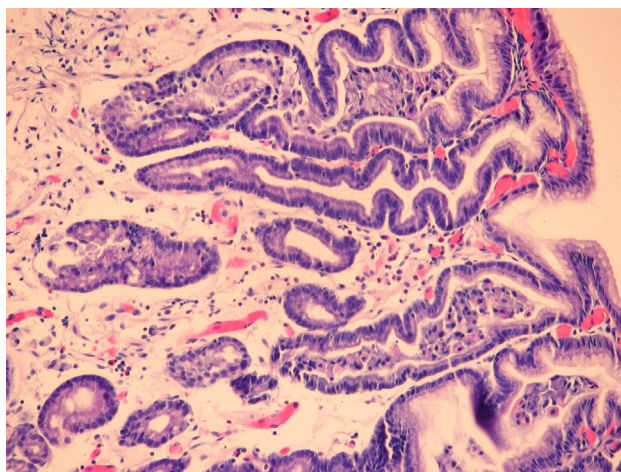


Figure 3 – PHG. Gastric mucosa: optic microscopy. Gastric mucosa with dilated glands, sinuous, lapped in chorion with rare hemorrhage, and blood vessels with stasis ($\times 132$).

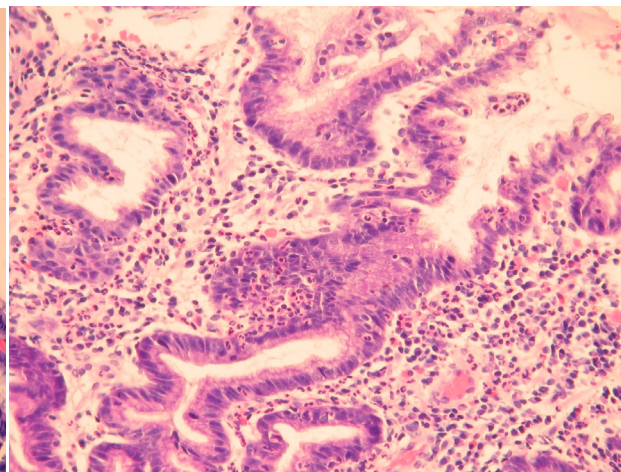


Figure 4 – PHG. Gastric mucosa: optic microscopy. Gastric mucosa presents glands with large lumen, lapped in chorion with inflammatory infiltrate and microhemorrhages. Up to surface, the glandular epithelium spoiled ($\times 132$).

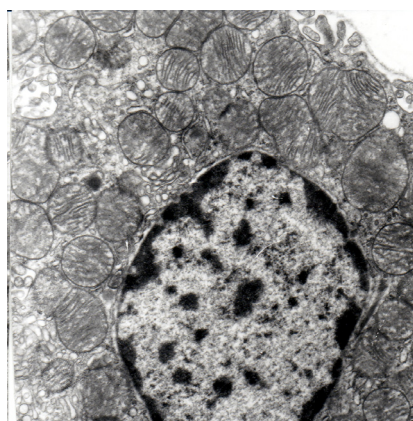


Figure 5 – Gastric mucosa. Electron microscopy. Cells with eu-chromatic nucleus, numerous mitochondria in the cytoplasm and frequent microvesicles ($\times 13\ 000$).

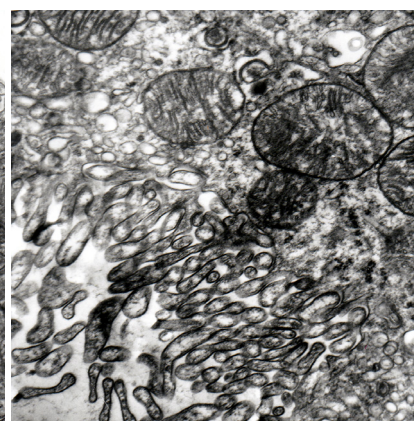


Figure 6 – Gastric mucosa. PHG: electron microscopy. Apical chief cell showing a great number of microvilli and an apical cytoplasm rich in mitochondria, clear microvesicles and dense bodies ($\times 12\ 000$).

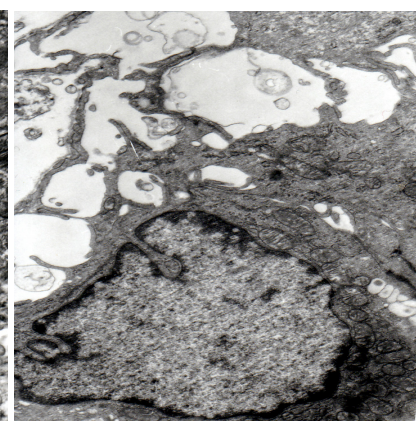


Figure 7 – Gastric mucosa. PHG. Electron microscopy of an active parietal cell with eu-chromatic irregular nucleus, and large intracellular canaliculi at the apical region below the plasmalemma ($\times 20\ 000$).

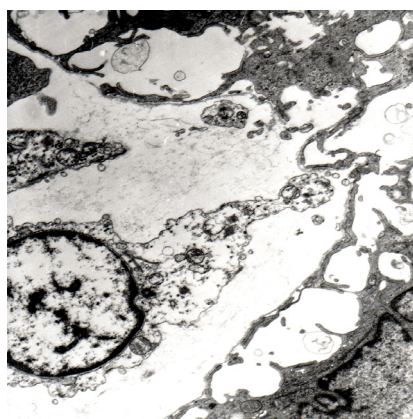


Figure 8 – Gastric mucosa. PHG. Electron microscopy of two adjacent parietal cells, with large tubulo-vesicles at the cell apical cytoplasm. Between them, frequent active fibroblasts surrounded by edema can be observed ($\times 20\ 000$).

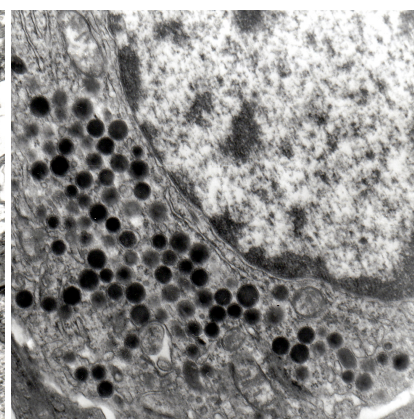


Figure 9 – Gastric mucosa. PHG. Electron microscopy of an entero-endocrine cell. The nucleus is rich in euchromatin and dark vesicles in the cytoplasm ($\times 20\ 000$).

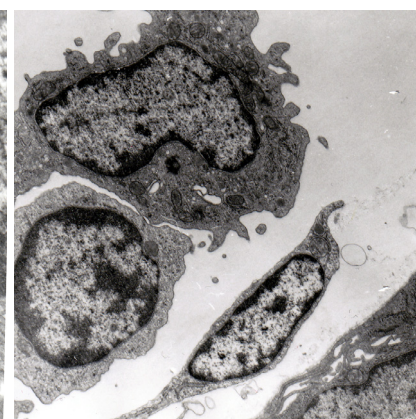


Figure 10 – Gastric mucosa. PHG. Electron microscopy: connective interstice with fibroblasts, lymphocytes, macrophages, embedded in edema ($\times 10\ 000$).

Thin and thick collagen bands, frequently with entrapped red blood cells, inflammatory cells, superficial mucosal capillaries, with unequal, dilated lumen and hypertrophic endothelium protruding in the vascular lumen, are present.

Descriptive statistics of the lots

Clinically, 22 patients of the group of 29 patients with portal hypertension were aged between 45 and 60 years. The most frequent risk factors in the portal hypertension and cirrhosis were alcohol and viral infection (15 patients), alcohol (five patients), and viral infection (two patients).

Upper gastro-intestinal endoscopy showed esophageal varices, portal hypertensive gastropathy. Fragments measuring 0.1–0.3 cm of gastric mucosa were biopsied and histopathologically analysed. The routine histological techniques (HE, Van Gieson) have been used, after the fixation of specimens in buffer formalin and embedding in paraffin.

From the group taken in study, seven cases have been selected for the electron microscopical study. The patients were studied after they were diagnosed by histopathological examinations and with their consent.

Discussion

In the present studied group, the number of male patients with cirrhosis/PHG was prevalent; the risk factors in decreasing order were the alcohol/viral infections, the alcohol and the viral infections separately.

The electron microscopical observations show great alterations of the gastric mucosa in all investigated patients.

The modifications appear in various grades, differing from one zone to the other. The apex of the mucosa, mostly affected, presents epithelial erosions, hemorrhage, micro-inflammation, alternative modifications of the undifferentiated cells found in the neck region.

The ultrastructural modification of the undifferentiated cells are followed by perturbation of their function, since these cells move upward and replace the pit and surface mucous cells, in a turn-over time of 3–7 days. Others of the undifferentiated cells migrate into the glands to give rise to the glandular neck cells, chief cells, parietal cells and enteroendocrine cells. Consequently, the glandular activities are slowed down or even stopped because the replacement of these cells is much slower than that of the surface mucous cells [11].

The modifications in the ultrastructural organizations appear in the different groups of the chief cells inserted among microsomes of quasi-normal zymogenic cells. Although chief cells, with normal histological aspect are present in microgroups, the altered cells are responsible for a decreased secretion of pepsinogen; its conversion into the high active proteolytic enzyme pepsin is shortening and represents, along with other enzymes disturbances (lipase, gastrin), factors of digestive troubles.

The parietal cells present vesicles of various diameters, with serious perturbation in their structural organization. The membrane changes are increased or diminished

even with a blockade of the secretion and the liberation of hydrochloric acid, producing hyperacidity or achlorhydria. Vitamin B₁₂ deficiency, the intrinsic factor deficiency, determines anemia in the great majority of PHG patients. In agreement with Agnihotri N *et al.* (1997) [12], we find a decreasing number of the parietal cells in PHG.

The membrane transport towards the gastric juice, depending of the K⁺ ions, and the presence of ATP-ase [13] factors affected in PHG [5] modify the intracellular pH and alter the physiological status of the mucosa.

The enteroendocrine cells, presenting low modifications, give the impression to have special etiopathogenic role, with special and precise target over the gastric mucosa.

The microclimate of connective-glandular tissue with active fibroblast, inflammatory cells, edema, capillary stasis, blood vessels with irregular lumen and hypertrophic endothelium add a great factor of unfavorable function in the gastric mucosa, favoring the appearance in different degrees of the hyaline process, of superficial micro-hemorrhage in the tissue, of reactions associated with numerous micro and macrophages.

We can appreciate, at the end of the present ultrastructural study, that the portal-hypertensive gastropathy is accompanied by alterations of the gastric mucosal histopathology, recognized also by others [14–16]. Good knowledge and precise information could be of real help for an efficient therapeutical comportment to prevent the mortality caused by the hemorrhage.

The patients diagnosed with severe hepatic disorders taken in observation give us the possibility to consider, along with others, acceptable the idea that PHG could be a marker for a grave hepatic malady, and a precursor of a variceal hemorrhage [18, 19], even for melena, frequently the cause to a chronic anemia [20].

Conclusions

The portal hypertensive gastropathy is associated with great structural and physiological modifications in the gastric mucosa which affect the structure and functions of the surface epithelium: erosions, hemorrhage, regeneration, mucous secretions, alterations of the chief cells (secretion of pepsinogen, lipase, gastrin), and of the parietal cells secretions (hydrochloric acid, intrinsic factors). They are involved in the stability and functionality of vascular endothelium, concomitant with the appearance of micro-hemorrhage in the inter-glandular connective tissue and the mucosal surface, edema and micro-zones of inflammations.

In consequence, almost the whole activity of gastric digestion is compromised. The present observations draw the attention to the important cause of digestive hemorrhage in hepatic cirrhosis, insufficiently well known and frequently ignored, with a high percentage of mortality and limited therapy.

References

- [1] PAGLIARO L, D'AMICO G, LUCA A, PASTA L, POLITI F, ARAGONA E, MALIZIA G, *Portal hypertension: diagnosis and treatment*, J Hepatol, 1955, 23(Suppl 1):36–44.

- [2] GHEORGHE C, Actualități în sindromul de hipertensiune portală. In: VOICULESCU M (ed), *Actualități în hepatologie*, InfoMedica, 1996, 165–200.
- [3] ATTERBURY CE, Portal hypertension. In: SPIRO HM (ed), *Clinical gastroenterology*, McGraw-Hill, New York, 1993, 1163–1177.
- [4] IWAO T, TOYONAGA A, YKEGAMI M, OHO K, SUMINO M, HARADA H, SAKAKI M, SHIGEMORI H, AOKI T, TANIKAWA K, *Reduced gastric mucosal blood flow in patients with portal-hypertensive gastropathy*, Hepatology, 1993, 18(1):36–40.
- [5] DRĂGHIA AC, *Histochemical and histopathological study of the gastric mucosa in the portal hypertensive gastropathy*, Rom J Morphol Embryol, 2006, 47(3):259–262.
- [6] MCCORMACK TT, SIMS J, EYRE-BROOK I, KENNEDY H, GOEPEL J, JOHNSON AG, TRIGER DR, *Gastric lesions in portal hypertension: inflammatory gastritis of congestive gastropathy?*, Gut, 1985, 26(11):1226–1232.
- [7] TAJIRI H, MATSUDA K, FUJISAKI J, *What can we see with the endoscope? Present status and future perspectives*, Dig Endosc, 2002, 14(4):131–137.
- [8] NAKAYOSHI T, TAJIRI H, MATSUDA K, KAISE M, IKEGAMI M, SASAKI H, *Magnifying endoscopy combined with narrow band imaging system for early gastric cancer: correlation of vascular pattern with histopathology*, Endoscopy, 2006, 36(12):1080–1084.
- [9] CALÈS P, OBERTE F, DELMOTTE JS, BASLÉ M, CASA C, ARNAUD JP, *Gastric mucosal surface in cirrhosis evaluated by magnifying endoscopy and scanning electronic microscopy*, Endoscopy, 2000, 32(8):614–623.
- [10] GHEORGHE C, COTRUTA B, IACOB R, BECHEANU G, DUMBRAVA M, HERLEA V, SUCIU S, BANCILA I, *The value of magnifying endoscopy with narrow band imaging in the prediction of malignancy in equivocal gastric lesions*, Endoscopy, 2008, 40(Suppl 1):A170.
- [11] JUNQUEIRA LC, CARNEIRO J, KEILEY RD, *Basic Histology*, Prentice-Hall International Ltd., London, 2000, 292.
- [12] AGNIHOTRI N, KAUR S, DILAWARI JB, BHUSNURMATH SR, KAUR U, *Diminution in parietal cell number in experimental portal hypertensive gastropathy*, Dig Dis Sci, 1997, 42(2):431–439.
- [13] GANONG WF, *Review of medical physiology*, 21st edition, Lange Medical Publishers, Los Altos, California, 2003, 402–403.
- [14] IWAO T, TOYONAGA A, TANAKA J, YAMASHITA F, IRIE A, HARADA H, MITSUYAMA K, SUMINO M, TAKAGI K, SASAKI E, TANIKAWA K, SHIMOTSUURA Y, IDE K, *Endoscopy and clinical study on hemorrhagic gastritis in patients with liver cirrhosis*, Dig Endosc, 1990, 2(1):28–39.
- [15] BECK PL, LEE SS, MCKNIGHT GW, WALLACE JL, *Characterization of spontaneous and ethanol-induced gastric damage in cirrhotic rats*, Gastroenterology, 1992, 103(3):1048–1055.
- [16] PAYEN JL, CALES P, PIENKOWSKI P, SOZZANI P, KERVAN A, FREXINOS J, PASCAL JP, *Weakness of mucosal barrier in portal hypertensive gastropathy of alcoholic cirrhosis. Effects of propranolol and enprostil*, J Hepatol, 1995, 23(6):689–696.
- [17] GUPTA R, SARASWAT VA, KUMAR M, NAIK SR, PANDEY R, *Frequency and factors influencing portal hypertensive gastropathy and duodenopathy in cirrhotic portal hypertension*, J Gastroenterol Hepatol, 1996, 11(8):728–733.
- [18] PRIMIGNANI M, CARPINELLI L, PREATONI P, BATTAGLIA G, CARTA A, PRADA A, CESTARI R, ANGELI P, GATTA A, ROSSI A, SPINZI G, DE FRANCHIS R, *Natural history of portal hypertensive gastropathy in patients with liver cirrhosis. The New Italian Endoscopic Club for the study and treatment of esophageal varices (NIEC)*, Gastroenterology, 2000, 119(1):181–187.
- [19] ZOLI M, MERKEL C, MAGALLOTTI D, MARCHESINI G, GATTA A, PISI E, *Evaluation of a new endoscopic index to predict first bleeding from the upper gastrointestinal tract in patients with cirrhosis*, Hepatology, 1996, 24(5):1047–1052.
- [20] SARIN SK, SHAHI HM, JAIN M, JAIN AK, ISSAR SK, MURTHY NS, *The natural history of portal hypertensive gastropathy: influence of variceal eradication*, Am J Gastroenterol, 2000, 95(10):2888–2893.

Corresponding author

Alina Drăghia, Assistant Professor, MD, PhD, Department of Cellular and Molecular Medicine, "Carol Davila" University of Medicine and Pharmacy, 8 Eroilor Sanitari Avenue, 050474 Bucharest, Romania; Phone +40721–183 270, e-mail: dfraghia@yahoo.com, dfraghia@gmail.com

Received: June 1st, 2009

Accepted: March 5th, 2010