Evseyev A.N., Plekhova N.G., Evseyeva A.A.

Morphological changes in gastric mucosa in hemorrhagic fever with renal syndrome

ABSTRACT

Gastric biopsy specimens in acute hemorrhagic fever with renal syndrome cases were examined using indirect immunolabeling of the viral antigen assisted by confocal laser scanning and electron microscopy. Degenerative and necrotic cellular changes combined with signs of focal hyperplasia of gastric focal epithelium and incipient shift of epithelial differentiation towards mucocytes to the profound epithelial proliferation combined with poor differentiation of highly specialized cells (chief and parietal cells) were revealed. Ultrastructural virus-specific inclusions in the cells were found and dependence thereof on the adaptive rearrangement of gastric mucosa was established.

Key words: Hemorrhagic fever with renal syndrome, immunofluorescence, immunoelectron microscopy, gastric mucosa, erosions, acute ulcers, endothelium, macrophages

Generalized microvessel lesion with damage to different tissues and organs and subsequent inflammatory and reparative processes represent a pathogenic pathway of hemorrhagic fever with renal syndrome (HFRS) (1, 2, 3, 4). No morphological study into the mechanisms of gastric mucosa (GM) damage in HFRS have ever been done till the present date. There are almost no data on the condition of different GM regions based on comprehensive morphological assessment using immunoelectron microscopy.

The aim is to provide comprehensive morphological assessment of GM changes at the acute stage of HFRS and establish the relationship between the changes and the antigen availability in different cells.

MATERIALS AND METHODS

Gastric biopsy specimens of ten HFRS patients obtained within 6 to 14 days from the onset of clinical manifestation of HFRS were fixed in 10% neutral formalin and embedded in paraffin. Serial sections were stained by Ehrlich's hematoxylin and eosin; neutral mucopolysaccharides were identified by PAS-reaction, acidic mucopolysaccharides – by Alcian blue staining with additional hematoxylin staining of nuclei. In addition, immunofluorescence by means of confocal laser scanning microscopy and immunoelectron microscopy of biopsy

specimens was used. Gastric biopsy specimens were studied with electron microscope JEM-100S (JEOL, Japan) with an accelerating voltage of 80 kV in a diffraction contrast mode.

RESULTS

Gastroscopy of HFRS patients at different stages of the disease revealed mucosal thickening of gastric body, cardia and antrum due to hyperemia and edemas. Here and there, gastric folds appeared ridged with punctate hemorrhage, acute erosions and ulcers. With light microscopy, gastric changes were found to be diffuse, involving superficial and deep layers. Degenerative changes and necroses of superficial epithelial structures and focal stromal hemorrhage were prevailing (see Fig. 1). Gastric foveolar epithelium was represented by tall columnar cells with pyknotic nuclei located in the basal part. Signs of hypersecretion were found in superficial-foveolar epithelium – a thick layer of PAS-positive mucin that was located on the cell surface (Fig. 2).

Foveolar cell cytoplasm was found to contain alcianophilic granules with glycosaminoglycans. Lamina propria of gastric mucosa presented with stromal edema, microvasculature hyperemia and stases, focal hemorrhage, polymorph cell infiltration.

Microscopic study: erosion base was found to be covered with mucus and fibrin and infiltrated with polymorphonuclear leukocytes. In addition, vascular hyperemia, stases, red blood cell aggregation (erythrocyte sludge) with microthrombi in stromal microvessels were identified.

Acute ulcers looked like oval defects of mucosa. The ulcer base was colored grayish-red; some ulcers presented with arrosion-damaged vessels. Histopathology of ulcer base revealed necrotic matter impregnated with fibrin and infiltrated with polymorphonuclear neutrophils. Areas of fibrinoid necrosis and granulations were found below the necrotic matter (Fig. 3).

Confocal microscopy revealed specific coarse-granular luminescence in GM capillary epithelium and endothelium, which was indicative of Hantavirus antigen (Fig. 4).

Electron microscopy study of gastric biopsy specimens revealed degenerative changes in GM superficial epithelial cells. Mucocytes were found to have lost their microvilli; the entire apical surface was occupied by vacuoles that pushed mucoid granules and the nucleus to the periphery of the cell; the cells were overloaded with mature mucoid showing degenerative changes (Fig. 5).

An ultrastructural study of gastric biopsy specimens revealed positive response to Hantavirus antigen in macrophage type cells. Hantavirus inclusion particles were found in cytoplasm areas of higher ribosome content and a great number of endoplasmic reticulum

cisterns (Fig. 6). Mucocyte and macrophage cytoplasm presented with solid viroplasts, double-layer membrane structures as well as laminar structures.

Clasmatotic outgrowths on the surface of the cells, expansion of endoplasmic reticulum, mitochondrial vacuolation with cytolemma rupture were observed. Vacuolated cells were observed on the top of the folds, the sides of the foveolae, and in the depth of the cristae. A part of the cells was found to lose microvilli and desmosomes and separate from the adjacent cells of the epithelial layer. Chromatin was often condensed into large dense lumps. Cellular nuclei were often subject to condensation and looked like apoptotic corpuscles. Intercellular spaces appeared extended with polynuclear leukocytes, lymphocytes and macrophages contained therein.

The cytoplasm of the principal cells presented with sites of both higher and lower electronic density with a great number of vacuoles varying in size. The number of rough-surface endoplasmic reticular structures appeared somewhat decreased, the nuclei acquired an irregular shape with a plicate surface and clusters of chromatin near the karyolemma. The cytoplasm also presented with solid viroplasts, double-layer membrane structures as well as laminar and tubular structures.

Ultrastructurally, accessory cells presented with reduction in the apical part of the cytoplasm, loss of microvilli, invagination in the intracellular tubules, virus-induced structures available, increased number of lysosomes and autophagosomes, rarefied matrix and reduced mitochondrial cristae, and extended areas of intercellular contact.

The proper lamina of gastric mucosa presented with cellular infiltrations of plasmacytes, lymphocytes, neutrophilic polynuclear leukocytes and erythrocytes. Endothelial cells of blood vessels appeared swollen, bearing signs of villous transformation, with extension of the granular ergastic reticulum, mitochondrial destruction, clusters of myelin-like structures, and virus-like particle bearing erythrocytes and macrophages in the vascular lumens.

Our investigation has shown that GM at the acute stage of HFRS presents with the prevalence of alterative ultrastructural changes in the cells combined with signs of focal hyperplasia of gastric focal epithelium, incipient shift of epithelial differentiation towards mucocytes secondary to the profound epithelial proliferation combined with poor differentiation of highly specialized cells (chief and parietal cells) were revealed. Ultrastructural virus-specific inclusions in the cells were found and dependence thereof on the adaptive rearrangement of gastric mucosa was established.

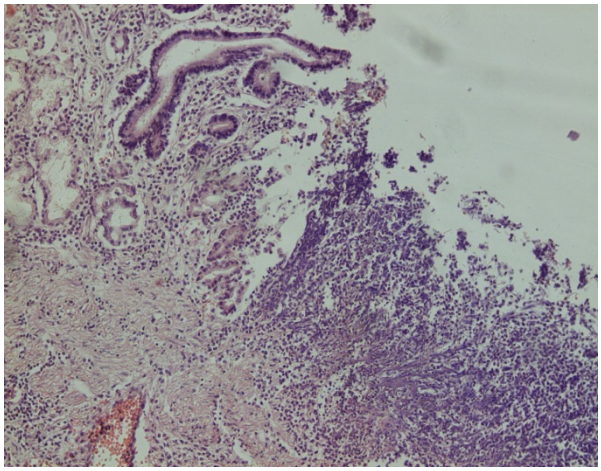

Fig. 1.General view of a biopsied fragmen to gastric mucosa: multiple superficial hemorrhage, degenerative and necrotic changes of foveolar epithelium, stromal edema. Disease duration: 7 days. H&E staining, x 80.

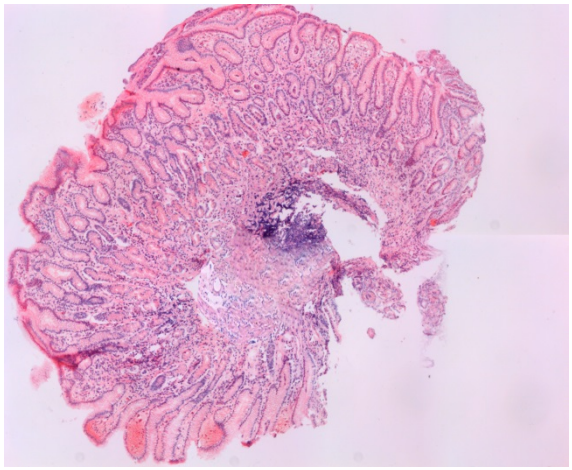

Fig. 2.Mucin hyperproduction in gastric mucosa. Biopsy. Disease duration: 9 days. PAS-reaction, x100

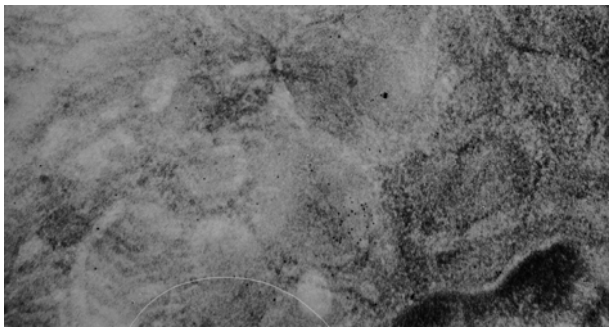

Fig. 3.Acute gastric ulcer: the area of fibrinoid necrosis extends to muscular coat, abundant infiltration with polymorphonuclear leukocytes, stromal edema, hyperemia and stases in the vessel. Biopsy. Disease duration: 10 days. H&E staining, x 100.

Fig. 4.Granular specific luminescence of Hanta virus antigen in GM epithelial and endothelial cells. Gastric biopsy specimen. Disease duration: 7 days. Laser scanning confocal microscopy. Indirect IFA, x 1000

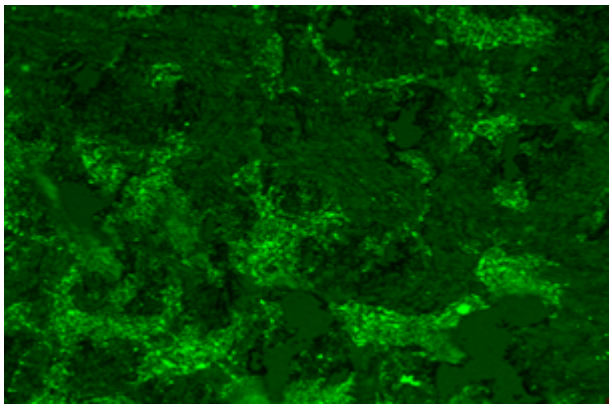

Fig. 5.Gastricmucosa: free surface of mucocytes contains single microvilli, close fitting secretion granules located in the apical part with destructive changes. Biopsy. Disease duration: 7 days. Electronogram, x 3000.

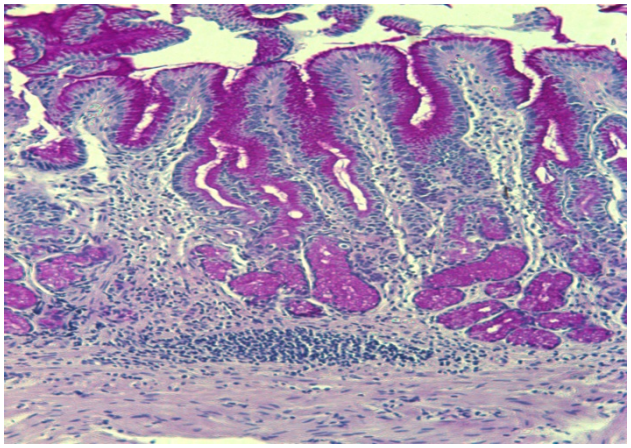

Fig. 6.Macrophage type cell in the gastric biopsy specimen of a HFRS patient. A good view of well-developed endoplasmic reticulum, chromatin condensation at the karyolemma, and virus-specific structures with a positive response to Hantavirus antigen (x50000)

References:

1. Zelenskiy A.I. Kovalskiy G.S. Konstantinov A.A. [et al.]. Gemorragicheskaya likhoradka na Dalnem Vostoke SSSR [Hemorrhagic Fever in the Russian Far East of the USSR]. Khabarovsk, 1979, P. 110.

2. Evseev A.N. Gemorragicheskaya likhoradka s pochechnym sindromom. Pato- i morfogenez, makro- i mikroskopicheskoe issledovanie: monografiya. [Hemorrhagic Fever with Renal Syndrome. Patho- and Morphogenesis, Macro- and Microscopic Study: Monograph]. Khabarovsk: OOO Omega-Press, 2010, 296 p.

3. Kaminskiy Yu.V. Klinicheskaya morfologiya byfektsionnykh zabolevaniy v Primorie (pod redaktsiey OG Polushina) [Clinical Morphology of Infectious Diseases in Primorye (Edited by: OG Polushin)]. Vladivostok: Meditsina DV, 2011, 216 p.

4. Sirotin B.Z. Ocherki izucheniya gemorragicheskoi likhoradki s pochechnym sindromom: Monografiya [Essays of Hemorrhagic Fever with Renal Syndrome Study: Monograph]. Khabarovsk: RIOTIP, 2005, 194 p.

Authors:

1. **Evseev AlexeyN.** «Far Eastern state Medical University» of Ministry of healthcare of the Russian Federation, Khabarovsk str. Muravyov-Amursky 35

candidate of medical Sciences , head of the course of pathological anatomy Department of pathological anatomy and forensic medicine

Contact phone 89141580564 slave. 8(4212)409564

E-mail alexevseyev@yandex.ru

2. **Plekhova NatalyaG.**

Head of Central research laboratory of «Pacific medical University» of Ministry of healthcare of the Russian Federation

690002, Vladivostok, ProspektOstryakova, 2

D. SC.N. Plekhova Natalya hundred 89623396391

e-mail: pl_nat@hotmail.com

3. **Evseeva Alena A.** senior laboratory of the Department of pathological anatomy and forensic medicine of the medical faculty «Far Eastern state Medical University» of Ministry of healthcare of the Russian Federation Khabarovsk street Muraveva-Amur 35
G. Khabarovsk St. Brick 36A D. 23 sq servant.8(4212)409564
e-mail: alenaevseeva.93@mail.ru