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FEATURES STATE OF THE MUCOUS MEMBRANE OF THE STOMACH AND DUODENUM WITH PEPTIC ULCER DISEASE WHEN IT COMBINED WITH DIABETES MELLITUS II TYPE.

Belousova Kateryna

студент

Sithinska Inna

к.м.н., ассистент

Higher State Educational Establishment of Ukraine "Higher State Medical University"

Chernivtsi, Ukraine

Annotation: The article presents data on histological changes of the gastric and duodenum mucosa with peptic ulcer disease (PUD) and second type of diabetes mellitus. The presence of toxigenic strains of HP infection contributes to the development of edema of the stroma, the sludge phenomenon, desquamation of the epithelium etc. These changes are detected on the background of low indicators of mucus formation (optical density in the cervical mucosa cells, covering epithelium, surface mucosa, goblet cells) considering the strains of toxigenicity of HP infection and combination the peptic ulcer disease of stomach and duodenum with diabetes mellitus second type.

Keywords. Toxigenicity of the strains of HP infection, peptic ulcer, stomach, duodenum, diabetes mellitus of the second type (DM2).

Relevance of the topic. At present, the basic cause of PUD is an infection of the mucous membrane of the stomach and duodenum by Helicobacter pylori (HP)[1], high virulence genes which determine such as cagA, vagA, babA, icaA [2, 3, 4, 5]. The presence of the association between infection with toxigenic HP strains (CagA+,VacA+) the severity of chronic gastroduodenal pathology [6]. The risk of HP

infection increases with type 2 diabetes, because hyperglycemia contributes to the development of HP infection, the impact which on chronic inflammation can lead to increased insulin resistance (IR) and progressing DM2.[5] It is known that the effectiveness of eradication of HP influence the compliance of the patient, the growth of acid production, a high degree of invasion of the mucosa, characteristics of the HP strains, the resistance to antibiotics, the severity of which depends on the choice of therapy regimens Helicobacter pylori infection [2,3].

Purpose of the work. To assess the condition of the mucous membrane (MM) of the stomach and duodenum, combined with DM2 considering the toxigenicity of strains of HP infection.

Materials and methods. 144 patients who gave consent to participate in the study and were divided into groups: 1 group – 53 patients with stomach (n=33) and duodenal ulcer (n=20) without signs of DM2, 2group – 67 patients with stomach (n=39) and duodenal ulcer (n=28) associated with DM2. NDA of HP was extracted from the biopsy (MM) specimens of the antrum of the stomach using special kits ("Litex".U Russia) The genes cagA and vacA of Helicobacter pylori from biopsy was determined using special kits such as "Helicopol" ("Litex" Russia). The intensity of the signal in the gel was determined using the following criteria signal: weak (+), moderate (++), strong (+++).In the development take the results with weak, moderate and strong signal. Evaluation of the detection of HP genes as cagA and vacA and theirs alleles carried out according to the instructions, namely the presence of a DNA fragment of the gene cagA 404 of the light filter and vacA (alleles s1+s2,m1,m2) on 259 +286, 290 and 352 accordingly. Blood glucose levels researched by glucose oxidase method using standard kits of production reagents by SPE "Filisit diagnosis" (Ukraine). Glycated hemoglobin (HbA1c) was determined using the photocolorimetric method by reagents "SpineLab" company (Ukraine, Kharkiv). To study oxidative modification of proteins suitable histochemical technique for acid and basic proteins with bromophenol blue according to Michael Calvo. With this technique, proteins which are dominated by carboxyl groups over amino groups, painted red, and proteins, which is dominated by amino groups over carboxyl groups,

in the blue color. Oxidative modification and glycosylation of the proteins leads to the transformation theirs amino groups, which leads to relative predominance of carboxyl groups over amino groups. Quantitatively and accurately with a high degree of reproducibility it can be assessed by method of computer micro-spectrophotometry on the basis of the R/B coefficient. The red color predominance, shows an increase in this indicator above the unit and blue in the color - reduction factor below units.

Research result. On the basis of staining with hematoxylin and eosin were obtained other morphometric indicators of the state of the mucous membrane of stomach (MMS) and duodenum (MMD) (Table 1-4). In General, the analysis of tables allows to conclude that in cagA+vacA+ patients state of the MMS and MMD worse, than in cagA+ or vacA+ patients. This is especially well illustrated by the manifestations of inflammatory reactions which appreciated not only by the level of inflammatory infiltration by polymorphonuclear leukocytes (PMNL), but also taking into account such phenomena of exudation as stromal edema, hemorrhage, blood stasis and sludge of erythrocytes. The level of desquamation of the surface epithelium indicated the level of alteration (damage) of these cells. In patients with peptic ulcer with DM2 the damage of MMS and MMD for individual indicators were more pronounced.

Table 1

Morphometric indicators of the condition of mucosa in patients with PUD of stomach without comorbidities, M±m

Indicators	cagA+vacA+ n=17	cagA+ or vacA+ n=13
Desquamation of the surface epithelium (Points from 0 to 5)	3,1±0,04	2,1±0,04 p<0,05
The percentage of blood vessels with symptoms of stasis and (or) sludge of erythrocytes (%)	34,0±1,8	12,0±0,7 p<0,05
Stromal edema	2,4±0,07	1,2±0,03

(Points from 0 to 5)		p<0,05
Hemorrhage in stroma (Points from 0 to 5)	1,8±0,04	1,3±0,03 p<0,05
The degree of infiltration of PMNL (Points from 0 to 5)	3,1±0,08	1,1±0,02 p<0,05

Note. p < 0.05 - reliability of differences of indicators between these groups.

Table 2

Morphometric indicators of the condition of mucosa in patients with PUD of duodenum without comorbidities, M±m

Indicators	cagA+vacA+ n=11	cagA+ or vacA+ n=7
Desquamation of the enterocytes (Points from 0 to 5)	2,4±0,05	1,8±0,04 p<0,05
The percentage of blood vessels with symptoms of stasis and (or) sludge of erythrocytes (%)	32,0±1,6	7,0±0,4 p<0,05
Stromal edema (Points from 0 to 5)	3,7±0,07	1,2±0,02 p<0,05
Hemorrhage in stroma (Points from 0 to 5)	2,4±0,05	1,1±0,02 p<0,05
The degree of infiltration of PMNL (Points from 0 to 5)	2,8±0,07	1,1±0,04 p<0,05
The percentage of goblet cells (%)	14,0±0,9	24,0±1,2 p<0,05

Note. p < 0.05 - reliability of differences of indicators between these groups.

Table 3

Morphometric indicators of the condition of mucosa in patients with PUD of stomach combined with DM2, M±m

Indicators	cagA+vacA+ n=13	cagA+ or vacA+ n=21
Desquamation of the surface epithelium (Points from 0 to 5)	3,8±0,05	2,6±0,02 p<0,05
The percentage of blood vessels with symptoms of stasis and (or) sludge of erythrocytes (%)	39,0±1,8	12,0±0,4 p<0,05
Stromal edema (Points from 0 to 5)	3,5±0,07	1,6±0,04 p<0,05
Hemorrhage in stroma (Points from 0 to 5)	2,5±0,04	1,9±0,03 p<0,05
The degree of infiltration of PMNL (Points from 0 to 5)	4,1±0,08	3,5±0,02 p<0,05

Note. $p < 0.05$ - reliability of differences of indicators between these groups.

Table 4

Morphometric indicators of the condition of mucosa in patients with PUD of duodenum combined with DM2, M±m

Indicators	cagA+vacA+ n=9	cagA+ or vacA+ n=17
Desquamation of the enterocytes (Points from 0 to 5)	3,2±0,05	2,0±0,04 p<0,05
The percentage of blood vessels with symptoms of stasis and (or) sludge of erythrocytes (%)	39,0±1,6	19,0±0,4 p<0,05
Stromal edema	3,9±0,07	2,8±0,02

(Points from 0 to 5)		p<0,05
Hemorrhage in stroma (Points from 0 to 5)	2,9±0,05	1,8±0,02 p<0,05
The degree of infiltration of PMNL (Points from 0 to 5)	3,9±0,08	2,1±0,05 p<0,05
The percentage of goblet cells (%)	12,0±0,8	21,0±1,1 p<0,05

Note. p < 0.05 - reliability of differences of indicators between these groups.

Also made separate studies of the processes of formation of mucus in MMS and MMD based on the histochemical methods (PAS-reaction), which allows to identify and quantify glycoproteins and polysaccharides mucus (Table 5-8).

Table 5

Morphological indicators of the formation of mucus in patients with peptic ulcer of the stomach without DM2, M±m

Indicators	cagA+vacA+ n=17	cagA+ or vacA+ n=13
The optical density of the PAS-reaction of the mucous cervical cells (relative optical density units)	0,324±0,0020	0,347±0,0025 p<0,05
The optical density of the PAS-reaction of the surface epithelium (relative optical density units)	0,263±0,0018	0,284±0,0020 p<0,05
The optical density of the PAS-reaction of the slime layer (relative optical density units)	0,186±0,0015	0,230±0,0017 p<0,05

Note. p < 0.05 - reliability of differences of indicators between these groups.

Table 6

Morphological indicators of the formation of mucus in patients with peptic ulcer of the duodenum without DM2, M±m

Indictors	cagA+vacA+ n=11	cagA+ or vacA+ n=7
The optical density of the PAS-reaction of the goblet cells (relative optical density units)	0,290±0,0019	0,291±0,0022[нв]
The optical density of the PAS-reaction in cells of the Brunner's glands (relative optical density units)	0,381±0,0028	0,394±0,0028 p<0,05

Note. p < 0.05 - reliability of differences of indicators between these groups. HB- the reliability between the indicators is not identified.

According to the data, the optical density of the PAS-reaction was reduced in patients with PUD in the presence of such genes like cagA+vacA+ in comparison with cagA+ or vacA+ patients, which indicates on a stronger violation of the mucus formation. Moreover, there is a further deepening of violations of the processes of slime formation in patient with peptic ulcer and DM2. (Table 5-6)

Table 7

Morphological indicators of the formation of mucus in patients with peptic ulcer of the stomach with DM2, M±m

Indicators	cagA+vacA+ n=13	cagA+ або vacA+ n=21
The optical density of the PAS-reaction of the mucous cervical cells (relative optical density units)	0,311±0,0021	0,328±0,0024 p<0,05
The optical density of the PAS-reaction of	0,250±0,0018	0,267±0,0021

the surface epithelium (relative optical density units)		p<0,05
The optical density of the PAS-reaction of the slime layer (relative optical density units)	0,148±0,0015	0,184±0,0014 p<0,05

Note. p < 0.05 - reliability of differences of indicators between these groups.

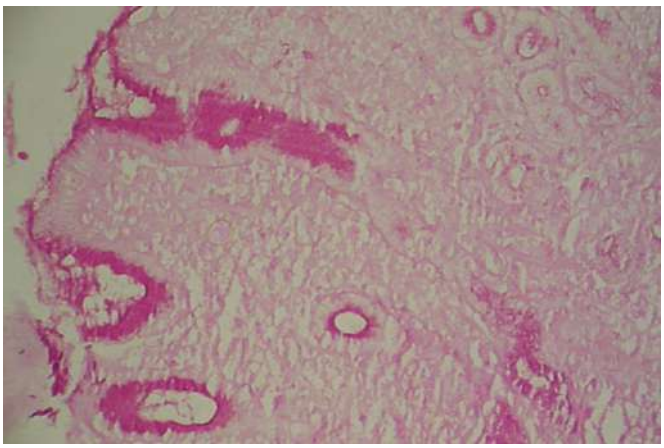
Table 8

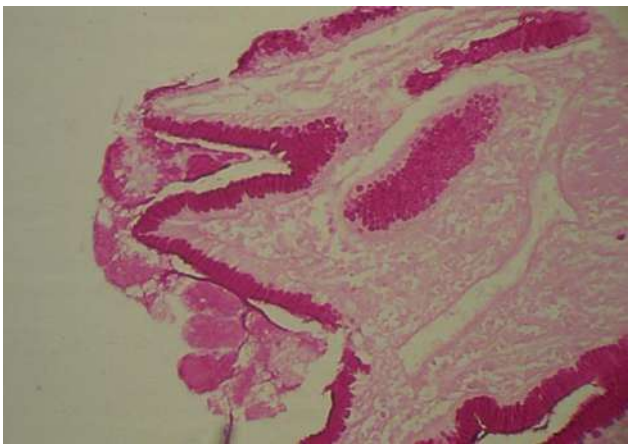
Morphological indicators of the formation of mucus in patients with peptic ulcer of the duodenum with DM2, M±m

Indictors	cagA+vacA+ n=9	cagA+ or vacA+ n=17
The optical density of the PAS-reaction of the goblet cells (relative optical density units)	0,264±0,0014	0,278±0,0024 p<0,05
The optical density of the PAS-reaction in cells of the Brunner's glands (relative optical density units)	0,342±0,0024	0,364±0,0021 p<0,05

Note. p < 0.05 - reliability of differences of indicators between these groups.

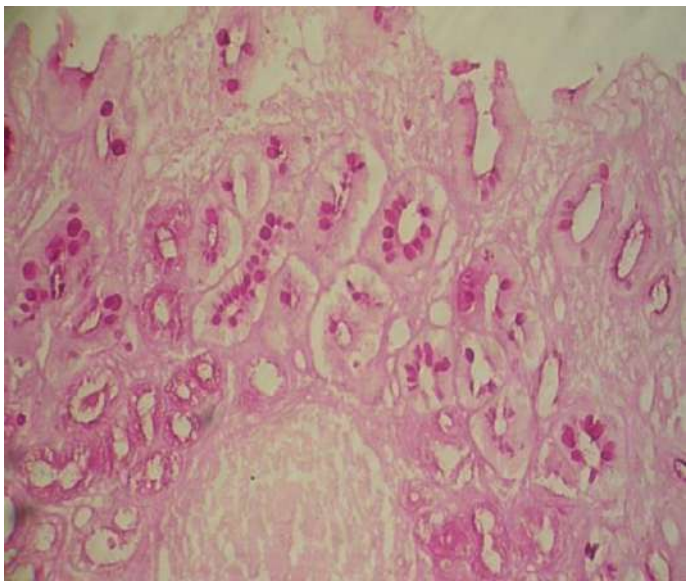
The data are shown in the pictures 1-6.

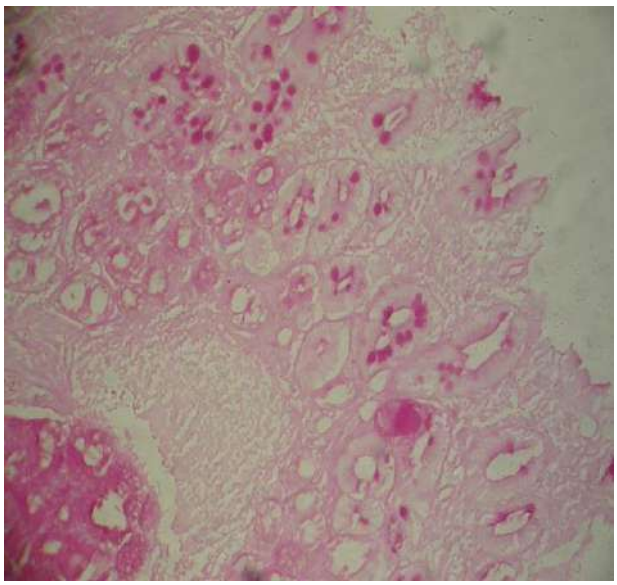




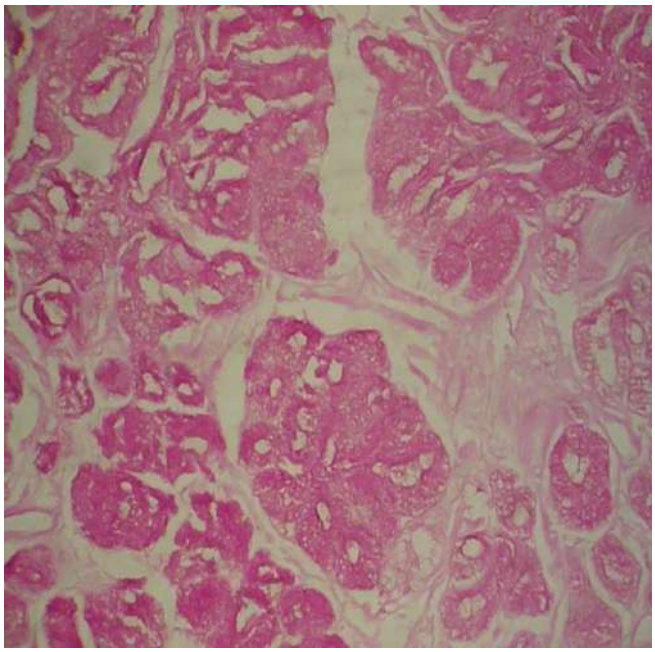
Picture 1. Surveillance of the gastric ulcer without combining with DM2

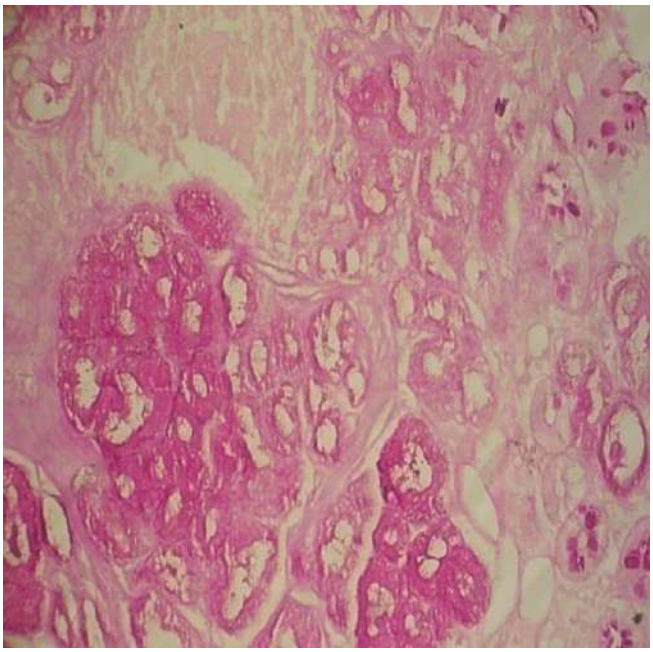
Gastric MM: A) in cagA+ vacA+ Helicobacter pylori; B)in cagA+ or vacA+ Helicobacter pylori; PAS-reaction.





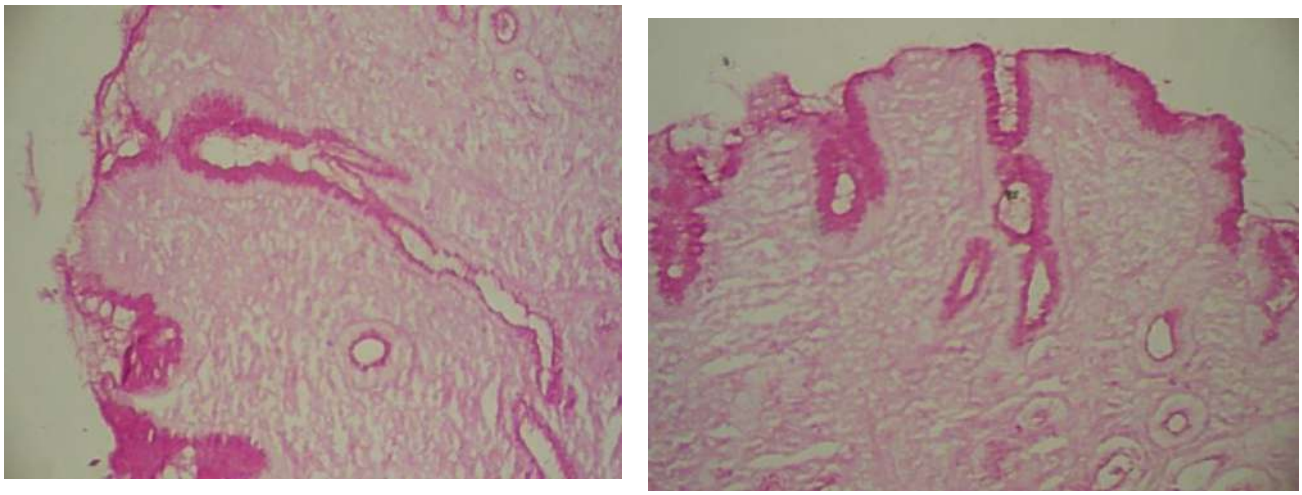
Picture 2. Surveillance of the duodenum peptic ulcer. Duodenum MM: A) in cagA+ vacA+ Helicobacter pylori; B)in cagA+ or vacA+ Helicobacter pylori; PAS-reaction.





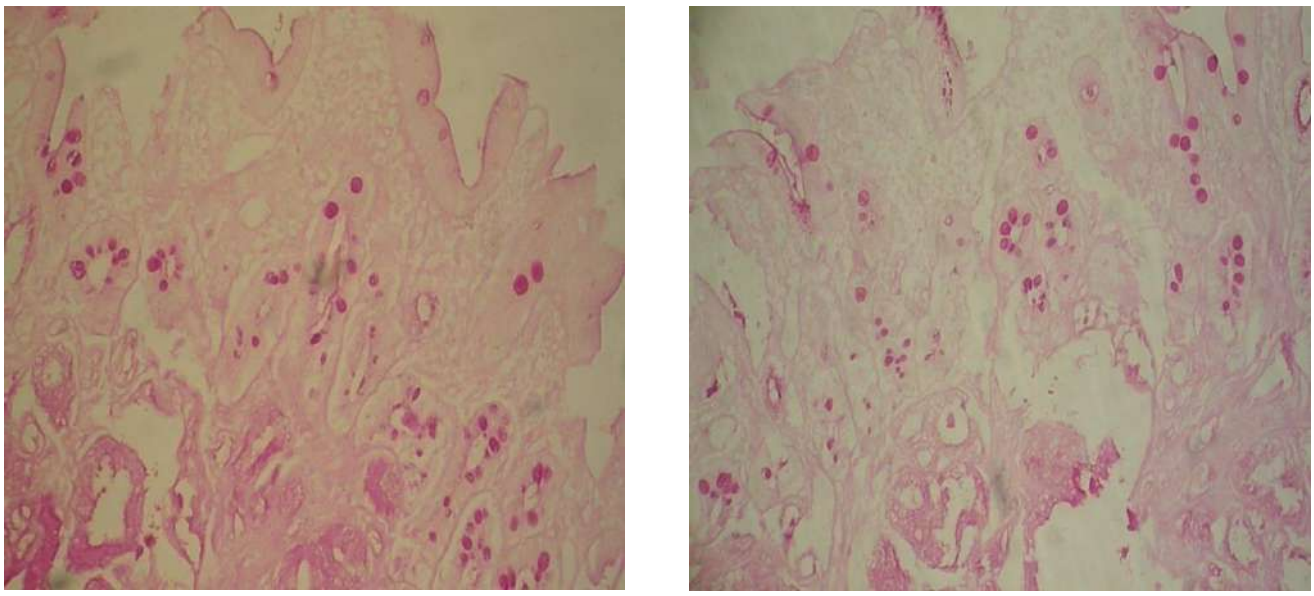
Picture 3. Surveillance of the duodenum peptic ulcer

The localization of Brunner's glands of the duodenum: A) in cagA+ vacA+ Helicobacter pylori; B)in cagA+ or vacA+ Helicobacter pylori; PAS-reaction.



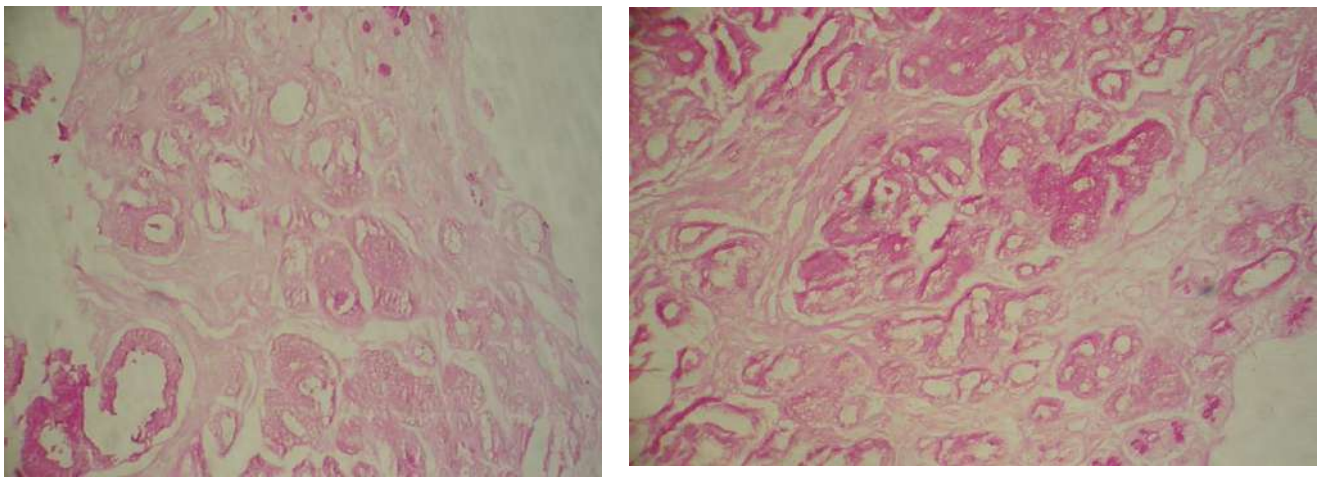
Picture 4. Surveillance of the gastric ulcer with DM2.

Gastric MM: A) in cagA+ vacA+ Helicobacter pylori; B)in cagA+ or vacA+ Helicobacter pylori; PAS-reaction.



Picture 5. Surveillance of the duodenum peptic ulcer with DM2.

Duodenum MM: A) in cagA+ vacA+ Helicobacter pylori; B)in cagA+ or vacA+ Helicobacter pylori; PAS-reaction



Picture 6. Surveillance of the duodenum peptic ulcer with DM2.

The localization of Brunner's glands of the duodenum: A) in cagA+ vacA+ Helicobacter pylori; B)in cagA+ or vacA+ Helicobacter pylori; PAS-reaction.

The analysis of the data obtained histological and histochemical studies have shown that PUD association with cagA+vacA+ Helicobacter pylori genotype observed more pronounced morphological changes in comparison with presence of cagA+vacA- or cagA-vacA+ genotype characterized by high percentage of blood vessels with symptoms of desquamation of endothelial (by 60% for gastric PUD , by 42,1% for duodenum PUD, by 41,7% for gastric PUD with DM2, by 19,43% for duodenum PUD with DM2, $p<0,05$); smaller volume of nuclei of endotheliocytes (by 36,3% for gastric PUD , by 36% for duodenum PUD, by 32,7% for gastric PUD with DM2, by 25,3% for duodenum PUD with DM2, $p<0,05$) that testifies to the deepening of alteration of the cells; the higher variation coefficient of the distribution of nuclear chromatin in the nuclei of endotheliocytes (by 41,7%,26,1%,28,6%,17,6% accordingly); with the decrease of the optical density of the surface mucus layer of the stomach (by 19% for gastric PUD, by 19,6% for gastric PUD with DM2, $p<0,05$) and optical density in the Brunner's cells of the duodenum.

Conclusion. The presence of combinations of cagA+vacA+ and cagA+ or vacA+ genes in patients with gastric and duodenal PUD leads to increased inflammatory infiltration of PMNL, edema of stroma, hemorrhages, blood stasis and sludge of erythrocytes. The level of desquamation of the surface epithelium indicated the level of alteration (damage) of these cells. The condition of MMS and MMD in

patients with gastric and duodenal PUD with DM2 is accompanied by pronounced changes in these parameters. When evaluating the optical density of the PAS-reaction in the presence of cagA+vacA+ genes in patients with gastric and duodenal PUD in comparison with the same disease patients with cagA+ or vacA+ genes in all studied structures indicates a more pronounced impaired of mucus formation, moreover there is a further deepening of violations of the processes of slime formation, in patients with gastric and duodenal PUD combined with DM2.

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