NÍVEIS SÉRICOS DE IL-8 EM PACIENTES COM GASTRITE CRÔNICA ATIVA E DOENÇA ÚLCERA PÉPTICA

SERUM LEVELS OF IL-8 IN PATIENTS WITH CHRONIC ACTIVE GASTRITIS AND PEPTIC ULCER DISEASE

ПОКАЗАТЕЛИ СЫВОРОТОЧНОГО ИЛ-8 У БОЛЬНЫХ ХРОНИЧЕСКИМИ АКТИВНЫМИ ГАСТРИТАМИ И ЯЗВЕННОЙ БОЛЕЗНЬЮ

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RESUMO

A maioria dos pesquisadores descobre associações entre *H. pylori* (Hp) e a produção sérica de IL-8 com o estágio da inflamação crônica. Os dados sobre os níveis de IL-8 na gastrite crônica (GC) e na úlcera gastroduodenal (GDU) são insuficientes, e frequentemente controversos. Todos os pacientes com gastrite crônica ativa (GCA) e GDU foram divididos em 4 subgrupos: pacientes com GDU e infecção por Hp de acordo com métodos comuns (GDU+Hp), pacientes com GDU e sem infecção por Hp documentada (GDU), pacientes com CAG e encontraram infecção por Hp de acordo com métodos comuns (GCA+Hp) e pacientes com CAG sem infecção por Hp documentada (GCA). Os níveis séricos mais altos de IL-8 foram observados em pacientes com GDU+Hp, enquanto os mais baixos foram observados em pacientes com CAG. Os homens têm os níveis mais baixos de IL-8 no subgrupo GCA+Hp em comparação com outros subgrupos. Os valores de IL-8 foram significativamente diferentes dos valores mais altos nas mulheres. A taxa de antígeno O fecal do *H. pylori* LPS encontrada no teste de coaglutinação é de 86%. Os níveis mais altos de antígeno O do *H. pylori* LPS foram detectados no subgrupo GCA. Em pacientes com GDU + Hp, os níveis de antígeno O do *H. pylori* LPS foram significativamente maiores em comparação com o subgrupo GDU. Portanto, pacientes com GDU, especialmente aqueles com GDU+Hp e com GCA, têm produção ativa de IL-8. A coaglutinação para detectar o antígeno O do *H. pylori* LPS nas fezes usadas junto com métodos comuns aumenta a probabilidade de confirmar que os pacientes (especialmente aqueles com gastrite crônica) estão infectados com esse micróbio.

Palavras-chaves: Infecção por *H. pylori*, gastrite crônica, úlcera péptica, interleucina 8, antígeno O de *H. pylori*, diagnóstico

ABSTRACT

The majority of researchers discover associations between *H. pylori* (Hp) and serum IL-8 production with the stage of chronic inflammation. Data on IL-8 levels in chronic gastritis (CG) and a gastroduodenal ulcer (GDU) are insufficient, and often controversial. All patients with chronic active gastritis (CAG) and GDU were divided into 4 subgroups: patients with GDU and found Hp infection according to common methods (GDU+Hp), patients with GDU and no documented Hp infection (GDU), patients with CAG and found Hp infection according to common methods (CAG+Hp), and patients with CAG with no documented Hp infection (CAG). The highest serum IL-8 levels were seen in patients with GDU+Hp, whereas the lowest ones were observed in patients with CAG. Men have the lowest IL-8 levels in the CAG+Hp subgroup as compared with other

subgroups. The IL-8 values were significantly different from higher values in women. The rate of fecal O-antigen of *H. pylori* LPS found using the coagglutination test is 86%. The highest levels of O-antigen of *H. pylori* LPS were detected in the CAG subgroup. In patients with GDU+Hp, the levels of O-antigen of *H. pylori* LPS were significantly higher as compared with the GDU subgroup. So patients with GDU, especially those with GDU+Hp, and with CAG, have active IL-8 production. Coagglutination to detect O-antigen of *H. pylori* LPS in feces used along with common methods increases the probability to confirm that the patients (especially those with chronic gastritis) are infected with this microbe.

Keywords: *H. pylori* infection, chronic gastritis, peptic ulcer disease, interleukin 8, O-antigen of *H. pylori*, diagnostics

АННОТАЦИЯ

Большинство исследователей отмечают связь присутствия *H. pylori* (Hp) и уровней IL-8 в сыворотке крови со степенью хронического воспаления. Несмотря на то, что IL-8 интенсивно изучается при различных заболеваниях, имеется недостаточно сведений о его уровнях при хронических гастритах (ХГ) и язвенной болезни желудка и двенадцатиперстной кишки (ЯБ), кроме того, полученные результаты порой носят противоречивый характер. У больных хроническими активными гастритами (ХАГ, включая эрозивные гастродуодениты) и ЯБ повышение сывороточных уровней IL-8 (выше 62 пг/мл) наблюдается у большинства больных (90%). Присутствие Hp общепринятыми методами установлено у 33,3% пациентов с хроническими гастритами и у 61,7% пациентов с ЯБ. Все больные ХАГ и ЯБ были разделены на 4 подгруппы: больные ЯБ с выявленной инфекцией Hp по данным общепринятых методов (ЯБ+Hp), больные ЯБ без подтвержденной Hp-инфекции (ЯБ), больные ХАГ с выявленной инфекцией Hp по данным общепринятых методов (ХАГ+Hp) и больные ХАГ без подтвержденной Hp-инфекции (ХАГ). Наиболее высокие уровни сывороточного ИЛ8 выявлены у больных ЯБ+Hp, наиболее низкие - у больных ХАГ. У мужчин, в целом, отмечены наиболее низкие уровни IL-8 в подгруппе больных ХАГ+Hp, в сравнении с другими подгруппами, и эти показатели IL-8 достоверно отличались от более высоких показателей у женщин. Частота выявления в кале ЛПС/О-антигена Hp методом коагглютинации - 86%. Наиболее высокие уровни ЛПС/О-антигена Hp выявлены у больных в подгруппе ХАГ. У больных ЯБ+Hp уровни ЛПС/О-антигена Hp были достоверно выше, чем в подгруппе ЯБ. Полученные данные свидетельствуют, что у больных ЯБ, особенно при ЯБ+Hp и больных ХАГ наблюдается активная продукция IL-8. Применение метода коагглютинации для выявления ЛПС/О-антигена *H. pylori* в кале, в дополнение к общепринятым методам, увеличивает вероятность подтверждения инфицирования этим микробом, особенно у больных хроническими гастритами.

Ключевые слова: инфекция *H.pylori*, хронический гастрит, язвенная болезнь, интерлейкин 8, О-антиген *H.pylori*, диагностика.

1. INTRODUCTION

Helicobacter pylori (Hp) is the most widespread infection in humans. This microaerophilic gram-negative bacterial infection causes chronic gastritis, gastroduodenal ulcer, stomach cancer, and MALT-lymphoma (McColl, 2010; Rehman, 2020). Gastric mucus is the basic primary colonization site of *H. pylori*. However, the microbe can penetrate even deeper tissues, submucous membranes of the stomach, where it is found next to endothelial cells (Kusters *et al.*, 2006).

Almost all people infected with *H. pylori* develop chronic gastric inflammation. In this case, the gastric mucus is infiltrated with neutrophils and macrophages (Sipponen, 1993; Vinagre *et al.*, 2018; Israel and Peek, 2001). In patients with chronic nonatrophic gastritis, the activity of inflammation depends on gastric mucus Hp

colonization; the inflammation intensity is decreased against the background of reduced bacterial content (Ageeva *et al.*, 2011).

This inflammation is caused by cytokines and chemokines, including interleukin-1 β , tumor necrosis factor- α (TNF- α), IL-8, and IL-6. IL-8 (Kononov, 2006; Sun *et al.*, 2014; de Brito *et al.*, 2018; Serrano *et al.*, 2007), which is a powerful angiogenic and chemoattractive agent, a key mediator of Th1-type immune response overexpressed in gastric epithelial cells during infection with *H. pylori*, is necessary to differentiate and recruit effector cells into the focus of Hp-associated inflammation (Crabtree, 1996; Naumann and Crabtree, 2004).

The mechanism used by *H. pylori* to stimulate IL-8 induction in gastric epithelial cells and chronic inflammation is examined intensively [3]. The stomach has a greater mucus surface;

thus, it is believed that chronic inflammation caused by *H. pylori* cannot be limited but have systemic effects due to the action of serum inflammatory cytokines (Bayraktaroglu *et al.*, 2004).

H. pylori of primary endothelial cells stimulate the secretion of key inflammatory cytokines in humans, IL-6, and IL-8. Endothelial cells play a decisive role in the innate immune response, wound healing, and oncogenesis. IL-8 is secreted by *H. pylori*-infected endothelial cells 10-20 times more actively as compared to gastric epithelial cells infected with *H. pylori* (Tafreshi *et al.*, 2018).

It must be noted that the level of interleukin depends not just on gene polymorphism (Saes *et al.*, 2017; Kang *et al.*, 2009) but also on a microorganism genotype. In particular, it is known that the highest IL-8 content is found when the organism is infected with Hp CagA-strains (Siregar *et al.*, 2015; Lee *et al.*, 2013; Higashi *et al.*, 2004).

IL-8 is significantly activated in tumors and their microenvironment. It also acts as a key regulator of proliferation, angiogenesis, and metastasis. IL-8 regulates the cellular transmission of signals in cancer development and progression irrespective of chemotaxis during an inflammatory process. The expression of IL-8 makes stomach cancer resistant to chemotherapy. There is a close association between gastric cancer and *H. pylori*, with IL-8 levels indicating the unfavorable prognosis (Pece *et al.*, 1997).

Although the majority of authors note an association between Hp, IL-8 levels and stage of chronic inflammation along with the stage of neutrophil infiltration (Kononov, 2006; Siregar *et al.*, 2015; Nagashima *et al.*, 2015), some data and opinions oppose these provisions (Bayraktaroglu *et al.*, 2004; Zimmerman, 2018).

Data on IL8 levels in various diseases are limited and sometimes controversial. Thus, we examined serum levels of IL-8 in patients with chronic gastritis and gastroduodenal ulcer depending on confirmation of Hp infection with common methods and based on the presence of O-antigen of *H. pylori* LPS in stools according to coagglutination tests on immunological plates.

Purpose: To determine the levels of serum IL-8 in in-patients with chronic gastritis and gastroduodenal ulcer when Hp-infection is confirmed with common methods and based on the presence of O-antigen of *H. pylori* LPS in stools.

2. MATERIALS AND METHODS

Ninety-five patients of the gastroenterological department were examined (37 men and 58 women with a mean age of 59). Fifty-three of them were diagnosed with chronic active gastritis (CAG, including erosive gastroduodenitis; 17 men and 36 women), whereas 42 had a gastroduodenal ulcer (GDU, 20 men and 22 women).

To confirm the diagnosis, the following laboratory and instrumental methods of examination were used: clinical blood analysis, blood chemistry, Helpful test (AMA, Russia), urea breath test, test for fecal occult blood, blood group and Rh factor, fibro gastro duodenoscopy (Olympus xq40, Japan) with a cytological examination of the biopsy specimen, and abdominal ultrasound imaging (Sonoscape s8, China, GE Vivid 7, USA). Special blood tests for detecting and determining the level of IL-8 using the enzyme-linked immunosorbent assay and analysis of feces for O-antigen of *H. pylori* LPS during the agglutination test were carried out.

To find the dependence between the found levels of serum IL-8 and fecal LPS/O-antigen levels all the patients with CAG and GDU were subdivided into 4 subgroups: patients with GDU and found Hp infection using common methods (GDU+Hp) (16 blood samples), patients with GDU but without confirmed Hp (GDU) (52 blood samples), patients with CAG and Hp found using common methods (GDU+Hp) (25 blood samples), and patients with CAG but without confirmed Hp (CAG) (58 blood samples).

Surface epithelium with signs of dystrophia, chronic inflammation, edematous mucous layer, and leucocytic infiltration was found during a microscopic examination of 5 biopsy samples taken from the affected site of the antral section and duodenal bulb in CAG.

Cellular detritus, degenerative changes, inflammatory infiltrate elements, focal necrosis limited with granulation tissue, stromal edema, layers of the surface-foveolar epithelium in GDU with proliferative and dystrophic changes, leucocytic infiltration, atrophic fragments, and mucus dysplasia were discovered during a microscopic examination of biopsy samples in GDU.

The patients obtained proton pump inhibitors (PPI), antacids, H-2 receptor blocking agents. When Hp infection was confirmed, eradication therapy was given according to the National recommendations on diagnostics and

treatment of acid-dependent and *Helicobacter pylori*-associated diseases (VI Moscow agreements, dated 24-25 November 2016).

One hundred fifty-one blood serum samples were examined to detect the level of IL-8 using the EIA and test systems produced by the «Cytokine» company. Paired blood samples were obtained from patients with CAG and GDU upon their hospitalization, and also in 3-4 days after the first blood test.

Detection of O-antigen of *H. pylori* LPS in coprofiltrates of patients (149 samples) was done during a coagglutination test (CAT) using diagnostic agents prepared in accordance with the patent (Belaia *et al.*, 2001).

The test was done using immunological plates with a round (U-shaped) bottom. Every coprofiltrate sample was titrated twice (in phosphate buffer saline, 0.15M, pH=7.4) with 2 rows of plates. Coagglutination diagnostic was added in one row, and 1% unsensitized staphylococcus suspension was added to the other row. The plate was covered and put on air-bath at 37°C for 12 hours. A positive reaction is indicated by the formation of visible agglutinate clumps at 2+. Four-fold or greater dilution with a diagnostic showed the presence of O-antigen of *H. pylori* LPS in the sample as compared to the control reagent.

All patients included in the study gave informed consent to the inpatient examination. Confidentiality was preserved.

The digital data underwent statistical processing using common methods of variation statistics, Student's t criterion, and programs of Microsoft Word and Microsoft Excel.

3. RESULTS AND DISCUSSION

Confirmation of Hp infection by conventional methods did not exceed 30%.

Increased levels of serum IL-8 (above 0.062 ng/ml) were found in the majority of patients (90%). As mentioned above, all patients with CAG and GDU were subdivided into 4 subgroups: patients with GDU and found Hp using common methods (GDU+Hp), patients with GDU and no confirmed Hp (GDU), patients with CAG and found Hp using common methods (CAG+Hp) and patients with CAG with no confirmed Hp (CAG).

During the study it was found out that the highest levels of serum IL-8 were detected in patients with GDU+Hp ($p \leq 0.05$) (the same

tendency was seen for the first stool sample), the lowest levels were found in patients with CAG (without detection of Hp using common methods). No significant differences were found between the mean IL-8 levels in the first and second stool samples due to a small time interval (3-4 days). Only a weak, insignificant tendency to lower IL-8 levels was detected during the second analysis (Table 1).

When results obtained during analyses 1 and 2 are combined, levels of IL-8 in patients with GDU+Hp were significantly higher than those in patients with GDU but without Hp detected using common methods (0.50 ± 0.09 and 0.32 ± 0.04 , respectively; $p \leq 0.05$), and higher than those in patients with CAG+Hp (0.50 ± 0.09 and 0.30 ± 0.05 , respectively; $p \leq 0.05$). In patients with GDU and CAG, the mean levels of IL-8 were similar (0.32 ± 0.04 and 0.30 ± 0.04 , respectively) and significantly lower than in patients with GDU +Hp ($p \leq 0.05$).

The levels of serum IL-8 were examined in patients of both sexes during two blood serum analyses (Table 2). In men with Hp detected using common methods, the levels of IL-8 in GDU+Hp were significantly higher than in CAG+Hp (0.46 ± 0.07 and 0.20 ± 0.04 , respectively, $p \leq 0.05$). This influenced the aggregate level of IL-8 (both in men and in women) where the levels of IL-8 in GDU+Hp were significantly higher than those in patients with GDU (0.50 ± 0.09 and 0.32 ± 0.04 , respectively, $p \leq 0.05$), CAG+Hp (0.50 ± 0.09 and 0.30 ± 0.05 , respectively, $p \leq 0.05$) and CAG (0.50 ± 0.09 and 0.30 ± 0.04 , respectively, $p \leq 0.05$).

According to the aggregate value obtained in two analyses, the levels of IL-8 in women were significantly higher than in men (CAG+Hp) (0.40 ± 0.07 and 0.20 ± 0.04 , respectively, $p \leq 0.05$). No significant differences were found between men and women with GDU, CAG, and GDU+Hp.

Thus, men with CAG+Hp have lower levels of IL-8 as compared with other subgroups. These IL-8 values were significantly different from those in women.

To establish the dependence between the detected levels of serum IL-8 and fecal LPS/O-antigen, the levels of the antigen were examined in 4 subgroups of patients mentioned above (Table 3).

The total rate of fecal LPS/O-antigen detection was 86%. The highest levels of fecal O-antigen of *H. pylori* LPS were found in CAG patients (lg10 reverse antigen titre – 1.473 ± 0.111) and in the general group of patients with CAG and

CAG+Hp (1.452±0.092). Patients with GDU+Hp and CAG+Hp had identical or lower levels of O-antigen of *H. pylori* LPS (1.400±0.180 and 1.400±0.167, respectively). Patients with GDU+Hp had significantly higher

Helicobacter pylori cause chronic gastritis, gastroduodenal ulcer, stomach cancer, and MALT-lymphoma (McColl, 2010). Chronic gastric inflammation, characterized by gastric mucous infiltration with neutrophils and macrophages (Kusters *et al.*, 2006), is due to cytokines and chemokines including interleukin-1 β , IL-8, and IL-6, tumor necrosis factor- α (TNF- α), which are produced under the influence of *H. pylori* pathogenicity factors (Siregar *et al.*, 2015; Naito *et al.*, 2005; Sharma *et al.*, 1995).

The majority of researchers notice a relationship between the presence of *H. pylori* alongside with serum IL-8 levels and the rate of chronic inflammation with the rate of neutrophil infiltration (Siregar *et al.*, 2015). IL-8 is a powerful angiogenic and chemoattractive agent, a key mediator of Th1-type immune response overexpressed in gastric epithelial cells influenced by *H. pylori* and epithelial cells (Sharma *et al.*, 1995). Although widely studied in any disease, data on IL-8 levels in chronic gastritis (CG) and a gastroduodenal ulcer (GDU) are insufficient and often controversial.

According to the current study of serum IL-8 levels in patients with chronic active gastritis (CAG, including erosive gastroduodenitis) and a gastroduodenal ulcer (GDU), its increased levels were found in the majority of patients (90%), while Hp-infection using conventional methods was detected in no more than 30% of patients. Thus, the increased concentration of serum IL-8 was seen even in those patients for whom the presence of Hp wasn't confirmed using common methods.

The highest levels of serum IL-8 were found in those with GDU+Hp ($p \leq 0.05$), the lowest ones in those with CAG. Men generally had the lowest levels of IL-8 in CAG+Hp as compared to other subgroups. These IL-8 values were significantly different from higher values in women.

The total detection rate of fecal LPS/O-antigen of *H. pylori* was 86%, and this level was higher than the confirmation of Hp infection by conventional methods. The highest levels of fecal O-antigen of *H. pylori* LPS were found in CAG patients, and this level of O-antigen was higher than that in patients with GDU.

Patients with GDU+Hp had significantly

higher levels of O-antigen of *H. pylori* LPS as compared to patients with GDU. Patients with GDU+Hp and CAG+Hp had similarly low levels of O-antigen of *H. pylori* LPS. In the aggregate, the levels of O-antigen of *H. pylori* LPS in GDU were significantly inferior to those in CAG. This could be due to more intense destruction of gastric Hp, making it impossible to detect whole microbial cells when using common methods of Hp diagnostics, while when they are destroyed, there is a greater amount of free O-antigen in the feces. This is probably the reason for significantly higher levels of O-antigen of *H. pylori* LPS in CAG as compared to GDU.

Thus, among patients with a peptic ulcer disease who had their *H. pylori* infection confirmed with the common methods, the mean levels of fecal O-antigen of *H. pylori* were higher in GDU+Hp than in GDU, whereas the levels of IL-8 were the highest in GDU as compared with other subgroups. Patients with CAG had the highest levels of O-antigen/LPS and relatively low levels of IL-8. We believe that the level of O-antigen in feces is not a determining factor in stimulating the production of IL-8 in patients with chronic active gastritis and peptic ulcer disease.

At the same time, it was found that an intermediate metabolite of LPS *Helicobacter pylori* inner core heptose biosynthesis (HBP) modulates host cell responses by CagT4SS-dependent translocation. The authors suggest that HBP is a major factor leading to the activation of cellular responses. This response is connected to the human cellular adaptor protein TIFA (Stein *et al.*, 2017).

As known from experiments in CagA and/or CagL knockout bacterial lines, extracellular CagA might induce IL-8 production by AGS cells (Fazelli *et al.*, 2016; Zeng *et al.*, 2020).

The C-terminal coiled-coil region of the *H. pylori* CagL protein, a protein described to be located on the tip of the T4SS-pilus, is responsible for *H. pylori*-induced IL-8 secretion via the transforming growth factor (TGF)- α activated epidermal growth factor-receptor (EGF-R) signaling pathway and the bridging of the T4SS to its human target cells. This novel bacterial-host recognition sequence allows a new insight into how *H. pylori* induce the inflammatory response in gastric epithelial cells and facilitate the development of precancerous conditions (Tafreshi *et al.*, 2018; Wiedemann *et al.*, 2016; Lee *et al.*, 2016). Outer inflammatory protein A (OipA) of *Helicobacter pylori* is regulated by host cell contact and mediates CagA translocation and interleukin-

8 response only in the presence of a functional cag pathogenicity island type IV secretion system (Horridge *et al.*, 2017).

There is insufficient data on the effect of LPS Hp on IL-8 levels (Stein *et al.*, 2017; Pece *et al.*, 1997). Despite the low immunological potential, an array of proinflammatory cytokines (IL-8, IL-1, and TNF- α) are produced both in vitro and in vivo following the stimulation of mucosal cells with *H. pylori* organisms (Pece *et al.*, 1997). The highest levels of fecal O-antigen of *H. pylori* LPS were found in CAG patients, while the level of IL-8 was the highest in the group of ulcers, indicating a mismatch between the levels of O-antigen in feces and IL-8 in serum. Nonetheless, the detection of *H. pylori* O antigen in feces has diagnostic value, especially in patients with CAG in the lack of detection of Hp by conventional methods.

4. CONCLUSION

The vast majority of patients (90%) have elevated levels of IL-8. According to the data obtained, more active IL-8 production was found in peptic ulcer disease, especially when *H. pylori* was detected using common methods.

The rate of fecal *H. pylori* O-antigen in the coagglutination test was 86%. The highest levels of O-antigen of *H. pylori* LPS were detected in the CAG subgroup, and in total in patients with CAG compared with DGU. In patients with GDU+Hp, the levels of O-antigen of *H. pylori* were significantly higher as compared with the GDU subgroup.

Simultaneous use of the coagglutination test to detect fecal O-antigen of *H. pylori* and common methods make the infection confirmation highly probable, especially in chronic gastritis (almost until the level of infection confirmation in peptic ulcer disease).

5. ACKNOWLEDGMENTS

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Table 1. Mean serum levels of IL-8 in the course of chronic gastritis and a gastroduodenal ulcer (ng/ml)

IL-8 ng/ml	GDU+Hp	CAG+Hp	GDU	CAG
Analysis 1	0.46±0.11 n=10	0.32± 0.07 n=13	0.32±0.05 n=29	0.34±0.05 n=33
Analysis 2	0.4±0.15 n=6	0.30±0.07 n=12	0.31±0.04 n=23	0.25±0.05 n=25
Total	0.50±0.09 n=16	0.30±0.05 * n=25	0.32±0.04 * n=52	0.30±0.04 * n=58
Overall	0.36±0.05 n=41		0,31±0,03 n=110	

Note: *) statistical significance in comparison with patients suffering from GDU+Hp ($p \leq 0.05$)

Table 2. Mean levels of serum IL-8 in patients with chronic active gastritis and gastroduodenal ulcer depending on the gender and in the aggregate

Patients:	Mean IL-8 levels in blood serum (ng/ml)				
	GDU	GDU + Hp	CAG	AG + Hp	Total
Men Analysis 1	0,32±0,09 n=13	0,43± 0,14 n=7	0,36±0,10 n=8	0,19±0,06 n=7	0,33±0,05 n=35
Men Analysis 2	0,35±0,06 n=11	0,45 ±0,23 n=4	0,30±0,06 n=6	0,22±0,18 n=4	0,33±0,05 n=25
Men Analysis 1+2	0,34±0,05 n=24	0,46±0,07 n=11	0,33±0,06 n=14	0,20±0,04 * n=11	0,33±0,03 n=60
Women Analysis 1	0,32±0,07 n=16	0,43±0,22 n=3	0,33±0,05 n=25	0,47±0,1 n=6	0,35±0,04 n=50
Women Analysis 2	0,26±0,06 n=12	0,42±0,01 n=2	0,24±0,07 n=19	0,40±0,10 n=8	0,28±0,04 n=41
Women Analysis 1+2	0,30±0,05 n=28	0,43±0,12 n=5	0,30±0,04 n=44	0,40±0,07 ** n=14	0,32±0,03 n=91
Total (M+F, 2 analyses)	0,32±0,04 & n=52	0,50±0,09 n=16	0,30±0,04 & n=58	0,30±0,05 & n=25	0,32±0,02 n=151
Total	0,32 ± 0,03 n = 68		0,31 ± 0,03 n = 83		0,32±0,02 n=151

Note. The significance of differences in comparison with: *) a subgroup of men with Hp GDU ($p = 0.004$); **) the respective subgroup of men ($p = 0.04$); &) the 'total' subgroup with Hp GDU ($p \leq 0.05$)

Table 3. Mean levels of fecal O-antigen of *H. pylori* LPS during the coagglutination test (Ig10 reverse antigen titer) in patients with CAG and GDU depending on the association of the disease with *H. pylori* (according to common methods)

Mean levels of LPS/O-antigen	GDU+Hp	GDU	CAG+Hp	CAG
	1,400 ± 0,180 n = 16	1,000 ± 0,080 * n = 52	1,400 ± 0,167 n = 23	1,473 ± 0,111 ** n = 58
Total	1,195 ± 0,076 n = 68		1,452 ± 0,092 # n = 81	

Note. The significance of differences in comparison with: *) patients with GDU+Hp ($p = 0.026$); **) patients with GDU ($p = 0.00$); &) all patients with GDU ($p = 0.00$)