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IMMUNOLOGICAL INDICES OF PATIENTS WITH CHRONIC GASTRITIS ASSOCIATED WITH HELICOBACTER PYLORI IN YAKUTIA

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In research presents the immunological characteristics of patients with chronic gastritis. Survey participate 88 patients with chronic gastritis type B, indigenous (yakut), in age 30 to 50 years. In research the revealed dependence of the levels of immunoglobulins and cytokines on the degree of contamination Helicobacter pylori in the serum of patients with chronic gastritis.

Keywords: chronic gastritis, Helicobacter pylori, immunoglobulins, cytokines.

Introduction

In the Republic of Sakha (Yakutia), chronic gastritis (CG) is the most common disease in the structure of gastroenterological diseases [2,11]. According to the data of Goskomstat in Republic of Sakha (Yakutia) the diseases of the digestive system in the structure of general morbidity took third place (8.4%) in 2009. In 2008, the incidence reached 148.2 per 1,000 of population, while in Russia it is 112.7.

The frequency of atrophic gastritis, antral form among the native-born population of Yakutia (38.5 to 57.1% people) characterized by a latent course is much higher than that of non-native population (28.5%). In this case it's noted that the incidence of atrophic form of gastritis among nonresidents increases with the time of residence in the North [5].

Opening of Helicobacter pylori (HP) revolutionized the understanding of the pathogenesis of chronic gastritis. However, despite the great number of researches, some aspects of pathogenesis are controversial and debatable. HP has a pathogenic effect on patients with insufficient protection, especially of the immune mechanisms in the stomach lining. As the lining of the gastrointestinal tract refers to the local factors of the immune system protection, in the process of damage that occurs during the invasion of HP, its barrier properties are violated, and the passage of antigens through the mucosa increases. In addition, HP has a low immunogenicity, which leads to long-term interaction of microorganisms with the immune system of mucous and subsequent persistence of infection [6].

A high degree of HP infection among native-born population of Yakutia, which correlates with high rates of stomach cancer was educed by Sosin, S.S., Ivanov P.M. (2005), (20.7 per 100,000 population) [8].

In the Far North the state of nonspecific and specific anti-HP has a number of features. They are due to climatic, social and environmental conditions of habitat, the degree of adaptation of native-born people, their genetic and ethnic characteristics.

The purpose of this research was to evaluate immunoglobulins and cytokines in patients with chronic gastritis, depending on the degree of contamination of the HP.

Material and methods:

We examined 88 patients of native-born nationality (Yakut), suffering from Helicobacter pylori gastritis with chronic type B, 17 men and 71 women aged 30 to 50 years (average age was 44,64 ± 5,82 years). The control group consisted of 20 healthy people without pathology of the gastrointestinal tract and the presence of HP (average age was 43,85 ± 3,84).

Surveyed patients had never received H. pylori therapy. The diagnosis was verified clinically, endoscopically with histological examination of biopsy samples of the mucosa and on the basis of ELISA to CagA Helicobacter pylori antigen.

The investigation material was blood taken from patients' cubital vein on an empty stomach.

Immunological data were determined depending on the severity of H. pylori infection according to the morphological and immunological studies. Surveyed patients were divided into three groups.

Immunological data were determined depending on the intensity of H. pylori infection according to the morphological and immunological studies. Surveyed patients were divided into three groups.

The first group consisted of 26 patients suffering from chronic gastritis, in morphological biopsies [4], of which were found up to 20 microorganisms in the field of vision, and the antibodies titer ratio to the CagA HP antigen was 1:5, 1:10.

The second group included 18 patients with moderate severity of HP infection, varying from 20 to 50 microorganisms in the morphological biopsy, the antibodies titer ratio to CagA HP antigen was 1:20,1:40. The third group consisted of 43 patients with severe degree, more than 50 microorganisms, with a positive titer of the antibodies' presence, the antibodies titer ratio to CagA HP antigen was 1:80.

The content of antibodies titer to CagA HP antigen in the serum was determined by ELISA test-system "HelikoBest - antibodies". C-reactive protein concentrations of immunoglobulins IgA, IgG, sIgA, the concentration of pro-inflammatory cytokine α-tumor necrosis factor, IL-6 and anti-inflammatory cytokine IL-4 in blood serum were measured by ELISA using kits of reagents JSC "Vector-Best" (Novosibirsk, Russia) according to the instruction.

Statistical analysis was performed with using the software packages and Statistica for Windows (version 6.0). Significant differences between groups was assessed using t-test (t). Data in tables are presented as M ± m, where M - medium, m - medium error. The probability of the null hypothesis was accepted at $p < 0,05$.

Results and discussion

ELISA data are presented in the Table 1. The content of IgA in the blood serum in the first group was 1.5 times above normal ($p \leq 0,05$), in the second group - 1.7 times above normal in the third - 1.8, compared with the control group (Table 1).

Increasing concentrations of IgA can be explained by intensified production mainly IgA-antibodies with the ability to prevent adhesion of microorganisms in the gastric mucosa, as the body protection against infection, particularly from HP, belongs to IgA.

Our results are consistent with published data. According to Lazebnik L.B. (2006) increased circulating of IgA in the blood serum of patients with chronic gastritis indirectly proves the severity of atrophic processes in the gastric mucosa [3].

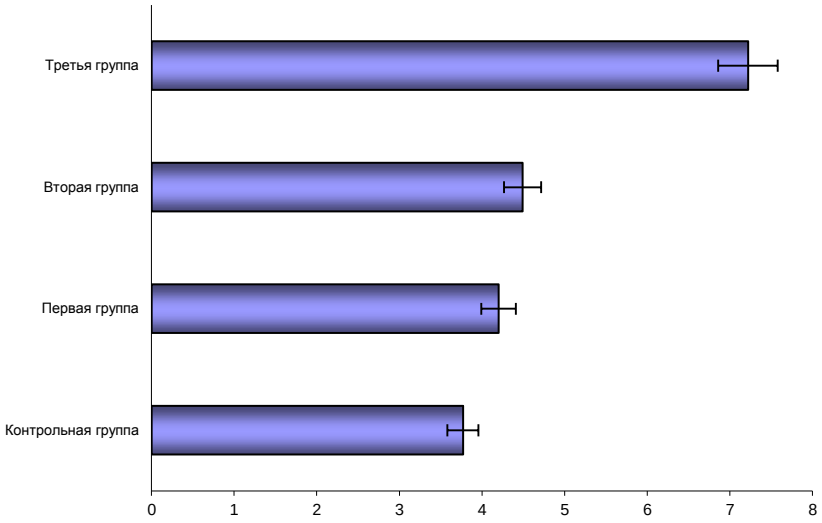
Our data showed that the concentration of IgG in the blood serum of the patients increased, depending on the degree of contamination of the HP. Thus, the content of IgG in the second group of patients was 1.5 times higher, in the third group - 1.2 times compared with the

control group ($p \leq 0,05$). Perhaps the rise of IgG antibodies in the blood serum reflects not only the presence of HP colonization and the intensity of infiltration of the gastric mucosa by leukocytes, but also systemic immune responses to local damage in the gastroduodenal system. By the high level of IgG in the blood serum of patients, suffering from chronic gastritis we can estimate the intensity of inflammation in the gastric mucosa caused by the pathogenic effect of HP. Significant increase of IgG level in patients with moderate contamination indicates the activation of the immune system, while in the third group of patients with a high degree of contamination, the reserves of the immune system can be reduced. Thus, the morphological study of Loskutova K.S. (2006) shows that that native-born people with less degree of contamination have more severe changes of the gastric mucosa, compared with the nonresidents [7]. The increase of serum immunoglobulin was shown in the researches of such authors as: Petrova P.G. (1993), Fedorov A.I., Osakovsky V.L. (2008). According to these authors, enhanced synthesis of antibodies is one of the components of the organism alteration to the natural conditions of the Far North [10, 11].

We found a dependence of sIgA concentration on the degree of HP contamination. The level of sIgA in the first group was significantly lower (2.9 times) compared with the control group. In the second group sIgA level in the blood of patients was lower ($p \leq 0,05$) 3.7 times below normal, in the third group 2.5 times ($p \leq 0,05$) below normal, compared with the control group.

Effective protection of the gastric mucosa is determined by a balanced feedback of all levels of tissue immunity, however, the leading factor, according to all researchers, is sIgA. The failure of this component involves the colonization of mucous membranes by the microbes, increase the flow of allergens through the epithelial barrier and, consequently, increase of the load on the "second line" protection. Despite the numerous studies on the mechanisms of sIgA failure, there is no generally accepted concept that explains the reason for reducing the concentration of immunoglobulin in the lumen of the digestive tube. A.V. Kononov proved the possibility of a local shortage of sIgA in the gastric mucosa as a result of HP's ability to disrupt disulfide bonds [6].

The level of C-reactive protein (CRP) of the surveyed patients ranged from $4,2 \pm 0,38$ to $7,22 \pm 0,48$ pg / ml. The concentration increasing (CRP) in the blood serum of patients in the first and second group was insignificant, and in the third group the level of CRP was 1.9 times higher than in the control group ($p \leq 0,05$). The concentration of CRP in the control group was $3,77 \pm 0,6$ pg / ml (picture).



CRP level in the blood of patients suffering from chronic gastritis according to the degree of HP contamination.

The picture shows that the increasing of CRP concentration in the blood of patients depends on the degree of HP contamination. It is known that C-reactive protein (CRP) provides antimicrobial effect by binding with pathogens and activating the complement system in the classical way, and takes part in the regulation of immune cells, leading to the increased transendothelial migration of leukocytes. Statistically, the significant increase of CRP in the third group testifies that the patients have the activation of nonspecific immunity.

The cytokines regulate immunopoiesis and effect on all parts of the immune system, acting as the main mediators of the immune response. They contribute to immune responses directed at the elimination of the infectious agent, damaged structures and restoration of constant internal environment.

In 1996 for the first time, the group of Italian researchers found increased content of IL-1β and IL-6 in the gastric mucosa with Hp-associated infection [15]. It was later established that cytokines play an important role in HP-induced inflammation. Taking into account the large area of HP extension in the stomach, as well as the recently identified possibility of bacteria's penetration into the cytoplasm of epithelial cells, the intercellular spaces and into the underlying lamina propria of the mucous, the systematic secretion of cytokines is not surprising in the HP-infection. It is unlimited with clearance and gastric surface epithelium.

An imbalance of pro-and anti-inflammatory cytokines, naturally circulating in the bloodstream plays an important role in the development of HP-associated pathology. In this regard, the content of anti-inflammatory cytokine IL-4 and pro-inflammatory cytokines IL-6 and α-TNF in the blood serum of patients with chronic gastritis was evaluated.

In the blood of the first group patients the concentration of IL-4 was significantly lower 1.6 times compared with the control group, results of the second group were not significantly different; results in the third group showed significant decrease in the concentration of IL-4 1.9 times below normal (Table 2). Anti-inflammatory cytokines, particularly IL-4, are involved in limiting the inflammatory response by inhibiting the secretion of proinflammatory cytokines and regulating, thus, the severity of tissue damage. The most marked decrease of IL-4 levels was detected in patients with severe degree of contamination.

Production of proinflammatory cytokine IL-6 in the blood of the patients had a tendency to increase, depending on the degree of HP infection. In the first group patients' blood the levels of IL-6 was 1.3 times higher, in the second group IL-6 had a tendency to increase (1.1 times), and the third was higher at 1.2 compared with the control group. In studied group a significant increase of proinflammatory IL-6 was detected, compared with the control group, which reflects the activation of the inflammatory process occurring in the gastric mucosa of patients with H. pylori gastritis.

Analysis of the data showed that the level of α-TNF in the blood of patients significantly increased in all groups of HP dissemination compared with the control group. The concentration of α-TNF in the first group significantly increased 1.4 times above normal, in the second group it was higher 1.5 times ($p \leq 0,05$), in the third there was an increase 1.3 times above normal ($p < 0,001$) compared with the control group. Cytokines Th1-polarized immune response (α-TNF) affect on the regulation of acid in the stomach, causing loss of cells and associated ulcerative defects or atrophy of glands, as well as cell proliferation, metaplasia, and epithelial dysplasia.

Thus, the experimental studies on mice with the transfer of T-lymphocytes demonstrate that the inflammatory response, with a dominant Th1-type results the stimulation of IFN-gamma G-cells. Gastrin activates the parietal cells, causing it to hyperfunction, which causes hyperacidity. As the depletion of the pool there is atrophy of the parietal cells of gastric mucosa [6]. Based on the data, increased α-TNF can be characterized as a predictor of possible destructive changes in the gastric mucosa of patients with severe degree of HP contamination.

By the content of α-tumor necrosis factor (Th-1) and IL-4 (Th-2) we can judge of the imbalance ratio of Th-1 and Th-2, particularly the activation of immune response of Th1. It is generally accepted that Th-2 reflects the persistence of chronic infection and colonization of HP, and Th-1 prevents the colonization of HP in gastric mucosa. According to some authors, the effectiveness of antibacterial response belongs to the Th-1 immune response, whereas humoral Th-2 pathway is not sufficient [1, 3, 15]. Others believe that the most appropriate immune response to HP would be a Th-2, which cause minimal damage to the gastric mucosa. Thus, Th-2

immune response explanation of the "African enigma": when an exceptionally high colonization of HP the natives rarely have a stomach ulcer and duodenal ulcer [13].

Our data indicate the dysfunction of the immune system of patients with chronic gastritis, depending on the degree of HP contamination. There is evidence of activation of immune response to Th1-type (anti-inflammatory cytokine reduction of IL-4 and increased pro-inflammatory α-TNF), with increased synthesis of immunoglobulins IgA and IgG and lower secretory of sIgA.

Conclusion

1. A significant reduction of secretory sIgA and increased serum IgA and IgG in the blood of patients with chronic gastritis associated with HP, compared with the control group is shown.

2. A significant increase in the concentrations of CRP inflammatory marker in patients with chronic gastritis with severe HP dissemination is found.

3. The increase of pro-inflammatory cytokines (IL-6, α-TNF) against decrease of anti-IL-4 in the blood of the patients with chronic gastritis, depending on the degree of HP contamination is revealed.

References

1. Alexandrova V.A., Kozlova I.P. Immune response of Helicobacter pylori infection/ V.A. Aleksandrova, I.P. Kozlova // Treating physician. – 2002. – № 11. –P. 13 – 16.
2. Androsov V.T. Clinico-pathogenetic variants of chronic gastritis in the Far North (Yakut ASSR): abstract. dis. ... candidate. med. science. / V.T. Androsov – Yakutsk, 1984. – 18p.
3. Antibodies to Helicobacter pylori for stomach diseases (Russian) / L.B. Lazebnik T.M. Tsaregorodtseva, T.I. Serov [and others] // Terapevt.arh. – 2006. – T. 78, № 2. – P.15 – 19.
4. Aruin L.I. Morphological diagnosis of the stomach and intestines diseases/ L.I. Aruin, L.L. Kapuller, V.A. Isakov – M.: Triada-X – 1998. – 496p.
5. Emelyanova E.A., Safonova S.L. Chronic gastritis and peptic ulcer disease in the North (Russian) / E.A. Emelianova, S.L. Safonova. – Yakutsk. – 2000. – 96p.
6. Kononov A.V. Inflammation as a basis for Helicobacter pylori-associated diseases/ A.V. Kononov // Archives of Pathology. – 2006. – T.68., № 5 – P. 3 – 5.

7. Loskutova K.S. Changes in the mucous membrane of antrum in Helicobacter pylori-associated gastritis in the population of Yakutia / K.S. Loskutova //Messenger of YSU. – 2006. – V.3., № 2. – P. 22 – 26.
8. Features of ulcer disease in the indigenous population of the North/ S.S. Sosina, P.M. Ivanov, A.I. Ivanov and [others] //Actual problems of clinical oncology and prekantserogeneza: interreg. conf. materials. – Yakutsk. – 2005. – P.75-76.
9. Ostanin A.A. Characteristics of apoptosis and the functional activity of lymphocytes in patients with peptic ulcer disease / A.A. Ostanin, A.I. Paltsev, A.G. Lebedev // Bulletin of Siberian Branch of RAMS. – 2004. – № 1 (111). – P. 129 – 134.
10. Petrova P.G. Ecology, adaptation, health/ P.G. Petrova; in red. of N.A. Aghajanian. – Yakutsk: Sakhapoligrafizdat. – 1996. – 269p.
11. Tikhonov D.G. Diseases of the esophagus, stomach and duodenum in the Republic of Sakha (Yakutia) (Clinical and epidemiological study. Outpatient care): abstract. dis.... dr. med. science/D.G. Tikhonov – M., 1993. – 39p.
12. Fedorov A.I. Features of the immunoglobulin status of the population of Yakutia, depending on the geographic latitude of residence (Russian)/ A.I. Fedorov, V.L. Osakovsky //Yakutsk Medical Journal. – 2008. - № 4(24). – P.29 – 31.
13. Activation of the complement system during and after cardiopulmonary bypass surgery: postsurgery activation involves C-reactive protein and is associated with postoperative arrhythmia/ P. Bruins, H. te Velthuis, A. P. Yazdanbakhsh [et al.] //Circulation. – 1997. – Vol. 96. – P. 3542-3548.
14. Local cytokine response in Helicobacter pylori-infected subjects/ C. Lindholm, M. Quiding-Jarbink, H. Lonroth [et. al.]//Infect. and Immun. –1998.– Vol. 66. –№12. – P. 5964–597.
15. Validation of a serological test for the diagnosis of Helicobacter pylori infection and the immune response to urease and CagA in children/ M. Camorlinga-Ponce, J. Torres, J. Perez-Perez [et. al.]// Am. J. Gastroenterol. – 1998. – Vol. 93. – P. 1264 – 1270.

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Table1

Indicators of humoral immunity in the blood of patients suffering from chronic gastritis according to the degree of HP contamination.

Indicators	M±m (mg/l)			
	control group (n=20)	I group (n=26)	II group (n=18)	III group (n=43)
Ig A	1,8±0,76	2,66±0,26*	3,19±0,03*	3,24±0,25
Ig G	14,58±1,59	14,7±0,03*	21,97±0,03*	17,46±0,04*
sIg A	3,58±1,89	1,22±0,04*	0,97±0,04*	1,46±0,03*

Note:* P ≤ 0,05 when compared with the control group

Table 2

Concentration of IL-4, IL-6 and α-TNF in the blood serum patients with chronic gastritis according to the degree of HP contamination

Показатели	M±m (pg/ml)			
	control group (n=20)	I group (n=26)	II group (n=18)	III group (n=43)
IL-4	0,22±0,01	0,14±0,01*	0,21±0,05	0,17±0,03*
IL-6	2,8±0,02	3,56±0,30*	3,08±0,18	3,31±0,26
α-TNF	2,5±0,06	3,56±0,12*	3,77±0,22*	3,34±0,15*

Note:* P ≤ 0,05 when compared with the control group