International Journal of Biomedicine 2(2) (2012) 117-123

INTERNATIONAL JOURNAL OF BIOMEDICINE

Clinical Research

Interleukin-1B and Interleukin-1 Receptor Antagonist in Patients with Helicobacter pylori Associated Diseases

Olga V. Shtygasheva, PhD, ScD, Elizaveta S. Ageeva, PhD*

Nicolay F. Katanov State University of Khakassia, Abakan, Russian Federation

Abstract

The ethnic people of the Republic of Khakassia (the Khakas) with ulcer disease show a significant T-cell activation and humoral immune response when compared with the Europoids. The reasons for such differences could be due to certain ethno -specific allelic variants of the interleukins, which considerably change the degree of cytokine expression. The aim was to study the peculiarities of the association of the interleukin-1 (IL-1) gene polymorphisms and interleukin-1 receptor antagonist (IL-1Ra). Patients with chronic gastritis and ulcer disease were examined using the restriction analysis method. The most wide-spread allelic variants among the Khakas were discovered to be CC IL-1β and R4R4 IL-1Ra. In this study, we suggest the necessity to define the population`s risk and the protective genotypes that promote Helicobacter pylori-associated ulcer disease among the Khakas people. IJBM 2012; 2(2):117-123.

Key words: *Helicobacter pylori, ulcer disease, the Khakas, Th1/Th2 immune response, gene polymorphism, interleukin-1.*

Introduction

The interaction of Helicobacter pylori (H. pylori) with the host is known to be marked by significant population differences. Phenomena such as "African enigma", "Asian enigma" [1] are described in some research works. They show the peculiarity of various H. pylori isolates and their attachment to the different ethnic groups [2].

The epidemiological research on the incidence of ulcer disease among the Khakassian population shows that the incidence of ulcer disease among the non-ethnic population is 8.9% and among the ethnic population – 4.5% with H. pylori contamination of 97.8 % and 95.2%, correspondingly. The research proves the ethno-ecological dependence in the distribution of the H. pylori strains: the non-ethnic people with ulcer disease have s1s2 VacA H. pylori subtype, while the ethnic people have CagA H. pylori [3, 4].

While analyzing the immunophenotype of the inflammatory cell infiltrate we discovered clear differences in the functional activity of the immune response. Compared with the non-ethnic people, the ethnic ones have a significant activation of T-cell and humoral immune responses. The reasons for such differences can be some ethno-specific allelic variants of interleukins which considerably change the expression level of the cytokines and the immune response character.

The important cytokine identified in the Helicobacter pylori-associated inflammation is interleukin-1 (IL-1), which links the key stages in the development of gastroduodenal disorders: the intensive character of the proinflammatory reaction, proapoptopic and hypoacidic effects [5].

The IL-1 functional system consists of the IL-1α, IL -1β molecules, IL-RI and IL-RII receptors, IL-Ra receptor antagonist. IL-1β is a predominant form and it is considered a multifunctional mixed-activity cytokine. The result of this cytokine`s effects is seen in the activation of the neutrophils and T- and B-lymphocytes, the stimulation of acute-phase proteins and prostaglandin synthesis, the promotion of chemotaxis, phagocytosis, hematopoiesis, hyperpermeability of the vascular wall, cytotoxic and bactericidal activity. Under the influence of IL-1β, the vascular endotheliocytes produce polypeptides which

Corresponding author: Elizaveta S. Ageeva, PhD, Head of Fundamental Medicine and Hygiene Department, Federal State Budgetary Educational Institution of Higher Professional Education "Katanov State University of Khakassia", 178, Pushkin str., Abakan, 655004, Khakassia, Russian Federation

Tel: 7-3902-342720

E-Mail: Ageevaeliz@rambler.ru

stimulate cell migration, proliferation, pro-inflammatory mediators` induction (IL-2, -3, -6, TNFα, E-selectins). IL-1β is a strong inhibitor of hydrochloric acid in the gastric juice. Its effect is 100 times higher than the action of the proton-pump inhibitors, and 6000 times higher than that of the histamine H2-receptor antagonists [6]. The activity of this cytokine leads to the atrophic process in the stomach mucosal lining.

The IL-1 (IL-1Ra) receptor antagonist shows an affinity for the cytokine receptor and performs the role of a regulator in the expression level and IL-1β function. The IL-1Ra fails to provide intracellular signaling. Thus, it suppresses the potentially deleterious pro-inflammatory effect of IL-1β.

The IL-1 family genes encoding IL-1β and IL-1Ra are mapped on the long arm of chromosome 2 in the locus of q13-21. Gene IL-1β contains 22 exons: 20 of them are alternative ones and 9 are introns, 8 of which are alternative ones. Biallelic polymorphisms have been well researched. They are a variation of the nucleotides in T–31C, C–511T, C+3953T. The IL-1 (IL-1Ra) receptor antagonist gene is located in the 2nd intron. It is a microsatellite polymorphism – a variable number of identical tandem repeats (VNTR). The polymorphism presupposes the existence of five alleles, each of which has a definite number of repeats (R).

Such gene polymorphism implies the existence of high- and low-producing variants. Heterozygous (CT –511, TC –31, TC +3953) or homozygous (CC –511, TT –31, TT +3953) individuals produce twice to four times more cytokine than the homozygous for the "wild type" allele individuals (TT –511, CC –31, CC +3953) [7]. R2R2 genotype of IL-1Ra gene is associated with a higher activity of the gene promoter that leads to the hyperproduction of cytokines [8, 9].

However, various researches of the exact allelic variants associated with this or that form of disease contradict each other. The possible explanation of it is the existence of population-dependent associations between the gene alleles and the peculiarities of clinical presentations [10, 11].

The aim of this work was to study the peculiarities of the C+3953T IL-1β gene polymorphisms and IL-1Ra VNTR receptor antagonist in the ethnic and non-ethnic Khakassian groups as well as to reveal the possible associations with H. pylori-associated ulcer disease and chronic gastritis.

Material and methods

The object of research

We examined patients with duodenal ulcer (Europoids – 21, Khakas – 25), and chronic antral gastritis (Europoids – 59, Khakas – 63). The control group included healthy donors (Europoids – 60, Khakas – 63). All the patients examined belonged either to the ethnic population (the Khakas people) or to the non-ethnic population (Russians or Europoids). A comparable number of men and women participated in the research. The average age of the Europoids was 43.6, while the average age of the Khakas people was 42.9.

This study on these patients was based on the ethical principles stated in Article 24 of the Constitution of the Russian Federation and the World Medical Association Declaration of Helsinki (1964). The patients were instructed and they signed an informed consent to confirm their voluntary participation in the research.

The diagnoses of ulcer disease and chronic gastritis were based on the results of endoscopic examination and morphometry.

Helicobacter pylori

We used the histoscopic Giemsa staining method and rapid urease test to define H. pylori infection. H. pylori -specific IgG was detected in the blood serum using the enzyme-linked immunosorbent assay (ELISA) according to the manufacturer`s instruction (ImDispektr, Novosibirsk).

The analysis of polymorphisms

Total DNA was extracted from the peripheral blood leukocytes using the phenol-chloroform method. Amplification to define IL-1β gene polymorphisms was done with the primers (Table 1).

The C C+3953T IL-1β allele creates the restriction site for Taq I [12]. In TT C+3953T IL-1β genotype the length of the gene product is 249 bps.; in CT C+3953T IL-1β genotype the lengths of the gene products are 114, 135 and 249 bps.; in CC C+3953T IL-1β genotype the lengths of fragments are 114 and 135 bps.

Next, IL-1Ra polymorphism analysis was performed using the polymerase chain reaction method (PCR) with the primers (Table 1) which flanked a polymorphic locus, with a variable number of tandem repeats – 86 bps. [13]. Each allele is connected with a variable number of repeats (VNTR) [14]. The repeat length (86 bps.) was marked as R (repeats), 2 repeats (240 bps.) –

Table 1
Primers and loci (according to L.Joos, M.L. Laine [11, 12]

Cytokine	Specific primers[1]	Mutation	Restriction enzyme
IL-1Ra	F 5′ CTC AGC AAC ACT CCT AT 3’ R 5′ TCC TGG TCT GCA GGT AA 3’	VNTR	-
IL-1β	F 5′ GTT GTC ATC AGA CTT TGA CC 3′ R 5′ TTC AGT TCA TAT GGA CCA GA 3′	C+3953T	TaqI

Note: *[1]- “Sibenzyme” primers, Novosibirsk*

R2, 3 repeats (326 bps.) – R3, 4 repeats (412 bps.) – R4 and 5 repeats (498 bps.) – R5.

The amplification mix contained the following: 0.5 µl of each primer (R and F), 1.5 µl 25 mM MgSO4, 1.5 µl dNTP, 1.5 µl 10x buffer, 0.15 µl Tag-polymerase (Sibenzyme, Novosibirsk), 8.35 µl diH20 and 1 µl of DNA, mineral oil.

Amplification was performed in the "Tercyc" PCR thermocycler (DNA-Technology Company, Moscow) (heated up to 92°C). Thirty cycles of amplification reaction gave the following results: 92°C – 1 min., 56°C – 1 min., 72°C – 2 min., the elongation period (72°C) – 5 min.

Electrophoresis

The PCR-products were analyzed using 4% agarose gel electrophoresis with a 10% ethidium bromide solution (Sigma, USA) followed by UV light visualization. The pUC19 plasmid cut with the MspI restriction enzyme (Sibenzyme, Novosibirsk) was used as a DNA length marker.

Statistic analysis

The genotype distribution at the analyzed polymorphic loci was checked to see whether the genotypes observed conformed to the Hardy-Weinberg equilibrium using a χ^2 test. The data were analyzed using the GENDIST statistical package created by V.A. Stepanov. Analysis of the association of polymorphisms with chronic gastritis and ulcer disease was performed using the odd ratio (OR).

Results

The C+3953T IL-1β frequency in the population sample of the Khakas and Europoids in the control group

The distribution of IL-1β C+3953T polymorphism in the Khakas patients of the control group is presented in Table 2. The most frequent genotype was CC +3953 IL-1β - 73.3%. The CT +3953 IL-1β genotype was identified in 23.3% of the patients. TT+3953 IL-1β was a rare genotype which occurred in 3.4%. The control group of the Europoids had the same tendency of C+3953T IL-1β genotype distribution as in the Khakas patients. The CC genotype occurred at a twice the lower frequency when compared with the Khakas patients. At the same time, the frequency of the CT heterozygotes and TT homozygotes +3953 IL-1β dominated in the Europoids.

The C+3953T IL-1β frequency identified in the population sample of the Khakas and Europoids with H. pylori-associated ulcer disease and chronic gastritis

The distribution of the IL-1β C+3953T polymorphism in the patients with H. pylori-associated ulcer disease and chronic gastritis is presented in Table 2.

Table 2

Genotype and C+3953T IL-1β gene alleles' frequency among the Europoids and Khakas people with Helicobacter pylori-associated chronic gastritis and ulcer disease

Groups		Europoids			Khakas people		
		Healthy donors	Patients with ulcer disease	Patients with chronic gastritis	Healthy donors	Patients with ulcer disease	Patients with chronic gastritis
Genotypes							
CC	%	46.7	70.0[1]	59.2	73.3[3]	83.9	63.1[2]
	n	14	14	29	22	26	36
CT	%	33.3	20.0[1]	24.5	23.3	16.1	36.9[1,2]
	n	10	4	12	7	5	21
TT	%	20.0	10.0[1]	16.3	3.4[3]	0	0
	n	6	2	8	1	0	0
Alleles							
C		63.4	80.0[1]	71.5	84.9[3]	92.0	81.6[2]
T		36.6	20.0[1]	28.5	15.1[3]	8.0 3	18.4[2,3]
PXB (χ^2)		2.39 4	2.39[4]	7.84	0.22[4]	0.24 4	2.91[4]

Note: *[1] – p<0.05 – in comparison with healthy donors, [2] – p<0.05 – in comparison with ulcer disease, [3] – p<0.05 – in comparison with the Europoids, [4] – p>0.05 – genotype frequency distribution according to Hardy-Weinberg equilibrium.*

The analysis showed that the CC homozygote frequency in the Khakas patients with ulcer disease was considerably higher than in the healthy donors. Furthermore, the CC +3953 IL-1β genotype in the patients with ulcer disease occurred more frequently than in the patients with chronic gastritis (83.9% and 63.1% correspondingly, p<0.05). The CT heterozygotes occurred more often in the Khakas patients with chronic gastritis (36.9%) compared with the control group (23.3% p<0.05) and the patients with ulcer disease (16.1% p<0.05). The TT homozygotes were found neither in the cases with chronic gastritis nor in those with ulcer disease (Table 2).

The CC +3953 IL-1β genotype was also found dominant in the Europoid patients. Its frequency was higher in the patients with chronic gastritis and ulcer disease compared with the control group (Table 2). The analysis showed that the risk of developing ulcer disease in the Europoid patients was associated with the CC +3953 IL-1β genotype (OR=2.9, 95% CI 1.6-5.6). Regarding the CT +3953 IL-1β heterozygotes and TT +3953 IL-1β homozygotes in the Europoid patients, the indices dropped lower when compared with the control group (Table 2). The CT and TT +3953 IL-1β genotypes in the patients with ulcer disease occurred less frequently than in the patients with chronic gastritis and in the control group ($p<0.05$).

During the interpopulation analysis of the genotype distribution in the healthy Khakas and Europoid patients, the CC +3953 IL-1β homozygotes were found to occur more often in the Khakas patients than in the Europoid group. At the same time, TT +3953 IL-1β homozygotes in the Khakas patients occurred rarely when compared with the Europoid patients (Table 2).

The IL-1RaVNTR frequency in the population sample of the Khakas and Europoid patients in the control group

The IL-1RA polymorphism distribution in the control group of the Khakas patients is represented in Table 3.

The R4R4 genotype occurred in 80% of patients in the control group, while R3R4 were found in 10.0% of the healthy donors.

The most frequent genotype in the group of healthy Europoids was R4R4 (33.3%). The rarest genotypes were

Table 3

Genotype and IL-1Ra VNTR gene alleles' frequency among the Europoids with Helicobacter pylori-associated chronic gastritis and ulcer disease

Groups		Europoids			Khakas people		
		Healthy donors	Patients with ulcer disease	Patients with chronic gastritis	Healthy donors	Patients with ulcer disease	Patients with chronic gastritis
Genotypes							
R2R2	%	6.7	10.0	10.2	3.3[3]	0.0	1.8[3]
	n	2	2	5	1	0	1
R2R3	%	3.3	20.0[1]	12.2[1]	0.0	0.0	0.0
	n	1	4	6	0	0	0
R2R4	%	20.0	25.0	14.3[2]	0.0	0.0	1.8[3]
	n	6	5	7	0	0	1
R3R3	%	10.0	15.0	10.2	6.7	3.2[3]	7.0
	n	3	3	5	2	1	4
R3R4	%	26.7	10.0[1]	20.4	10.0	19.4	14.0
	n	8	2	10	3	6	8
R4R4	%	33.3	20.0[1]	32.7[2]	80.0[3]	77.4[3]	75.4[3]
	n	10	4	16	24	24	43
Alleles							
R2		18.4	32.5[1]	23.5	3.3[3]	2.7[3]	0
R3		25.0	30.0	26.5	11.7	14.0[3]	12.9
R4		56.6	37.5[1]	50.0	85.0[3]	83.3[3]	87.1[3]
PXB (χ^2)		2.81[4]	2.70[4]	6.26[4]	37.88[4]	0.77[4]	34.49

Note: *[1] – $p<0.05$ –in comparison with healthy donors, [2] – $p<0.05$ – in comparison with ulcer disease, [3] – $p<0.05$ – in comparison with the Europoids, [4] – $p>0.05$ – genotype frequency distribution according to Hardy-Weinberg's equilibrium.*

R2R2 (6.7%) and R2R3 (3.3%), although their frequency of occurrence was higher than that of the Khakas patients.

The IL-1Ra VNTR frequency in the population sample of the Khakas patients with H. pylori associated ulcer disease and chronic gastritis

The basic genotype of the IL-1Ra gene polymorphism, which dominated in the groups of the Khakas patients examined, was the R4R4 variant (75.4% - in the patients with chronic gastritis and 77.4% - in the patients with ulcer disease) (Table 2). The second most frequently occurring variant was R3R4 (19.4% in the patients with ulcer disease and 14.0% in the patients with chronic gastritis, $p>0.05$). In our study, the rare genotypes were identified as R3R3, R2R2 and R2R4 IL-1Ra (Table 3).

While studying the IL-1Ra gene polymorphism in the Europoid patients, it was found that R4R4 IL-1Ra was the dominant genotype in all the groups examined. The occurrence of R2R2 and R2R3 IL-1Ra in the patients with ulcer disease and chronic gastritis was higher when compared with the control group (see Table 3). It must be noted that there was a clear association between the R2R3 IL-1Ra genotype and the risk of developing ulcer disease (OR=7.1, 95% CI 1.9-31.4).

The frequency of the R4R4 IL-1Ra in the Khakas patients was several times higher than in the Europoid patients. At the same time, the R2R2 and R2R3 IL-1Ra were more frequent in the Europoid patients with ulcer disease and chronic gastritis compared with the analogous group of the Khakas patients (Table 3).

Discussion

The essence of the "African enigma", "Asian enigma" lies in the fact that gastric ulcers or duodenal ulcers seldom develop in the case of a higher level of H. pylori colonization, which means we are dealing with the conservation of the definite pathogenic strains in the population. The association of the various subtypes of H. pylori (s1s2 subtypes VacA H. pylori in the non-ethnic patients, CagA – in the ethnic patients) influences the formation of essential differences in the pathogenesis of H. pylori-associated stomach diseases. The explanation of this phenomenon probably lies in the unique character of the main pathologic factors, including the genetically determined character of the immune response, which leads to the binding of particular H. pylori strains in the organism. As a result, some populations of clinicopathologic peculiarities are formed.

The proinflammatory effect of the IL-1β depends not only on the rise or on drop in the expression of the IL-1β or IL-1Ra but also on the combination of the IL-1β/IL-1Ra. The cytokine network is a complex of intercellular interactions of interleukins, receptors and their antagonists. The IL-1β effects are realized only after their interaction with a specific membrane receptor. At the same time, the IL-1Ra antagonist regulates the IL-1β effects. It suppresses the potentially deleterious pro-inflammatory effect of IL-1β by binding to the receptor.

We isolated considerable differences in the clinical presentations of ulcer disease in the Europoid and Khakas patients [15]. In the group of Europoid men, we identified the syndrome of ulcerous dyspepsia three times more frequently than in the Khakas men group. Heartburn was registered 2.8 times more often, correspondingly. Right hypochondrium syndrome was registered among the Europoid women 3.5 times more often than among the Khakas women. The frequency of esophagitis and incompetence of cardia were definitely higher in the patients with ulcer disease in both populations when compared with patients without any pathology. The incidence of gastroduodenal erosion was 36.2% in the Europoid patients with ulcer disease and 17.8% in the control group ($p < 0.001$). The corresponding indices in the Khakas patient group were 13.5% and 11.3% ($p > 0.5$). Ulcer disease in the Europoid patient group was aggravated more often and could be characterized as more aggressive both in the number of clinical syndromes and in the character of their complications. The Europoid patient group was marked by bleeding ulcers (17.3%), gastric perforation (4.3%), and pyloric stenosis (4.9%). The indices in the Khakas patient group were 8.2% ($p<0.05$), 4.7% ($p>0.5$) and 2.4% ($p>0.1$) correspondingly [15]. It could be connected with the R2R2 IL-1Ra genotype in the Europoids and its spread could reach 23.0-26.0% of the population [16,17]. The IL-1Ra carriers possess a genetically determined inflammatory reaction with a capacity for self-maintenance that results in the disturbing cell turnover and developing mucosal coat atrophy. The high IL-1Ra production suppresses the IL-1β effects, and at the same time, through the negative feedback loop, the IL-1β production increases along with the development of chronic persistent inflammation [18, 19]. The IL-1Ra production also increases.

The high-producing R2R2 IL-1Ra genotype was rarely found to occur in the Khakas patient group and did not occur at all in the patients with ulcer disease. The data obtained on gene polymorphism distribution in the Khakas patient group concur with the data presented in the medical literature: The R2R2 IL-1Ra genotype occurs in only 6% of cases in the Mongoloids [20]. The most widespread variants among the Khakas patients are the CC IL-1β and R4R4 IL-1Ra genotypes associated with a low expression level of the corresponding protein molecules. Accordingly, the proinflammatory effect may be milder.

The data obtained earlier show that the Europoid patients with ulcer disease have a low acidity level (pH) in the body of the stomach. In the Khakas patient group, those with ulcer disease high acidity in the body of stomach were identified as occurring 36% more often than in the patients without ulcer disease, although these differences cannot be considered accurate [15]. On the one hand, these data contradict the character of disease distribution in the ethnic and non-ethnic patients because the high acidity proves to be an "aggressive factor" [21]. However, the peculiarities of acid production in the Khakas and Europoid patients can also be connected with the expression of IL-1β, one of the acid inhibitors. The IL-1β initiates a strong inflammatory response, which results in significant damage of the epithelium, and the destruction of the gastric glands. The atrophic process in the mucosal stomach lining leads to hypochlorhydria, which contributes to H. pylori colonization in the stomach, thereby potentiating inflammatory changes [22]. The interleukin-1 level considerably influences the pH level of the gastric contents. It leads to the elimination of the pathogen. However, on leaving the antrum, the H. pylori migrated to the body of the stomach showing the population peculiarities of ulcer defects` localization .

A relatively higher rate of the incidence of ulcer disease (compared with the incidence of duodenal ulcer) was registered among the Khakas patients (in relative value units). The proportion of the duodenal ulcers, and ulcers in the antrum and body of the stomach was 3.5:0.8:1.0 among the Russians and 1.7:1.0:1.0 – among the Khakas [15].

Overall, on comparing our results with the data in other medical research, we concluded that the associations of VNTR IL-1Ra and C+3953T IL-1β polymorphisms in the Khakas patients with ulcer disease and chronic gastritis

have the same distribution that is characteristic of other ethnic groups living in this Siberian territory. The Teleuts of Kemerovo Region, particularly, show similar peculiarities of allele distribution and VNTR IL-1Ra genotype frequencies as do the Khakas [23].

The immunogenetic peculiarities analyzed appear to be one of the explanations for the differences in the immune response and clinical presentations of the ulcer disease among the Mongoloid and Europoid populations of Khakassia. To summarize, in the analysis of the population peculiarities associated with interleukin-1 gene polymorphism, it must be noted that the most widespread low-producing polymorphic gene variants of interleukin in the Khakas patients form the immune response with a more significant activation of the T-cell and humoral immune response compared with the non-ethnic patients. It proves that the Khakas people have a more effective antimicrobial immunogenotype. More aggressive forms of the ulcer disease, characteristic of the Europoids can be connected with different factors that promote the local inflammation including the peculiarities of the distribution of the IL-1 polymorphic genes.

Conclusion

The individual gene expression defines the character and intensity of the inflammatory response, and therefore, the prognosis of the interaction between H. pylori and the host. The data obtained from our research proves the presence of some significant differences in the ulcer disease characteristics and its most important pathogenetic factors in the Mongoloid and Europoid populations living in Khakassia. The data also proves our hypothesis regarding the causes of the ethnically determined differences in the development of H. pylori-associated diseases among the Khakas and Europoid people. The basis on the differences occur is the genetic peculiarities connected with the interleukin gene polymorphism such as IL-1β and its functional IL-1Ra antagonist.

Acknowledgment

This research was conducted under the instructions of the Ministry of Education and Science of the Russian Federation «Studying the genetic basis of multifactorial diseases» (2012).

References

1. Perez-Perez GI, Rothenbacher D, Brenner H. Epidemiology of Hlicobacter pylori Infection. Helicobacter 2004; 9:1-6.
2. Yamaoka Y, Malaty HM, Osato MS. Concervation of Helicobacter pylori genotypes in different ethnic groups in Huoston, Texas. J Infect Dis 2000; 181 (6):2083-2086.
3. Tsukanov VV, Barkalov SV, Tonkikh YuA, Shtygasheva OV. et al. The incidence rate of CagA-strains of Helicobacter pylori and ulcer disease among the population of Eastern Siberia. Therapeutic Archive 2007; 2:15-18.
4. Shtygasheva OV, Tsukanov VV. The association of cagA and vacA Helicobacter pylori strains and ulcer disease in the organized population of Abakan. The Russian Journal of gastroenterology, hepatology and coloproctology 2004; 2:84-87.
5. Kononov AV. Molecular genetics and inflammatory phenotype caused by Helicobacter pylori. Omsk Scientific Journal 2007; 3(61):17-23.
6. Wolfe MM. Hypergastrinemia and colonic neoplasia: coincidental or related? Gastroenterology 1992; 103:1361-1363.
7. Liou JM, Lin JT, Chang CY, Chen MJ et al. Levofloxacin-based and clarithromycin-based triple therapies as first-line and second-line treatments for Helicobacter pylori infection: a randomised comparative trial with crossover design. Gut 2010; 59:572-578.
8. Gromova AYu, Simbirtsev AS. The polymorphism of IL-1 gene family. Cytokines and inflammation 2005; 2:24-35.
9. Wilkinson RJ, Patel P, Llewelyn M et al. Influence of polymorphism in the genes for the interleukin (IL)-1 receptor antagonist and IL-1β on tuberculosis. J Exp Med 1999; 189:1863-1873.
10. Landi S, Moreno V, Gioia-Patricola L et al. Association of common polymorphisms in inflammatory genes interleukin (IL)6, IL8, tumor necrosis factor-α, NF-κB1, and peroxisome proliferator-activated receptor γ with colorectal cancer Cancer Res 2003; 63:3560-3566.
11. McCarron SL, Edwards S, Evans PR et al. Influence of cytokine gene polymorphisms on the development of prostate cancer. Cancer Res 2002; 62:3369-3372.
12. Laine ML, Farre MA, Bart J et al. The Mouthwash: A Non-Invasive Sampling Method to Study Cytokine Gene Polymorphisms. Periodontol 2000; 71(8):1315-1318.
13. Joos L, McIntyre L, Ruan J, et al. Association of IL -1B and IL-1 receptor antagonist haplotypes with rate of decline in lung function in smokers. Thorax 2001; 56:863-866.
14. Tarlow JK, Blakemore AI, Lennard A et al. Polymorphism in human IL-1 receptor antagonist gene intron 2 is caused by variable numbers of an 86-bp tandem repeat. Hum Genet 1993; 91:403-404.
15. Shtygasheva OV. Clinicopathologic characteristics of ulcer disease among the population of the Republic of Khakassia. Abstract of the thesis for a doctor`s degree. Krasnoyarsk 2005; 48 p.
16. Lee SG, Kim B, Choi W, et al. Lack of association between pro-inflammatory genotypes of the interleukin-1 (IL-1B -31 C/ and IL-1RN *2/*2) and gastric cancer/duodenal ulcer in Korean population. Cytokine 2003; 21:167-71.
17. Palli D, Saieva C, Luzzi I, et al. Interleukin-1 gene polymorphisms and gastric cancer risk in a high-risk Italian population. Am J Gastroenterol 2005; 100:1941-1948.

18. Bidwell J, Keen J, Gallagher G et al. Cytokine gene polymorphism in human disease: on-line database. Genes and Immun. 1999; 1:3-19.
19. Witkin SS, Gerber S, Ledger WJ. Influence of interleukin-1 receptor antagonist gene polymorphism on disease. Clin Infect Dis 2002; 34 (2):204-209.
20. Chen A, Li CN, Hsu PI, et al. Risks of interleukin-1 genetic polymorphisms and Helicobacter pylori infection in the development of gastric cancer. Aliment Pharmacol Ther 2004; 20:203-211.
21. Goodwin CD. Duodenal ulcer, Campylobacter Pylori and the "leaking roof" concept. Lancet 1988; 2(8626/8627):1467-1469.
22. Kononov AV. Genetic regulation and inflammatory phenotype in the case of Helicobacter pylori-infection. Pathology Archive 2009; 71(5):57-63.
23. Shabaldin AV, Ostaptseva AV, Akhmatyanova VR. et al. Polymorphism of interleukin-1 receptor antagonist in the non-ethnic and ethnic people of Kemerovo Region. Immunology 2007; 2:72-74.