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ANALYSIS OF THE CLINICAL OUTCOMES OF SOME GASTRODUODENAL DISEASES IN YAKUTIA: ICEA1 STRAINS OF HELICOBACTER PYLORI RELATIONS WITH THE EARLY ONSET OF CHRONIC GASTRITIS

The article presents the results of a study of clinical outcomes in patients with several gastroduodenal diseases, depending on the allelic variants of the gene iceA Helicobacter pylori. The iceA1 strains were identified in 65.2% cases and iceA2 – in 34.7% cases. We found no associations according to gender of patients, their place of birth or residence. However, statistically significant differences in the distribution of iceA gene alleles were found depending on age of patients with chronic gastritis. Obtained results may indicate the early onset of chronic gastritis on carriers of iceA1 strains circulating in Yakutia.

SUMMARY

It is known that the clinical outcome of gastroduodenal diseases may depend on the virulence and pathogenicity factors of *Helicobacter pylori* strains. One of these factors is *iceA* gene which has two allelic variants – *iceA1* and *iceA2*. Earlier *iceA1* strains of *H. pylori* were associated with gastric ulcer in some populations of Europe, Asia and America. In Yakutia clinical outcomes of some gastroduodenal diseases depending on virulence and pathogenicity factors of *iceA* gene of *H. pylori* strains previously has not been studied. The aim of this work is to study clinical outcomes in patients with some gastroduodenal diseases depending on the allelic variants of *H. pylori iceA* gene in the Sakha Republic (Yakutia). Study sample totaled DNA samples of *H. pylori* isolated from biopsies of 92 Yakut patients with gastroduodenal diseases confirmed by histological examination (chronic gastritis, ulcers and erosions of the stomach), of which 43 was adolescent and 49 adults. The *iceA1* strains identified in 65.2% cases and in 34.7% *iceA2*. We found no association between strains *iceA1* and *iceA2* in patients with erosions and ulcers ($p > 0.05$), and did not found associations according to the gender of patients ($p>0.05$), and their place of birth or residence (urban or rural population) ($p>0.05$). However in Yakut population in adolescents with chronic gastritis is more common *iceA1* allele (79.0%), than in adults (53.0%) ($\chi^2=6.83$, $p<0.01$). The obtained results may indicate the early onset of chronic gastritis carriers *iceA1* strains, which generally proves more pathogenic properties of *iceA1* strains compared with the *iceA2* strains circulating in Yakutia.

Keywords: *Helicobacter pylori*, chronic gastritis, duodenal ulcer, Sakha Republic (Yakutia), *iceA* gene.

INTRODUCTION

Helicobacter pylori infection (*H. pylori*) has been recognized as the main cause of chronic gastritis (CG). Several epidemiological studies have shown that *H. pylori* infection is also associated with serious gastroduodenal diseases, including with peptic ulcer disease (PUD) and gastric cancer [17]. In 1994, the International Agency for Research on Cancer classified *H. pylori* infection to the I group carcinogen (obvious carcinogens), along with some of the radionuclides, radiation and certain chemicals [28]. The infection remains latent in most patients, and only about 20% of infected individuals develop serious diseases [16]. Manifestations of the diseases probably depend on environmental factors, lifestyle and eating habits, and also likely clinical outcomes can be affected by other factors such as the virulence and pathogenicity of *H. pylori* strains themselves.

At the moment known several virulence and pathogenicity factors of H. pylori associated with the ulcer and gastric cancer which are encoded by genes: *cagA*, *iceA*, *vacA*, *babA* and *oipA* [7, 9, 17, 19, 21, 30]. One of the most important factors of virulence and pathogenicity is *iceA* gene. The first series of studies have shown that the *iceA* gene (induced by contact with epithelium) has two variants – *iceA1* and *iceA2* [5]. The *iceA1* allele associated with gastric ulcer (GU) and duodenal ulcer (DU) [5, 8, 29]. Allelic variant of *iceA2* has no homology with known genes and it is still not clear function of *iceA2* product. Yet, some researchers have linked it with asymptomatic gastritis and non-ulcer dyspepsia [17]. Nevertheless, the role of *iceA* gene remains controversial, since some studies have failed to reproduce the observation of other samples of patients [7, 10, 26, 27, 29].

Earlier in Yakutia were performed researches dedicated on study of gastroduodenal pathology, which were mainly focused on the analysis of changes in the mucous of the antrum, and morphological pattern characteristic of *H. pylori*-associated gastritis in adults, children and adolescents [1-4]. Clinical outcomes of some gastroduodenal diseases, depending on the availability of *iceA* gene *H. pylori* strains, circulating in the Yakutia have not been studied.

The aim of this work is to study clinical outcomes in patients with several gastroduodenal diseases, depending on the allelic variants of *iceA* gene *H. pylori* in the Republic of Sakha (Yakutia).

MATERIALS AND METHODS

Study design

In total 144 patients were examined (mean age 34.97 ± 16.21 years) with gastroduodenal diseases, including 43 adolescents (mean age 15.05 ± 1.41 years) and 101 adults (mean age 43.45 ± 12.42 years). Patients with a previously diagnosis of CG were sent for analysis by doctors (pediatrician, therapist and gastroenterologist) in the endoscopic department of the Republican Hospital №1 – the

National Ministry of Health Medical Center of the Sakha Republic (Yakutia) on fibrogastroduodenoscopy (EGD). During EGD was performed fence gastrobiopsies. Subsequently, gastrobiopsies were sent for histological examination of the gastric mucosa in pathology department of Republican Hospital №1.

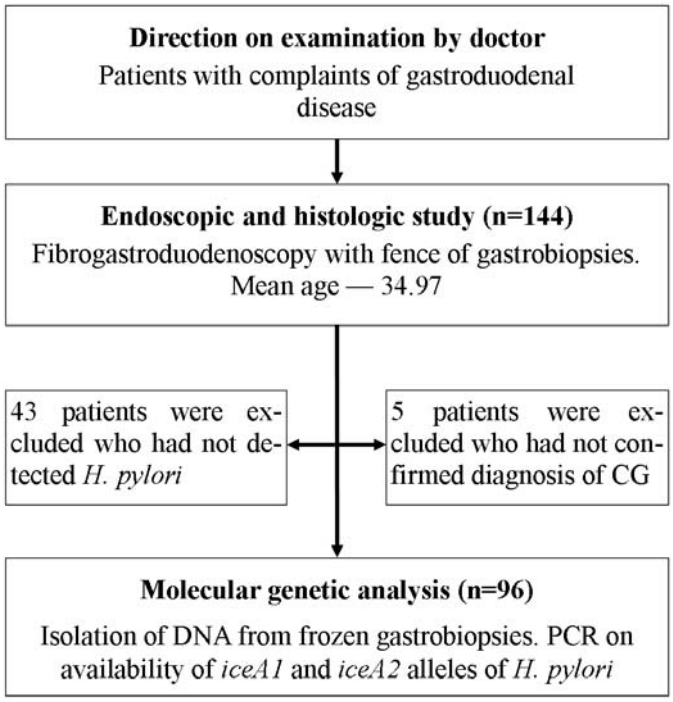

Fig. 1. Scheme of samples formation for molecular genetic assay. Note: CG – chronic gastritis, n – number of patients.

According to results of histological studies, among 144 patients, 43 had not been found *H. pylori* and in 5 patients did not confirm the diagnosis of CG (excluded from further analysis).

Among the remaining 96 patients with confirmed diagnosis of CG (n=46) and CG with erosions and ulcers (n=50) was performed molecular genetic analysis.

Endoscopic and histological examination

Fibrogastroduodenoscopy was held in the morning on an empty stomach. The fence of pieces was made from gastric antrum in an amount of 2-3 biopsies using fiberscope GIF-P3 of the "Olympus" company (Japan).

The obtained biopsy samples of gastric mucosa were fixed in 10% formalin solution. Deparaffinization of shear and staining by hematoxylin and eosin performed according to standard procedures. For sighting microscopy, shears were stained by the Romanovsky-Giemsa method. The study was performed under magnification x100, x400 and x1000 on the microscope "Axioskop" of the

"Opton" company. Morphological criteria of CG evaluated in accordance with the visual analog scale for the modified Sydney system (Houston, USA, 1996).

Molecular genetic analysis

From frozen gastrobiopsies in patients with confirmed histologic diagnosis of CG and CG with erosions and ulcers *H. pylori* genomic DNA was isolated by phenol-chloroform extraction.

Detection of iceA gene Helicobacter pylori

Amplification of the required DNA fragments of *H. pylori* was performed using PCR thermocycler «Bio-Rad». Detection of *iceA* gene was performed using the original sequence of oligonucleotide primers previously proposed (Table 1), which flank DNA region containing the *H. pylori iceA* gene.

Table 1

Design of oligonucleotide primers for *iceA* gene alleles detection

Gen, fragment	Name of oligonucleotide primer	Sequence 5 '→ 3'	Size of amplified fragment	Reference
iceA	*iceA1*	F5'-GTGTTTTTAACCAAAGTATC-3' R5'-CTATAGCCAGTCTCTTTGCA-3'	247 bp	9]
	iceA2	F5'-GTTGGGTATATCACAATTTAT-3' R5'-TTRCCCTATTTTCTAGTAGGT-3'	334 bp	9]

Note: R – any nucleotide

Separation of amplification products was carried in the horizontal electrophoresis chamber in a 3% agarose gel. Visualization of PCR products was performed by gel video documentary device «Bio-Rad» using software Image Lab ™ Software.

Statistical analysis

The results of molecular genetic studies were estimated by the test χ-square using Biostatd software (McGraw-Hill, Inc. Version 3.03). Differences considered statistically significant at $p<0.05$.

Ethical approval

Written informed consent was obtained from all individuals. This study was approved by the local Committee on Biomedical Ethics of the Federal State Budgetary Scientific Institution of the Federal State Budgetary Scientific Institution "YNC CMP" (Yakutsk, Russian Federation, Protocol No 41, November 12, 2015. Decision №5).

RESULTS AND DISCUSSION

In result of endoscopic and histological study from examined 144 patients, 96 individuals have been confirmed diagnosis of CG associated with the presence of *H. pylori*. The *iceA1* allele was identified in 65.2% cases (60 samples), and *iceA2* allele was detected in 34.7% cases (32 samples) (Table 2). Four samples were excluded from the study because three of them were negative on both alleles *iceA1* and *iceA2*, and one sample was positive on both alleles *iceA1* and *iceA2*.

Comparative analysis of *iceA1* and *iceA2* strains depending on the availability of ulcers and erosions

According to the results of molecular genetic studies comparing allele frequencies of *iceA* gene was carried out according to the availability of erosions and ulcers. As a result, no statistically significant differences in the distribution of alleles *iceA* gene between CG and erosions and ulcers were found (χ^2=0.11, p> 0.05) (Table 2). Several authors demonstrated that clinical outcomes with gastroduodenal diseases associated with specific alleles of *iceA* gene *H. pylori* [8, 16, 20, 22]. A recent study based on meta-analysis of *iceA* gene alleles on clinical outcomes showed that the *iceA1* allele was weakly but significantly associated with PUD, particularly DU, while *iceA2* showed no such association [16]. However, there are studies that did not confirm the existence of the association between allelic variants *iceA* gene and clinical outcomes [8, 18, 23, 24, 26, 27, 29]. In our study, also association of *iceA1* alleles with PUD was not received, and probably can be explained due to small number of our sample, or the lack of such association in the Yakut population.

Table 2

Comparison of the frequency of *iceA* alleles depending on the presence of erosions and ulcers, age and sex and demographic factors

Factors	n (92)	*iceA1* (%)	*iceA2* (%)	χ^2	*p*
Dependence on the presence of erosions and ulcers					
CG with erosions and GU/DU	42	27 (64.2%)	15 (35.7%)	0.11	>0.05
CG	50	33 (66.0%)	17 (34.0%)		
Dependence of age					
Adolescences	**43**	**34 (79.0%)**	**9 (20.9%)**	**6.83**	**<0.01**
Adults	**49**	**26 (53.0%)**	**23 (46.9%)**		
Comparison by gender					
Male	42	28 (66.6%)	14 (33.3%)	0.07	>0.05
Female	50	32 (64.0%)	18 (36.0%)		
Comparison on place of residence					
Urban population	12	8 (66.6%)	4 (33.3%)	0.01	>0.05
Rural population	80	52 (65.0%)	28 (35.0%)		

Note: GU/DU – gastric ulcers/chronic ulcers, CG – chronic gastritis.

Comparative analysis of *iceA1* and *iceA2* strains depending on age and gender and demographic factors

When comparing the distribution of *iceA* gene alleles statistically significant differences was not found, depending on the gender of patients (χ2=0.07, *p*>0.05) and also when comparing urban and rural population (χ2=0.01, *p*>0.05) (Table 2). Statistically significant differences in the distribution of *iceA* alleles were found among adolescents, where *iceA1* allele was identified in 34 cases out of 43 (79.0%) and in adults *iceA1* allele detected much less frequently, in 26 cases out of 49 (53.0%) (**χ^2=6.83,**

***p*<0.01**) (Table 2). In our study association of *iceA1* allele was received with early onset of CG, which in general can confirm the more pathogenic strains of properties *iceA1*. This association with age was previously shown in Tunisia, where they were obtained statistically significant differences between adolescent and adult patients [25]. However, the average age of patients in Tunisia (39.9, from 2 years to 88 years) was slightly higher than in Yakutia (29.8 years in age rank between 13 to 67 years). In similar studies associations with age on *iceA* gene has not been shown.

Comparative analysis of *iceA1* and *iceA2* strains on the degree of contaminations and intensity of inflammation

Clinical outcomes of patients with CG were evaluated by comparing the allelic variants according to the degree of contamination and the degree intensity of inflammation.

According to the degree of contamination in a sample of patients with CG who have been identified ulcers and erosion, as well as in the sample of patients without ulcers and erosions, often observed the first degree of contamination (up to 20 microbial bodies in the field of view). According to the degree of contamination showed general trend in the distribution of *iceA* gene alleles in both groups of patients (Fig. 2, A B).

According to the intensity of inflammation in a sample of patients with CG who have been identified ulcers and erosions, dominated the second degree of intensity (Fig. 2, B). Another picture shows the intensity of inflammation with CG without ulcers and erosions – on the intensity of inflammation distribution trends of occurrence *iceA1* gene alleles and *iceA2* not coincide, since the strains *iceA1* significantly dominated the first degree of intensity of inflammation (Figure 2, D.).

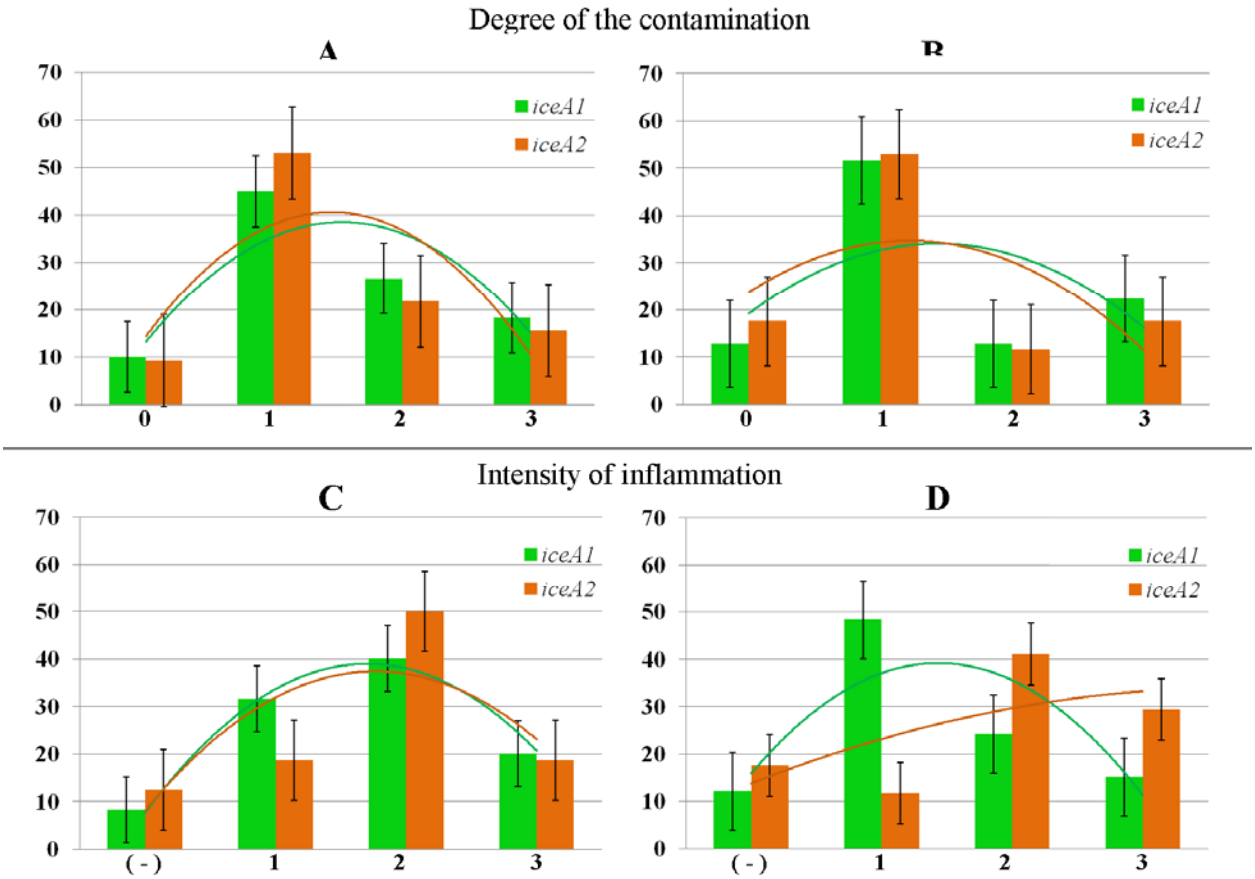

Figure 2. Comparison of allelic variants in the degree of contamination of the sample with the CG and ulcers and erosions with sample with CG to the absence of ulcers and erosions. A, B – CG with ulcers and erosions; B, D – CG without ulcers and erosions. Note: (-) – the absence of inflammatory activity.

Summarizing the comparison of the degree of contamination and inflammation activity we can conclude that in studied sample of patients was found weak intensity of inflammation in patients with *iceA1* strains and vice versa more severe degree of inflammation in *iceA2* In studies of Peek et al. [5] was shown the opposite trend – in patients with *iceA1* strains of *H. pylori* inflammatory infiltration of the gastric mucosa lamina propria were higher than in the presence of *iceA2* [5]. Authors explain this fact that possible *iceA1* genotype is associated with elevated levels of interleukin-8 and, therefore, with a more pronounced immune response to local microorganism [5, 31]. Probably weak inflammatory intensity in patients we studied colonized by *iceA1* strains of *H. pylori* can be explained as the structural features of genes and features in Yakut patients immune system. Obtained data about mismatch of inflammation intensity in carriers *iceA* strains in Yakutia requires further study.

CONCLUSIONS

Thus, we have found that in a population of Yakuts in adolescents with CG is more common *iceA1* allele (79.0%), than in adults (53.0%) (χ^2=6.83, p<0.01). The obtained results may indicate the

early onset of CG carriers *iceA1* strains, which generally proves more pathogenic properties of *iceA1* strains compared with the iceA2 strains circulating in Yakutia.

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