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APPROBATION OF THE MOLECULAR GENETIC METHOD FOR THE DIAGNOSIS OF *HELICOBACTER PYLORI* INFECTION IN YAKUTIA

ABSTRACT

In this paper we presented the results of approbation of the PCR method for the diagnosis of Helicobacter pylori infection based on the amplification of the *16S rRNA* marker gene of bacterial DNA isolated from samples of gastric mucosa tissue from patients with gastroduodenal diseases in Yakutia.

Keywords: Helicobacter pylori, gastroduodenal diseases, PCR, *16S rRNA* gene, histology, Yakutia.

INTRODUCTION

Helicobacter pylori (*H. pylori*) as considered is a main cause of the development of various gastroduodenal diseases, such as chronic gastritis, erosion and stomach ulcer in humans [1, 3, 5]. In 1994, the International Agency for Research on Cancer classified *H. pylori* infection to the I group of carcinogen (obvious carcinogens), along with some of the radionuclides and radiation [23]. Due to the fact that *H. pylori* is currently associated not only with certain gastroduodenal diseases, but also with severe oncological pathologies, it becomes necessary to specifically diagnose this infection. At present, in clinical practice, there are many different methods for diagnosing *H. pylori* [7, 11-13]. The variety of methods for detection of this infection can be divided into invasive (require fibrogastroduodenoscopy) and non-invasive. The main and most frequently used methods for diagnosing *H. pylori* infection are presented in Table 1. In addition, each of the method has its advantages and disadvantages [11-13]. The disadvantage of many non-invasive methods is their inaccuracy, and the invasive methods – risks of complications, as well as their duration and labor intensity. In clinical practice, the histological examination method is widely used, which allows at the same time to

Table 1

The main detection methods of *Helicobacter pylori*

Invasive methods*	Non-invasive methods**
Histological method: examination of a tissue sample of the gastric mucosa for *H. pylori*	ELISA: a study of feces for the presence of *H. pylori* antigens (using monoclonal antibodies)
Microbiological method: cultivation of *H. pylori* on substratum from a sample of gastric mucosa tissue	ELISA: detection of IgG antibodies to *H. pylori* in serum
PCR method: investigation by polymerase chain reaction on the presence of *H. pylori* DNA from a sample of the gastric mucosa tissue	Rapid urease test (CLO-test, Campylobacter-like organism test)
	Urea breath test (13C, 14C carbamide)
	PCR method: investigation by polymerase chain reaction for the presence of *H. pylori* DNA in saliva or feces

*- Require an endoscopic examination with a targeted biopsy and further study of gastrobiopsies;
** - Not require an endoscopic examination.

detect *H. pylori* and carry out a morphological evaluation of the gastric mucosa status [17]. The histological method for the detection of *H. pylori* is considered to be the "gold standard" to diagnose of this infection [8, 17], since histological sensitivity compose from 72 to 100%, and the specificity begins from 81 to 97% [18].

At present there are new approaches to the diagnosis of *H.* pylori infection, which include molecular genetic research methods by PCR, which is based on the amplification of the marker gene *16S rRNA* [10, 21]. This method excludes the possibility of amplification of homologous regions of the *16S rRNA* gene of the closest related species and *H. pylori* strains (*Campylobacter jejuni*, *Helicobacter cinaedi*, *Helicobacter mustelae и Wolinella succinogenes*) [10]. According to some authors the diagnostic sensitivity of PCR for detecting *H. pylori* in the gastric mucosa biopsies is 88-95,4% and specificity – 100% [13, 14].

Identification of *H. pylori* infection by PCR methods was not performed earlier in Yakutia. In earlier studies detection of this infection was performed by histological and cytological methods on gastrobiopsy specimens obtained at endoscopy [1, 4, 6, 7]. The aim of this study is approbation of the PCR method for the detection of *Helicobacter pylori* in patients with gastroduodenal diseases in Yakutia.

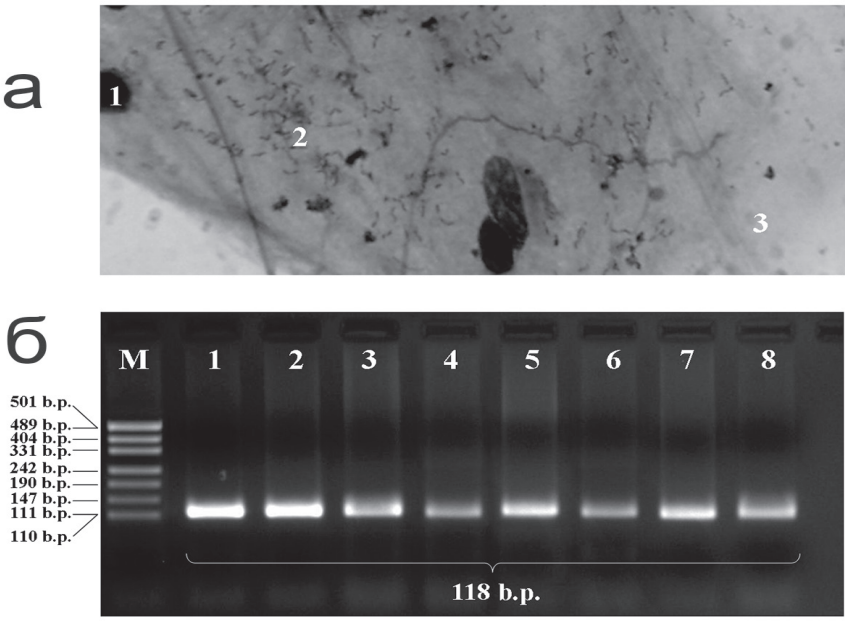

Figure 1. Examples of *H. pylori* detection using histological and molecular genetic studies.
A) Cytological micropreparation (Gram staining) of the patient's gastric mucosa with the third degree of dissemination (more than 50 microbial bodies in one visual field). 1 – cells of the epithelium of the stomach, 2 – congestion of *H. pylori*, 3 – mucous membrane;
B) Results of electrophoregram visualization after PCR analysis of *H. pylori 16S rRNA* gene. Lane 1-8 - samples of patients who gave a positive result for the presence of marker gene *16S rRNA H. pylori* (118 b.p.); M – is a mass molecular marker of pUC19/MspI

MATERIALS AND METHODS

Design of study

The sample of the study included 156 Yakut patients (from 6 to 70 years old, mean age 36.2 ± 17.5 years) with chronic gastritis. The patients observed at endoscopic department for fibrogastroduodenoscopy of the Republican Hospital No. 1 - the National Center of Medicine of the Ministry of Health of Sakha Republic (Yakutia) (RB No. 1 NCM). From 156 patients, 40 were children and adolescents (from 6 to 17 years, mean age 13.6 ± 2.6 years), the remaining 116 were adults (from 19 to 70 years, mean age 44.22 ± 13.84 years).

Endoscopic and histological examination

Fibrogastroduodenoscopy was performed in the morning, on an empty stomach. The biopsy sampling was carried out from the antral part of the stomach in an amount of 2-3 pieces during endoscopic examination with GIF-P3 fiberscope ("Olympus", 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 Romanowsky-Giemsa method. The study was performed under magnification x100, x400 and x1000 on the microscope "Axioskop" ("Opton"). Morphological criteria of chronic gastritis evaluated in accordance with the visual analog scale for the modified Sydney system (Houston, USA, 1996).

Detection of 16S rRNA gene of H. pylori

Genomic DNA of *H. pylori* was isolated from frozen gastrobiopsies of the examined patients by using phenol-chloroform extraction. Amplification of the required DNA fragments that flanking *16S rRNA* gene of *H. pylori* was performed using of the oligonucleotide primers described previously (Table 2). PCR was performed on «Bio-Rad» thermocycler. Separation of amplification products was carried in the horizontal electrophoresis camera in a 3% agarose gel (Fig. 1, B). Visualization of PCR products was performed by «Bio-Rad» gel video documentary device using Image Lab ™ Software.

Informativeness of PCR analysis for detecting H. pylori

For analysis of PCR informativeness we compared the PCR results with results of histological method and performed calculations such informative parameters as sensitivity (Se) and specificity (Sp) [2].

Sensitivity was calculated by the formula:

$$Se = (TP/D^{-}) \times 100\%$$

TP – true positive samples (74 positive patients by histology and PCR);

D^{-} – infected patients (86 positive by histology).

Specificity was calculated by the formula:

$$Sp = (TN/D) \times 100\%$$

TN – true negative samples (30 negative patients by histology and PCR);

D – not infected patients (70 negative by histology).

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).

Table 2

The oligonucleotide primers for detection of the of *16S rRNA* marker gene of *H. pylori*

Gene, fragment	Name of the oligonucleotide primer	Sequence from 5' → 3'	The size of the amplified fragment.
16S rRNA	*16S rRNA*	F5′-TGCGAAGTGGAGCCAATCTT-3′ R5'-GGAACGTATTCACCGCAACA-3'	118 п.н.

RESULTS

The cross method of *H. pylori* detection by PCR and histology was performed in 156 patients with gastroduodenal diseases (chronic gastritis, erosion and gastric ulcers). In 104 out of 156 examined patients (66.6%), the results of PCR completely coincided with the results of histology. 52 of 156 (33.3%) of the examined patients had mixed results (the results of PCR did not coincide with the results of histology). The results of a cross-sectional PCR and histology study are shown in Table 3.

To evaluate the informativeness of the PCR method, we analyzed the main operational characteristics such as sensitivity and specificity. The results of informativeness of the PCR analysis in relation to the histological method showed that the sensitivity of the PCR was 86.0% (p> 0.05) and specificity was 42.8% (p <0.05). The parameters of the PCR informativeness compared with the histological method are presented in Figure 2.

DISCUSSION

In this paper we presented the results of approbation of the PCR method for the diagnosis of *Helicobacter pylori* infection based on the amplification of the *16S rRNA* marker gene of bacterial DNA isolated from samples of gastric mucosa tissue from patients with gastroduodenal diseases in Yakutia. To evaluate the PCR informativeness for the detection of *H. pylori*, we compared PCR results with the histological method which has high sensitivity and specificity [18] and is considered the "golden standard" for the detection of *H. pylori* [8]. The results of histological studies conditionally were accepted by us for 100% for both sensitivity and specificity. In our study, the sensitivity of the PCR method was 86.0% and was almost inferior to the histological method (100%, p> 0.05). The specificity of the PCR method was significantly lower (42.8%) compared to the histological method (100%, p <0.05) (Fig. 2).

To evaluate the data obtained on the sensitivity and specificity of the PCR method of *H. pylori* detection, we performed a comparison analysis with the studies of other authors. Comparative analysis of the data showed that the sensitivity of the PCR method in different studies was from 55% to 100% (Table 4). The sensitivity of the PCR method in our study was 86.0% and takes an intermediate value among earlier studies (Table 4). The specificity of the PCR method among the analyzed data is from 80% to 100% (Table 4). The specificity of the PCR method of our study was significantly lower – 42.8%.

Probably, low specificity of PCR method in relation to histology is caused by a large number of false positive results (40 false positive versus 12 false negative results). This assumption is confirmed by earlier studies, in which was showed the high sensitivity of the PCR method [21]. Thus, in the work of Ramírez-Lázaro et al. [21] was studied biopsies of patients which histology was negative for *H. pylori* (n=52). In 25 of 52 patients, real-time PCR gave positive results for the presence of *H. pylori* (48%) [21]. This result shows that the histological method of study does not always reveal the presence of this infection. This phenomenon probably can be explained by the fact that the PCR method, is based on DNA detection and does not require viable bacteria and can give positive results with negative histology results.

Table 3

Cross method of *H. pylori* detection using PCR and histology

Obtained results	Cross-matches*		Mixed values**		Total
	PCR (+)/H(+)	PCR (-)/H(-)	PCR (+)/H(-)	PCR (-)/H(+)	
Number of samples, (%)	74 (47,4%)	30 (19,2%)	40 (25,6%)	12 (7,7%)	156 (100%)
In total	104 (66,6%)		52 (33,3%)		

Note: Cross-matches * – number of matches with histology; Mixed values** – number of mismatches with histology; PCR (+) – PCR positive results; PCR (-) – PCR negative results; H (+) –positive results of histology; H (-) – negative results of histology.

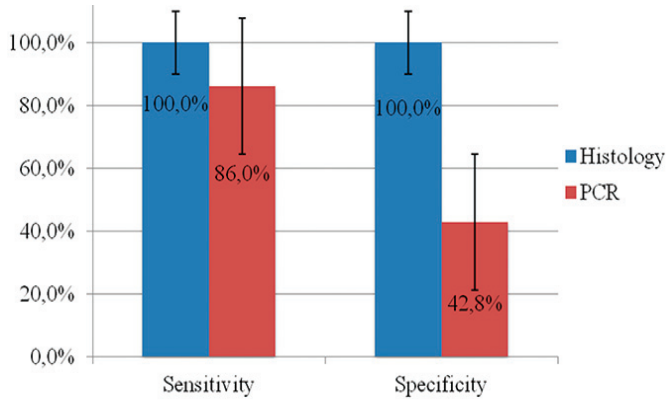

Figure 2. The parameters of the PCR informativeness compared with the histological method.

Thus, at present there is still no single method for detection of *H. pylori* with 100% sensitivity and specificity. To achieve the effectively confirm the presence or absence of this infection, it is necessary to use not one but several methods for diagnosing *H. pylori* [12]. In this regard, for the successful diagnosis of *H. pylori* in Yakutia, several cross-methods are recommended.

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Table 4

Comparative analysis of the sensitivity and specificity of the PCR method for the detection of *H. pylori*

The studied gastroduodenal diseases	Number of patients	Parameters of informativness	Molecular genetic analysis (gastrobiopsy)	Reference
Chronic gastritis, gastric ulcer, gastric adenocarcinoma	78	Sensitivity	100,0	[12]
		Specificity	90,0	
Superficial gastritis, chronic gastritis, lymphoid follicles, atrophy, metaplasia	52	Sensitivity	55,0	[18]
		Specificity	80,0	
Dyspepsia, upper gastrointestinal tract diseases	328	Sensitivity	98,	[19]
		Specificity	-	
Dyspepsia, chronic active gastritis	95	Sensitivity	94,0	[11]
		Specificity	100,0	
Chronic gastritis, erosion and stomach ulcers	156	Sensitivity	86,0	Current study
		Specificity	42,8	

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