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ОРИГИНАЛЬНЫЕ СТАТЬИ

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NON-INVASIVE DIAGNOSTIC OF HELICOBACTER PYLORI INFECTION

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SUMMARY: In this article, we told about three main non-invasive diagnostic methods for verification *Helicobacter pylori* infection. First method — breath tests: C^{13}-urease breath test and by ammonium breath test. We estimated frequency of *H. pylori* infection in patients with dyspepsia by ammonium breath test in St-Petersburg, Russia in three periods: 134 patients in 2008–2009, 37 patients in 2012–2013,50 patients in 2015–2016. Detection of *H. pylori* was made by non-invasive ammonium breath test (HELIC ABT, Association of Medicine and Analytic, St-Petersburg). The process of diagnostic: 1. Drinking of carbamide solution: 0,5 g of carbamide in 50 ml of still water; 2. Hydrolysis of carbamide by urease of *H. pylori*: $(NH_2)2CO + H_2O \leftrightarrow 2NH_3\uparrow + CO_2\uparrow$; 3. Detection of loading level of ammonium in oral cavity. In result of this analysis we saw that in 2008–2009 frequency of *H. pylori* infection in patients with dyspepsia was 78 %, in 2012–2013– 56 %, in 2015–2016–45 % ($p < 0.05$). It can be associated with widely administered eradication therapy. Second method — serological test with detection IgG to *H. pylori*. We used GastroPanel test for detection of IgG anti-*Helicobacter pylori.* Prevalence of *H. pylori* is high enough in dyspeptic patients by this method — 63 %. Serological test detect antibodies to *H. pylori* (not antigen) so it can explain the higher rate of *H. pylori* frequency by this test in compare with breath tests. Third method — enzyme-linked immunoelectrodiffusion essay of *H. pylori* in the faeces.

KEY WORDS: *Helicobacter pylori*, ammonium breath test, serology test

НЕИНВАЗИВНАЯ ДИАГНОСТИКА ИНФЕКЦИИ HELICOBACTER PYLORI

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РЕЗЮМЕ: В статье представлена информация о трех основных методах неинвазивной диагностики, используемых для верификации инфекции *Helicobacter pylori*. Первый метод —

дыхательные тесты: С13-уреазный дыхательный тест и аммонийный дыхательный тест. Мы оценили частоту встречаемости инфекции *H. pylori* с помощью аммонийного дыхательного теста у пациентов с диспепсией в Санкт-Петербурге за три временных периода: было обследовано 134 пациента в 2008–2009 годах, 37 пациентов в 2012–2013 годах, 50 пациентов в 2015–2016 годах. Определение *H. pylori* проводилось с использованием неинвазивного аммонийного дыхательного теста (HELIC ABT, Ассоциация медицины и аналитики, Санкт-Петербург). Процесс диагностики состоят из трех этапов: 1. Прием карбамида (мочевины) нормального изотопного состава: 0,5 г карбамида на 50 мл воды; 2. Гидролиз мочевины уреазой *H. pylori* (NH2)2CO+H2O ↔ 2NH3↑+CO2↑; 3. Определение прироста уровня аммиака в воздухе ротовой полости. В результате проведенного исследования было установлено, что частота встречаемости *H. pylori* у пациентов с диспепсией составляла в 2008–2009 78 %, в 2012–2013–56 %, in 2015–2016–45 % ($p<0,05$). Это может быть связано с широким использованием антихеликобактерной терапии. Второй метод — серологический тест с определением IgG к *H. pylori*. Мы использовали тест «Гастропанель» для выявления IgG к *H. pylori*. Частота встречаемости инфекции по данным серологического теста составила 63 %. При использовании данного метода имеет место определение антител (не антигена) к микроорганизму, что может объяснить более высокие показатели по распространенности инфекции по результатам серологического теста по сравнению с аммонийным дыхательным тестом. Третий метод — иммуноферментный анализ антигена *H. pylori* в кале.

КЛЮЧЕВЫЕ СЛОВА: *Helicobacter pylori*, аммонийный дыхательный тест, серологический тест

Helicobacter pylori infection is one of the most famous and serious gastroenterology problems as the prevalence of *Helicobacter pylori* infection is very high in the world, the *Helicobacter pylori* associated disease is now often diagnosed in young people of employable age, and as this microorganism is recognized to be a Group 1 carcinogen. Therefore, the development of algorithms for early and accurate diagnostics of *Helicobacter pylori* infection will make it possible to improve the quality of treatment and follow-up care for this category of patients.

The C13-urease breath test and the enzyme-linked immunoelectrodiffusion essay of *H. pylori* in the faeces were approved as the recommended diagnostic methods for identification of *H. pylori* at the Maastricht Consensus V [13]. However, the existence of a large number of different methods for diagnostics of the *H. pylori* infection supports the postulate that there is not yet a unique method, a so-called "gold standard" for the diagnostics of this infection. The complex of the diagnostic methods for this microorganism can be divided into invasive (which require to perform gastroduodenoscopy) and non-invasive (which do not require to perform gastroduodenoscopy), direct (actual identification of *H. pylori*) and indirect (the identification of *H. pylori* metabolic products) methods. The basic and most frequently used methods for the diagnostics of *H. pylori* are presented in Table 1 [1].

Invasive methods are generally used when performing the complex of the primary diagnostic procedures on the patient, as in this case it is mandatory to perform a gastroduodenoscopy. Potential indications for the use of **noninvasive methods** are somewhat broader. These indications include the primary diagnostic in case it is not need to perform a gastroduodenoscopy, the screening of adults, the examination of children complaining of periodic abdominal pain, the evaluation of the eradication success, and scientific indications (the evaluation of infection prevalence, the investigation of the association

Table 1

Diagnostic Methods for the *Helicobacter pylori* infection

Invasive methods	Non-invasive methods
A) bacteriological test (culture test) B) histological test C) rapid urease tests D) molecular genetic test (polymerase chain reaction) as a biopsy material examination	A) C13-urease breath test B) ammonium breath test C) serological test D) enzyme-linked immunoelectrodiffusion essay of *H. pylori* in the faeces E) molecular genetic test (polymerase chain reaction) as an examination of the faeces

between the presence of *H. pylori* and extra-digestive disorders) [11].

As mentioned above, none of the diagnostic methods for identification of *H. pylori* can be considered universal. Each diagnostic method for identification of *H. pylori* has its advantages and disadvantages, these methods vary in sensitivity and specificity. During numerous comparative investigations it was determined that the results of different methods are not always identical, therefore, to avoid false positive or false negative results and to more accurately detect the presence of infection, it is necessary to use at least two methods and to consider the obtained results as positive or negative if the results of both investigative methods coincide. Some authors even recommend using three methods to confirm the absence of infection [9].

When speaking about **noninvasive diagnostic methods** for identification of *H. pylori* special attention should be paid to the breath tests, the identification of the microorganism in the faeces, and the serological test. Comparative data about results, sensitivity and specificity of different non-invasive methods are in table 2.

The **C13-urease breath tests** are based on the biochemical method of identification of the *H. pylori* infection of the gastric mucosa based on the

Table 2

Results, sensitivity and specificity of different non-invasive diagnostic methods of *H. pylori* infection [7]

Method	Advantage	Disadvantages
Polymerase chain reaction in faeces: specificity — 92 % sensitivity — 94 %	— possibility to diagnose active infection and also latent infection — possibility to detect a few amount of bacteria — possibility to detect genes coding synthesis of pathogenicity factors of *H. pylori* — possibility to devote relapse from reinfection — possibility to detect genetic mutations coding resistance to antibiotics — express diagnostic — 5–6 hours	— technical difficulties to transportation and storage of faeces — false-negative results due to constipation
Ammonium breath test specificity — 92 % sensitivity — 95 %	— high speed of getting results — results do not depend on the age and type of gastroduodenal pathology — a cheaper in compare with C13-urease breath test — simple to perform test — In ward test — suitable for population screening	— some difficulties in children under 5 years — possible to get false-negative results if two week before testing patient received any medicines which can inhibit urease activity of *H. pylori*
C13-urease breath test specificity — 93–99 % sensitivity — 95–97 %	— accuracy of getting results — easy to perform — recognized as the standard in the diagnosis of *H. pylori* in adults both before and after eradication therapy	— some difficulties in children under 5–8 years (specificity и sensitivity can decrease) — possible to get false-negative results if two week before testing patient received any medicines which can inhibit urease activity of *H. pylori* — expensive method — results depend of physical activity of patient
Enzyme-linked immunoelectrodiffusion essay of *H. pylori* in the faeces specificity — 90–95 % sensitivity — 80–90 %	— accuracy of getting results — easy to perform — recognized as the standard in the diagnosis of *H. pylori* in adults both before and after eradication therapy	— expensive method — false-negative results due to constipation
Serological test sensitivity — 59–71 %	— easy to perform — suitable to use as population screening method	— false-negative results in children due to low immune response — not recommended for evaluating the effectiveness of the *H. pylori* eradication

microorganism's urease activity, namely on the ability of urease to decompose urea to $NH^{4+}$and HCO^{3-}, with subsequent formation of CO_2 from HCO^{3-}, which after entering the blood stream is then discharged through the lungs and can be determined in the expired air. Radioisotope urea breath test with urea marked with radioactive carbon ^{13}C is considered to be the most accurate of noninvasive methods for the diagnostics of *H. pylori* and has been known since 1987 [10]. Sensitivity and specificity of radioisotope urea breath test is 90 % according to most investigations, but for this test, in some cases false positive results are obtained as compared to the histological method [11]. However, the way of performing this test is still not well standardized, and used reagents are expensive [11].

In ammonium breath test we can to use the detection of ammonia vapour (the second metabolite from hydrolysis of urea) in the breath air after oral administration of urea with normal isotopic composition has significantly increased the frequency with which the breath tests are performed, because it allows to reduce the cost of this method, as well as to increase its safety, as it does not use radioactive isotopes. According to this principle in Russia in 1997, Association of Medicine and Analytics Company (Saint Petersburg) developed "Helic-test", the results of which do not depend on the duration and nature of gastroduodenal pathology and only show the presence or absence of *H. pylori* [5]. It should be noted that the breath tests are not recommended in patients who receive antisecretory treatment, bismuth medicines and antibiotics two week before tests perform to avoid the risk of false negative results connected with the possible interaction of hydrochloric acid and ammonia and to avoid risk of critically decrease of urease activity of *H. pylori* [4]. Particularly relevant is the use of breath tests in children due to limited use of invasive diagnostic methods for identification of *H. pylori*.

For identification of *H. pylori* in the faeces, the enzyme-linked immunoelectrodiffusion essay for identification of the *H. pylori* antigen and polymerase chain reaction with detection of the *H. pylori* (*ureC*, c*agA*) pathogenicity island genes are used. The **enzyme-linked immunoelectrodiffusion essay for identification of the** *H. pylori* **antigen in the faeces** is a high-sensitivity and specificity method, and it is recognized as the standard method in the *H. pylori* diagnostics in children and adults both before and after eradication therapy. The only two limitations for the widespread use of this method: is its high price and possible to have false-negative result in case of severe constipation. The **polymerase chain reaction (PCR) with detection of the** *H. pylori* **pathogenicity island genes** is a relatively new experimental method, the sensitivity and specificity of which is not clearly defined.

The **serological diagnostic method** is based on the identification of IgG antibodies to *H. pylori* and IgG antibodies to cytotoxin CagA of *H. pylori* in the blood. This method is intended for the screening in the population and for the primary diagnostics of the *H. pylori* infection, but it is less informative in children due to a low immune response. As by using this method one cannot distinguish between past and current infection, it is not recommended for evaluating the effectiveness of the *H. pylori* eradication [3, 12].

According to numerous published data, the evaluation of the effectiveness of the *H. pylori* eradication should be conducted not earlier than 1.5–2 months after the end of therapy, and the preference should be given to non-invasive methods of investigation if there is no need to perform a control gastroduodenoscopy, which is especially important in children [2, 4, 5, 6, 11].

We perform two studies for investigate the prevalence of *H. pylori* infection in St-Petersburg, Russia. In first study we estimated frequency of *H. pylori* infection in patients with dyspepsia by ammonium breath test in St-Petersburg, Russia. Methods: 221 patients with dyspepsia were observed: 134 patients in 2008–2009, 37 patients in 2012–2013, 50. Detection of *H. pylori* was made by non-invasive ammonium breath test (HELIC ABT, Association of Medicine and Analytic, St-Petersburg). The process of diagnostic: 1. Drinking of carbamide solution: 0,5 g of carbamide in 50 ml of still water; 2. Hydrolysis of carbamide by urease of *H. pylori*: $(NH_2)2CO + H_2O \leftrightarrow 2NH_3\uparrow + CO_2\uparrow$; 3. Detection of loading level of ammonium in oral cavity (fig. 1,2). Results: in 2008–2009 frequency of *H. pylori* infection in patients with dyspepsia was 78 %, in 2012–2013–56 %, in 2015–2016–45 % ($p < 0.05$) (fig. 3). It is similar with results of differ Russian studies (38.5–43.0 %) [8, 14]. Therefore, we can see a progressive decreasing of frequency of *H. pylori* infection in patients with dyspepsia in St-Petersburg, Russia. It can be associated with widely administered eradication therapy. Also this fact can be a promising for decreasing of frequency a stomach cancer in future in our region.

In the second study, we used GastroPanel test for detection of IgG anti-*Helicobacter pylori*.

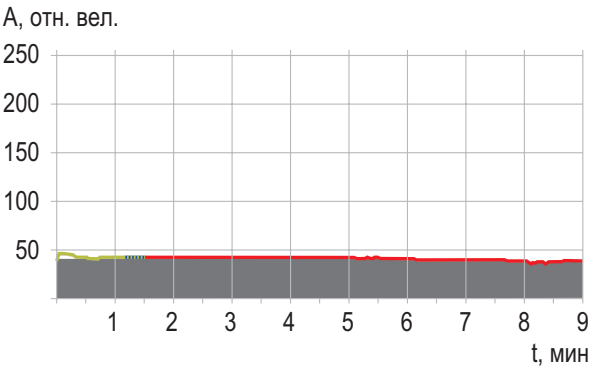

Fig. 1. Results in the level of ammonia in the air of the oral cavity in a patient with *Helicobacter pylori* (-) (ammonium breath test — "Indicator computerized HELIC®-device»).

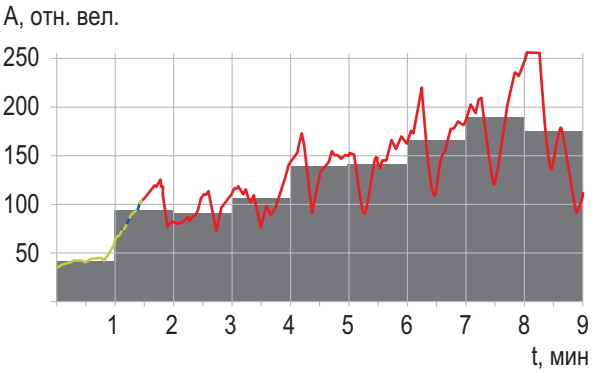

Fig. 2. Results in the level of ammonia in the air of the oral cavity in a patient with *Helicobacter pylori* (+) (ammonium breath test — «Indicator computerized HELIC®-device»).

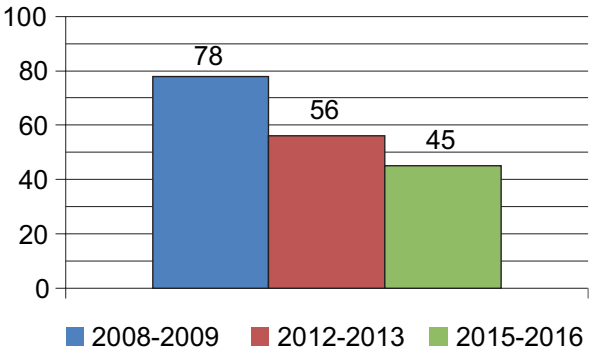

Fig. 3. Prevalence of *Helicobacter pylori* infection in dyspeptic patients in dynamic by ammonium breath test, %

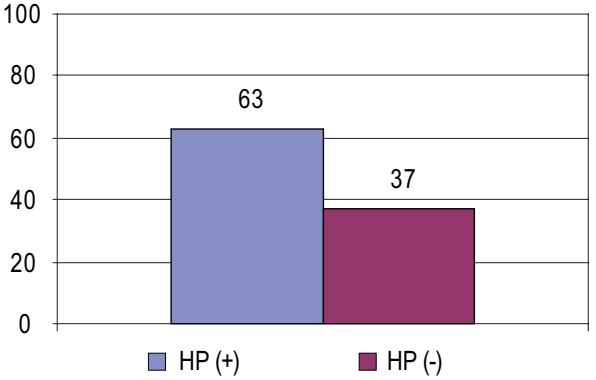

Fig. 4. Prevalence of *Helicobacter pylori* infection in dyspeptic patients by detection of IgG against *H. pylori*, %

Prevalence of *H. pylori* is high enough in dyspeptic patients by this method — 63 %. Serological test detects antibodies to *H. pylori* (not antigen) so it can explain the higher rate of *H. pylori* frequency by this test in compare with breath tests (fig. 4).

In conclusion, it is necessary to discuss about mail topics in non-invasive diagnostic of *H. pylori* infection:

1. Non-invasive methods of *H. pylori* diagnostic are the methods of first choice in case when it is no need to perform gastroduodenoscopy (primary and control), which is especially important in children
2. Results of breath tests and faeces test depend from taking any medicines with anti-helicobacter activity.
3. Serology test not recommended for control of eradication efficacy because IgG antibodies against *H. pylori* can stay in human blood for years.

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