
IMMUNOLOGY AND MICROBIOLOGY

Application of Electrochemical Breath Test for Detection of *Helicobacter Pylori* in Screening of Moscow Students

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The incidence of *Helicobacter pylori* infection is analyzed by the results of screening of first- and fourth-year students of Moscow Institute of Foreign Affairs using HelicoSense Scientific breath test system. Age-related dynamics of the infection in patients examined for the first time has been traced. The data on infection rates in patients after eradication therapy are presented.

Key Words: *Helicobacter pylori*; *Helicobacter pylori* infection; breath test; infection index

Infection caused by *Helicobacter pylori* (HP) is highly prevalent. It is proved to contribute to the development and relapsing of chronic gastritis, duodenitis, gastroduodenal ulcer, gastric B-cell lymphoma (maltoma), non-cardia gastric cancer. These diseases became curable after discovery of HP, and prevention of cancer became feasible and effective due to it, and hence, the need in timely detection of HP in the microecological niche of the stomach and duodenal bulb is obvious. Studies carried out in many countries have shown that the prevalence of HP in probands varies from 7 to 87% [4]. The incidence of this infection is the lowest in European countries (7-33%) and the highest in South Africa (87%). In South America, the incidence of HP infection is 48-78%, in Asia 37.5-66.0%. In Russia, the incidence of HP is 60-70%, but in some regions it reaches 98% [1,2]. Evaluation of the prevalence HP

infection in various population groups suggests regarding it as a disease with an epidemic pattern. Despite numerous methods for HP detection, the problems of diagnosis of HP infection in large groups of population and of eradication therapy monitoring remain unsolved. Evaluation of HP urease activity by ammonium concentration in exhaled air has been introduced for screening of patients in Russia in recent years. The method is realized by means of HelicoSense Scientific gas analyzer, developed at TKA company [1,2]. This paper presents the results of evaluation of HP infection prevalence by means of this electrochemical breath test. The study was carried out in first- and fourth-year students of one of the Moscow Universities, *i.e.* in individuals exposed to close lasting contacts and similar communal conditions.

MATERIALS AND METHODS

First- and fourth-year students of Institute of Foreign Affairs (Faculty of External Economic Relations) were examined over time for detection of gastroduodenal

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diseases. All studies were carried out in accordance with the informed consent protocol. Planned check-ups were carried out during 1 month in spring and 1 month in fall. Electrochemical breath test was included in the traditional examination algorithm during both seasons. The HelicoSense Scientific device (TKA company) with disposable mouthpiece was used. After oral carbamide, ammonium concentration was measured in exhaled air by an electrochemical pickup. The results were interpreted by the infection index (II) in accordance with methodological recommendations on the use of HelicoSense Gas Analyzers [1].

Prophylactic checkup of first-year students covered 235 men and 359 women ($N=594$) aged 17-30 years. Primary testing results were analyzed by Information System for Gastroenterologist's Decision-Making software [1]. During the fourth year, the same students were repeatedly examined with consideration for 2-year history and cases of deviations from the normal.

In cases with positive breath test, pathogenic activity of *Helicobacter pylori* strains was evaluated by EIA in serum specimens from infected subjects. In some students with positive II, blood (5 ml) specimens were collected from the ulnar vein, centrifuged (15 min, 2000g), inactivated (30 min, 56°C), and frozen. Each serum was then tested twice by enzyme immunoassay. Pathogenic activity was evaluated by the presence of antibodies using HelicoBest antibodies enzyme immunoassay kits (Vector-Best). The test system was a recombinant *Helicobacter pylori* CagA antigen immobilized on the surface of wells of a polystyrene plate. The results were evaluated by optical density, measured by a vertical spectrophotometer. Antibody titers in each sample were estimated as follows: dilutions $<1/5$ were considered negative, dilutions $1/5$ ambiguous, and dilutions $>1/5$ were considered positive.

According to modern requirements, HP infection is diagnosed on the basis of at least two positive tests. Therefore, after testing on a HelicoSense gas analyzer the students were recommended serological EIA and PCR detection of HP in the saliva and feces. In case

of typical gastroduodenal complaints, accessory studies were recommended: enterogastroduodenoscopy (EGDS) and PCR testing of gastric mucosa biopsy specimens for HP. Four biopsy specimens were collected for morphological diagnosis: 2 from the antral mucosa, 1 from the lesser, and 1 from the greater curvature.

Helicobacter infection, chronic gastritis, chronic gastroduodenitis, or peptic ulcer of the gastric lesser curvature were diagnosed by the results. Morphological verification and positive EIA and PCR results were indications for eradication therapy in 36 patients in accordance with WHO recommendations [3]. Repeated testing 4-6 weeks after a course of eradication therapy in case of negative breath test served the basis for conclusion about elimination of the agent.

Eradication protocol included a 7-day course of omeprazole (20 mg twice daily) in combination with 2 antibacterial drugs clarithromycin (500 mg twice daily) and amoxicillin (1000 mg twice a day). This therapy was assumed to be effective in 90% cases. This treatment was supplemented by probiotics (spirobacterin and lactobacterin) according to the standard protocols for microflora correction.

The data were statistically processed by variation statistical methods. Correlations (r) were evaluated by the type and strength of relationship between parameters A (breath test) and B (serological test). The relationship of correlation coefficient (ρ) and probability of correct results (A and B) was evaluated using Monte Carlo software. Selected means were compared by Student's t test. The differences were considered significant at $p<0.05$.

RESULTS

According to electrochemical breath testing, II was positive in 359 students, ambiguous in 16, and negative in 219 (Table 1). According to interviews, 18.8% students had gastrointestinal complains; 12.3% respondents complained of painful syndrome, 14.9% of dyspeptic syndrome, and 8.6% complained of both. Com-

TABLE 1. *H. Pylori* Infection Rate in First-Year Students (Checkup 1)

II	Men		Women		Total	
	abs.	%	abs.	%	abs.	%
Positive	136	22.9±3.6	223	37.5±3.2	359	60.4±2.6
Ambiguous	5	0.8±3.9	11	1.9±4.1	16	2.7±4.1
Negative	94	15.8±3.7	125	21.0±3.6	219	36.9±3.3
Total	235	39.6±3.2	359	60.4±2.6	594	100

plaints were recorded in 16.2% examined subjects in the group with positive diagnosis and in 14.7% of those with negative results.

High incidence of HP infection (60.4%) during the first year of studies at the Institute is explained by close contacts between the students during studies, in hostel, and during rest hours. The agent is transmitted by not only fecal and oral, but also by the oral-oral mechanism of infection. In addition, HP can be disseminated through mucus and secretion droplets during coughing, talking, *etc.* The absence of typical complaints in the presence of positive test results can be explained by the fact that HP infection is a chronic slowly unfolding gastroduodenal infection, and the time of testing could coincide with incubation or prodromal period of the disease.

The prevalence of HP infection in different age groups was evaluated separately for girls and boys (Fig. 1). The initial total infection rate was about the same: 57.9 boys and 62.1% (slightly more) girls. With aging, HP infection rate tended to increase, reaching 80% by 22-30 years in both groups.

Repeated examinations during the 4th year showed that a trend to HP prevalence in the students was retained. It increased in older students (20, 21, 22, 23, 24, 25, 26 years old and older). In comparison with the first preventive examination it increased, 1.4 times in boys and 1.2 times in girls. The HP infection rate in girls aged 25 years and more reached 90%, in boys of the same age 88%.

The results of repeated checkups of the same students ($n=534$) are summed up in Table 2. Positive tests were recorded in 413 subjects (30.9% men and 46.4% women). The incidence of negative results decreased to 21.6% (115 subjects), while that of positive results increased to 77.3% (413 students).

Hence, after 4 years the infection rate increased in a group of probands united by common everyday work and close contacts.

The diagnostic efficiency of HelicoSense Scientific gas analyzer was evaluated in individuals with manifest clinical picture of chronic gastritis, gastro-

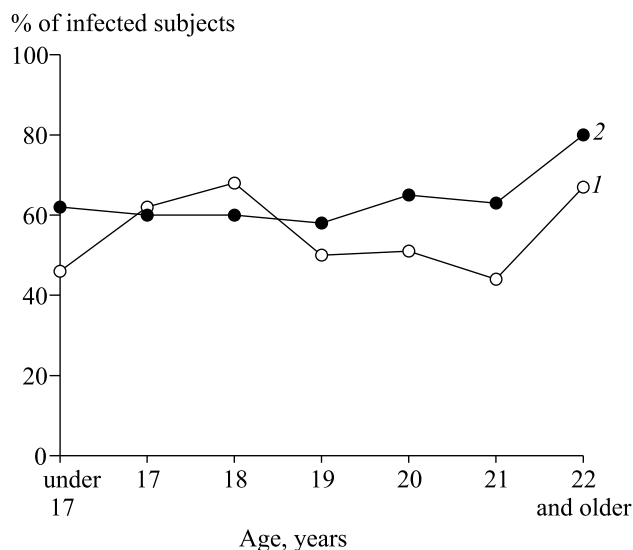


Fig. 1. Prevalence of *Helicobacter pylori* infection in different age groups of students – men (1) and women (2) – according to electrochemical breath test.

duodenitis, and duodenal ulcer in order to detect patients in need of eradication therapy. It was carried out in a group of 36 students aged 17-30 years, who complained of abdominal pain and agreed to repeated diagnosis and therapy of HP infection.

The breath test was positive at first checkup in probands from this sample. The HP infection was confirmed by morphological methods and/or PCR and EIA (evaluation of HP pathogenic activity).

All 36 patients received a three-component therapy [3]. Antibiotic sensitivity of HP was unknown in this study and hence, the therapeutic protocol was prescribed empirically.

After eradication therapy the breath test was negative in 31 of 36 patients. In the remaining patients the treatment was ineffective according to this test: in 2 patients it decreased but did not reach the negative level, in 1 patient it increased, and in 2 remained the same as previously. The PCR was carried out with salivary, fecal, and gastric mucosa biopsy specimens. The pathogenic strain was found in the saliva of only

TABLE 2. Prevalence of *H. Pylori* Infection in Fourth-Year Students (Checkup 2)

II	Men		Women		Total	
	abs.	%	abs.	%	abs.	%
Positive	165	30.9±3.6	248	46.4±3.2	413	77.3±2.1
Ambiguous	2	0.4±4.5	4	0.7±4.2	6	1.1±4.2
Negative	41	7.7±4.2	74	13.90±4.02	115	21.6±3.8
Total	208	39.0±3.4	326	61.0±2.7	534	100

one patient. Testing of fecal specimens gave negative results. Four gastric biopsy specimens were HP-positive. The results of eradication in 31 patients indicated the treatment efficiency. In the rest cases the treatment failed, and these patients are still observed, receive adequate therapy (second-line drugs), probiotics (spirobacterin and lactobacterin) according to appropriate protocols, and are regularly checked up.

The results of our study recommend the HelicoSense Scientific breath analyzer for epidemiological and prophylactic screening of large population groups for detection of HP infection. It is advisable to include this method in protocols of complex etiological diagnosis of gastritis and peptic ulcer in examinations of patients with gastroduodenal diseases. Importantly that only the use of this device allows monitoring of HP eradication without invasive procedures. Significant advantages of gas analyzer are its simple use, safety, comfort for the patient, and rapid (10 min) results.

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