

Immunocytochemical Study of *Helicobacter Pylori* in the Mucosae of the Gastric Antrum and Rectum

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We present the results of bacterioscopic and immunocytochemical study of *Helicobacter pylori* in biopsy specimens from the gastric antrum and smears from the rectum. Predominance of spiral-shaped vegetative form of *Helicobacter pylori* in the antrum and the presence of cocci with *Helicobacter pylori* antigens in smears from the rectum were demonstrated in patients infected with *Helicobacter pylori*. The diagnostic sensitivity of non-invasive immunocytochemical *Helicobacter pylori* test in rectal smears was 90%, specificity 76%, and efficiency of the test 84%.

Key Words: *Helicobacter pylori*; coccoid forms; immunocytochemistry; rectum

Colonization of the gastric mucosa (GM) with bacterial cells *Helicobacter pylori* (HP) plays an important role in the development of gastroduodenal diseases. It is assumed that HP infection is primarily transmitted by the fecal-oral route with coccoid forms of HP that result from transformation of bacillary forms [3,4].

Here we performed immunocytochemical analysis of biopsy specimens from the stomach and rectal smears from the same patients with acid-related disorders using antibodies against *Helicobacter pylori* and compared the results of detection of rectal bacteria positive for HP-antigen (coccoid forms of HP) with HP infection in the gastric antrum.

MATERIALS AND METHODS

The study included 109 individuals, patients of A. M. Nikiforov Russian Center of Emergency and Radiation Medicine, Ministry of Emergency Situations, S. M. Kirov Military Medical Academy (St. Petersburg), and Clinical Hospital No. 20 (St. Petersburg). Male (41%) and female (59%) patients suffered from chronic gastritis and erosive-ulcerative diseases of the stomach and duodenal bulb. Gastric and rectal examination for HP infection was

performed in each patient. At the time of examination, the patients did not yet receive eradication therapy.

HP in GM was examined in stamp-smears of the antral biopsy specimens obtained during fiberoptic upper endoscopy. HP in the rectal tissue were identified on the brush cytological smears where both fecal material and rectal mucosa cells were always present. The samples (antral biopsy specimens and anal smears) were taken within 2-3 days.

Immunocytochemical staining of smears was performed as described previously [2]. Polyclonal antibodies to antigens of HP cell wall (Novocastra, Dako) were used.

For detection of HP in rectal smears, 300 fields of view were examined under oil-immersion in each case. The sample were considered HP-positive if at least 5 bacteria carrying HP antigen were detected in 300 fields of view. HP contamination of antral GM after immunocytochemical staining was assessed as described previously [2].

The obtained data were analyzed statistically using Student's *t* test.

RESULTS

In biopsy specimens of the gastric antrum, bacteria immunohistochemically positive for HP stained brown.

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In most cases, spiral forms of HP were detected in the stomach. Coccoid and intermediate U-shaped forms of HP were detected far less often. HP spiral forms were 3-5 μ long (including the flagella) and about 0.5 μ in diameter. Coccoid forms of HP were from 0.5 to 1 μ in diameter, perfectly round and stained. It should be noted that in the antrum, coccoid forms of HP constituted 5% of the entire microbial population of bacterial cells HP infecting GM.

Spiral HP forms, *i.e.* bacteria immunocytochemically positive for HP antigens and having spiral shape and flagella, or at least a spiral shape, were not found in the rectal samples (in mucous epithelium and feces) throughout the research. Among HP-positive bacterial cells which retained the shape, only cocci were detected in the rectal smears. It is important to note that two types of coccoid HP forms were detected in the rectal samples. The first type was presented by small (0.5-1.0 μ) uniformly stained cells like in GM gastrobiopsies. HP-antigen positive cocci of the second type were larger (up to 2 μ) and less intensively stained cells, sometimes oval with clear center.

The percentage of HP revealed by immunocytochemistry in the surveyed group of patients was 56.9% in antral GM and 61.5% in rectal smears.

Table 1 shows the incidence of all variants of HP. Total positive coincidence (in the stomach and rectum) was the most common variant: 51.4 \pm 4.7%. Total negative coincidence was recorded less frequently. In 6 of 109 cases, HP were detected only in the stomach. Most likely, this variant was caused by methodological errors. HP were not found in the stomach, but were present in the rectum in 10.1 \pm 2.9% cases ($p < 0.05$).

Thus, the results of immunocytochemical study of the stomach and rectum coincided in 92 out of 109 patients (84.4 \pm 3.5% cases). In other cases, HP was either found or not found in one of the two examined organs.

If the bacterioscopic method of HP detection in stomach biopsy specimens obtained during endoscopy

can be considered the gold standard, let us examine how immunocytochemical test of HP detection in rectal samples can be evaluated in terms of sensitivity and specificity used in clinical and laboratory practice.

For calculation of sensitivity, specificity, and efficiency of the test for non-invasive immunocytochemical HP detection in the rectum, the following formulas were used [1]:

$$\text{Diagnostic sensitivity (DS)} = \frac{TP}{S} \times 100\%,$$

$$\text{Diagnostic specificity (DS)} = \frac{TN}{NS} \times 100\%,$$

$$\text{Diagnostic efficiency of the test (DE)} = \frac{TP+TN}{TP+FN+FP+TN} \times 100\%,$$

where TP are true positive specimens, *i.e.* HP-positive stomach specimens and HP-positive rectal smears; FN are false negative specimens, *i.e.* HP-positive stomach specimens, but HP-negative rectal smears; FP are false positive specimens, *i.e.* HP-negative stomach specimens, but HP-positive rectal smears; TN are true negative, *i.e.* HP-negative stomach specimens and HP-negative rectal smears; S are sick individuals, *i.e.* HP-positive stomach specimens; NS not sick individuals, *i.e.* HP-negative stomach specimens.

The data for the calculation are given in Table 2. Thus, we have

$$DS = \frac{TP}{S} \times 100\% = \frac{56}{62} \times 100\% = 90\%;$$

$$DS = \frac{TN}{NS} \times 100\% = \frac{36}{47} \times 100\% = 76\%;$$

$$DE = \frac{TP+TN}{TP+FN+FP+TN} \times 100\% =$$

$$\frac{56+36}{56+6+11+36} \times 100\% = 84\%.$$

In general, we can conclude that non-invasive immunocytochemical test for the diagnosis of HP in the rectum in 84.4 \pm 3.5% cases coincides with the results of HP assessment by the same method in stomach biopsy specimens obtained during endoscopy. As for discrepancies, they are mostly of diagnostic nature.

We used polyclonal antibodies to antigens of HP cell wall as first antibodies. Therefore, it is possible that false-positive results of HP detection in the rectum are due to cross-reactivity of polyclonal antibodies with other bacteria. Immunocytochemical method of HP detection can be probably improved by the use of monoclonal antibodies to HP antigens obtained to the

TABLE 1. Coincidence of Invasive (Stomach Biopsy Specimens) and Non-Invasive (Rectal Smears) Immunocytochemical Tests for HP Detection

Variants of detection	Cases of detection	
	N	%
Stomach, rectum (+)	56	51.4 \pm 4.7
Stomach, rectum (-)	36	33.0 \pm 4.5
Stomach (+), rectum (-)	6	5.5 \pm 2.2
Stomach (-), rectum (+)	11	10.1 \pm 2.9

Note. «+», found, «-», not found.

TABLE 2. Parameters for Calculation of Sensitivity, Specificity, and Efficiency of Non-Invasive Immunocytochemical HP Test in Rectal Smears

Group examinees	Research results		Total
	positive	negative	
Sick (S)	56 (TP)	6 (FN)	62 (TP+FN)
Not sick (NS)	11 (FP)	36 (TN)	47 (FP+TN)

coccoid forms of HP. Nevertheless, the results of our histocytochemical studies indicate high percentage of coincidence of HP detection in gastrobiopsies and rectal smears. This fact gives us reason to believe that HP bacteria are transformed from spiral into the coccoid forms as they move from the stomach into the intestine. Hence, it confirms the assumption that Helicobacter infection is transmitted with coccoid forms by the fecal-oral route [3,4]. Coccoid forms of HP are probably a way to preserve the bacteria and exist outside the body.

Similar parallel studies were conducted with immunoenzyme detection of HP antigens (HpSA) in stool samples and bacterioscopic detection in stomach biopsy specimens [5]. Of 62 HP-positive and 34 HP-negative patients by the data of histological analysis, 34 were HP

stool antigen positive and 27 had negative stool test. Thus, the sensitivity and specificity of HpSA compared with the gold standard were 54.8 and 79.4% respectively. Nevertheless, it was concluded that the test might be useful for mass screening for HP [5]. Presented in this paper immunocytochemical test for the diagnosis of HP infection in the rectal smears proved to have more coincidences with the gold standard than the HpSA enzyme immunoassay test. Immunohistochemical assay for HP in the rectal samples can be recommended in the cases when fiberoptic upper endoscopy cannot be performed as well as to control HP eradication.

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