

Immunohistochemical detection of *Helicobacter pylori* infection in gastric biopsies of urea breath test-positive and -negative pediatric patients

Diclehan Orhan¹, Gülsev Kale¹, İnci Nur Saltık-Temizel², Hülya Demir², Almıla Bulun¹, Ergun Karaağaoğlu³, Melda Çağlar¹

Divisions of ¹Pediatric Pathology and ²Gastroenterology, Hepatology and Nutrition, Department of Pediatrics, ³Department of Biostatistics, Hacettepe University Faculty of Medicine, Ankara, Turkey

SUMMARY: Orhan D, Kale G, Saltık-Temizel İN, Demir H, Bulun A, Karaağaoğlu E, Çağlar M. Immunohistochemical detection of *Helicobacter pylori* infection in gastric biopsies of urea breath test-positive and -negative pediatric patients. Turk J Pediatr 2008; 50: 34-39.

Helicobacter pylori (*H. pylori*) is a common cause of gastritis in both children and adults, and its incidence increases every year. The aims of this study were to evaluate the histopathologic features of *H. pylori* gastritis and to compare immunohistochemical with histochemical [hematoxylin-eosin (HE) and Giemsa] staining of gastric biopsy specimens for the detection of *H. pylori* infection from urea breath test (UBT) (-) and UBT (+) children. Seventy-eight gastric biopsies from pediatric patients who were administered UBT were included in this study. Gastric biopsy specimens were evaluated histopathologically and graded according to the Sydney system. HE, Giemsa and immunohistochemical staining was performed for the identification of *H. pylori*.

The frequency of *H. pylori* gastritis was higher in the antrum than corpus. All biopsies with *H. pylori* colonization showed chronic inflammation with activity. By using immunohistochemical method, coccoid forms of *H. pylori* and spiral bacteria with low density were observed easily. With histochemical staining, 1/10 (10%) UBT (-) biopsies were *H. pylori* (+), while with immunohistochemical staining, 3 of the biopsies from UBT (-) patients were found to be *H. pylori* (+). Biopsies from 65 of 78 (83.3%) UBT (+) patients were *H. pylori* (+) with histochemical staining, but only 53 of these biopsies were found to be *H. pylori* (+) immunohistochemically. We conclude that immunohistochemical staining is more specific than histochemical staining and UBT for the detection of *H. pylori* infection.

Key words: *Helicobacter pylori*, immunohistochemistry, gastritis, histochemistry, urea breath test.

Helicobacter pylori (*H. pylori*) is now accepted as a very common cause of gastritis in adults and children¹⁻². It is reported that *H. pylori* infection is almost always acquired in childhood and usually persists for life unless specifically treated³. Accurate detection of the organism is essential for correct patient management^{1, 3}. In adults, there are several noninvasive diagnostic tests to diagnose *H. pylori* infection, including serology and indirect detection of urease activity (¹³C- and ¹⁴C- urea breath test-UBT)⁴. However, the gold standard for the diagnosis of *H. pylori* infection is the histologic examination

of stomach biopsy specimens obtained by the use of esophagogastroduodenoscopy⁵⁻⁷. *H. pylori* colonized with high density can be observed in routine hematoxylin-eosin (HE)-stained sections, but the detection of a lower density of organisms requires special stains such as Gram, Giemsa, Genta, Dieterle or Warthin-Starry stains⁷⁻¹⁰. The Warthin-Starry silver stain, which is considered to be the most sensitive histochemical stain for the detection of *H. pylori*, is technically difficult and frequently not reproducible. Moreover, none of the stains mentioned above is specific for the organism⁸.

Immunohistochemical staining for the detection of *H. pylori* has been available since 1988. Immunohistochemical methods are highly specific. There is a limited number of studies comparing immunohistochemistry with histochemistry for the identification of *H. pylori* in pediatric gastric biopsies^{8,10-12}.

Few publications have analyzed the significance of histopathologic findings of *H. pylori* gastritis in the pediatric population. In this study, we have evaluated the histopathologic features of *H. pylori* gastritis in children. We compared immunohistochemistry with histochemistry for the detection of *H. pylori* in pediatric gastric biopsies.

Material and Methods

The material for this study was paraffin-embedded tissues from 78 pediatric patients who had undergone a gastric biopsy and UBT. There were 33 boys and 45 girls with an age range of 4-12 years (mean 11.12).

After informed consent was obtained from the parents and/or the children, an esophagogastro duodenoscopy was performed with an Olympus GIF P100 scope. Intravenous sedation with midazolam and pethidine was administered to all 78 children before endoscopic examination. Endoscopic findings were recorded. At least four antral gastric biopsies were obtained from each patient for histology, culture and rapid urease test medium (Dio-Helico, Diomed). Corpus biopsies were also taken from 63 of these patients.

Urea breath test (UBT) was performed in 78 patients. After a fasting baseline sample breath collection, 50 mg (<30 kg) or 75 mg (>30 kg) ¹³C-labeled urea was administered orally in orange juice. A second breath sample was collected 30 minutes after urea ingestion. Breath samples were analyzed by isotope ratio mass spectrometry (IRIS IEC 601-1; AYKA, Germany) and the cut-off value was 48 0%.

All biopsy specimens were fixed in 10% buffered formalin and embedded in paraffin. Standard HE and Giemsa stains were performed on 5 μm thick sections. All sections were evaluated for the presence of chronic inflammation, activity of inflammation, atrophy, lymphoid follicles, intestinal metaplasia and the presence of *H. pylori*. Each parameter was graded according to the updated Sydney system¹³. Immunostains

were performed using rabbit anti-*H. pylori* antibody (dilution: 1:100, Dako, Glostrup-Denmark, Code: B0471, Lot: 31). Streptavidin-biotin peroxidase technique (Dako, Glostrup-Denmark-Universal LSAB2 Kit/HRP) was used to visualize immunostaining.

All statistical analyses were performed by SPSS for Windows 12.0. The results of histologic analysis were compared with UBT results. Statistical analysis was performed using the kappa statistics for the strength of agreement between histochemical and immunohistochemical methods for the detection of *H. pylori*. These two methods were also compared to UBT results.

Results

Helicobacter pylori was observed as small, slightly curved bacillus with HE and Giemsa stains (Figs. 1, 2). No coccoid forms were identifiable with these stains. By using immunohistochemical method, we were able to detect *H. pylori* at low magnification even if they were small in number (Fig. 3). Additionally, coccoid forms of *H. pylori* not identified by HE or Giemsa stains were also observed easily by immunohistochemistry (Fig. 4). *H. pylori* density was scored according to the Sydney system. The number of biopsies with *H. pylori* density score 3 (+) with immunohistochemistry [25% of *H. pylori* (+) biopsies] was more than those with HE or Giemsa stains [12.1% of *H. pylori* (+) biopsies] (Table I). This result showed that the organisms with low density could be detected by immunohistochemistry more easily than by HE and Giemsa stains.

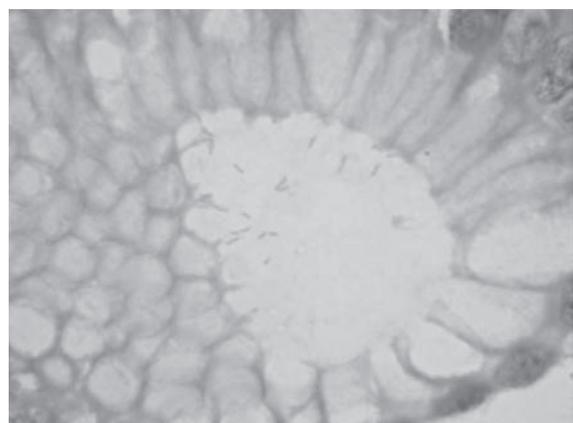


Fig. 1. The curved bacteria visualized with hematoxylin-eosin (x400).

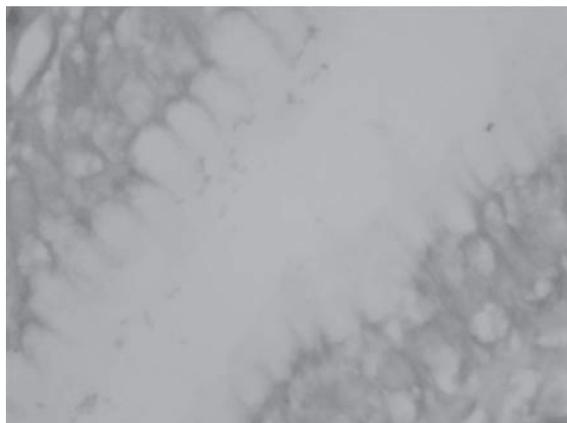


Fig. 2. *Helicobacter pylori* observed with Giemsa stain (x400).

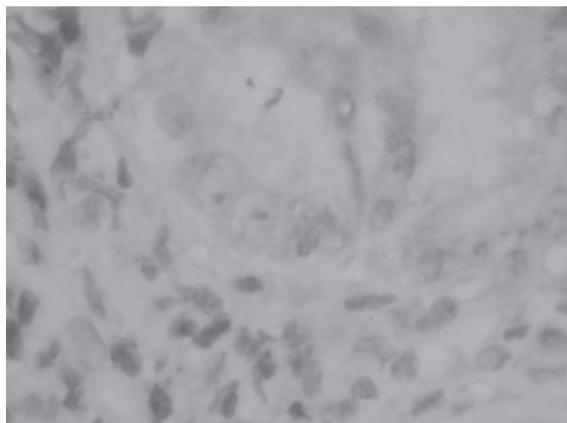


Fig. 3. A single *Helicobacter pylori* in the glandular lumina with immunohistochemical staining (x400).

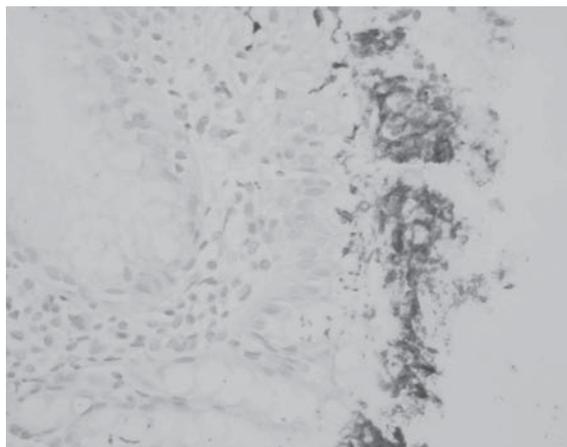


Fig. 4. Coccoid and bacillus forms of *Helicobacter pylori* within the mucus layer in close contact with the epithelial cells (x400).

Table I. Histochemical and Immunohistochemical Staining Results for the Detection of *H. pylori*

Histochemistry	Immunohistochemistry				Total
	0	1 (+)	2 (+)	3 (+)	
0	10	2	0	0	12
1 (+)	6	7	10	5	28
2 (+)	5	8	10	7	30
3 (+)	1	2	3	2	8
Total	22	19	23	14	78

The frequency of *H. pylori* gastritis was higher in the antrum than corpus both histochemically and immunohistochemically. As all analyses of the parameters evaluated in this study gave similar results for both antrum and corpus biopsies, we present the results of the antrum biopsies of 78 patients.

By using HE and Giemsa stains, 66 of 78 (84.6%) antrum biopsies were found to contain *H. pylori*. Immunohistochemically, 56 of 78 (71.8 %) antrum biopsies were *H. pylori* (+) (Table II). To measure the agreement between histochemical (HE and Giemsa) and immunohistochemical methods, data obtained from these methods were analyzed using kappa statistics. The agreement between these methods for the detection of *H. pylori* in biopsies was statistically significant, but the agreement was poor ($k=0.148$, $p=0.022$).

Table II. Comparison of Histochemistry and Immunohistochemistry for the Detection of *H. pylori* Density in Gastric Antral Biopsies

Histochemistry	Immunohistochemistry		Total
	Negative	Positive	
Negative	10	2	12
Positive	12	54	66
Total	22	56	78

When compared with immunohistochemistry results, the sensitivity and specificity of histochemistry for the detection of *H. pylori* were 45% and 96%, respectively, meaning that the probability of false positivity is 4% and the probability of false negativity is 55%.

All antrum and corpus biopsies with *H. pylori* colonization showed chronic inflammation with activity. There was atrophic gastritis in 16 antral biopsies and 12 of them were found to contain *H. pylori* histochemically and immunohistochemically. Seven corpus biopsies showed atrophic gastritis, with five of them positive for *H. pylori*.

There was no correlation between the presence of lymphoid follicles and *H. pylori* positivity.

Urea breath test (UBT) was positive in 68 of 78 patients. Only one antrum biopsy with (-) UBT was found to be *H. pylori* (+) histochemically and three immunohistochemically *H. pylori* (+) biopsies were taken from patients with (-) UBT. These three UBT (-) biopsies showed the coccoid forms of *H. pylori* immunohistochemically. Correlation of histochemistry and UBT is shown in Table III. Table IV summarizes the results comparing immunohistochemistry and UBT. Eight patients with gastritis in corpus biopsies had (-) UBT. None of these patients' biopsies showed *H. pylori* with HE and Giemsa but *H. pylori* was detected in one immunohistochemically.

Table III. Correlation of UBT and Histochemical *H. pylori* Detection

Histochemistry	UBT		Total
	Negative	Positive	
Negative	9	3	12
Positive	1	65	66
Total	10	68	78

UBT: Urea breath test.

Table IV. Correlation of UBT and Immunohistochemical *H. pylori* Detection

Immunohistochemistry	UBT		Total
	Negative	Positive	
Negative	7	15	22
Positive	3	53	56
Total	10	68	78

UBT: Urea breath test.

Ninety percent of UBT (-) cases were also negative for *H. pylori* in antrum biopsies with histochemistry and this ratio was 70% for immunohistochemistry. Among *H. pylori* (+) antrum biopsies, 98.5% had (+) UBT.

Discussion

Helicobacter pylori is one of the commonest infectious agents worldwide. This gram-negative spiral organism, which colonizes the gastric mucosa, causes chronic active gastritis and gastric ulcer disease. Furthermore, the World Health Organization has classified this organism as a carcinogen for gastric cancer^{1,14,15}. Hence, proper methods for the detection and accurate

treatment are of great importance. Many invasive and noninvasive methods have been developed for the detection of *H. pylori*. Noninvasive tests such as serology and UBT are recommended for the diagnosis of *H. pylori* infection in adult patients under the age of 45 years⁴. There are limited studies on the diagnostic value of these tests and it is reported that 10% false-positive and false-negative results must be accepted¹⁰. The histologic examination of gastric biopsy specimens is currently accepted as the gold standard^{16,17}. In addition to the detection of *H. pylori*, endoscopic biopsies can provide information about the type, grade, and extent of inflammation, atrophy and intestinal metaplasia in the stomach. It is reported that in clinical practice, therapeutic decision-making and in the planning of follow-up arrangements, knowledge of the microscopic appearance of the gastric mucosa is helpful^{6,17-19}.

In studies performed on adult gastric biopsies, it is reported that *H. pylori* gastritis is more prevalent and more severe in the antrum than in the corpus¹⁶. In our study performed on the pediatric gastric biopsies, the frequency of *H. pylori* gastritis was higher in the antrum than corpus, similar to the adult biopsies.

The significant association between chronic active gastritis and *H. pylori* infection has been reported in several studies. In our study, all biopsies with *H. pylori* colonization had chronic active inflammation with both mononuclear cells and neutrophils^{1,11,16,17}. Although atrophy is also known to be associated with *H. pylori* gastritis, in this study only 21.4% of biopsies with *H. pylori* gastritis showed atrophy. This finding may be because some of the biopsies did not contain the muscularis mucosa layer, in which case it is not appropriate to evaluate the presence of atrophy.

In histologic sections, *H. pylori* is observed as a curved bacterium mostly found beneath the mucus layer in close relation to the luminal surface of epithelial cells lining the superficial foveolae^{1,16}. Several staining methods, such as HE, Giemsa, Genta, silver stains and acridine orange, have been proposed for visualizing the curved bacteria in the tissue sections. Some of these stains like silver stains are time consuming and difficult to perform⁷⁻¹⁰. Furthermore, none of them is specific for *H. pylori*, and *H. pylori*-like

organisms may cause false-positive results with these stains. Since 1988, immunohistochemical staining using specific polyclonal antibodies to visualize *H. pylori* in sections is recommended for the diagnosis of *H. pylori* infection in gastric biopsies. So far, no cross-reactions to any other curved bacteria have been documented^{8,10-12}. In our study, we found that *H. pylori* positivity was 84.6% in antrum biopsies with histochemistry, but immunohistochemical staining showed 71.8% of antrum biopsies as positive for *H. pylori*. Furthermore, with HE and Giemsa stains, curved bacteria are only detected when found in large numbers. By immunohistochemical method with specific antibodies, it is possible to identify *H. pylori* in small numbers¹⁶. In our study group, it was impossible to identify the coccoid forms of the organism with HE and Giemsa stains. Coccoid forms were specifically stained and visualized by immunohistochemistry.

Statistical analysis of the results of this study revealed that histochemically *H. pylori* (-) biopsies should be investigated immunohistochemically for the presence of *H. pylori*. Since we found 12 biopsies with *H. pylori* positivity with histochemistry to be *H. pylori* (-) with immunohistochemistry, we recommend immunohistochemical staining on gastric biopsy sections for the detection of *H. pylori* gastritis. This will protect from false-positive results and from useless treatment. Immunohistochemical staining is simple, sensitive, specific, reproducible, and easy to perform.

Seven biopsies obtained from 68 UBT (+) patients did not show *H. pylori* positivity histochemically or immunohistochemically. This suggests that UBT can give false-positive results in children as reported for adults. Three immunohistochemically *H. pylori* (+) biopsies were taken from patients with (-) UBT, and *H. pylori* was observed mostly in the coccoid form. UBT is based on the urease activity of *H. pylori*, but coccoid forms of the organism cannot produce urease¹⁶. This may be the cause of false-negative results with UBT in these three cases.

Based on these results, we suggest performing endoscopic biopsy for the diagnosis of *H. pylori* gastritis in pediatric cases. We conclude that immunohistochemical staining with a specific antibody is an accurate technique to detect *H. pylori* infection in gastric biopsies.

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