Helicobacter pylori in Gastric biopsy: A Histochemical and Immunohistochemical Assessment

Zainab Waleed Aziz*, Shuaib Hashim Saleem**, Hatim Abdulmajeed Al-Nuaimy***
*Department of Pathology, College of Medicine, Ninevah University, Mosul, Iraq, **Department of Pathology, College of Medicine, University of Mosul, Mosul, Iraq, ***Senior Histopathology, Al-Jumhuri Teaching Hospital, Mosul, Iraq.

Correspondence: zainabwaleed90@yahoo.com


ABSTRACT

Background: Helicobacter species pylori represent one of the medically prominent and most common infections in the world. Contamination with this microbe has set as a causal factor in the development of gastritis, peptic ulcer, and gastric neoplasia. Consequently, prompt diagnosis is essential.

Objectives: This study was conveyed to detect H. pylori in gastric biopsies specimens by using routine Hematoxylin, Modified Giemsa dye as well as immunohistochemical stain, besides to assess the specificity and sensitivity of Helicobacter microbe detection in each method.

Patients and methods: The research was both prospective and retrospective, carried out on 100 cases of endoscopically obtained gastric biopsies. Data obtained from archives of the pathology department, at AL-Jamhuri Teaching Hospital, Mosul city, and collected in a period spanning from April 2013 to March 2014. The information included; Age, sex, gastric biopsy location, inflammation status, the presence of dysplasia or carcinoma. Helicobacter pylori infection was assessed histochemically and immunohistochemically.

Results: In a total of 100 gastric samples, patients’ age range was 11 to 82 years (mean age of 46.5 years), with a male to female ratio of 1.38:1. Helicobacter pylori bacilli were positive with H&E/MGS in 71 (71%) of cases, increased to 75 (75%) case with IHC. Chronic gastritis noticed in 85 biopsy specimens, 74% were positive for H.pylori. There was a statistically significant difference between IHC and H&E/MGS (p=0.04) for detection of H.pylori. The sensibility and specificity of the H&E/MGS were measured compared with the recommended standard sensitive and specific IHC test; they were 95% and 100% respectively.

Conclusion: The routine ancillary stains request for the detection of H.pylori remains a laboratory and an institution right. This study revealed that, in our laboratory, the regular application of ancillary dyes is not obliged for the description of H.pylori because it was readily recognizable in the bulk of sections with haematoxylin staining. However, we recommend the use of IHC in specific circumstances.

Keywords: Helicobacter pylori, Gastric biopsy, Modified Giemsa stain, Immunohistochemistry, Cancer.

عبصات الملونية البوابية في خزعة المعدة: تقييم كيميائي نسيجي
ومناعي كيميائي نسيجي

زيينب ولد عزيز*، شعب هاشم سالم**، حاتم عبد المجيد النعيمي***
*فرع الأمراض، كلية الطب، جامعة تفتون، الموصل، العراق، **فرع الأمراض، كلية الطب، جامعة الموصل، الموصل، العراق، ***اختصاص علم الأمراض، مستشفى الجمهورى التعليمى، الموصل، العراق

الخلاصة

معلومات أساسية: عصبات الملونية البوابية تمثل واحدة من الأمراض الأكثر شيوعاً في جميع أنحاء العالم. العدوى بهذه البكتيريا سلبية الغرام قد أنشئ كعامل مسبب في حدوث التهاب المعدة، اورام المعدة، بما في ذلك سرطان غشاء المعدة والأورام الليمفاوية

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Helicobacter pylori (H. pylori) represent one of the medically prominent and most common infections worldwide. It is a curved rod gram-negative multi-flagellated bacterium observed virtually solely attached on the gastric mucosa of human beings. Under unfavorable circumstances, it can become viable but non-culturable cocoid form. In gastric biopsies, Helicobacter microbes are 0.5 to 1.0 Mm wide and 2.5 to 5.0 Mm long; they carry up to six unipolar wrapped flagella which are crucial for bacterial movement. Flagella manifests a specific end bulb, which is an expansion of the flagellar case. In developing countries, 70% to 90% of the people carries H. pylori before the age of 10 years. In developed countries, the prevalence of infection is lower, varying from 25% to 50%. The rate of new H. pylori infections among adults in the Western world is less than 0.5% per year. Since spontaneous elimination of the infection is rare, it proceeds in most cases to chronic gastritis. However, the cytokine response and the gastric acid secretion are bound for an individual’s ultimate clinical outcome. It has found that a reduction in the production of gastric acid predisposes to corpus gastritis or pangastritis which in turn leads to gastric ulcer, atrophy, and carcinoma.

In gastric cancer, irrespective of their histology, most of these tumors originate from mucosa infected by H. pylori and very rarely occur without inflammation. Therefore, H. pylori are considered to be a strong factor in the evolution of gastric cancer. Therefore, regarding all facts about the strong correlation between H. pylori and different upper gastrointestinal lesions, it is very crucial to specify the presence of this bacteria in gastric biopsy reports as it has an important role in the therapeutic implications too. Since, over years pathologists have sought different reliable methods for detecting H. pylori in biopsy specimens, including special stains, immunohistochemistry (IHC), polymerase chain reaction (PCR) and lately, in situ hybridization.
PATIENTS AND METHODS

Patient Selection
This study was a prospective and retrospective carried out in a series of 100 consecutive endoscopically obtained gastric biopsies during a period spanning from April 2013 to March 2014. A tissue block of each case was chosen for histochemistry and IHC.

We maintained a routine protocol approval to access the clinicopathological data from archives of the department of pathology laboratory, at AL-Jamhuri Teaching Hospital, Mosul City, and these included: Age (ranged from 11 to 82 years with a mean of 46.5 years), sex (58 males and 42 females with a male to female ratio of 1.38:1), gastric biopsy location.

Patients with chronic gastritis, gastric ulcers, adenocarcinoma, and MALToma also included. The studied cases of chronic gastritis were reviewed according to the recommended table by the Sydney system

Histological and IHC Staining
All the obtained gastric biopsies were collected, placed on filter paper, fixed in 10% formalin overnight, processed, and embedded in paraffin wax. After that 4micron-thick tissue sections taken.

Once the slides prepared, H. pylori status analyzed by three methods: H&E, modified Giemsa stain (MGS) using 1:9 dilution (Sheehan’s modified may) and immunohistochemical stains (IHC) applying a rabbit polyclonal antibody against H. pylori (1:160 dilution; Cell Marque, Ventana, catalog: 760-2645, Rocklin, Calif) according to the manufacturer’s guidance using automated BenchMark instrument (Ventana). Antigen retrieval was performed by microwave heating in a sodium citrate buffer. An avidin-biotin detection method used with 3,39-diaminobenzidine tetrahydrochloride visualization

Scoring and Analysis of H pylori Staining
All cases microscopically examined for interpretation of histochemical and immunohistochemical stains of H pylori infection. Each set of histologic sections calculated, and the results inscribed.

H. pylori categorized as either positive or negative. The presence of any stained organisms resembling H. pylori bacteria designated as positive. The lack of any H. pylori like microbe stain assigned as negative. H. pylori is typically a curved rod microbe that is 2.5-4.0 microns long and 0.5-1.0 microns thick. It is observed on the lumen or epithelial surface of the gastric mucosa; the organisms are infrequently seen in within epithelial cells or gastric crypts

Statistical Analysis
The Chi-square test was performed to analyze negative and positive cases as defined by H&E/MGS and IHC for pathologic hallmarks. A cut level of p<0.05 was used for separation of cases.

RESULTS
The patients’ age range was 11 to 82 years (mean of 46.5 years) most of them were in the third decade. There were 58 (58%) males and 42 (42%) females with a male to female ratio of 1.38:1, Figure 1&2.

The presence of H. pylori was significantly correlated with male sex and with young age group (p<0.01), as shown in Table 1.

Gastric biopsies were obtained from the antrum (81%) and the corpus (19%) only. The frequency of H. pylori was higher in the antrum than corpus both histochemically and immunohistochemically, Table 2.

The histopathological findings of endoscopically obtained gastric biopsies were all illustrated in Table 3. Cases of gastritis were classified and graded, according to the updated Sydney system, Table 4.

The detection rate of H. pylori was different with different stains used (H&E, MGS or IHC). The bacilli were positive with H&E/MGS in 71 (71%) of cases, increased to 75 (75%) by IHC stain, with a degree of colonization graded into mild, moderate and marked, Table 5 & Figure 3. There were 8 (11%) cases of mild colonization detected by IHC; of these 8 cases, only 4 were positive by the H&E/MGS Figure 4&5. On the other hand, all moderate and marked cases were positive by all stains, Figure 3&6. All parenchyma infected with H. pylori manifested variable active and chronic gastritis. With a sample of 85 chronic gastritis biopsy specimens, 63 (74%) maintained active inflammation, and 22 (26%) kept chronic inactive inflammation. The presence of H. pylori significantly linked with active (p < 0.0001) and chronic (p < 0.0001) inflammation. Among H. pylori

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definite chronic gastritis, there was no significant
difference between the infiltration of _H. pylori_ in the
mucosa and the degree of severity (mild, moderate, or severe). Intestinal metaplasia was
present in 3(4%) cases of gastritis; one was of a
severe type. Two of them were positive for Helicobacter microbe by IHC; both displayed
colonization of bacteria in areas other than metaplasia. Five gastric biopsies exhibited features
of peptic ulcer, _H. pylori_ were positive in 3 of them.
Gastric cancer (adenocarcinoma and lymphoma)
detected in 10% of the cases, 50% were
associated with _H. pylori_ positivity. The presence
of _H. pylori_ significantly correlated with dysplasia
and gastric cancer in spite of the small sample in
this study (p < 0.04). Figure 7.

The sensitivity and specificity of the H&E/MGS
were measured compared with the recommended
standard sensitive and specific IHC test; they were
95% and 100% respectively. Ultimately, this survey
noticed a statistically significant difference between
IHC and H&E/MGS (p= 0.04) for the detection of
_H. pylori._

### Table 1: _H. pylori_ density in relation to age and sex
in the study group.

<table>
<thead>
<tr>
<th>Age</th>
<th>Male</th>
<th>Female</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 40</td>
<td>38</td>
<td>33</td>
<td>71</td>
</tr>
<tr>
<td>&gt; 40</td>
<td>28</td>
<td>33</td>
<td>61</td>
</tr>
<tr>
<td>Positive</td>
<td>43</td>
<td>33</td>
<td>76</td>
</tr>
<tr>
<td>Negative</td>
<td>14</td>
<td>9</td>
<td>23</td>
</tr>
</tbody>
</table>

*p value<0.01*.

### Table 2: Correlation between histochemistry and
IHC for detection of _H.pylori_ in relation to the
location of the gastric biopsy.

<table>
<thead>
<tr>
<th>H. pylori presence</th>
<th>Corpus (n=19)</th>
<th>Antrum (n=81)</th>
<th>Total (n=100)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>11</td>
<td>60</td>
<td>71</td>
</tr>
<tr>
<td>Negative</td>
<td>8</td>
<td>21</td>
<td>29</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>IHC</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>12</td>
<td>63</td>
<td>75</td>
</tr>
<tr>
<td>Negative</td>
<td>6</td>
<td>19</td>
<td>25</td>
</tr>
</tbody>
</table>

### Table 3: Histopathological findings of
endoscopically obtained gastric biopsies.

<table>
<thead>
<tr>
<th>Chronic gastritis</th>
<th>85</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gastric ulcer</td>
<td>5</td>
</tr>
<tr>
<td>Gastric cancer</td>
<td>10</td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>7</td>
</tr>
<tr>
<td>Intestinal type</td>
<td>3</td>
</tr>
<tr>
<td>Diffuse type</td>
<td>4</td>
</tr>
<tr>
<td>Lymphoma(MALToma)</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
</tr>
</tbody>
</table>
Table 4: *H. pylori* colonization and histological grades of gastritis by updated Sydney system.

<table>
<thead>
<tr>
<th>Sydney system</th>
<th><em>H. pylori</em> +</th>
<th><em>H. pylori</em> -</th>
<th>Total</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chronic inflammation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>6</td>
<td>6</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>2</td>
<td>38</td>
<td>16</td>
<td>54</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>25</td>
<td>0</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>PMN inflammation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>13</td>
<td>9</td>
<td>22</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>1</td>
<td>7</td>
<td>5</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>34</td>
<td>8</td>
<td>42</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>9</td>
<td>0</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Glandular atrophy</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>42</td>
<td>7</td>
<td>49</td>
<td>P=0.258</td>
</tr>
<tr>
<td>1</td>
<td>14</td>
<td>10</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>7</td>
<td>2</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>3</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Intestinal metaplasia</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>63</td>
<td>19</td>
<td>82</td>
<td>P=0.08</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

Table 5: *H. pylori* colonization in correlation to histochemistry and IHC

<table>
<thead>
<tr>
<th><em>H. pylori</em> colonization</th>
<th>H&amp;E/MGS (n)</th>
<th>IHC (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 None</td>
<td>29</td>
<td>25</td>
</tr>
<tr>
<td>1 Mild</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>2 Moderate</td>
<td>41</td>
<td>41</td>
</tr>
<tr>
<td>3 Severe</td>
<td>26</td>
<td>26</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

p value = 0.04

Figure 3: The typical spiral curved *H. pylori* bacterium can be clearly seen using the three staining methods. (100X oil objective).

Figure 4: Chronic gastritis with H&E/MGS negative for *H. pylori*; however, IHC was positive for the coccoid form (arrow), (10X40HPF).
**DISCUSSION**

*Helicobacter pylorus* is a major human pathogen for which an accurate detection required for proper patient management. In most cases, *H. pylori* recognized in a good hematoxylin and eosin stain. However, the sensitivity of this remains low, especially when there are no many bacteria.

None one method has sufficient sensitivity and specificity to be considered a gold standard, therefore, most laboratories use an additional staining method in the identification of the organism.

In the present study, we adopted three methods (H&E, MGS, and IHC) for *H. pylori* revealing. The mean age of sampled patients was 46.5 years, being more prevalent between 21 and 30 years. The *H. pylori* appearance significantly correlated with a young age group, a finding similar to the fact that the incidence of *H. pylori* infection is higher at a younger age in the developing world.

This study displayed *H. pylori* infection more common in male sex which is similar to a systematic review and meta-analysis of 244 studies analyzed by Ibrahim et al. This explained by that, in practice, most gastric biopsies obtained from the antrum.

Similar to our research, most studies confirmed the high prevalence rate of antral *H. pylori*, utilizing various identification methods, explained by that, in practice, most gastric biopsies obtained from the antrum.

The detection rate of *H. pylori* is variable and dependent on the stain used (H&E, special stain).
or IHC). It has reported that hematoxylin-eosin stain alone can detect *H. pylori* in 66% of cases with many false positives and false negatives.

In this study, we introduced a modified Giemsa stain (MGS) and immunohistochemistry (IHC) stain for detection of *H. pylori*. The bacilli were positive with H&E/MGS in 71% of cases which rate increased to 75% by using IHC. The sensitivity and specificity of the H&E/MGS were measured compared with the recommended standard sensitive and specific IHC test; they were 95% and 100% respectively. This high sensitivity attributed to patient used during the examination of the H&E and Giemsa stained sections in addition to the use of at least 15 high power fields looking for *H. pylori* organisms, a similar finding reported in Smith et al. However, the false negative results in H&E/MGS explained by the fact that the mildly colonized or the singly scattered bacteria easily be lost by the H&E/MGS. In extension to the point that many *H. pylori* organisms transformed into coccoid forms, after therapy, which may pass by the routine dyes undetected. Those single or modified organisms were visualized obviously by IHC. Based on these results it is clear that immunohistochemical staining could marginally enhance the detection rate of the organisms.

In the current research with an 85 chronic gastritis biopsy samples, the significant association between chronic active gastritis and *H. pylori* infection has been previously analyzed in many other studies. Hence, the chronic active inflammation should prompt a careful search of the sections for the presence of the *H. pylori*.

Five percent of dyspeptic patients confirmed to have gastric ulcer disease in this study, three of them were positive for *H. pylori* microbe, a conclusion that similar to a study in Iran, with a percentage of 71%. Chronic *H. pylori* gastritis leads in more than half of the affected subjects to gastric atrophy. In this survey, glandular atrophy present in 36 (42.4%) cases of chronic gastritis, most were of a mild degree, results similar to those reported by previous studies done in Iraq. On the other hand, *H. pylori* were present in 49.5% of atrophic gastritis just comparable to those reported in Turkey and Iran, (43% and 68%) respectively.

Despite some doubts, it globally believed that *H. pylori* have a fundamental role in the pathogenesis of gastric cancer. Gastric cancer (adenocarcinoma and lymphoma) detected in 10% of the studied sample, 50% were associated with *H. pylori* similar to Taiwanese study. The residence of Helicobacter microbe was positively associated with gastric dysplasia and cancer in spite of the small sample. However, further studies are needed to confirm the role of this microorganism in the gastric carcinogenesis in our locality.

The results of this study revealed that IHC is a highly specific and sensitive method for the identification of *H. pylori* as compared to H&E and MGS. However, in our laboratory, *H. Pylori* almost readily viewed in the majority of cases with haematoxylin stain which comparable to a recent study suggesting that pathologists’ are able to identify these bacilli regardless their training level therefore ancillary staining for *H. pylori* is not indicated in our practice. However, in a small number of cases, an immunohistochemical stain can be particularly useful like in severe active gastritis in which no helicobacter microbes could be detected on haematoxylin stains, to avoid the false-negative results, and for the follow-up biopsies to confirm the absence of *H. pylori* regardless of the number of the organisms present or the shape it chooses.

**CONCLUSION**

The routine ancillary stains request for the detection of *H. pylori* remains a laboratory and an institution right. This study revealed, in our laboratory, a regular application of ancillary dyes for the description of *H. pylori* not obligated because it was readily recognizable in the bulk of sections with haematoxylin staining. However, we propose IHC for samples with severe chronic severe active gastritis in which *H. pylori* not distinguished by H&E dyes, post-treatment biopsy samples, and when particles “suspicious” but not conclusive for *H. pylori* viewed on haematoxylin stains.
REFERENCES


