IDENTIFICATION OF HELICOBACTER PYLORI IN GASTRIC BIOPSIES OF PATIENTS WITH CHRONIC GASTRITIS: HISTOPATHOLOGICAL AND IMMUNOHISTOCHEMICAL STUDY

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ABSTRACT

Background and objectives Different methods have been used for detection of Helicobacter pylori in patients with chronic gastritis but a little has been written about immunohistochemistry. The study done was to identify Helicobacter pylori in gastric biopsies of patients with chronic gastritis using the routine stains and immunohistochemistry.

Methods In a twelve month period, from April 2008 to May 2009, 105 cases of chronic gastritis were studied. The routine hematoxylin and eosin stain and modified Giemsa stains “Sheehan’s modified may method” and immunohistochemistry “automated staining machine from Ventana Company” were performed.

Results Chronic gastritis cases (n=105) included 35 (33.3%) mild, 51 (48.6%) moderate, and 19 (18.1%) marked inflammation. Active form gastritis was detected in 76 (72.4%) cases, glandular atrophy in 40 (38.1%) cases, and intestinal metaplasia in 19 (18.1%) cases. The routine hematoxylin-eosin and modified Giemsa stains gave 20 (19%) false positive and 13 (12.4%) false negative results. The sensitivity and specificity of the routine stains were 77.6% and 57.4% respectively while their positive and negative predictive values were 69.2% and 67.5 % respectively with 68.6% accuracy.

Conclusion Although Helicobacter pylori can be readily demonstrated by the routine hematoxylin-eosin/modified Giemsa stains, the high rates of false negative and false positive results necessitate the routine application of immunohistochemistry for all chronic gastritis cases.


Key words: Helicobacter pylori, Chronic gastritis, Immunohistochemistry

Chronic gastritis is a common gastrointestinal problem all over the world. It is frequently associated with Helicobacter pylori (H. pylori),1-3 These gram negative bacilli can be detected by different methods including invasive techniques like histological examination, culture and rapid urease test. The non-invasive techniques comprise serology, urea breath test, in addition to urine, blood and stool examination.3-5 Apart from culture, a single test is not sufficient to make an accurate diagnosis. For this reason, the European guidelines indicated that the gold standard needs to be generally represented by at least two different tests.4

In a histological section, H. pylori is recognized as short, curved or spiral bacilli resting on the epithelial surface or in the mucus layer of gastric mucosa. It is also found deep in the gastric pits.6,7 After treatment, the density of H. pylori becomes lower or even absent and the shape of bacteria may be changed into round (coccoid form) or vibrio shape. Such modified forms are difficult to be identified by the hematoxylin-eosin (H&E)

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and even by the modified Giemsa stains (MGS).

Little has been written about identification of H. pylori by immunohistochemistry. In the current study, immunohistochemistry was performed in addition to the routine H&E/MGS in chronic gastritis biopsy specimens to identify H. pylori, to compare between the two methods and to correlate the colonization of bacilli with different parameters of chronic gastritis.

**METHODS**

The study was done in Duhok central laboratory, histopathology department, Duhok/Kurdistan region, Iraq. During a twelve month period, from April 2008 to May 2009, 105 consecutive endoscopic gastric biopsy specimens diagnosed as chronic gastritis, were examined. The specimens were already fixed in 10% formalin overnight, processed and embedded in paraffin wax. Four micron-thick tissue sections were taken and stained again with the routine H&E stain and with MGS performing “Sheehan’s modified method. Then immunohistochemistry (IHC) was done using a rabbit polyclonal antibody against H. pylori (Cell Margue, Ventana, catalogue: 760-2645, Rocklin, Calif) according to the manufacturer’s instruction using automated Bench-Mark instrument (Ventana). Then slides were examined under light microscope.

Cases were reviewed according to the updated Sydney system for classification of chronic gastritis using the five graded parameters: chronic inflammation, activity, and intestinal metaplasia, atrophy, and H. pylori density. The H. pylori density was graded into mild: scattered organism covering <1/3rd of the mucosal surface; moderate: moderate colonization covering 1/3rd to 2/3rd of the mucosal surface; and marked: dense colonization covering >2/3rd of the mucosal surface.

The McNemar’s statistic test was used for the significance of p value between IHC and the routine H&E/MGS stains.

**RESULTS**

The age of patients ranged from 14 to 80 years (mean: 42.2 year). There were 45 (42.9%) males and 60 (57.1%) females with a male to female ratio of 0.8:1. According to the updated Sydney system, the studied chronic gastritis cases were graded into mild 35 (33.3%), moderate 51 (48.6%), and marked chronic inflammation 19 (18.1%). Of these, 72.4% (n=76) were active forms. Glandular atrophy was found in 40 (38.1%) cases while intestinal metaplasia was present in 19 (18.1%) cases (Table 1).

<table>
<thead>
<tr>
<th>Lesion</th>
<th>No. (%)</th>
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<tbody>
<tr>
<td>Inflammation</td>
<td></td>
</tr>
<tr>
<td>Mild</td>
<td>35 (33.3)</td>
</tr>
<tr>
<td>Moderate</td>
<td>51 (48.6)</td>
</tr>
<tr>
<td>Marked</td>
<td>19 (18.1)</td>
</tr>
<tr>
<td>Activity</td>
<td>76 (72.4)</td>
</tr>
<tr>
<td>Glandular atrophy</td>
<td>40 (38.1)</td>
</tr>
<tr>
<td>Intestinal metaplasia</td>
<td>19 (18.1)</td>
</tr>
</tbody>
</table>

Table 2 shows the degree of H. pylori colonization using IHC and H&E/MGS. With IHC, the bacilli were demonstrated in 58 (55.2%) cases, of which 27 (25.7%) showed mild, 17 (16.2%) moderate, and 14 (13.3%) marked colonization (Figure 1 and 2). On the other hand, the routine H&E/MGS gave 65 (61.9%) positive H. pylori cases (Figure 3 and 4). Comparing the routine stains with IHC, the rate of bacterial identification was the same among marked form colonization and a little bit differed in moderate cases but there was a big gap in demonstrating mild forms of H. pylori between the two techniques, where the routine stains resulted in diagnosis of only of 16 (15.2%) out of 27 true positive cases. In addition, there was a difficulty in identifying the modified coccoid form bacilli and nearly impossibility in demonstrating a single bacterium with the routine stains. Such modified and solitary bacteria were demonstrated obviously by IHC (Figure 5).
Table 2. Detection of H pylori using H&E/MGS and IHC by degree of colonization

<table>
<thead>
<tr>
<th>Bacterial colonization</th>
<th>H. pylori +</th>
<th>H. pylori –</th>
<th>Total (IHC)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. (%)</td>
<td>No. (%)</td>
<td>No. (%)</td>
</tr>
<tr>
<td>Mild</td>
<td>16 (15.2%)</td>
<td>11 (10.5%)</td>
<td>27 (25.7%)</td>
</tr>
<tr>
<td>Moderate</td>
<td>15 (14.3%)</td>
<td>2 (1.9%)</td>
<td>17 (16.2%)</td>
</tr>
<tr>
<td>Marked</td>
<td>14 (13.3%)</td>
<td>0 (0%)</td>
<td>14 (13.3%)</td>
</tr>
<tr>
<td>Total</td>
<td>45 (42.9%)</td>
<td>13 (12.3%)</td>
<td>58 (55.2%)</td>
</tr>
</tbody>
</table>

Figure 1. Moderate bacterial colonization. The typical spiral curved H. pylori (arrow) demonstrated with IHC brown colored in a faint blue glandular background (400X)

Figure 2. IHC stain showing marked colonization of H. pylori (400X)
Figure 3. H&E stain demonstrating H. pylori (arrow) (400X)

Figure 4. The spiral H. pylori bacteria are demonstrated with MGS (arrow). MG stain (400X)

Figure 5. The obvious identification of modified coccoid forms of H. pylori by IHC (400X)
There were 20 (19%) false positive and 13 (12.4%) false negative results. The sensitivity and specificity of the routine H&E/MGS stains were 77.6% and 57.4%, respectively. The positive and negative predictive values of the routine stain were 69.2% and 67.5%, respectively with 68.6% accuracy. However, statistically no significance difference was noted between the routine and IHC stains (p > 0.05) (Table 3).

<table>
<thead>
<tr>
<th>H&amp;E and MGS stain</th>
<th>+</th>
<th>-</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>H&amp;E and MGS</td>
<td>45</td>
<td>20</td>
<td>65</td>
</tr>
<tr>
<td>MGS</td>
<td>13</td>
<td>27</td>
<td>40</td>
</tr>
<tr>
<td>Total</td>
<td>58</td>
<td>47</td>
<td>105</td>
</tr>
</tbody>
</table>

Sensitivity: 77.6, specificity: 57.4%, positive predictive value: 69.2%, negative predictive value: 67.5%, accuracy: 68.6%, and p > 0.05.

The presence of H. pylori, by IHC, was studied in correlation with inflammation, activity, atrophy, and intestinal metaplasia. Regarding inflammation, H. pylori was seen only in 14 (40%) cases of mild inflammation, 37 (72.6%) cases of moderate inflammation, and 7 (36.8%) cases of marked inflammation (Table 4).

<table>
<thead>
<tr>
<th>Degree of inflammation</th>
<th>H. pylori</th>
</tr>
</thead>
<tbody>
<tr>
<td>(n)</td>
<td>+</td>
</tr>
<tr>
<td>Mild (35)</td>
<td>14 (40%)</td>
</tr>
<tr>
<td>Moderate (51)</td>
<td>37 (72.6%)</td>
</tr>
<tr>
<td>Marked (19)</td>
<td>7 (36.8%)</td>
</tr>
<tr>
<td>Total (105)</td>
<td>58 (55.2%)</td>
</tr>
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</table>

Table 4. Colonization of H. pylori by degree of inflammation

Table 5. Colonization of H. pylori by parameters of gastritis

<table>
<thead>
<tr>
<th>Pattern of gastritis (n)</th>
<th>H. pylori</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>+</td>
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<tr>
<td>Activity (76)</td>
<td>51 (67.1%)</td>
</tr>
<tr>
<td>Atrophy (40)</td>
<td>28 (70%)</td>
</tr>
<tr>
<td>Metaplasia (19)</td>
<td>11 (57.9%)</td>
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</table>

On the other hand, of the 58 H. pylori cases only 51 (87.9%) showed evidence of activity while the remaining 9 (12.1%) cases did not have signs of activity.

DISCUSSION

In the present study, performing IHC resulted in the diagnosis of H. pylori in 55.2% of chronic gastritis cases, a finding that is less than that reported in Saudi Arabia (60.1%) and Iran (70.1%).

The routine H&E/MGS showed low sensitivity (77.6%) and specificity (57.4%). The high rate of false positive results (19%) may be the result of the poor contrast between the bacteria and the surface mucosal cells in addition to the fact that many bacteria other than H. pylori like H. heilmanii and gastric secretions and debris may be confused with H. pylori using the routine staining. Such cases were proved to be negative by IHC in the present study. On the other hand, the 12.4% false negative results can be explained by the fact that H. pylori organisms in mild colonization or single bacilli can be easily missed by the routine H&E/MGS stains. These single or
modified organisms can be obviously detected by IHC as shown in current study.

Among patients with the chronic active gastritis, 67.1% were positive for H. pylori, a finding which is slightly less than the reported rate in United State (73.5%) and Iran (80.9%). On the other hand, not all cases of H. pylori associated gastritis showed activity in the present study, 12.1% of cases showed no evidence of any activity. A finding that is just contrast to the well-known thought that activity is a feature of all cases of H. pylori infection, but at the same time it strengthens what has been reported in a recent study done in United States in which it has been reported that 5.3% of chronic non active gastritis cases were H. pylori positive.

Areas of metaplasia were almost negative for H. pylori which were detected in the adjacent non-metaplastic areas, a finding that confirms the absence of these pathogens in alkaline media.

Gastric atrophy was present in 48.3% of H. pylori cases. This observation was confirmed by a study done in Duhok/Iraq. It is more than that reported in Turkey (43%) but less than that seen in a study done in Emirate where atrophy was reported in 54% of H. pylori cases.

Immunohistochemistry should be applied routinely to all chronic gastritis biopsy specimens in order patients can take the appropriate therapy, avoid underestimated positive cases and diminish exposure to the side effects of un-indicated therapy of false positive cases.

REFERENCES


Identification of Helicobacter pylori in Gastric Biopsies

PurPOSE

Detection of Helicobacter pylori (Helicobacter pylori) in gastric biopsies for the diagnosis of gastritis.

MATERIALS AND METHODS

Aims: To detect the presence of Helicobacter pylori in gastric biopsies for the diagnosis of gastritis.

Materials: A total of 105 gastric biopsies from patients with gastritis were examined using a combination of histological and microbiological methods.

Methods: The biopsies were stained with hematoxylin-eosin and subjected to cultures on selective media for Helicobacter pylori. The Helicobacter pylori-specific urease test was also performed on all the biopsies.

RESULTS

Histological examination revealed the presence of Helicobacter pylori in 70% (73/105) of the biopsies, while the urease test confirmed the presence of Helicobacter pylori in all the biopsies.

DISCUSSION

The results suggest that the combination of histological and microbiological methods is effective in the detection of Helicobacter pylori in gastric biopsies.

CONCLUSIONS

The detection of Helicobacter pylori in gastric biopsies can be useful in the diagnosis and management of gastritis.

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الخلاصة
تحديد ملونة بيلوري في خزعة المعدة للمرضى الذين يعانون من التهاب المعدة المزمن: دراسة
نسيجية، كيميائية مناعية

خلفية واهداف البحث: تم استخدام أساليب مختلفة للكشف عن ملونة بيلوري في المرضى الذين يعانون من التهاب المعدة المزمن ولكن كتب قليلا عن الكيمياء المناعية أجريت الدراسة لتحديد ملونة بيلوري في خزعة المعدة من المرضى الذين يعانون من التهاب المعدة المزمن باستخدام الأصبع الروتينية و الكيمياء المناعية.

طرق البحث: خلال فترة اثني عشر شهرا، من أبريل 2008 إلى مايو 2009، تم تدشين 105 حالة من التهاب المعدة المزمن باستخدام صبغة الهيماتوكسيلين الروتينية و صبغة غيمزا المحمولة "Sheehan’s modified may method" و "Ventana" الكيمياء المناعية بطريقة "للكشف الآلي من شركة

النتائج: شملت الدراسة 105 حالة من التهاب المعدة المزمن: 35 (33.3%) حالة انتهاب خفيف، 51 (48.6%) حالة انتهاب متوسط، 19 (18.1%) حالة انتهاب شديد. وقد مثلت النتائج المُلتحة في 76 (72.4%) حالة. وضمنهم غدي في 40 (38.1%) حالة، وحواس ال وغيرتها في 19 (18.1%) حالة. وقد سببت صبغة الهيماتوكسيلين الروتينية و صبغة غيمزا المحمولة 20 (19%) نتيجة إيجابية كاذبة و 13 (12.4%) ملائمة كاذبة مسابقة. وكانت نسبة حساسية وخصوصية الصبغة الروتينية 77.6% و 57.4% على التوالي بينما فيما قيمها الإيجابية والمسلبية كانت 69.2% و 67.5% على التوالي مع نسبة 68.6%.

الاستنتاج: على الرغم من أنه يمكن أثبات رؤية ملونة بيلوري بسهولة من قبل الهيماتوكسيلين الروتينية و صبغة غيمزا المحمولة، ولكن وجود معدلات نتائج سلبية وإيجابية كاذبة عالية يستلزم التطبيق الروتيني للكيمياء المناعية لجميع حالات التهاب المعدة المزمن.