Diagnostic Efficacy of Different Techniques for Detection of Helicobacter. Pylori in Upper Gastrointestinal Tract Pathologies

A.R.Piyush, Roobina Khan, Hasan Harris, Veena Maheshwari

Senior Resident ,Department of Pathology, Jawaharlal Nehru Medical College, AMU

Accepted 2017-05-20; Publish 2017-06-09

Abstract:

Aim:

Helicobacter pylori infection is very common worldwide and causes a wide spectrum of diseases. A reliable diagnosis is crucial for patients with H. pylori-related diseases. Hence comes the role of detection of H.pylori using various invasive and noninvasive methods.

Material and Methods:

We conducted a study on 176 patients suffering from various gastrointestinal complaints who were screened for H. pylori infection using invasive tests like rapid urease test (RUT) on fresh biopsy and histological examination using H & E, Giemsa and polyclonal H.pylori antibody.

Results:

Results were derived in terms of sensitivity and specificity with each test and compared. Rapid urease test gave positivity in 40 cases with Sensitivity and Specificity of 87% and 100% respectively while H&E showed sensitivity of 96% and specificity of 98%.Giemsa was positive in maximum number of cases with no false negative hence Sensitivity and Specificity calculated is 100% and 98% respectively. Immunohistochemistry with polyclonal H.pylori antibody gave no false positive or false negative result leading to highest rate of sensitivity and specificity of 100% each.

Conclusion:

Our study concluded that Giemsa and Polyclonal H.pylori antibody both have a very high sensitivity in detecting H.pylori. While giemsa is inexpensive, less time consuming and is sufficient for routine diagnosis of the bacteria the latter has maximum specificity and differentiates H.pylori from its other morphological mimickers.

Key words: Helicobacter.pylori, Detection, Giemsa, Immunohistochemistry.

Introduction:

Helicobacter pylori are a gram-negative, microaerophilic, urease producing motile bacteria, which was discovered in 1982 by Marshall and Warren \[1\]. More than 50% of world’s population is infected by these bacteria \[2\], which is usually acquired in childhood. However greater than 70% of patients are asymptomatic, which if not treated persist for life-long\[3\]. The prevalence of H. pylori colonization is chiefly related to age, socioeconomic status and geographic location \[4\]. The bacteria usually colonises in mucus layer of human, only reservoir of infection.
The most commonly recognized manifestation of H. pylori infection in India is peptic ulcer disease, particularly duodenal ulcer in the ratio of 8:1 to 30:1 with gastric ulcer. Other lesions include chronic gastritis, atrophic gastritis, gastric adenocarcinoma, MALT lymphoma & atrophic gastritis. Atrophic pangastritis caused by this bacteria is the underlying precursor lesion leading to gastric cancer. H. pylori therefore is classified as Class I carcinogen by International Agency for Research on Cancer, hence highlighting its importance for detection.

Various tests are employed for detection of H. pylori associated with numerous advantages and disadvantages. Noninvasive test includes Serology (Enzyme-linked immune sorbent assay, Polymerase chain reaction, Reverse transcription polymerase chain reaction, Latex agglutination test etc) testing, 13-C Urea breath test, Faecal antigen tests etc. While, invasive methods includes Endoscopic evaluation, Culture, Rapid urease test, Histo pathological studies using Routine, Special stains and Immuno histochemistry. Variation exists in different studies evaluating the relation between H. pylori infection and various gastrointestinal tract pathologies which may be due to differences in the methodology for detection of H. pylori.

The present study was performed to evaluate the efficacy of various invasive methods like rapid urease test, histo pathologically with Haematoxylin and Eosin, Giemsa and immune histochemistry using polyclonal H. pylori antibody in the detection of H. pylori.

Material and methods:
A prospective study was conducted over a period of 2 years on 176 patients including both male and female of all age groups. A detailed history & examination of the patients presenting with long standing complaints of upper gastrointestinal symptoms like dysphagia, dyspepsia, nausea and vomiting, bloating, loss of weight, haematemesis were recorded. The duration, family history, past medical history, environmental and hereditary factors, along with preliminary investigations were taken into account. The patients receiving proton pump inhibitors, antibiotics, or bismuth subsalicylate in the previous 6 wks or those with a history of using nonsteroidal anti-inflammatory drugs and medication for H. pylori infection were excluded from the study. The patients were subjected to endoscopy and multiple biopsies of the suspected lesions were taken from oesophagus and stomach. An informed consent was taken before performing endoscopic biopsies.

**Rapid Urease Test (RUT):**
A small piece of fresh biopsy specimen was treated with commercially available rapid urease test kit. H.pylori releases enzyme urease that splits urea into ammonia & carbon dioxide which turns colour of the indicator (kit) from yellow to red which is taken as a positive result. Although colour may vary from faint orange to bright red depending on the bacterial density in the biopsy specimen. The remaining biopsy pieces were then, sent for histopathological examination.

**Histopathology:**
The biopsy pieces were processed and histopathological examination was done using Haematoxylin and eosin and Giemsa stains. In addition to detection of the bacteria, Haematoxylin and Eosin helps in evaluation of severity of inflammation and other significant changes produced by the bacteria like intestinal metaplasia and atrophy. Giemsa stain is more feasible in detection of the bacteria apart from being simple and less expensive.

**Immunohistochemistry:**
Immunohistochemistry was performed on the biopsy specimen using Polyclonal Helicobacter pylori antibody of Ig G isotype and which was heat stable and reacted with somatic antigens of the whole H. pylori organism. This antibody stains The individual H. pylori bacterium whether it presents on the surface of the epithelium or in the

International Journal of Contemporary Research and Review, Vol. 8, Issue. 6, Page no: MS 20225-20231
doi: [http://dx.doi.org/10.15520/ijcrr/2017/8/06/201](http://dx.doi.org/10.15520/ijcrr/2017/8/06/201)
cytoplasm of the epithelial cells in biopsy tissue sections.

**Control:**
Various studies have taken histology as the gold standard while few preferred rapid urease test, but variation are seen usually with every method. Hence in our study cases showing positivity to H. pylori by any of the two methods were considered as control. (Gold standard cases).

![Figure 1: H. pylori showing Marked positivity. (Giemsa: 1000X)](image1)

![Figure 2: H. pylori showing Moderate positivity. (Hematoxylin & Eosin: 1000X)](image2)

![Figure 3: H. pylori showing Marked positivity. (Immunohistochemistry; Polyclonal H. pylori Antibody: 1000X)](image3)

![Figure 4: Rapid urease test using pylo dry kit showing positive (red) and negative (yellow) result.](image4)

**Result:**
Out of the total 176 biopsies processed and studied, 112 were from gastric region, 56 were from oesophagus and 8 were from gastroesophageal junction. The study showed male preponderance with male patients being 109
and 67 female patients with the predominant complaint of dyspepsia and nausea.

Out of total 176 cases studied, the maximum number of 47 cases showed positivity by both giemsa and immunohistochemistry, which were taken as the gold standard cases (Table 1).

**Table 1: Total No of cases showing positivity to H.pylori by various tests:**

<table>
<thead>
<tr>
<th>Tests</th>
<th>Total No of cases positive</th>
<th>% of positivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rapid urease test</td>
<td>40/176</td>
<td>23%</td>
</tr>
<tr>
<td>H &amp; E</td>
<td>47/176</td>
<td>27%</td>
</tr>
<tr>
<td>Giemsa</td>
<td>49/176</td>
<td>28%</td>
</tr>
<tr>
<td>Polyclonal H.pylori antibody</td>
<td>47/176</td>
<td>27%</td>
</tr>
</tbody>
</table>

Rapid urease test gave relatively fewer positivity amounting to 40 cases, having 7 false negative cases. No false positive case was noted. Hence Sensitivity and Specificity calculated was 87% and 100% respectively. However total no of cases positive on H& E was 47. There were 2 false positive cases as they were not positive by any other test while 2 cases showed negative result(false negative). Hence sensitivity calculated was 96% while specificity was 98%. Giemsa was positive in maximum no of cases leading to the figure of 49, while true positive cases were 47. There was no false negative result with this method, highlighting its high sensitivity against other routine methods. So the Sensitivity and Specificity calculated was 100% and 98% respectively. Immunohistochemistry with polyclonal H.pylori antibody gave no false positive or false negative result leading to highest rate of sensitivity and specificity of 100% each.

**Discussion:**

The prevalence of H. pylori in the Indian subcontinent is very high, especially in rural areas leading to wide spectrum of diseases. Various studies have shown its association with peptic ulcer disease, chronic gastritis, atrophic gastritis, gastric adenocarcinoma and MALT lymphoma. H.pylori has been classified as Class I carcinogen by International Agency for Research on Cancer. Numerous detection methods for the presence of H.pylori have been developed. Each one of them has been associated with advantages and disadvantages. Noninvasive tests like Serology has limitation in endemic areas while ¹³C Urea BreathTest is technically very demanding. The stool antigen detection method, although specific, has poor sensitivity\[10\]. The ¹³C UBT is believed to be specific, but because the stomach is colonized by many other urease producing bacteria this test becomes questionable. Histopathology is again a dilemma as, bacteria like H. heliominii, H. bizzozeroni, Pseudomonas fluorescence have a similar morphology \[11\]. The sensitivity of Rapid Urease Test is influenced by the bacterial density and the forms (spiral or coccoid) of bacteria present in the biopsy. The minimum of 10⁴ organisms per biopsy piece are required for a positive RUT result, but a good proportion of patients may harbor lower densities\[12\].

Therefore in our study, cases with result of 2 positive tests are taken as the positive case/gold standard. Similar standard has been taken/advocated by other studies\[10],[13],[14\]. Various studies have been performed till now comparing the sensitivity and specificity of various tests in detecting H.pylori and hence contributing in understanding the spectrum of pathologies caused by these bacteria. The comparision of result with various studies in case of rapid urease test is given in Table 2.

The result is largely dependent on H. pylori density and presence of other urease producing bacteria. It requires the presence of minimum of $10^4$ organisms for a positive result. The sensitivity of histology is influenced by the site, number and size of the biopsies collected. Also, patchy colonization sometimes cause misdiagnosis while the presence of other bacterial species like H. heilimanii, H. bizzozeroni, Pseudomonas fluorescence having similar morphology leads to overdiagnosis[11]. Furthermore, prior antibiotics and PPI may transform the typical shape of H. pylori from spiral to coccoid which becomes undetectable by the routine microscopy technique[19]. The sensitivity and specificity given by Hematoxylin & Eosin and Giemsa, with various studies has been compared(Table-2).

<table>
<thead>
<tr>
<th></th>
<th>Rapid urease test</th>
<th>Hematoxylin and Eosin</th>
<th>Giemsa</th>
<th>Polyclonal H.pylori antibody</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sensitivity</td>
<td>Specificity</td>
<td>Sensitivity</td>
<td>Specificity</td>
</tr>
<tr>
<td>Our study</td>
<td>87%</td>
<td>100%</td>
<td>96%</td>
<td>98%</td>
</tr>
<tr>
<td>Dandin et al[2013][13]</td>
<td>89%</td>
<td>94%</td>
<td>90%</td>
<td>98%</td>
</tr>
<tr>
<td>Redeen et al[2011][16]</td>
<td>90%</td>
<td>98%</td>
<td>97%</td>
<td>90%</td>
</tr>
<tr>
<td>Kobayashi et al[2002][14]</td>
<td>94%</td>
<td>100%</td>
<td>98%</td>
<td>100%</td>
</tr>
<tr>
<td>Kalan and Caulhair[2004 ] [17]</td>
<td>97%</td>
<td>80%</td>
<td>97%</td>
<td>90%</td>
</tr>
<tr>
<td>Tzeng et al,2005[18]</td>
<td>98%</td>
<td>100%</td>
<td>99%</td>
<td>98%</td>
</tr>
<tr>
<td>Riba et al,2011[20]</td>
<td></td>
<td>98.5%</td>
<td>100%</td>
<td></td>
</tr>
</tbody>
</table>

Our study showed highest level of sensitivity and specificity achieved by Immunohistochemistry which was in concordance with other studies(Table-2). Further Jonkert and Stobbering (1997) quoted polyclonal h.pylori antibody to be highly specific & associated with low interobserver variation in comparision to modified Giemsa & Warthin-silver staining. Diagnostic efficacy of immunohistochemistry was also highlighted by Vaira and Gatta( 2001). Above data is due to the fact that it identifies H. pylori even after specific therapy like clusters of round or vibrio-shaped bacteria. Also it has low interobserver variation discreetly distinguishes H.pylori from its other morphological mimickers.

**Conclusion:**

Giemsa and Polyclonal H.pylori antibody both have a very high sensitivity in detecting H.pylori. But, Polyclonal H.pylori antibody has maximum specificity which plays role in detecting coccoid or vibroid forms(formed in older cultures and post treatment) which are unidentified by other stains. While giemsa is inexpensive, less time consuming and is sufficient for routine diagnosis of the bacteria, polyclonal H.pylori antibody helps in differentiating H.pylori from its other morphological mimickers.
References:


19. Marzio L, Angelucci D, Grossi L. Anti-Helicobacter pylori specific antibody immunohistochemistry improves the diagnostic accuracy of Helicobacter pylori in biopsy specimen from patients treated
