EVALUATION OF ENDOSCOPY BASED METHODS (HISTOPATHOLOGY, CYTOLOGY AND UREASE TEST) FOR THE DETECTION OF HELICOBACTER PYLORI

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Abstract

This study aimed to assess the accuracy of histopathology, brush cytology, and urease test in the diagnosis of Helicobacter pylori and to evaluate the effect of test duration on the sensitivity and specificity of positive urease test for the detection of H. pylori.

Fifty patients [25 patients with gastritis group A and 25 patients with duodenal ulcer group B] selected from those attending endoscopy unit for dyspeptic symptoms, were enrolled in the study. Four endoscopic biopsies were taken from each patient. One biopsy from each of antrum and body were obtained for urease test (Urease test was read at 30 min, 1, 4 and 24 hour after biopsy insertion into the reagent), and one biopsy from each of antrum and body were used for histopathological examination. Antral brush cytology was taken also from each patient. The patients were considered H. pylori positive when minimum concordances of 2 out of 3 tests (Histopathology, brush cytology, and urease test) were positive.

Fourteen patients were positive for H. pylori in group A, in comparison to seventeen patients in group B. The sensitivities of the histopathological examination, brush cytology, and urease test at 24 hours in group (A) were 58%, 79%, and 93% respectively. Corresponding figures for the specificity were 100%, 91%, and 46% respectively. While in group (B) the sensitivities were 82%, 82%, and 100% and the specificities were 100%, 100%, and 88% respectively.

It is concluded that among the invasive methods, the association of the urease test with brush cytology constituted the best choice for confirming the diagnosis of H. pylori, due to the high sensitivity of the urease test and high specificity of brush cytology.

Introduction

Helicobacter pylori are spiral shaped, microaerophilic, gram negative bacteria measuring approximately 3.5 microns in length and 0.5 microns in width. H. pylori are the most common chronic bacterial infection in human. It has been demonstrated worldwide and in individuals of all ages. Conservative estimates suggest that 50% of the world’s population is affected. Infection is more frequent and acquired at an earlier age in developing countries compared to industrialized nations.

H. pylori infection, which has a very narrow host range, colonizing and persisting in only the gastric mucosa, generally manifests as gastroduodenal disorders. These conditions include gastritis and peptic ulcer disease (primarily duodenal ulcers (DU), and to a lesser extent gastric ulcers) in which the ulcers are present as an infection sequelae in approximately 15% of those infected.
Diagnostic testing for \textit{H. pylori} can be divided into invasive and non invasive techniques based upon the need for endoscopy.

I- Invasive testing:

Biopsy urease testing: the sensitivity of biopsy urease tests is approximately 90 to 95 percent, and specificity is 95 to 100 percent\textsuperscript{8}. Thus, false positive tests are unusual.

Histology: Potential problems with histologic examination include: The density of \textit{H. pylori} can vary at different sites, possibly leading to sampling error, and interobserver variability\textsuperscript{9}.

Brush cytology: a sensitivity of 98% and specificity of 96% have been reported\textsuperscript{10}. It is suggested and found to be more sensitive in some studies in comparison to biopsy histology\textsuperscript{11-13}.

Bacterial culture and sensitivity testing, String test\textsuperscript{14}, Brushing urease test\textsuperscript{15}.

II- Non invasive testing

A variety of non invasive tests for the diagnosis of \textit{H. pylori} are available or being evaluated. These include:

Urea breath test (UBT)\textsuperscript{16-17}, serology\textsuperscript{18}, \textit{13C} bicarbonate assay\textsuperscript{19}, stool antigen\textsuperscript{20-23}, saliva\textsuperscript{24-25} and urinary assays\textsuperscript{26-27}.

Aims of the study: Since peptic ulcer disease and gastritis are not uncommon in Basrah and since they have a strong association with \textit{H. pylori} infection\textsuperscript{28}, this study was carried out to achieve the following aims:

1. To assess the accuracy of three commonly used tests for \textit{H. pylori} diagnosis (Histopathology, brush cytology, and urease test) in symptomatic patients. The patient was regarded as \textit{H. pylori} infected if two or more of the above tests were positive.

2. To evaluate the effect of test duration on the sensitivity and specificity of positive urease test for the detection of \textit{H. pylori}.

Patients and Methods

Fifty patients (27 males and 23 females) selected from those attending the endoscopy unit in Al- Sadar Teaching Hospital in Basrah for various dyspeptic symptoms during the period from May 2006 to November 2006 were enrolled in this study.

Eligibility criteria were the following: patients who have positive endoscopic findings (DU or gastritis), absence of upper gastrointestinal malignancy, no prior gastric surgery, no acute upper gastrointestinal bleeding, and no consumption of non steroidal anti inflammatory drugs (NSAIDs), protons pump inhibitors (PPIs), anti-\textit{H. pylori} antibiotics or bismuth preparations within four weeks of endoscopy.

Patients were divided into two groups according to endoscopic findings: \textit{Group A}: 25 patients with gastritis (12 males and 13 females) aged (17-72) years old (Mean age = 40 years old).

\textit{Group B}: 25 patients with DU (15 males and 10 females) aged (14-82) years old (Mean age = 43 years old).

Upper GI endoscopy with biopsies for histopathological examination and urease test (UT), and antral brushings was performed with fibre optic endoscope (GIF Type 2T200, model Olympus) after an overnight fasting. Four biopsies were pinched from each patient with the help of three biopsy forceps, and as following: first one for taking biopsies from the antrum and body for histopathological examination. The second and third forceps were used for taking biopsies from antrum and body for UT respectively. Each forceps was sterilized with 10%
formaldehyde for at least 30 minutes before use.

Histopathology: One biopsy specimen from each antrum and body were fixed in a tube containing 10% formaldehyde solution. The specimen then stained by haematoxylin and eosin, and modified Giemsa to be used for histopathological examination. The pathologist characterized the presence of spiral bacteria in the superficial mucous layer or along the luminal surface of the gastric epithelial cells as a positive test.

Brush cytology was performed with cytology brush with the endoscopic tip in the antrum by gentle rubbing the surface of brush with mucosal wall in all direction for 10-12 times, brushing smears were then spread on two glass slides, and are air dried and labelled for patient’s no. Each slide stained by Giemsa stain to be used for cytological examination by the pathologist for detection of *H. pylori*. The brush tips was sterilised with formaldehyde 10% for at least 30 minutes before use.

Cytological and histopathological slides were examined blindly by the pathologist, who was also blind for endoscopic diagnosis.

Urease test: One biopsy specimen from each antrum, and body was used immediately for UT detection, each biopsy specimen was put in two different tubes, each contains 1 ml. of modified urea broth medium and labelled for site of biopsy, and time of taking biopsy. Each tube was maintained at ambient temperature and followed for change in colour in four periods: at 30 minutes, 1 hour, 4 hours, and 24 hours. The test was considered positive for *H. Pylori* when the colour of urea broth changed from yellow-orange to pink. Used tubes were washed with water and sterilised with autoclave for at least 20 minute before use.

*H. pylori* infection was considered positive when two or three tests (UT, brush cytology, and histopathologic observation) were positive.

Appropriate statistical methods were used to assess sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of these diagnostic modalities with the help of SPSS program version 15.

Results
Fifty patients, 27 (54%) males and 23 (46%) females with dyspeptic symptoms were enrolled in this study and subjected to various investigations for diagnosis of *H. pylori* infection (Histopathology, brush cytology and UT). The ages of patients ranged from 14-82 years (Mean age is 41.5 year).

In patients with gastritis, 14 out of 25 cases were positive for *H. pylori* (the prevalence rate = 56%). The sensitivity, specificity, positive and negative predictive values of each diagnostic method (Histopathology, brush cytology, and UT) in patients with gastritis are shown in Table I.

<table>
<thead>
<tr>
<th>Methods</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV*</th>
<th>NPV+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histopathology</td>
<td>57%</td>
<td>100%</td>
<td>100%</td>
<td>65%</td>
</tr>
<tr>
<td>Brush cytology</td>
<td>79%</td>
<td>91%</td>
<td>92%</td>
<td>77%</td>
</tr>
<tr>
<td>UT (at 24 hours)</td>
<td>93%</td>
<td>46%</td>
<td>68%</td>
<td>83%</td>
</tr>
</tbody>
</table>

*PPV= Positive predictive value
+NPV= negative predictive value
In patients with DU, 17 out of 25 cases were positive for *H. pylori* (the prevalence rate = 68%). The sensitivity, specificity, positive and negative predictive values of each diagnostic method (Histopathology, brush cytology, and UT) in patients with DU are shown in Table II.

<table>
<thead>
<tr>
<th>Methods</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV*</th>
<th>NPV+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histopathology</td>
<td>82%</td>
<td>100%</td>
<td>100%</td>
<td>73%</td>
</tr>
<tr>
<td>Brush cytology</td>
<td>82%</td>
<td>100%</td>
<td>100%</td>
<td>73%</td>
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<tr>
<td>UT (at 24 hours)</td>
<td>100%</td>
<td>88%</td>
<td>94%</td>
<td>100%</td>
</tr>
</tbody>
</table>

*PPV= Positive predictive value  
+NPV= negative predictive value

To evaluate the effect of UT duration on the sensitivity and specificity of positive UT for the detection of *H. pylori*, four intervals (At 30 min, 1 hour, 4 hours, and 24 hours) were studied. The sensitivities, specificities, positive and negative predictive values of UT in the four intervals are shown in Table III.

In patients with gastritis: UT tended to increase in sensitivity if the incubation period was increased. The sensitivity of UT at 30 min, 1 hour, 4 hour, and 24 hours were 36%, 64%, 79%, and 93% respectively, while UT specificity tended to drop beyond 4 hours incubation from 73% to 46%.

<table>
<thead>
<tr>
<th>Methods</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV*</th>
<th>NPV+</th>
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</thead>
<tbody>
<tr>
<td>UT at 30 min.</td>
<td>36%</td>
<td>73%</td>
<td>63%</td>
<td>47%</td>
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<tr>
<td>UT at 1 hour</td>
<td>64%</td>
<td>73%</td>
<td>75%</td>
<td>62%</td>
</tr>
<tr>
<td>UT at 4 hours</td>
<td>79%</td>
<td>73%</td>
<td>79%</td>
<td>73%</td>
</tr>
<tr>
<td>UT at 24 hours</td>
<td>93%</td>
<td>46%</td>
<td>68%</td>
<td>83%</td>
</tr>
</tbody>
</table>

*PPV= Positive predictive value  
+NPV= negative predictive value

While the sensitivity, specificity, positive and negative predictive values of UT in the four intervals are shown in Table IV.

<table>
<thead>
<tr>
<th>Methods</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV*</th>
<th>NPV+</th>
</tr>
</thead>
<tbody>
<tr>
<td>UT at 30 min.</td>
<td>59%</td>
<td>88%</td>
<td>91%</td>
<td>50%</td>
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<tr>
<td>UT at 1 hour</td>
<td>71%</td>
<td>88%</td>
<td>92%</td>
<td>58%</td>
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<tr>
<td>UT at 4 hours</td>
<td>82%</td>
<td>88%</td>
<td>93%</td>
<td>70%</td>
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<tr>
<td>UT at 24 hours</td>
<td>100%</td>
<td>88%</td>
<td>94%</td>
<td>100%</td>
</tr>
</tbody>
</table>

*PPV= Positive predictive value  
+NPV= negative predictive value
In patients with DU: the duration of incubation for UT increased the sensitivity from 30 min to 24 hours. The sensitivity of UT at 30 min, 1 hour, 4 hour, and 24 hours were 59%, 71%, 82%, and 100% respectively, while the UT specificity is constant at 88% through 24 hours.

Discussion

This study showed that in patients with gastritis, histopathological examination has the highest rate of false negative results; the sensitivity was only 58% in comparison to urease and brush cytology tests 79% and 93%, respectively. However, its specificity was high 100%, incontestable proof of the presence of bacteria. The false negative results seen in histopathological examination were possibly due to obtaining a fragment without \( H.\ pylori \) because of its patchy distribution in gastric mucosa, as well as the colonized surface of epithelial cells might be lost in fixative or other solutions during processing of biopsy sample. Moreover, brush cytology screens a much larger area of antral mucosa than a grasp biopsy.\(^{29-30}\)

The UT presented only one false negative result, demonstrating its high sensitivity 93%, which was similar to that observed by Cifuentes P et al\(^{31}\) study, but disagreeing with it by its low specificity 46%.

Our results showed a fairly good sensitivity 79% with high specificity 91% of brush cytology. Brush cytology sensitivity was observed to be better than histology because brush cytology allows sampling of a large area of the mucosal surface and preserves the mucin layer, in which many organisms are present. The sensitivity of histopathological examination in patients with DU was 82% which was similar to that of brush cytology, and both methods showed 100% specificity, while the sensitivity and specificity of UT were 100% and 88% respectively, Table II.

The sensitivities of histopathological examination, brush cytology and UT in patients with DU were higher than that in patients with gastritis, this is possibly because the ability of each of these tests to detect evidence of \( H.\ pylori \) depend on a given threshold of colonization, which is higher in patients with DU than in patients with gastritis. Tables I&II. Saksena S et al\(^{32}\) concluded that in patients with DU the most sensitive test was brush cytology 100% followed by UT 94.5% while histopathology had the highest specificity 89.3%.

Rde O Custodio et al\(^{33}\) also concluded that brush cytology was superior to histopathology in patients with non ulcer dyspepsia and the percentage of positive cases detected by brush cytology was similar to histopathologic examination in patients with DU, while CY Ho et al\(^{34}\) stated that UT has higher detection rate than histopathological examination of the biopsy specimens obtained from the margins of gastric ulcer in the diagnosis of \( H.\ pylori \) infection.

Neeraj Goel et al\(^{30}\) also concluded that brush cytology was the most sensitive and specific test. UT also had good sensitivity but lower specificity. Histopathology was very specific but there was a possibility of missing the diagnosis in 33.3% cases. So for the assessment of \( H.\ pylori \) in the gastric mucosa, brush cytology was more sensitive than histopathology, as well as faster and cheaper, particularly when the density of the bacteria is relatively low. It is a quick method, with results available before the patient leaves the endoscopy unit, whereas the average turnaround time for histology usually ranges from 2 to 4 working days. Also UT is a dependable, quick, and cost effective method for the detection of \( H.\ pylori \), but lacks specificity.

In patients with gastritis the UT tend to increase in sensitivity if incubation
period was increased beyond 30 min, to reach 93% at 24 hours, but this was accompanied by dropping in UT specificity beyond 4 hours incubation from 73% to 46%. While in patients with DU the UT tended to increase in sensitivity if the incubation period was increased beyond 30 min. to reach 100% at 24 hours, and this was accompanied by constant UT specificity at 88% in all four intervals, Tables III&IV.

These results were similar to studies of Ho KY et al, Lim LL et al, and Neeraj Goel et al who observed that the UT tended to increase in sensitivity if incubation period was increased but dropped in the specificity with increasing the time of incubation. Ho KY et al also concluded that better results could be obtained if the UT continued to be read over a 24 hour period, but Neeraj Goel et al concluded the optimum duration of incubation for UT for a good sensitivity and specificity was 4 hours.

The sensitivities of UT in patients with DU were higher than that in patients with gastritis in all four intervals; this may be attributed to high infection rate of DU patients with H. pylori in comparison to patients with gastritis, Tables III&IV.

The increase rate of false positive reading of UT in patients with gastritis could be attributed to the instability of the reagent, or from autolysis of the tissue by chemical reaction or due to the presence of a contaminate with lower urease activity than H. pylori which need prolong incubation to produce a positive test such as Gastrospirillum hominis, Pseudomonas and Proteus.

References

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