

Comparison of Different Methods for Diagnosis of *Helicobacter pylori* Infection

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A number of diagnostic tests have been developed and are currently in use for detection of *H. pylori* infection. The present investigation was conducted to evaluate and correlate various tests used for detection of *H. pylori* infection such as specific anti-IgG test and other biopsy dependent procedures. From every patient four antral biopsies were taken and subjected to direct urease test, second to culture, third to direct immunofluorescent antibody staining (DFA), and fourth to histological-histopathological examination. Sera from each patients were estimated for specific IgG against *H. pylori*. Histopathology, rapid urease test, culture and direct fluorescent antibody staining (DFA) had *H. pylori* detection range of 90%, 70%, 56.7% and 63.3% respectively, while IgG test against *H. pylori* was positive with 83.3% efficiency. Sensitivity and specificity of various laboratory tests compared to histopathology exhibited values of 70.4%, 33.3% for direct urease test, 59.3%, 66.7% for culture, 66.7%, 66.7% for DFA and 88.9%, 66.7% for *H. pylori* IgG. Similarly, sensitivity and specificity of various laboratory tests to culture showed results of 88.2%, 53.8% for direct urease test, 94.1%, and 76.9% for DFA, 82.4%, 15.4% for *H. pylori* IgG and 94.1%, 15.4% for histopathology test. Histopathology is a rapid, sensitive and easy method for rapid detection of *H. pylori* and although culture based method is expensive and time consuming; it still remains a viable method for diagnosis of *H. pylori* infection.

Key words: *H. pylori*, antibodies, Histpathology, Culture, rapid urease test, culture and direct fluorescent antibody staining.

Helicobacter pylori (*H. pylori*) is a gram negative, spiral, flagellated bacterium produce abundant urease. It was first discovered by Warren and Marshall, in 1983 that set a basic idea of management to diagnose dyspepsia¹. *H. pylori* is involved in several upper gastrointestinal diseases that present as dyspepsia and often observed colonizing the human gastroduodenal mucosa^{2,4}. The organism is usually found side by side to gastric epithelial cells, under the mucus layer in

the gastric pits where it causes damage to the cells⁵. It is a causative agent of chronic gastritis, peptic ulcer disease, gastric carcinoma, and gastric mucosal associated lymphoid tissue (MALT) lymphoma^{2,3}. Peptic ulcer disease is now viewed as an infectious disease since eradication of *H. pylori* leads to its cure⁵. There is a close association between *H. pylori* and gastric inflammatory diseases. *H. pylori* are responsible for more than 95% of duodenal ulcer and in about 70% of gastric ulcer⁶.

Various different diagnostic tests for *H. pylori* have been developed and are being performed for detection of *H. pylori* infection

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includes histopathological examination, direct stained smears from the biopsy specimens, culture, direct urease test, and estimation of the specific immunoglobulins⁷. They can be broadly classified into invasive and non-invasive tests⁵. Invasive tests uses endoscopic biopsy samples for histology examination, culture, rapid urease test (RUT) and polymerase chain reaction. These methods have high sensitivities and specificities⁸. The non-invasive tests do not require endoscopy, include urea breath test (UBT), immunoglobulin G and M serology, stool antigen test, saliva antibody test and urinary antibody test⁵. The non-invasive tests are not generally preferred except Immunoglobulin G (IgG) serology because of their low discriminatory power between previous and current infection.

The aim of our study was to find the relation between *H. pylori* and histo-pathological picture of gastritis, also to validate estimation of anti-*H.pylori* specific IgG as diagnostic test by comparing it with other biopsy dependent procedures, e.g. rapid urease test, culture on specific media, direct immunofluorescent antibody (DFA) staining and histopathology.

METHODS AND MATERIALS

Subjects

30 patients admitted with suffering from dyspeptic symptoms with positive endoscopic findings were selected from Endoscopy department at al Eman Hospital, Riyadh. All patients were subjected to a full research sheet and blood samples before endoscopy. All patients did not receive any specific treatment against *H.pylori* before the study. Consent was obtained from all the patients as well as from the ethics committee of Hospital for the study.

Biopsy specimens

From every patient four antral biopsies were taken using gastroscope Olympus, fibroptic videoscope (Japan) with sterile channel, tip and biopsy forceps. One of the biopsies was subjected to direct urease test, the second to culture, the third to direct immunofluorescent antibody staining (DFA), and the fourth to histological-histopathological examination.

- a) Direct urease test was done using Christensen's medium according to *Mackie*

and *McCartney* (9).

- b) Culture: The biopsy was dipped into a sterile small screw capped bottle containing 1ml of brain heart serum (Oxoid) and 2 sterile glass beads. Bottles were vortexed at 10,000 rpm for 2 minutes, 500ul of the tissue suspension were inoculated onto selective Skirrow's media. The plates were incubated micro-aerophilically at 37°C for 4 days. Any growth was identified by its colonial morphology and characteristic morphology by Gram stain (Figure 1).
- c) Detection of *H. pylori* was done by direct immunofluorescent antibody staining using kits supplied from Pathfinder, Kallestad Diagnostic Kits, Chaska as per manufacture instruction and kit protocol.
- d) Histological-histopathological examination: The biopsy was immediately fixed in buffered neutral formaline, and then embedded in paraffin. Sections of 5 mm thickness were stained with H. &E., and examined for the presence of characteristic *H. pylori*. The stained sections were also examined from the histopathological point of view (figure 2 & 3).

Serological examination

Sera were estimated for specific IgG against *H. pylori* using (Enzygnost, Anti-*H. pylori*/IgG, BEHRING, Germany) as per manufacture instruction and kit protocol. The cutoff is obtained by calculating the mean absorbance of the control sera and borderline. Samples with absorbance below the cutoff value are considered negative (and vice versa).

RESULTS

The present study was conducted on 30 patients with upper gastrointestinal symptoms, who were subjected to upper gastrointestinal endoscopy examination.

The mean age was 44.8 year (+13.4), and 80% of the patients were from rural areas, and 66.7% of low socioeconomic state. Male to female ratio was 3:1

H. pylori assessment

H. pylori status was assessed by culture and histology. Diagnosis of *H. pylori* infection was made if culture, histology, or both were

Cultures	Histopathology		Rapid urease	Histopathology		Total	Histopathology		Total	IgG against <i>H. pylori</i>		Total
	Positive	Negative		Positive	Negative		DFA	Positive		Negative	Positive	
Positive	16	1	Positive	19	2	21	Positive	18	1	19	Positive	24
Negative	11	2	Negative	8	1	9	Negative	9	2	11	Negative	3
Total	27	3	Total	27	3	30	Total	27	3	30	Total	27
Sensitivity	59.30%		70.40%		70.40%		66.70%		66.70%		88.90%	
Specificity	66.70%		66.70%		66.70%		66.70%		66.70%		66.70%	
PPV	94.10%		90.50%		94.70%		94.70%		96%		96%	
NPV	15.40%		11.1%		18.20%		18.20%		40%		40%	

Table 1. Comparison of other test for identification of *H. pylori* with Histopathology results.

Histopathology	Culture		Rapid urease	Culture		Total	DFA		Total	Culture		Total	IgG against <i>H. pylori</i>		Total
	Positive	Negative		Positive	Negative		Positive	Negative		Positive	Negative		Positive	Negative	
Positive	16	11	Positive	15	6	21	Positive	16	3	19	Positive	16	11	27	
Negative	1	2	Negative	2	7	9	Negative	1	10	11	Negative	1	2	3	
Total	17	13	Total	17	13	30	Total	17	13	30	Total	17	13	30	
Sensitivity	94.10%		88.20%		94.10%		94.10%		82.40%		82.40%		82.40%		
Specificity	15.40%		53.80%		76.50%		76.50%		15.40%		15.40%		15.40%		
PPV	59.30%		71.70%		84.20%		84.20%		56%		56%		56%		
NPV	66.70%		77.85		90.90%		90.90%		40%		40%		40%		

Table 2. Comparison of other test for identification of *H. pylori* with Culture results.

positive. *H. pylori* negative subjects were negative for both tests.

Maximum 27 were detected positive by histopathological examination followed by 25 by IgG for *H. pylori*, whereas only 17 were culture positive, 21 by rapid urease test and DFA was

positive for 19. Three and 13 patients were negative by Histopathological examination and culture respectively.

Comparison of histopathology and Culture with other laboratory tests in terms of sensitivity and specificity is shown in Table 2-3.

DISCUSSION

The close association between gastric inflammatory disease and *H. pylori* is now well established¹⁰. A rapid, sensitive, specific and quantitative test for the detection of the *H. pylori* infection would be of great value. We compared and evaluated different diagnostic tests for the detection of *H. pylori* infection including histopathological sections, DFA, culture, urease test, and detection of the specific IgG for *H. pylori*. Culture isolation of *H. pylori* is very less when compare to histopathology examination, attributed the lower level of isolation of *H. pylori* by culture may be due to either sampling error or technical difficulties¹¹. Also, in patients with chronic atrophic gastritis and those with achlorohydra, may have heavy colonization in the stomach with commensal flora interfering with the growth of the organism. Even in some cases, the biopsy samples do not contain a sufficient amount of *H. pylori* organisms to allow their isolation, since the distribution of *H. pylori* in the stomach is patchy. This patchy distribution was proved by *Hazell et al.*, (1987) who stated that *Campylobacter pylori* are likely to be found in one biopsy specimen examined histopathologically and not from another obtained from the same individual and examined by the same histopathologist¹².

The sensitivity and specificity of the serological test (IgG against *H. pylori*) compared to the culture method were low. Three patients were positive by culture and negative serologically, these false negative were probably due to either early stage of infection, as IgG, does not appear until several weeks, or they may be IgA positive (if done) but IgG negative, as what has been reported by some study that about 2% of investigated patients produce an IgA response in absence of an IgG response¹³.

On the other hand, IgG level was positive in 11 cases whose biopsies proved to be *H. pylori* negative by culture, these patients may have had

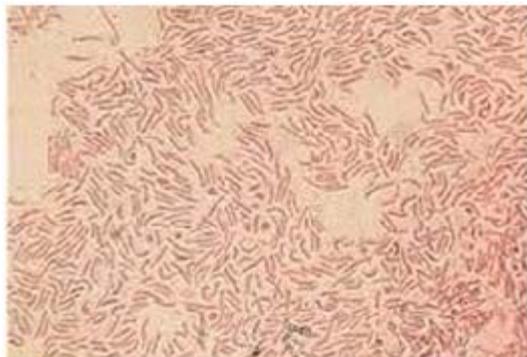


Fig. 1. Gram stain of *H. pylori* from culture plate

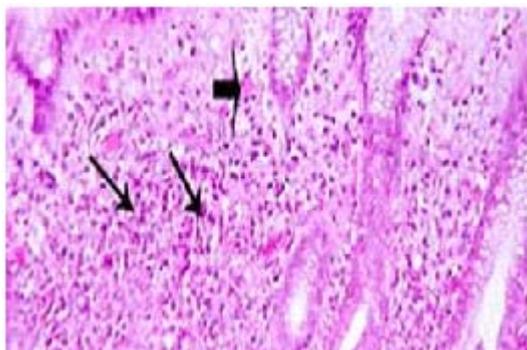


Fig. 2. Histology of *H. pylori* in gastrointestinal tract

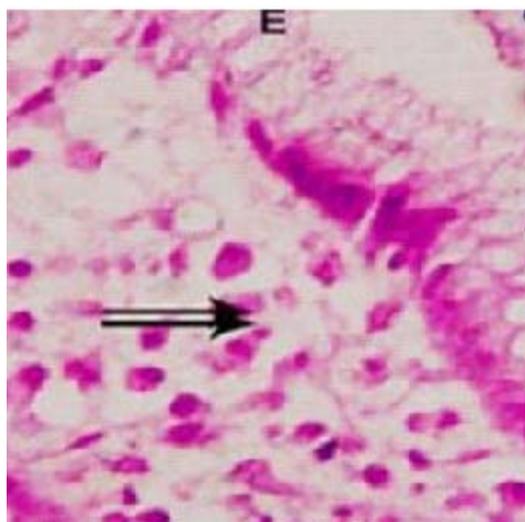


Fig. 3. Histology of *H. pylori* in gastrointestinal tract

previous infection with *H. pylori* and their antibody levels were still positive. The antibody level needs up to 6 months after eradication of the organism for a significant drop in titre to occur⁸. On comparison of IgG against *H. pylori* levels with histopathology, sensitivity and specificity was found to be 89 % was 67 %. Variance in the seroprevalence and the frequency of infection detected by biopsy methods suggest that *H. pylori* may have been eradicated or suppressed or that serology was a more sensitive and less specific method to detect infection in this population. Further studies are needed to examine the factors that may affect the detection of *H. pylori* infection. In comparing the DFA with culture they found that, there was a concordance of 83% between the combined morphology and the bacteriologic culture and it can be a rapid diagnosis of *H. pylori*. As regards to the results of culture method in our study; sensitivity of direct urease test was 88.2% and specificity was 53.8%. The cause of these differences could be because human gastric mucosa might become colonized with urease positive bacteria other than *H. pylori*, and this explained the 6 specimens with positive rapid urease test encountered in this study, with no *H. pylori* detection in culture^{2,4}.

Comparing the results of DFA and culture in the present study, 16 patients were positive by both tests while 3 were positive by DFA only and one patient was positive by culture only. It suggest that DFA staining is sensitive and specific for detection of *H. pylori* in biopsy specimens and also suggested that the DFA test may be sensitive than tissue culture and can detected *H. pylori* antigen irrespective of the viability of the organism, while culture isolation requires viable organism.

It is concluded that the specimen which was culture positive and DFA negative may be due to sampling error or due to obtained independent biopsy which may have no organisms. The two positive patients by DFA and negative by culture may be due to the presence of non-viable organism in the biopsy specimen or due to non-specific reaction.

H. pylori have a great association with several causes of dyspepsia, and must be considered in all cases of dyspepsia. *H. pylori* specific IgG may give helpful information about primary infection diagnosis, and prognosis.

Serological evidence of *H. pylori* infection was greater than the prevalence of infection documented by culture methods in our study, suggesting suppression or recent clearance of infection. Although, culture is expensive, requires good skills, time consuming, it remains an important method for strain typing, and detection of pathogenic properties and virulence factors of *H. pylori*.

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Disclosure

The authors certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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