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Comparison between Invasive and Noninvasive Tests in Diagnosis of *Helicobacter pylori* Infection

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Abstract: In this study, the invasive and noninvasive diagnotic tests were compared to choose the appropriate test for diagnostice of *H. pylori* infection. *Helicobacter pylori* (*H. pylori*) is a human pathogen that causes chronic gastritis, has a role in gastric and duodenal ulcer, is involved in gastric carcinogenesis and is regarded as a possible important factor in at least a subset of patients with functional dyspepsia. The diagnosis of *H. pylori* is an essential element in the management of many common gastrointestinal pathologies. The assessment of each routine invasive and noninvasive test is important. We studied a total of 127 outpatients for the detection of *H. pylori* infection. The presence of *H. pylori* infection by invasive tests containing the Rapid Urease Test (RUT), histology (Giemsa staining) and culture in 127 patients. Patients who were positive in culture, or two tests from four tests, invasive and noninvasive, were considered to have *H. pylori* infection. In noninvasive tests, we evaluated anti-*H. pylori* IgG and anti-CagA antibodies using commercial Enzyme-Linked Immunoassay (ELISA) and Western blot in dyspeptic patients. Eighty five out of the 127 patients were positive for *H. pylori*. *Helicobacter pylori* IgG seropositivity and 35 out of the 127 patients were positive for immunoblot. RUT had sensitivity, specifity and accuracy of 96, 80 and 90.5%, respectively; for Elisa these were 85.2, 33 and 70.5%, respectively and for ELISA with immunoblotting they were 65, 45 and 58.8%, respectively. The results of this study suggest that noninvasive tests (ELISA, immunoblotting) have the lowest and RUT with histology have the highest accuracy. These earlier tests can not be used for accurate infection diagnosis.

Key words: *Helicobacter pylori*, serological test, chronic gastritis, immunoblotting, diagnostic

INTRODUCTION

The diagnosis of *H. pylori* is an essential element in the management of many common gastrointestinal pathologies. The assessment of each routine invasive and noninvasive test is important. In this research, the invasive and noninvasive diagnotic tests in kashan were compared to choose the appropriate test for diagnostice of *H. pylori* infection.

Helicobacter pylori play a pivotal role in the pathogenesis of several gastro duodenal pathologies makes its diagnosis necessary in many different circumstances (Fock and Ang, 2010). Numerous reliable invasive and noninvasive diagnostic tests have been developed. Each has advantages and disadvantages which will make it more or less appropriate depending on the clinical situation.

Invasive tests were the first to be used in the diagnosis of *H. pylori* (Dondi *et al*., 2006).

The stomach is usually accessed by fiber optic endoscopy and biopsy specimens are obtained. Unfortunately, with standard technology the endoscopic features of *H. pylori* infection are not specific.

The non-invasive tests obviate the need for endoscopy and comprise serology and the urea breath test, using either 13 or 14°C (Mana *et al*., 2005). In view of the patchy distribution of *H. pylori*, all biopsy-based tests may theoretically fail to diagnose the infection. The inherent risk of sampling error can, however, be virtually eliminated by obtaining several biopsy samples from the gastric corpus as well as the from the antrum. In contrast to biopsy-based methods, non-invasive tests assess the global presence of *H. pylori* in the stomach even when the bacteria are irregularly distributed on the gastric mucosa. Non endoscopic tests, particularly serology, are cheaper and more convenient and thus should be preferred in situations where the additional information yielded by an endoscopy is not needed (Korstanje *et al*., 2008).

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Each of the available diagnostic techniques has advantages as well as disadvantages. It is now clear that the discussion over the different diagnostic methods cannot be oversimplified by reasoning only in terms of which is the best diagnostic tool? (Chong, 2007; Iwanczak *et al*., 2005).

The aim of the study was comparative evaluation of diagnosing of *H. pylori* infection on the invasive method including histology, culture, rapid urease test and noninvasive method including Elisa and immunoblot. The primary goal of this study was to determine the accuracy of these tests.

MATERIALS AND METHODS

Endoscopy and blood sampling: We studied a total of 127 patients (57.5% male and 47.5% female with a mean age of 46.3 years) undergoing endoscopy at the university Hospital of kashan. At endoscopy, multiple antrum biopsy specimens were used for the rapid urease test and the others were immediately fixed and transported in 10% phosphate-buffered formalin solution for histopathologic examination.

The 5-10 cc blood were taken from each patient after separated red blood cell (3000 rpm for 5 min) Sera were collected and frozen at -20°C. This research project was conducted from April 2001 to December 2002.

Histopathology examination of biopsy specimens: Paraffin-embedded gastric biopsy specimens were routinely processed. Hematoxylin and eosin and Giemsa stains (Meark, Germany) were used for morphologic examination of Helicobacter-like organisms (HLO). *Helicobacter pylori* infection was defined as positivity of histopathology and rapid urease test. Histology was performed by a specialized pathologist. A patient was defined as

Helicobacter pylori negative when both histological examination and urease test were negative and as *H. pylori* positive when both histological examination and urease test were positive.

Culture: Two biopsies were processed for culture on to 5% packed cell *Columbia agar* addition 2-4% fetal calf serum (oxoid-Basingstoke, United Kingdom) containing skirrow's antibiotic supplements (oxoid). The plates were incubated at 37°C for 5-7 days in microaerophilic conditions. The isolates were identified as *H. pylori* by urease, catalase and oxidase reactions and gram staining.

Serological tests: IgG ELISA was used to detect the presence of *H. pylori*-specific serum antibodies according to the manufacturer's instructions (Gene probe Swiss). The recommended cut-off values were used.

Immunoblot assay: In immunoblot assay, the Helico-blot 2.1 system (Genelabs Diagnotice, Singapore) were used. Briefly, membrane strips were incubated in wash buffer, after which sera were added to each strip and then the strips were washed three times. After washing, alkaline phosphatase goat anti-human IgG conjugate was added and incubated for 1 h then substrate solution was added and the reaction was stopped with dd H_20. The recommended criteria for determining a sample as *H. pylori* seropositive is any one of the following criteria: 116 kD (CagA) positive with one or more 89 kD (VacA), 37, 35, 30 and 19.5 kD together, or with current infection marker; presence of 89, 37 or 35 kD; presence of both 30 and 19.5 kD.

Statistical analysis: The statistical analysis of data was made using Student's t test for unpaired data, Receiver Operating Characteristic (ROC) curves, linear regression, Bland Altman analysis and one-way Analysis of Variance (ANOVA).

Ethics: The research protocol was approved by the Ethical Committee of the Kashan Medical University, Faculty of Medicine, Kashan, Iran. All patients gave their written consent to participate in the study.

RESULTS

Helicobacter pylori infection was diagnosed in 58 patients by histopathology. In culture of 43 patients, *H. pylori* was grown and Rapid urease tests (RUT) in 71 patients were positive.

ELISA showed that *H. pylori* seropositivity was in 68 patients 82.1% for IgG antibodies.

Anti *H. pylori* Western blot of IgG antibodies also showed reactivity with 116 kD (CagA), 89 kD (VacA), 37, 35, 30 and 19.5 kD were positive in 35 patients.

Helicobacter pylori infection was diagnosed by means of histology in which 58 patients. By means of culture 43 patients were positive. RUT 71 patients were *H. pylori* positive. Sixty eight patients diagnosed by ELISA and 35 patients were detected by immunoblot assay.

Based on the current golden standard i.e. positive culture [C^+] or positive giemsa [G^+]with RUT[R^+], 55.1% patients were *H. pylori* infection.

In the pathological studies, 10 patients had metaplasia and adenocarcinoma, 47 patients had chronic gastritis, 5 patients had normal Biopsies and 8 patients had acute on chronic gastritis.

DISCUSSION

The discovery of *Helicobacter pylori* in 1982 was the starting point of a revolution concerning the concepts and management of gastroduodenal diseases. It is now well accepted that the most common stomach disease, peptic ulcer disease, is an infectious disease and all consensus conferences agree that the causative agent, *H. pylori*, must be treated with antibiotics. Furthermore, the possibility emerged that this bacterium could be the trigger of various malignant diseases of the stomach and it is now a model for chronic bacterial infections causing cancer (Megraud and Lehours, 2007).

Helicobacter pylori infection can be diagnosed by invasive and non-invasive tests. Serology can be performed on noninvasively collected clinical samples. Serological detection of infection with a CagA containing strain of *H. pylori* by anti-CagA ELISA and Western blot of CagA is the only noninvasive diagnostic test at present available for assessing strain virulence potential and possible disease risk. The reliability of CagA serology as a predictive test for determining the CagA genotype of the infecting strain is important because various serological assays are now available (Radosz-Komoniewska *et al*., 2004).

The invasive biopsy-based tests which include rapid urease test, histology and culture are important in the assessment of *H. pylori* status pre-treatment, as endoscopy allows assessment of treatment indications such as ulcer disease.

In contrast to biopsy-based methods, non-invasive tests assess the global presence of *H. pylori* in the stomach even when the bacteria are irregularly distributed on the gastric mucosa. Nonendoscopic tests, particularly serology, are cheaper and more convenient and thus should be preferred in situations where the additional information yielded by an endoscopy is not needed (Yilmaz *et al*., 2006).

To avoid endoscopy, other less invasive paths to the stomach have been proposed. It is possible to obtain gastric juice using a nasogastric tube. Gastric juice allows the detection of *H. pylori* by culture, staining, urease test and PCR, but it is less reliable than gastric biopsy specimens.

Gastroduodenal disease due to *H. pylori* infection is a significant health problem (Zambon *et al*., 2004).

In present study, *H. pylori* prevalence in subject population was 55.3%. Reflecting the importance of diagnosis and treatment of *H. pylori* infection properly. In this study, 33% of patients found to be *H. pylori* positive in culture, 46.5% in histology, 56% in RUT, 80% in ELISA and 85.7% in immunoblotting.

We used different golden standards with which each test (Table 1). We identified Sensitivity (SN), Specifity (SP), Positive Predictive value (PP), Negative Predictive value (NP) and Accuracy (AC) for each test (Table 1).

These findings indicated that noninvasive tests have lower specifity than histology and RUT. The culture had low sensitivity and high accuracy. Histology with the RUT had specifity, sensitivity and accuracy higher than others.

Noninvasive methods are the simplest and most widely available diagnostic test in the epidemiological studies and the value of them i.e., immunoblot and ELISA in diagnosis of *H. pylori* is controversial (Iwanczak *et al*., 2005).

In other study, the combination of ELISA and immunoblot was detected as more sensitive than culture and histology. It seems that Immunoblot test is useful noninvasive tool in children (Ogunc *et al*., 2003) and can give seropositivity and determine anti-CagA, VacA virulence factor status of patients (Yilmaz *et al*., 2006). In all serologic tests have a lower diagnostic accuracy and should only be used for screening *H. pylori* infection or after careful local validation (Leodolter *et al*., 2001). It seems that using highly conserved antigen in developing countries is essential.

At present, culture with or without invasive or noninvasive test (Bravo and Realpe, 1999), RUT with histology (Korstanje *et al*., 2008), invasive test with one noninvasive test, UBT with histology or culture and HpsA with RUT or histology (Chong, 2007), UBT with ELISA, ELISA with helicon blot 2.1 system (Monteiro *et al*., 2001) and three positive of the five tests; RUT, culture, histology, UBT and serology (Yanez *et al*., 2000) and detection of antigen in stool (Tanaka *et al*., 2003) are widely used for diagnosis of *H. pylori*. While a significant number of *H. pylori* infections that would not be detected by gold tests analysis(Weiss *et al*., 2008).

The lower specifity of noninvasive test may be partially attributed to latent infection so that patients

Table 1: Different tests compared with current various golden standard in diagnosis of patients

Name of test	Comparison with golden standard	Sensitivity (%)	Specifity (%)	PP (%)	PN (%)	Accuracy
Culture	R^+, E^+ or G^+, E^+	54.0	83.0	89.1	41.6	62.3
RUT	C^+ or G^+, E^+	96.0	80.0	89.8	92.3	90.5
Histology	C^+ or R^+, E^+	73.6	71.4	84.0	57.0	72.9
ELISA	C^+ or G^+, E^+ or G^+ R^+	85.2	33.0	76.5	47.0	70.5
Immunoblot assay	C^+ or G^+, R^+ or G^+, E^+ or R^+	81.0	28.5	85.7	22.2	72.7
ELISA with immunoblot	G^+, R^+ or C^+	65.0	45.0	71.4	38.4	58.8

R^+: Positive RUT, G^+: Positive giemsa (histology), E^+: Positive elisa, C^+: Positive culture; PP: Positive predictive value PN: Negative predictive value

previously infected but cleared with *H. pylori*, continued having seropositive for a prolonged period.

It is concluded that in our subject population, the latent infection rate is high and routine serological tests are nonreplicable and no accurate. In addition immunoblot test is expensive, difficult and has low specifity. We suggested that histology with RUT is the best in Iran while it needs more improvement. It is recommended to do more research on conducting other new tests e.g., HpsA, UBT and DNA detecting in Iran.

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