Original Article

Comparison of an (HM-CAPs) ELISA Test and the Gold Standard Tests for Detecting *Helicobacter Pylori* Infection

Mohammad Reza Naficy, Behnam Zamanzad, Hedayatollah Shirzadeh
Department of Microbiology and Immunology, Shahrekord University of Medical Sciences, Shahrekord, Iran

Kuwait Medical Journal 2005, 37 (2): 94-97

ABSTRACT

Background and objectives: *Helicobacter pylori* (*H. pylori*) is one of the main causes of gastritis and is highly related to gastric and duodenal cancer. Different methods are introduced for diagnosis of bacterial infections, but serologic tests are more common. In this study, an ELISA (Enzyme linked immunosorbent assay) test was performed using bacterial high molecular cell-associated proteins (HM-CAPs) containing urease. The sensitivity and specificity of this method was then compared with those using histological (Giemsa and/or modified Gram staining) as well as bacterial culture findings, both considered as gold standard tests for diagnosis.

Materials and methods: HM-CAPs containing bacterial urease, were extracted by NOG (n-octyl-ß-D-glycopyranoside) detergent and purified on sephacryl gel S-300 HR. Besides, biopsy specimens from antral portion of gastric mucosa together with sera of 168 patients (85 male and 83 female), suspected of *H. pylori* infection, were obtained. The results of culture and/or histological examinations (gold standard tests) were compared with those of serum ELISA test.

Results: Sensitivity and specificity of HM-CAPs ELISA test as compared with histological findings and/or bacterial culture were 93.3% and 95.1% respectively.

Conclusion: ELISA test using HM-CAPs antigen is highly specific and sensitive. Therefore, it could be used for serological diagnosis of *H. pylori* infection.

KEYWORDS: *Helicobacter pylori*, HM-CAPs ELISA, urease enzyme

INTRODUCTION

H. pylori is a predominately extracellular, Gram-negative, short, S-like shaped, flagellated and motile bacterium[1]. Nowadays, there is general consensus that *H. pylori* infection is the main etiological factor of primary gastritis in children[2] and adults[3]. Significant correlation between *H. pylori* - associated gastritis and peptic ulcer has been found, especially with duodenal ulcer[3,4]. Gastric cancer and lymphoproliferative gastric diseases also have been correlated with *H. pylori* infection[5]. Australian researchers isolated and identified the bacterium using microaerophilic conditions and enriched media. They showed a relationship between *H. pylori* and gastritis type B, which was previously reported as idiopathic[6].

A reliable test for detection of *H. pylori* infection is crucial but none of the available tests are appropriate for all cases[7]. Invasive tests have been considered as the gold standard tests, but these biopsy-based methods may suffer from sampling error, because of the patchy nature of the infection and low concentration of bacteria in fragments[7,8]. Culturing also has a low sensitivity, therefore a combination of the tests is recommended as gold standard[7,8].

Currently, there are several non-invasive methods for diagnosis of the infection, each having its own advantage, disadvantage and limitation[7-9]. Grabtree showed the difference between titers of antibodies in individuals who were *H. pylori* negative as compared with infected patients and showed that the IgG and IgA levels in *H. pylori* positive and negative individuals were significantly different[10]. Basically, there is general consensus that non-invasive serologic tests are reliable methods for diagnosis of *H. pylori* gastric infections[7,9,11]. The majority of these tests have some limitations due to their low specificity and sensitivity. Since agglutination, complement fixation and immunoflouresence tests are not more sensitive for detection of antibodies against H. pylori, attention was driven to more sensitive methods such as enzyme linked immunosorbent assay (ELISA) using different bacterial antigens[11,12]. For example, due to cross-reactivity of *H. pylori* whole cell antigens with those of other bacterial species, especially *Campylobacter jejuni*, false positive results were observed[13,14]. In contrast, using appropriate antigen like urease enzyme will not cause cross-

Address correspondence to:
Dr. MR Naficy, Department of Microbiology and Immunology, Faculty of Medicine, Shahrekord University of Medical Sciences, Shahrekord, Iran.
Fax: 0098 381 3335776, E-mail:mrnaficy@yahoo.com

reactivity[15]. *H. pylori* urease forms about 6% of cell bacterial proteins and is important for bacterial survival and colonization in gastric epithelial cells[16,17]. Due to the genetic and biochemical aspects of *H. pylori* urease enzyme, which is different from those of other bacteria such as *Proteus, Providencia and Morganella* species the cross, -reactivity does not occur[18,19].

ELISA is an easily performed low cost method for detection of *H. pylori* infections. But due to some differences in *H. pylori* antigenic properties in different parts of the world[20,21], this study was aimed to test a HM-CAPs antigen in an ELISA method for detection of *H. pylori* infection. The results were then compared with those of culture and/or histological reports in patients' gastric biopsy specimens as gold standard tests.

MATERIALS AND METHODS

One hundred and sixty eight patients (85 male and 83 female) suspected to have *H. pylori* infection, admitted in internal medicine ward of Isfahan Alzahra hospital, with a median age of 44.8 years were investigated. Endoscopy was performed with the Olympus opticfiber after overnight fasting. Patients' sera as well as biopsy specimens were obtained simultaneously. Antral biopsies were taken from the antrum within about 2 cm of the pyloric channel for histology and bacterial culture .

Histology:

The biopsy specimens were crushed and then smeared on slide glass, fixed in 10% formaldehyde solution and stained by Giemsa and modified Gram (10% Ziehl-Neelsen's carbol fuchsin in water for up to three minutes as counterstaining)[22].

Isolation of bacterial cells:

The gastric specimens were crushed and after homogenization, inoculated in blood BHIA (Brain Heart Infusion Agar [Oxoid]) medium containing skirrow's antibiotics (Vancomycin 10 mg/l, Polymyxin B 2500 IU/l, Trimethoprim 5 mg/l, Amphotericin B 5 mg/l [Merck]) using microaerophilic conditions (6% O_2, 10% $C0_2$ with 100% humidity (Anoxomat system, MART) and incubated at 37 °C for 3-5 days. After incubation period, some bacterial isolates were selected randomly and identified by their reactivity to Gram staining, urease, catalase and oxidase tests. To obtain sufficient bacteria as the source of antigen, the isolated bacteria were subcultured in BHIB (Oxoid) medium containing 5% horse serum and 2% bovine fetal serum.

Extraction of HM-CAPs proteins:

The harvested bacterial cells were washed twice in Phosphate Buffered Saline (PBS pH: 7.4) by centrifugation (8000 rpm for 12 min). The bacterial pellet was then suspended in 1% n-octyl-ß-D-glycopyranoside in PBS and centrifuged at 15000 rpm for 15 min. The supernatant, containing cell membrane-associated proteins was dialyzed overnight against two liters of PBS and then centrifuged at 15000 rpm for 20 min. The cell extract was concentrated using polyethylene glycol 40000.

Table 1
The results of HM - CAPs ELISAas compared to gold standard tests in 168 patients

Diagnostic Tests/ No. of cases	Culture results	Histologic findings	HM-CAP ELISA
75	+	+	+
42	-	-	-
40	-	+	+
6	-	+	-
2	+	-	+
3	-	-	+

Purification of HM-CAPs:

Five millilitre of concentrated extract (700 g/ml) was placed on a Sephacryl S-300 HR (Sigma) column and eluted with 0.05 mol/liter Tris-Hcl buffer (pH 8.0) (22). The fractions with high optical density (280 nm in UV spectrophotometry) and maximum urease activity (Christensen's medium [Merck]) were pooled and used as HM-CAPs source.

Standardization of ELISA test:

The different stages of standardization of HM-CAPs ELISA test, were performed as described by Carpenter[23]. This procedure was carried out to indicate the proper concentration of both prepared antigen (homemade) and conjugated antibody (anti-human IgG-HRP provided by Biogen, Mashhad, Iran) with the aim of standardizing a test for patients' sera. Criss-Cross checker board titration was carried out using different concentrations of antigen (1-8 g/ml) in PBS and dilutions of conjugated antibody were 1/500 - 1/16,000. The antigen concentration (8 g/ml) and conjugated antibody dilution (1/12,000) were selected for ELISA experiments. One hundred and sixty eight sera from the patients were tested and the sensitivity and specificity of the test were compared with those of the gold standard tests.

RESULTS

The results of HM-CAPs ELISA test as compared to those of the gold standard tests (culture and/or histological examination of gastric specimens) are listed in Table 1. Culture and/or histological examinations of gastric samples in 123

Table 2
False positive and false negative results of ELISAtest compared with gold standard tests in 168 patients

Gold Standard tests/ELISA test	Positive cases	Negative cases
Positive cases	TP[1] = 117	FP[2] = 3
Negative cases	FN[3] = 6	TN[4] = 42

[1]True positive : Positive in both two tests, [2]False positive : Positive in ELISA and negative in gold standard tests, [3]False negative : Negative in ELISAand positive in gold standard tests, [4]True negative: Negative in both two tests

patients were positive. The numbers of false positive and false negative results for HM-CAPs ELISA method were three and six respectively (Table 2). Further, specificity and sensitivity of this method as compared with those of gold standard tests were about 93.3% and 95.1% respectively.

DISCUSSION

The results of patients, sera tested by ELISA, using homemade HM-CAPs antigen compared with gold standard tests (culture and/or histological examinations) showed a sensitivity and specificity of 93.3% and 95.1% respectively. Previous studies showed similar findings using commercial HM-CAPs ELISA[20,24]. Evans *et al* reported that HM-CAPs ELISAtest and urea breath test had equal sensitivity and specificity[15]. Further, it seems that HM-CAPs ELISA test is more economical and easier to use. Besides, some drugs such as bismuth components and antibiotics do not affect the results of this method[15]. These drugs suppress the bacterial multiplication temporarily and falsely change the urea breath test into negative[15].

Despite of the current usefulness of bacterial whole cell antigens for diagnostic purposes, many researchers have reported false positive results with whole cell ELISA test[25,26]. These results can be due to common antigenic epitopes between *H. pylori* flagellin proteins and *Campylobacter* species and other bacteria[13,14]. This is more important in developing countries with high prevalence of *C. jejuni* infection[14]. As in one survey, the isolation rate of *C. jejuni* from the stool of Isfahanian children was reported as 8.8%[27]. On the other hand, Jones has suggested that, high antibody titer against *Campylobacter jejuni* in an ELISA test, have cross-reaction with *H. pylori* antigens. Such reactions are not seen in low sensitivity tests like complement fixation test[28]. Further, in one study it was specified that patients with high antibody titer against *Campylobacter sputorum, Campylobacter jejuni, Campylobacter fetus* and *Campylobacter faecalis* react with *H. pylori* antigens in ELISA[29].

Several other methods were used by different investigators. Simple urea breath tests (UBT), serology, stool and urine assays are sometimes used[30,31]. Trials investigating PCR amplification of saliva, feces, and dental plaque to detect the presence of *H. Pylori* are on going[1,32]. ^{14}C-Urea Breath test is one of the common methods for detection of *H. pylori* infection. ^{14}C is a radio-isotope with a very long half life. Therefore, the ^{14}C - UBT cannot be used in children and women of childbearing age[33]. The ^{13}C-UBT is similar to the ^{14}C- UBT except that ^{13}C is non-radioactive isotope of ^{12}C. It can also be used for monitoring and follow up of the therapy for *H. pylori* infection[33].

Serologic tests offer a fast, easy and relatively inexpensive means of identifying patients who have been infected with the organism. Since, the antibody levels do not decrease rapidly after treatment, these methods are not useful means for confirming eradication of *H. pylori* infection[34]. However, ELISA testing is preferable when prevalence rate is very high (90%) as seen in developing countries. Using a confirmatory urease breath test for negative ELISA test increases the diagnostic accuracy to 96% with modest incremental costs[34].

In summary our results showed that homemade HM-CAPs ELISA test as compared with gold standard tests, is highly sensitive and specific. Therefore, it could be used for serological diagnosis of *H. pylori* infection. Since this method fails to show the immune cellular and mucosal responses in infected tissues as well as neoplastic changes, endoscopic and histological studies are recommended in suspected cases.

REFERENCES

1. Williams CL. Helicobacter pylori: Bacteriology and Laboratory Diagnosis. J Infec 1997; 34:1-5.
2. Blecker U, Helicobacter pylori disease in childhood. Eur J Pediat 1996; 155:733-735.
3. Blaser MJ. Helicobacter pylori; Its role in disease. Clin Infe Dis 1992; 15:386-393.
4. Rautelin H, Kosunen TU. Helicobacter pylori and associated gastroduodenal diseases. APMIS 1991; 99:677-695.
5. Wotherspoon AC, Ortiz - Hidalgo C, Falzon MR, *et al*. Helicobacter pylori associated gastritis and primary B - cell gastric lymphoma. Lancet 1991; 338: 1175-1176.
6. Marshall BJ, Warren JR. Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. Lancet 1984; 1:1311-315.
7. Megraud F. Advantages and disadvantages of current diagnostic tests for the detection of Helicobacter pylori. Scand J Gastroenterol 1996; 31:57-62.
8. Morris A, Ali MR, Brown P, *et al*. Campylobacter pylori infection in biopsy specimens of gastric antrum: laboratory diagnosis and estimation of sampling error. J Clin Pathol 1989; 42:727-732.
9. Cutler AF, Haustad S, Ma CK, *et al*. Accuracy of invasive and non - invasive test to diagnose Helicobacter pylori

infection. Gastroenterol 1995; 109:136-141.

10. Crabtree JE, Phil D, Shallcross TM, *et al*. Mucosal humoral immune response to Helicobacter pylori in patients with duodenitis. Diges Dis & Sci 1991; 36:1266-1273.
11. Barthel JS, Everett ED. Diagnosis of Campylobacter pylori infections; the Gold Standard tests and the alternatives. Rev Inf Dis 1990; 12:107-114.
12. Nilius M, Wex T, Muller-Dietz G, *et al*. Comparative evaluation of two hemagglutination tests for the detection of anti - Helicobacter pylori antibodies. Diagn Microbiol Infect Dis 2001; 41:221-223.
13. Salama SM, Wefuan JN, Shiro-KoullaS, *et al*. Value of whole - cell antigen extract for serologic detection of Helicobacter pylori. J Cli Microbiol 1993; 31:3331-3332.
14. Newell DG. Identification of the outer membrane protein of Campylobacter pyloridis and antigenic cross-reactivity between C. pyloridis and C. jejuni. J Gen Micro 1987; 133:163-170.
15. Evans DJ, Evans DG, Graham DY, *et al*. A sensitive and specific serologic test for detection of Campylobacter pylori infection. Gastroenterol 1989; 96:1004-1008.
16. Harris PR, Mobley HLT, Perez-Perez GI, *et al*. Helicobacter pylori urease is a potent stimulus of mononuclear phagocyte activation and inflammatory cytokine production. Gastroenterol 1996; 111:419-425.
17. Rektorschek M, Weeks D, Sachs G, *et al*. Influence of pH on metabolism and urease activity of Helicobacter pylori. Gasteroenterol 1998; 115:628-641.
18. Mobley HLT, Cortesia MJ, Rosenthal LE, *et al*. Characterization of urease from Campylobacter pylori. J Cli Micro 1988; 26:834-836.
19. Dunn BE, Sung CC, Taylor NS, *et al*. Purification and Characterization of Helicobacter pylori urease. Inf & Immun 1991; 59:3343-3345.
20. Leung WK, Ng EK, Chan FK, *et al*. Evaluation of three commercial enzyme-linked immunosorbent assay kits for diagnosis of Helicobacter pylori in Chinese patients. Diagn Microbiol Infect Dis 1999; 34:13-17.
21. Obata Y, Kikuchi S, Miwa H, *et al*. Diagnostic accuracy of serological for Helicobacter pylori infection with the same assay system but different antigen in a Japanese patient population. J Med Microbiol 2003; 52:889-892.
22. Duguid JP. Staining methods, in : Collee JG, Duguid JP, Fraser AG, Marmion BP, editors. Practical Medical Microbiology. 13th edition. New York : Churchill Livingstone; 1984, p 43-94.
23. Carpenter AB. Enzyme-linked immunoassay; In Rose NR, de Macario EC, Fahey JL, editors. Manual of clinical laboratory immunology. 4th ed. American Society for Microbiology 1996:2-9.
24. Meijer BC, Thijs JC, Kleibeuker JH, *et al*. Evaluation of eight enzyme immunoassays for detection of immunoglobulin G against Helicobacter pylori. J Clin Microbiol 1997; 35:292-294.
25. Emami H, Fazeli A, Hazeghi K. Application of ELISA for diagnosis of H.pylori infections in patients with upper gastrointestinal infections. Isfahan University press 1996 June.
26. Buchvald D, Maeland JA. Characterization of a 25,000 - dalton Helicobacter pylori protein, cross-reacting with a Campylobacter jejuni protein. APMIS 1992; 100:470-478.
27. Fazeli A, Hazeghi K, Poursina M. Isolation and identification of Campylobacter jejuni from diarrheal stools. Isfahan Med J 1995; 41:42-47.
28. Jones DM, Eldridge J, Fox AJ, *et al*. Antibody to the gastric Campylobacter-like organism (Campylobacter pyloridis); clinical correlation and distribution in the normal population. J Med Microbiol 1986; 22:57-62.
29. Rathbon BJ, Wyatt JI, Worsley BW, *et al*. Systemic and local antibody responses to gastric Campylobacter pyloridis in non-ulcer dyspepsia. Gut 1986; 27:642-647.
30. Vaira D, Vakil N, Menegatti M, *et al*. The stool antigen test for detection of Helicobacter pylori after eradication therapy. Ann Intern Med 2003; 136:280-287.
31. Adachi K, Kawamura A, Ono M, *et al*. Comparative evaluation of urine - based and other minimally invasive methods for the diagnosis of Helicobacter pylori infection. J Gastroenterol 2002; 37:703-708.
32. Braden B, Caspary WF. Detection of Helicobacter pylori infection: when to perform which test? Ann Med 2001; 33:91-97.
33. Vakil N, Vaira D. Non - invasive tests for the diagnosis of H. pylori infection. Rev Gastroenterol Disord 2004; 4:1-6.
34. Bergey B, Marchildon P, Peacock J, Megraud F. What is the role of serology in assessing Helicobacter pylori eradication? Aliment Pharmacol Ther 2003; 18:635-639.