Clinical significance of PCR in *Helicobacter pylori* DNA detection in human gastric disorders

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Abstract

**AIM:** To investigate the clinical significance of the PCR assay in the diagnosis of gastric *Helicobacter pylori* (*Hp*) infection.

**METHODS:** *Hp* infection in gastric antral biopsied specimens was identified by using the polymerase chain reaction (PCR) to amplify the specific *Hp* urease gene fragments (PCR-*Hp*-DNA) in 154 patients with gastrointestinal disorders. *Hp* urease gene oligonucleotide primers specific for *Hp* (16s rRNA) were used. Urease test and enzyme-linked immunosorbent assay (ELISA) for anti *Hp*-IgG serum were also used as controls.

**RESULTS:** PCR-*Hp*-DNA was detected in 140 (91%) of the 154 patients, where patients 114 and 125 were found infected with *Hp* by urease test and ELISA *Hp*-IgG, respectively. There was a marked difference in the *Hp*-positive rate between the PCR-*Hp*-DNA and the urease test or ELISA-*Hp*-IgG (P < 0.05). The *Hp* infection rate increased with age, although a minority of infected people developed signs and symptoms of gastric disorders. *Hp* infection is closely related to adenocarcinoma in both the gastric antrum as well as the down body of the stomach.

**CONCLUSION:** PCR is a sensitive and specific method for the detection of *Hp* in human gastric tissues. Detection of *Hp* DNA in *vivo* using this approach might improve the clinical diagnosis and epidemiological research related to *H. pylori* infection.

**Key words:** Peptic ulcer; Gastritis; Stomach neoplasms; *Helicobacter pylori*; *Hp* infections; Polymerase chain reaction (PCR)

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INTRODUCTION

*Helicobacter pylori* (*Hp*) is now recognized as the major etiologic agent of chronic active gastritis, peptic ulceration and a risk factor for the development of adenocarcinoma of the distal stomach[1]. Currently, the phenotypic characteristics known to differ among strains include the production of VacA and the presence of CagA[2]. Mucosal and systemic immunologic recognition of *Hp*-infected individual is associated with peptic ulcer disease. However, expression of *Hp* virulence factors in *vivo* may not accurately reflect the expression profiles in host tissues. In *vivo* detection of the *Hp* gene may improve the accuracy of clinical diagnosis[3].

We employed the polymerase chain reaction (PCR) assay using primers specific for *Hp* 16s rRNA to detect *Hp* infection. We then compared our results with those from the rapid urease test and enzyme-linked immunosorbent assay (ELISA) performed on serum *Hp* IgG and evaluated the sensitivity and specificity of PCR for the detection of *Hp* infection in clinical practice.

MATERIALS AND METHODS

**Clinical specimens**

A total of 154 patients undergoing gastric endoscopy from the Department of Gastroenterology of the Shanghai Changhai Hospital were studied retrospectively. Patients were excluded if they had a history of gastric surgery, receiving steroids or other immunomodulating drugs, abusing alcohol or illicit drugs, or were HBsAg-positive.

Among the 154 patients (95 male and 59 female; mean age 51 years, range between 18-63 years) with gastric disorders, 40 had chronic superficial gastritis (CSG), 12 had chronic atrophic gastritis (CAG), 44 had duodenal ulcers, 16 had gastric ulcers and 42 had gastric carcinoma, which was determined based on histological examination.
Gastric biopsy specimens from gastric antrum (15 μg) were placed immediately in normal saline at 4 °C and were usually homogenized in 400 μL TE buffer (10 mmol/L Tris-HCl, 0.1 mmol/L EDTA, pH7.8) with a tissue grinder. In an Eppendorf, (50 μL) of homogenized mucosa was then mixed with 50 μL of lytic solution and proteinase K added to reach a final concentration of 200 μg/mL. The mixture was incubated at 50 °C for 30 min until the tissue pellets were completely digested, and they were then boiled for 10 min. The samples were centrifuged at 20000 × g for 1 min at 4 °C and the supernatants were stored in sterile vials at -75 °C until they were used as PCR templates.

Peripheral blood samples were obtained to measure the immunoglobulin G (IgG) response to Hp with ELISA.

### PCR amplification

PCR primers were designed based on published sequences of Hp 16s rRNA. The primers were used as follows: Primer 1: 5'-CGGCAATCAGTGTCAGGTTAG-3'; Primer 2: 5'-GCTAAAGATACCCATGGTGCC-3'. These primers were synthesized using the automated phosphoramidite coupling method. Amplification of Hp genomic DNA sequences was carried out in 25 μL PCR buffer (50 mmol/L KCl, 10 mmol/L Tris-HCl (pH8.3)), 1.5 mmol/L MgCl2, 200 μmol/L deoxyribonucleotides, and 1 μL of boiled Hp supernatant as DNA template as well as the control. The 16s rRNA primers were each used at a final concentration of 0.5 μmol/L. Each reaction was amplified for 36 cycles as follows: 1 min at 94 °C for denaturation, 45 s at 60 °C for annealing and 90 s at 72 °C for extension. PCR of cDNA from gastric biopsied specimens was performed exactly as described above. Agarose gel electrophoresis with ethidium bromide staining was performed from each PCR mixture. Negative and positive comparisons were made for each experiment.

### Enzyme linked immunosorbent assay (ELISA)

The purified Hp crude preparation of urease (CU) antigen was diluted in 0.1 mol/L carbonate buffer (pH9.6) to a final concentration of 0.5 mg/L. Polystyrene microtest plates were coated with 100 μL/well of antigen solution and incubated overnight at 4 °C. 100 μL of each serum sample was added to wells and incubated at 37 °C for 2 h after the plates were washed. After three washings, 100 μL/well of a substrate solution was added to each well. Each plate contained a positive and a negative control serum.

### Rapid urease test

A 2-mm pinch biopsy was taken from the prepyloric mucosa (within 5 cm of the pylorus, at an angle of about ten o'clock), and the tissue was pushed beneath the surface of the reactive solution. In positive cases a red tinge developed around the biopsy at one minute. There was no color changes if Hp was absent.

### RESULTS

There were marked difference in positive rates between various methods in the determination of Hp infection (Table 1).

Hp was detected in 114 (74%) of 154 patients using rapid urease test, and all of these samples showed positive PCR results in gastric mucosa.

Thirty of 40 rapid urease test-negative cases were PCR positive. Out of 125 (81%) of ELISA-positive cases, 123 were PCR positive and 2 were negative (a CSG and a CAG, respectively). However, 16 of 29 ELISA-negative cases had positive PCR results. Among the 154 patients with antral gastritis, peptic ulcer or gastric carcinoma, Hp was found in 140 (91%), 114 (74%) and 125 (81%) by Hp PCR, rapid urease test and ELISA-Hp IgG, respectively, which were significantly different from each other (P < 0.05). There was, however, a positive correlation among these three methods. The Hp-PCR was the most sensitive and specific. There was no difference between the males (89, 93%) and the females (51, 87%) in Hp-PCR positive results. The age distribution of gastric mucosal Hp infection with polymerase chain reaction was shown in Table 2. The positive rates in various age groups were 96% for males and 79% for females (P < 0.05). Daytime Hp infection was found in gastric mucosa more frequently at all ages in the Chinese population compared to Western populations.

PCR-Hp: Polymerase chain reaction to amplify the specific Helicobacter pylori urease gene fragments.

### DISCUSSION

Hp infection has been implicated in the pathogenesis of active chronic gastritis and peptic ulcer. Recently, it has also been identified as a risk factor for gastric cancer. The diagnosis of Hp infection is usually based on invasive methods such as biopsy with histological examination, culture and urease test of the gastric biopsy specimens, as well as noninvasive methods such as the 13Curea and 14Curea breath tests. Due to the fastidious growth of Hp and the prolonged incubation period required, several alternative approaches have been developed for the accurate and rapid detection of Hp in gastric mucosa[4-6]. The urease test appeared to have a low sensitivity in detecting Hp when compared with other diagnostic tech-

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**Table 1** Comparison of urease test, enzyme-linked immunosorbent assay and polymerase chain reaction for detection of Helicobacter pylori in gastric mucosa

| Diagnosis                        | No. of patients | Rapid urease test (%) | ELISA-Hp-IgG (%) | PCR-Hp (%) |
|----------------------------------|-----------------|-----------------------|------------------|------------|
| Chronic superficial gastritis    | 42              | 28 (71)               | 34 (81)          | 37 (88)    |
| Chronic atrophic gastritis       | 12              | 7 (58)                | 8 (67)           | 10 (83)    |
| GU                               | 16              | 13 (81)               | 13 (81)          | 14 (88)    |
| DU                               | 44              | 37 (84)               | 37 (84)          | 42 (98)    |
| GC                               | 42              | 24 (58)               | 33 (78)          | 37 (88)    |
| Total                            | 154             | 114 (74)              | 125 (81)         | 140 (91)   |

**Table 2** The age distribution of gastric mucosal Helicobacter pylori infection with polymerase chain reaction

| Age   | No. studied | Positive number | Positive rate (%) |
|-------|-------------|-----------------|-------------------|
| 16-18 | 15          | 12              | 80                |
| 18-20 | 12          | 9               | 75                |
| 20-25 | 19          | 15              | 79                |
| 25-30 | 50          | 48              | 96                |
| 30-35 | 42          | 40              | 95                |
| 35-40 | 26          | 25              | 96                |
| Total | 154         | 140             | 91                |

**Table 3** Stratified analysis of Helicobacter pylori infection with respect to tumor location

| Size of tumors | No. studied | PCR-Hp positive (%) |
|---------------|-------------|---------------------|
| Gastric antral| 15          | 14 (93)             |
| Gastric corpus| 17          | 8 (47)              |
| Gastric cardia| 12          | 4 (33)              |
| Esophageal    | 5           | 5 (100)             |
| Total         | 42          | 37 (88)             |

**Table 4** Stratified analysis of Helicobacter pylori infection with respect to tumor histological type

| Tumors                  | No. studied | Helicobacter pylori positive (%) |
|-------------------------|-------------|---------------------------------|
| Adenocarcinoma          | 30          | 30 (100)                        |
| Myoepithelioma          | 2           | 2 (100)                         |
| Undifferentiated cancer | 3           | 3 (100)                         |
| Myxoma/myosarcoma       | 2           | 2 (100)                         |
| Benign tumors           | 5           | 2 (40)                          |
| Total                   | 42          | 37 (88)                         |
type of gastric carcinoma may be linked with environmental factors. As chronic atrophic gastritis (type B) has mainly been linked to the intestinal type of gastric carcinoma, Hp infection would be expected to show the same relationship. In this study, Hp infection was found to be associated with an increased risk of gastric intestinal adenocarcinoma, and it seems to be an independent risk factor for gastric carcinoma. There are several possible mechanisms by which Hp infection may be involved in gastric carcinogenesis. Hp adversely affects the chemical and physical properties of the mucous layer, which may make the mucosa susceptible to carcinogenic factors. Moreover, Hp seems to promote the progression from a normal to a metaplastic epithelium, possibly by inducing a hyperproliferative state in the inflamed gastric mucosa. There has been more evidence that Hp is a major risk factor for human gastric adenocarcinomas and all low-grade B cell gastric lymphomas. The International Agency for Research on Cancer categorized Hp as a carcinogen.

In conclusion, PCR is a highly sensitive and specific method for the detection of the presence of Hp in human gastric tissues. Detection of Hp DNA in vivo by this approach may improve the clinical diagnosis and molecular epidemiological research of Hp infection.

REFERENCES

1. Tompkins LS, Falkow S. The new path to preventing ulcers. Science 1995; 267: 1621-1622 [PMID: 7886448 DOI: 10.1126/science.7886448]
2. Telford JL, Ghiara P, Dell'Orco M, Comanducci M, Baroni D, Bugnoli M, Tecece MF, Censini S, Covacci A, Xiang Z. Gene structure of the Helicobacter pylori cytotoxin and evidence of its key role in gastric disease. J Exp Med 1994; 179: 1653-1658 [PMID: 8163943 DOI: 10.1084/jem.179.5.1653]
3. Peek RM, Miller GG, Thamm KT, Pérez-Pérez GI, Cover TL, Atherton JC, Dunn GD, Blaser MJ. Detection of Helicobacter pylori pylori gene expression in human gastric mucosa. J Clin Microbiol 1995; 33: 28-32 [PMID: 7969000]
4. Xiang Z, Bugnoli M, Ponzetto A, Morgando A, Figura N, Covacci A, Petracca R, Pennati C, Censini S, Armellini D. Detection in an enzyme immunoassay of an immune response to a recombinant fragment of the 128 kilodalton protein (CagA) of Helicobacter pylori. Eur J Microbiol Infect Dis 1993; 12: 739-745 [PMID: 8307041 DOI: 10.1007/BF02098460]
5. Foxall PA, Hu LT, Mobley HL. Use of polymersome chain reaction-amplified Helicobacter pylori urease structural genes for differentiation of isolates. J Clin Microbiol 1992; 30: 739-741 [PMID: 1313051]
6. Marshall BJ, Warren JR, Francis GJ, Langton SR, Goodwin CS, Hinchow Editor. Rapid urease test in the management of Campylobacter pyloridis-associated gastritis. Am J Gastroenterol 1987; 82: 200-210 [PMID: 3548326]
7. Xiang Z, Censini S, Bayed PF, Telford JL, Figura N, Rappuoli R, Covacci A. Analysis of expression of CagA-VacA virulence factors in 43 strains of Helicobacter pylori reveals that clinical isolates can be divided into two major types and that CagA is not necessary for expression of the vacuolating cytotoxin. Infect Immun 1995; 63: 94-98 [PMID: 7863980]
8. Marchetti M, Arocio B, Baroni D, Figura N, Rappuoli R, Ghiaia P. Development of a mouse model of Helicobacter pylori infection that mimics human disease. Science 1995; 267: 1655-1658 [PMID: 7886456 DOI: 10.1126/science.7886456]
9. Crabtree JE, Farmery SM, Lindley IJ, Figura N, Pechl P, Tompkins DS. CagA/cytotoxin strains of Helicobacter pylori and interleukin-8 in gastric epithelial cell lines. J Clin Pathol 1994; 47: 945-950 [PMID: 8262690 DOI: 10.1136/jcp.47.10.945]
10. Hansson LE, Engstrand L, Nyrén O, Evans DJ, Lindgren A, Bergström R, Andersson B, Athlin L, Bendsten O, Tracz P. Helicobacter pylori infection: independent risk indicator for gastric adenocarcinoma. Gastroenterology 1993; 105: 1089-1103 [PMID: 8405854]
11. Graham DY, Go MF, Genta RM. Helicobacter pylori, duodenal ulcer, gastric cancer: tunnel vision or blinders? Ann Med 1995; 27: 589-594 [PMID: 8541037 DOI: 10.3109/07873899509002474]
