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ORIGINAL RESEARCH

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*; Helicobacter 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 *Vac*A and the presence of *Cag*A[2]. Mucosal and systemic immunologic recognition of *Hp*-infected individual is associated with peptic ulcer disease. However, expression of *Hp* virulence factors *in vitro* 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.

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
		No. (%)	No. (%)	No. (%)
Chronic superficial gastritis	40	28 (70)	34 (85)	37 (93)
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 (96)
GC	42	24 (68)	33 (78)	37 (88)
Total	154	114 (74)	125 (81)	140 (91)

ELISA-Hp-IgG: Enzyme-linked immunosorbent assay for anti *Helicobacter pylori*-IgG; PCR-Hp: Polymerase chain reaction to amplify the specific *Helicobacter pylori* urease gene fragments.

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

Age	No. studied	Positive number	Positive rate (%)
10-	5	3	60
20-	12	9	75
30-	19	15	79
40-	50	48	96
50-	42	40	95
≥ 60	26	25	96
Total	154	140	91

Gastric biopsy specimens from gastric antrum (15 μg) were placed immediately in normal saline at 4 ℃ and were coarsely 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 mucus was then mixed with 50 μL of lytic solution and proteinase K was added to reach a final concentration of 200 mg/L. The mixture was incubated at 50 ℃ 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 ℃ and the supernatants were stored in sterile vials at -75 ℃ 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 used were as follows: Primer 1: 5′-GCGCAATCAGCGTCAGGTAATG-3′ Primer 2: 5′-GCTAAAGAGATCAGCCTATGTCC-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 Tri HCl (pH8.3)], 1.5 mmol/L $MgCl_2$, 200 μmol/L deoxynucleotides, and 1 μL of boiled *Hp* supernatant as DNA template as wel 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 ℃ for denaturation, 45 s at 60 ℃ for annealing and 90 s at 72 ℃ 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 (CPU) 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 ℃. 100 μL of each serum sample was added to wells and incubated at 37 ℃ 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.

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

Site of tumors	No. studied	PCR-*Hp* positive	
		No.	(%)
Gastric antral	15	14	-93
Gastric corpus	17	16	-94
Gastric cardia	5	4	-80
Esopheageal	5	3	-60
Total	42	37	-88

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

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

Tumors	No. studied	*Helicobacter pylori* positive	
		No.	(%)
Adenocarcinoma	30	29	-97
Myxoepithelioma	2	1	-50
Undifferentiated cancer	3	2	-67
Myoangiosarcoma	2	1	-50
Benign tumors	5	4	-80
Total	42	37	-88

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* detected with PCR is shown in Table 2. The positive rates in various age groups with gastric disorders by PCR method are higher than those by other methods. *Hp* infections occur earlier and more frequently at all ages in the Chinese population compared to Western populations.

These studies of *Hp* infection in patients with gastric carcinoma indicated that there was a relationship between *Hp* infection and tumor location. *Hp* infection was found in 35 of 42 (83%) patients with gastric carcinoma using PCR. Gastric carcinoma patients were more likely to be infected by Hp than normal controls. In a stratified analysis of tumor location within the upper gastrointestinal tract (Table 3, Table 4), *Hp* infection in noncardia tumors was significantly increased (29/32, 91%). Twenty eight of 30 *Hp* positive gastric carcinoma cases (93%) had intestinal adenocarcinoma.

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 ^{13}Curea and ^{14}Curea 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-

niques[5]. The sensitivity and specificity of the urease test ranged from 60%-93% and 96%, respectively, but with false positive and false negative results[6]. The sensitivity and specificity of the ELISA are 81%/97% and 78%/97%, respectively[7].

Current diagnostic tests for *Hp* infection, however, still involve invasive gastric endoscopy and detection of the organism in gastric biopsy specimens[1,5]. Molecular biological techniques have been applied to the diagnosis of *Hp* infection and both a genomic *Hp* DNA probe and an oligonucleotide probe developed for an Hp-specific 16s rRNA sequence have been found to detect as few as 10^4 *Hp* cells. These nucleic and hybridization methods, however, require the use of ^{32}P radioactivity and take up three days to provide autoradiographic results. PCR can selectively amplify the copy number of a target gene more than 10^6-fold. PCR, therefore, has great potential to improve the ability to diagnose infectious diseases caused by fastidious or slow-growing microorganisms.

A rapid, sensitive and specific test for *Hp* would be of great value because of the clinical importance of this pathogen and the large number of laboratory identifications being routinely undertaken around the world. The reference "gold standard" used for the comparative detection of *Hp* in clinical biopsy samples was bacteriological culturing[3]. PCR-detected *Hp* in all biopsy samples was found to contain culturable *Hp*. The fact that some samples were negative by rapid urease test and ELISA for *Hp*-IgG and positive by PCR may reflect the high sensitivity of this method. PCR, however, detected *Hp* in two of the patients found not to have *Hp* by ELISA for *Hp*-IgG. The results may represent either false positive PCR results or the time difference between the eradication of *Hp* in gastric mucosa and the turnover of antibodies against *Hp* in serum. PCR analysis of clinical biopsy samples by the boiling DNA extraction method is convenient and sensitive for routine use. Currently, the phenotypic characteristics among strains include both the production of a *vacuolating cytotoxin* (*Vac*A) and the presence of a high molecular weight protein encoded by *Cag*A gene (*Cag*A)[8,9]. Although present in most cytotoxic strains, *Cag*A is not necessary for *Vac*A expression. Expression of these virulence factors *in vitro* may not accurately reflect the expression profile in host tissues. Therefore, *in vivo* detection of the *Hp Cag*A or *Vac*A genes and their expression levels may improve the diagnosis of toxic *Hp* strain infection[2]. PCR of genomic DNA for the *Hp Cag*A gene in the corresponding bacterial isolates was closely correlated with either *Cag*A or *Vac*A gene expression in gastric tissue. In the near future, PCR for the *Cag*A or *Vac*A genes in gastric mucosa would prove very useful in both routine diagnosis of *Hp* as well as in *Hp*-related molecular epidemiology research.

Another main finding in this study was the significantly increased prevalence of *Hp* infection among patients with noncardia gastric cancer compared with other patients. This difference could not be explained by dietary or socioeconomic factors. Our findings were in agreement with previous results of studies on association between *Hp* infection and gastric cancer[10]. In patients with carcinoma located at the gastric cardia, we found no significant association with *Hp* infection based on *Hp* previous epidemiological and clinical studies. It has been hypothesized that the pathogenesis of the intestinal 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[11].

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.

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