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Short Reports

Diagnostic accuracy of PCR based method using four gene primers to detect Helicobacter pylori infection in gastric tissues: Report from Iran

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Introduction

Precise detection of *H. pylori* infection is relevant for clinical practice as well as for research purposes, and several invasive and noninvasive tests are currently available for diagnosis of *H. pylori*. Culture has long been the method of choice to detect infectious agents. However, culture has some limitation in a prompt detection as *H.pylori* is a very slow growing bacteria.. The sensitivity and specificity of different diagnostic tests for *H. pylori* detection varies widely.[2] Additionally, serology cannot detect the clearance of *H. pylori* and urease assays can lead to non-specific results due to the presence of other urease-positive bacteria and false negative results have also been reported in individuals taking proton pump inhibitors.[3,4] Methods based on molecular biology are considered highly specific and sensitive tests, and many PCR-based assays have been developed to detect *H. pylori* DNA in gastric biopsies, saliva and stool samples.[5,6] However, this technique is able to detect specific fragments but not viable bacteria, and its sensitivity also depends on several factors.[6]

Recently, we established a PCR based method for detection of *Helicobacter pylori* at our institution. The present study was therefore aimed to determine the sensitivity and specificity of PCR primers to diagnose Helicobacter pylori infection.

Methods

Patients

All patients who attended the outpatient clinic of gastroenterology with dyspepsia and underwent a diagnostic endoscopy evaluation with biopsy from March 2005 to March 2006 were consecutively included into this analysis. A total of 664 specimens from 166 patients (four biopsies each) were assessed. Biopsies were taken from the antrum of the patients for rapid urease test, histo-pathological examinations, and DNA analysis. Three specimens were sent for rapid urease test and histopathology and the remaining specimen was frozen for PCR analysis.

As culture of *H. pylori* from biopsy specimens was not performed in our study, any sample positive on histological examination as well as rapid urease test was considered as the gold standard for determination of the sensitivity and specificity of the PCR methods.

Histological examination

Paraffin-embedded tissue sections were stained with hematoxylin and eosin and the severity of gastritis was graded, according to the Sydney system.[7] Giemsa stain was used to to detect *H. pylori*.

Rapid-urease test

One antrum biopsy specimen was introduced with a sterile needle into a semisolid 2% urea agar and incubated at room temperature. Results were recorded up to 4 h after inoculation.[8]

Preparation of samples for PCR amplification

Genomic DNAs were extracted from all strains by method of Marais et al.[9] The extracted DNAs were dissolved in water, and solutions were prepared and used throughout the study. Briefly, the biopsy samples were ground and centrifuged for 5 min at 10,000×g. After the supernatants were discarded, biopsy specimens were resuspended in extraction buffer (20 mmol/L Tris-

HCl, pH 8.0; 0.5% Tween 20) and proteinase K (0.5 mg/mL final concentration). The mixture was incubated at 56 °C for one hour after which the enzyme was inactivated by boiling for 10 min.

Using our nested assay, we were able to detect *H. pylori*-specific sequences at an estimated concentration of 20 picomoles. Five µL of DNA was used as the template for each PCR. Each sample was examined by four different PCRs. Primers used in this study were from, 16S rRNA (bp:521), Urease A (bp:411), Cag A (bp: 400), 26kDa (bp: 303). The primer sequences and PCR conditions are listed in **Table 1**.

Table 1: Primer sequences and expected lengths of amplified DNA products

Primer	Length	Sequence
16S rRNA	521bp	F:5ð-GCAATCAGCGTCAGTAATGTTC-3ð
		R:5ð-GCTAAGAGATCAGCCTATGTCC-3ð
UreA	411bp	F:5ð-GCCAATGGTAAATTAGTT-3ð
		R:5ð-CTCCTTAATTGTTTTTAC-3ð
Cag A	400bp	F:5ð-AATACACCAACGCCTCCAAG-3ð
		R:5ð-TTGTTGCCGCTTTTGCTCTC-3ð
26kDa	303bp	F:5ð-TGGCGTGTCTATTGACAGCGAGC -3ð
		R:5ð-CCTGCTGGGCATACTTCACCATG -3ð

Statistical analysis

SPSS software (Statistical Product and Services Solutions, version 13.0, SPSS Inc, Chicago, IL, USA) was used to analyze the data. Statistical differences between patients' subgroups were assessed using the chi-square test, the Fisher exact test for proportions, and the t test for continuous data. Values for P less than .05 were considered statistically significant.

Results

Complete data for this study was obtained on 166 patients (78 males, 88 females; age: 41.7±15.7 years). Of these, a total of 55 (33%) patients gave positive results for at least one of the PCR methods employed and by any methods (including PCR), respectively. Thirty seven samples (22%) were positive both by rapid urease test and by nested PCR. Eighty nine biopsies were positive by histologic staining (48.7%), of which 49 gave negative results by PCR.

Patient samples were considered to be positive for *H. pylori* by PCR amplification if any of the 521bp, 411bp, 400bp, 303bp bands was seen in the reaction. Of the 55 PCR positive samples, 23 (41.8%) showed 400bp band, 31 (56.4%) represented 303bp band, 20 (36.4%) revealed 521bp band, and 23 (41.8) presented 411bp band.

32 (19%) samples were positive by both PCR and pathological assessment, while 49 (29.5%) of pathologically positive subjects were negative for *H. pylori* DNA by PCR methods and 23 (13.9%) of PCR positive samples were negative by pathology. Nine samples were negative by pathology while positive by both rapid urease test and PCR methods; 41 cases were negative for PCR evaluations while they were positive by both rapid urease test and pathology; as well, only 4 cases who were positive by both PCR and pathology were negative by rapid urease test.

The degree of gastritis based on pathological evaluations using Sydney system was not associated with a positive result on PCR for H.pylori (p>0.1). However, patients with a positive rapid urease test had significantly higher grade of gastritis (Grade of Gastritis mean±SD: 2.3±1.3 vs. 0.3±0.8; respectively; p<0.0001).

Based on the gold standard, 37 of the patients examined as part of this study (22.3%) were diagnosed as *H. pylori* infected. **Table 2** shows different variables of the study with respect to the study's gold standard result. Computed sensitivity for 521bp, 411bp, 400bp, 303bp bands for detection of *H. Pylori* and exclusion of negative cases in this study were 41%, 46%, 38%, and 32%, respectively; the attributed specificity for each of the PCR methods were 85%, 86%, 84%, and 83%, respectively (**Table 3**).

Table 2: Frequencies of different test results endoscopic disease states with respect to *H. pylori* presence (as per gold standard)

Laboratory methods		Gold standard	
		Positive	Negative
Any of PCR	Positive	37(22%)	18(11%)
	Negative	-	111(67%)
PCR 16SrRNA	Positive	15(9%)	5(3%)
	Negative	22(13%)	124(75%)
PCR Ure A	Positive	17(10%)	6(4%)
	Negative	20(12%)	123(74%)
PCR CagA	Positive	14(9%)	9(6%)
	Negative	23(14%)	119(72%)
PCR 26kDa	Positive	12(7%)	5(3%)
	Negative	25(15%)	124(75%)
Rapid Urease test	Positive	37(22%)	56(34%)
	Negative	-	73(44%)
HP positive in	Positive	28(17%)	49(29%)
pathological assessment	Negative	8(5%)	76(46%)
	Unremarkable	1(0.6%)	4(3%)
Duodenal ulcer	Positive	30(19%)	109(69%)
	Negative	4(3%)	14(9%)
Gastric ulcer	Positive	1(0.6%)	1(0.6%)
	Negative	33(21%)	122(78%)
Gastritis	Positive	26(17%)	85(54%)
	Negative	4(3%)	27(17%)
Hiatus hernia	Positive	11(7%)	36(23%)
	Negative	23(15%)	87(55%)
Distal esophagitis	Grade I	7(9%)	27(33%)
	Grade II	10(12%)	32(40%)
	Grade III	20(25%)	61(75%)

Table 3: Diagnostic accuracy of PCR primers in detecting H. pylori infection

Statistical indices	PCR 16S rRNA	PCR CagA	PCR 26kDa	PCR UreaA
Sensitivity	40.5%	37.8%	32.4%	45.9%
Specificity	96.1%	92.9%	96.1%	95.3%
Positive predictive value	75.0%	60.9%	70.6%	73.9%
Negative predictive value	84.9%	83.8%	83.2%	86.0%

Discussion

Appropriate diagnostic tools for diagnosis of a probable *H. pylori* infection in patients with dyspepsia is of utmost importance for physicians as well as for patients. The rapid urease test is the most frequently used diagnostic test for the diagnosis of *H. pylori* infection in patients with chronic gastritis in gastroenterology clinics. This method of diagnosis has several advantages including giving a prompt result for *H. pylori* infection even before the patient leaves the clinic, high diagnostic accuracy as well as its low economical strain on both patients and the health systems.[10-12] Histological diagnosis of *H. pylori* infection is the reserved method especially for patients with a negative rapid urease test and a high suspicion of infection or for exclusion of malignancy.

The general concept is that PCR is the most sensitive technique for the detection of microorganisms such as *H. pylori*. The detection of *H. pylori* in gastric biopsy samples by PCR has been assessed by several researchers representing high sensitivity and specificity usually over 95% as compared to other invasive methods.[13-16]

In this study, we examined the strength of PCR methods in the detection of four *H. pylori* recognized alleles. We found that PCR has a very low power to detect *H.pylori* infection among our patients. There may be several reasons for this; first, is the low technical ability of our laboratory staff; second is not using proper materials or techniques for this purpose; moreover, our pathologists had complained that the biopsy samples were too small for a proper PCR evaluation; although the general assumption is that PCR can detect the infection even in extremely limited volume samples. Ideally proton pump inhibitors should be discontinued before the endoscopy;[12,18] it was demonstrated that after 4 weeks of omeprazole treatment, the histological density of *H. pylori* in the antrum and corpus was reduced, while in the fundus was increased.[19] With a suspicion of high self medication among our patients, we hypothesize that drugs may cause an adverse impact on the accuracy of PCR test results.

Several previous studies have assessed the sensitivity and specificity of PCR methods to detect several primers in *H. pylori*'s gene loci. The results were very diverse in different reports. Lu et al[20] in their study comparing the power of five different PCR methods to detect *H. pylori* DNA in gastric tissues of patients with chronic gastritis, found that sensitivity of PCR methods is not satisfactory in detecting *H pylori*; they finally concluded that this observation can be related to sequence polymorphism in the specified loci. Smith et al[21] in their study of PCR methods for diagnosis of *H. pylori* infection in gastric tissues also reported a low sensitivity of 56% for glmM gene, but they found a sensitivity of 100% for 26kDa gene primer, but specificity of 44% for ureA. They also found that 68% of biopsies that showed positive amplification in all three genes were positive for the cagA gene while this proportion was just 43% in our survey. On the other hand, Lage et al[13] reported that there were no false positive or negative biopsies amplified by the glmM in their study. In this study, however, we reached to a sensitivity of 32% for 26kDa and specificity of 83% for ureA gene primer and comparable results for all the other gene primers investigated.

The high number of false negative results reported in this study, can not be solely interpreted by gene polymorphism; although, one may claim that the gold standard criteria used in this study for determination of positive and negative cases may not be of enough accuracy, our reason for using these criteria was that they were previously reported as high sensitivity and specificity methods and were also used as gold standard criteria for the same purpose by previous investigators.[22] PCR is a time consuming and expensive procedure with need for highly trained staff performing it. In the developing countries, the advantages of using PCR for detecting microorganisms such as *H. pylori* may be lost owing to the scenario of low healthcare funds as well as apaucity of well-trained experts. Our study demonstrated that using PCR methods for detection of *Helicobacter pylori* does not have a high diagnostic accuracy rate.

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