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Detection of Helicobacter pylori using nested polymerase chain reaction and rapid urease test in gastric biopsy samples

Gastrik biyopsi örneklerinde Helicobacter pylori'nin "nested" polimeraz zincir reaksiyonu ve hızlı ürea testi ile belirlenmesi

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Background/aims: *The purpose of this study was to compare nested polymerase chain reaction and rapid urease test findings in the diagnosis of Helicobacter pylori infection.* ***Methods:*** *Two gastric biopsy specimens were obtained from each of 64 patients and polymerase chain reaction and rapid urease test were performed. DNA extraction was followed by amplification with two primer pairs from the urease A gene of Helicobacter pylori genome.* ***Results:*** *Fourty two patients (65.6%) had a positive rapid urease test result while 22 (34.4%) had a negative result for Helicobacter pylori. Sixty of 64 patients (93.8 %) had a positive result with the nested polymerase chain reaction method. Four patients with negative nested polymerase chain reaction results also had negative rapid urease test results. All of the eighteen patients with rapid urease test-negative results were positive with nested polymerase chain reaction. The number of nested polymerase chain reaction-positive patients (93.8%) was significantly higher than rapid urease test-positive (65.6%) and first round polymerase chain reaction-positive (53.1%) patients (P< 0.001).* ***Conclusions:*** *Our results indicate that the nested polymerase chain reaction is more specific and sensitive than the rapid urease test for detecting Helicobacter pylori in gastric biopsy samples.*

Key words: Rapid urease test test, Helicobacter pylori, nested polymerase chain reaction, urease A gene.

Amaç: *Altmışdört gastrik biyopsi örneğinde nested polimeraz zincir reaksiyonu ve hızlı ureaz testi yöntemleri kullanılarak Helicobacter pylori varlığı değerlendirildi. Bu çalışmanın amacı, Helicobacter pylori enfeksiyonunun teşhisinde nested polimeraz zincir reaksiyonu ve hızlı üreaz test sonuçlarını karşılaştırmaktır.* ***Yöntem:*** *"Nested" polimeraz zincir reaksiyonu ve hızlı ureaz testi için ayrı ayrı olmak üzere, tüm hastalardan iki gastrik biyopsi örneği alınmıştır. DNA izolasyonunu takiben Helicobacter pylori genomunun ureaz A genine uygun iki primer çiftiyle amplifikasyon yapılmıştır.* ***Bulgular:*** *Hızlı ureaz testi sonucuna göre, Helicobacter pylori için 42 hasta pozitif (%65.6) ve 22 hasta da negatif (%34.4) bulunmuştur. 64 hastanın altmışı (%93.8), "nested" polimeraz zincir reaksiyonu metodu ile pozitif bulunmuştur. "Nested" polimeraz zincir reaksiyonu ile negatif olan dört hasta hızlı ureaz test ile de negatiftir. Hızlı ureaz testi ile negatif olan 18 hastanın hepsi, "nested" polimeraz zincir reaksiyonu ile pozitif bulunmuştur. "Nested" polimeraz zincir reaksiyonu -pozitif hastalar (%93.8), hızlı ureaz test-pozitif (%65.6) ve ilk tur polimeraz zincir reaksiyonu -pozitif (%53.1) hastalara göre önemli derecede yüksektir (P< 0.001).* ***Sonuç:*** *Sonuçlarımız, gastrik biyopsi örneklerinde Helicobacter pylori'nin belirlenmesi için hızlı ureaz teste göre "nested" polimeraz zincir reaksiyonu metodunun daha spesifik ve duyarlı olduğunu göstermektedir.*

Anahtar kelimeler: CLO test, Helicobacter pylori, nested PZR, ureaz A geni.

INTRODUCTION

Helicobacter pylori (H. pylori) is a small, spiral-shaped, Gram-negative bacterium that has been implicated in the pathogenesis of gastritis, peptic ulcer and gastric cancer of humans (1-3). Several techniques have been used for the detection of H. pylori, different sensitivity and specificity. The routine invasive diagnostic assays consist of culture, urease tests, and histological examination of gastric biopsy sections requiring endoscopy (4-6). Urea breath tests and serologic detection of antibodies are used as non-invasive methods (7-9).

The rapid urease test (CLO test) measures ammonia produced from urea by the bacterial enzyme urease for diagnosing H. pylori infection, and it has high sensitivity and specificity (10). There are also molecular techniques that are based on nucleic acid hybridization and polymerase chain reaction (PCR) and which target different H. pylori genes (11-18). The PCR is also an extremely sensitive and specific method of detecting H. pylori in gastric biopsy and juice samples (11, 13, 19-21).

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Table 1. Results of nested PCR and CLO test to detect Helicobacter pylori infection.

	Nested PCR		CLO test	
Diagnosis	*Positive (%)*	*Negative (%)*	*Positive (%)*	*Negative (%)*
Gastric ulcer (n=4)	4 (100%)	0 (0.0%)	3 (75.0%)	1(25.0%)
Duodenal ulcer (n=18)	18 (100%)	0 (0.0%)	13 (72.2%)	5 (27.8%)
Gastritis(n=42)	38 (90.5%)	4 (9.5%)	26 (61.9%)	16 (38.1%)
Total (n=64)	60 (93.8%)	4 (6.2%)	42 (65.6%)	22 (34.4%)

In this study, the efficiency of these two techniques (PCR and CLO test) was compared and the sensitivity of a nested PCR method in detecting H. pylori in gastric biopsy specimens evaluated.

MATERIALS AND METHODS

Gastric biopsy samples were obtained from 64 patients (33 male and 31 female) with an age range of 20-69 years (mean, 37.95) who underwent endoscopy for upper gastrointestinal complaints at the Gastroenterology Clinic of Erciyes University Hospital. Patients who had received non-steroidal anti-inflammatory drugs, bismuth compounds, proton pump inhibitors, oral anticoagulants or antibiotics known to be effective against H. pylori within the previous three months were excluded, as were those who had recently received blood transfusions, undergone gastric surgery, or had bleeding diathesis. The 64 patients were diagnosed as gastric ulcer (n=4), duodenal ulcer (n=18) and gastritis (n=42) based on endoscopic examination. For each patient, two gastric biopsy specimens were collected from the antrum of the stomach and DNA extraction and CLO test was performed.

One specimen was minced and digested overnight (at 37°C) in a solution containing 100 µg/ml proteinase K, 100 mM Tris-HCl (pH 8.0), 40 mM EDTA and 0.2% SDS. DNA was isolated from the homogenate by extraction with phenol/chloroform and ethanol precipitation and dissolved in 100-200 µl of Tris-EDTA buffer (TE; 10 mM Tris-HCl and 1 mM EDTA [pH 8.0]) (22). DNA extracts were stored at –20°C until used for PCR.

The other specimen was rapidly examined for the presence of H. pylori by CLO test (Delta West Ltd., Bentley, Western Australia). In the CLO test, the biopsy material was placed into gel containing a pH indicator that changed colour from yellow to red within 24 hours if H. pylori was present, due to the production of ammonia by the organism's urease enzyme.

Five microliters of each DNA solution were subjected to a two-step nested PCR using two primer pairs from the urease structural gene of the H. pylori genome (23). The outer primer pair was 5'-GCCAATGGTAAATTAGTTCC-3' and 5'-CTCCTTAATTGTTTTTACAT-3', and amplified a 411-bp product from urease A gene (nucleotides 304 to 714) (11, 13). The amplification cycle consisted of 40 cycles at 96°C for 30 seconds, 56°C for 15 sec-

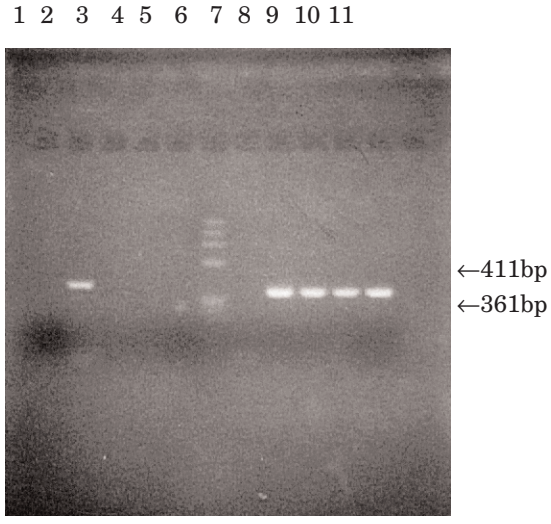

Figure 1. First and second PCR round results for H. pylori in gastric biopsy samples. Lane1: negative control; lane 2:positive control (411 bp); lanes 3-5: first PCR round products in patient samples; lane 6:f x 174/Hae III; lanes 7-11: second PCR round products of samples in lanes 1-5 (361 bp), respectively.

onds and 74°C for 30 seconds. The final cycle included extension for 10 min at 74°C to ensure full extension of the product. Negative reagent control reactions were performed with each batch of amplifications, consisting of tubes containing distilled water in place of the DNA samples. After PCR, 1 microliter of the reaction mixture was transferred to the second round reaction mixture containing 0.6 µM of each inner primer and the same buffer as in the first round. The nested inner primer pair was 5'-AGTTCCTGGT-GAGTTGTTCT-3' and 5'-AGCGCCATGAAAAC-CACGCT–3', and amplified a 361-bp product from urease A gene (nucleotides 318 to 678). The amplification cycle of the second round PCR was the same as in the first round (13). Ten microliters of the first and second round PCR products were electrophoresed on a 2 % agarose gel containing 0.5 µg of ethidium bromide per ml.

Data were analyzed by t test for proportions.

RESULTS

Table 1 presents the results obtained with nested PCR and CLO test used for the detection of H. pylori infection in gastric biopsy samples. Of the 64 biopsy samples, 42 were positive (65.6%) and 22 were negative (34.4%) by CLO test. Of the 42 patients with CLO test-positive results, three had gastric ulcer, 13 had duodenal ulcer and 26 had non-ulcer or gastritis (75.0%, 72.2% and 61.9% respectively). Of the 22 patients with CLO test-negative results, one (25.0%) had gastric ulcer, five (27.8%) had duodenal ulcer and 16 (38.1%) had gastritis.

Sixty patients (93.8%) were positive for H. pylori by nested PCR, while 34 patients (53.1%) were positive for H. pylori in the first round PCR (Figure 1). Of the 60 nested PCR-positive patients, four (100%) had gastric ulcer, 18 (100%) had duodenal ulcer and 38 (90.5%) had gastritis. All of the four nested PCR-negative patients had gastritis and they also had negative CLO test results. All of the 18 patients with CLO test-negative results were positive by nested PCR and six of them were positive by first round PCR. Fourteen CLO test-positive patients were negative by first round PCR. The percentage of nested PCR-positive patients appeared to be higher among patients with both CLO test-positive ($P<0.001$, $Z:3.954$) and first round PCR-positive ($P<0.001$, $Z:5.203$) results. No false positive result was observed in the negative controls in the first and second PCR rounds, and positive control for H. pylori was successfully detected by both the PCR rounds.

DISCUSSION

H. pylori infection may cause various gastroduodenal diseases, such as gastritis, gastric or duodenal ulcers and gastric cancer (1-3). Although there are various tests for the detection of H. pylori, it is important that accurate diagnostic methods are used.

In this study, the sensitivities of the CLO test and nested PCR results were evaluated. Both of these methods require biopsy samples, but not all biopsy samples contain a sufficient amount of H. pylori organisms to allow detection. Since the one step PCR is not sufficiently sensitive and nested PCR has been shown to have a higher specificity and sensitivity in the detection of H. pylori (13, 16, 21) the latter method was used in the present study. Nested PCR can also be applied to the analysis of H. pylori in any biological sample, such as gastric tissue, saliva, fecal, etc. as long as DNA can be extracted from the sample. The nested PCR showed high sensitivity (93.8%) in our study while the single PCR round and CLO test demonstrated less sensitivity at 53.1% and 65.6% respectively ($P<0.001$). The CLO test results were found to be more sensitive (65.6%) than one step PCR results (53.1%), but this difference was not significant ($P>0.05$, $Z:1.44$). False negative CLO test results might have occurred when only a small amount of H. pylori was present in the samples although this could then detected by subsequent nested PCR assays using the same biopsy samples. In the present study, the CLO test and PCR assays were performed with the same gastric biopsy specimens taken from the same site of the antrum. Lin et al. (10) reported that among 82 gastric biopsy samples, 56 were positive by CLO test and 52 were positive by PCR; it was suggested that PCR might be used as a complementary assay for CLO test. A comparison of the PCR, histology, culture and CLO test methods shows that PCR had the highest diagnostic sensitivity (99.4%) for H. pylori infection (24). Archimandritis et al. also demonstrated that rapid urease (CLO) test was less sensitive than histology in diagnosing H. pylori infection (25). In this study, histological examination of obtained clinical samples could not be evaluated. However, in comparison with four diagnostic methods (CLO test, PCR assay, culture and histological examination), it has been suggested that

CLO test is a rapid and sensitive method of screening for H. pylori infection and that PCR assay is more sensitive than other methods for detecting infection following treatment (26).

It is concluded that the PCR assay with nested primers is a specific and sensitive method for detecting H. pylori DNA in gastric biopsy samples. This technique could thus be used to evaluate for continuing infection after antibacterial therapy of H. pylori-infected patients.

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