Detection of Helicobacter Pylori in dental plaque and gastric biopsy samples of Turkish patients by PCR-RFLP

F. İ. Şahin[1], A. C. Tınaz[2], İ. Ş. Şimşek[1], S. Menevşe[1], A. Görgül[3]

(1) Gazi University Faculty of Medicine, Department of Medical Biology and Genetics ; (2) Gazi University Faculty of Dentistry Department of Operative Dentistry and Endodontics ; (3) Gazi University Faculty of Medicine, Department of Gastroenterology.

Abstract

Helicobacter pylori is regarded as an important pathogen playing a key role in the pathogenesis of peptic ulcer. Different studies about the mode of transmission of the microorganism report conflicting results about dental plaque as the source of _H. pylori_ infection. In the present study we aimed to detect the presence of _H. pylori_ in dental plaque of Turkish patients by polymerase chain reaction (PCR) and if any to do typing by restriction fragment length polymorphism (RFLP) analysis. Fifty dyspeptic patients, to whom upper gastrointestinal endoscopy was performed, were included in the study. Dental plaques were obtained before endoscopic examination. Both dental plaque and gastric biopsy samples were amplified with Ure A and Cag A gene primers. There were no positive dental plaque samples even in the 23 patients whose gastric biopsy specimens were positive. Our findings showed that there is not a correlation between dental presentation of the microorganism and _H. pylori_ gastritis. (Acta gastroenterol. belg., **2001**, 64, **150-152**).

Key words : *H. pylori,* dental plaque, gastric biopsy, PCR, RFLP.

Introduction

Helicobacter pylori (H. pylori) is considered as a common worldwide gastric pathogen. *H. pylori* gastritis is associated with duodenal ulcer, gastric ulcer, gastric carcinoma and primary gastric B-cell lymphoma (1). The detection of *H. pylori* by assays based on PCR technology has been used in the recent years by amplification of several genes (2).

Although several studies have been reported about the mode of transmission of the microorganism and natural reservoirs have been investigated, there are conflicting results about dental plaques as sources of *H. pylori* infection (3).

In Turkey, *H.pylori* infection is a common health problem with its 35-56% frequency (4). In a previous study, we used PCR-RFLP analysis in order to detect and type *H. pylori* strains in gastric biopsy specimens (5). In the current study we aimed to detect *H. pylori* in gastric biopsy specimens of dyspeptic patients by PCR-RFLP analysis and also our aim was to investigate dental plaque as a reservoir for *H. pylori* infection.

Material and methods

Subjects : 24 male, 26 female 50 dyspeptic patients, aged between 21 and 76 years, referred for upper gastrointestinal endoscopy were included in the study. Dental plaque samples were obtained before endoscopy at the same day. The patients were asked about the frequency of dental visits and oral hygiene. Subjects taking antibiotics and who have total denture were not included in the study. Before samples were taken the subjects were informed about the procedure and their approval was taken.

Specimen collection

Gastric Biopsy Samples : Two antral biopsy specimens were obtained from each patient and the samples were frozen at -70°C until processing.

The endoscopic apparatus (Olympus GIF –XQ 200, London, UK), all channels and biopsy forceps were carefully cleaned and disinfected by immersion in 2% steranious solution (Anious, Lille Hellemmes, France) for 15 minutes, rinsed in water and dried at the end of each endoscopic session.

Dental Plaque Samples : Dental plaque samples were collected from upper incisor and first molar teeth's mesiobuccal surface. Before plaque collection, the area was isolated with cotton rolls and supragingival plaque was removed with sterile gauze to prevent contamination. Subgingival plaque was collected by inserting sterile endodontic absorbing paper points (XX-fine, Roeko, Ulm, Germany) to the bottom of the periodontal pocket for 10 seconds (6). The samples were put into sterile PBS and kept at -20°C until DNA isolation. Samples were collected with great attention to prevent bleeding.

DNA Isolation : Gastric biopsy samples were minced in a petri dish under sterile conditions and were taken into 1,5 ml eppendorf tubes. 100 µlt distilled water was added to the samples. After boiling for 15 minutes, centrifugation at 14000 g for 5 minutes was performed and supernatant was taken into another tube to be kept at -20°C prior to PCR amplification.

DNA from dental plaques was obtained with a similar protocol.

PCR Amplification : Two pairs of primers were used to amplify *H. pylori* urease A (Ure A) and Cytotoxin

Corresponding author : Feride İffet Şahin, M.D., Ph.D., Department of Medical Biology and Genetics, Faculty of Medicine, Gazi University, 06510, Beşevler, Ankara, Turkey. E-mail : feridesahin@hotmail.com.

associated antigen A (Cag A) genes. HPU 1 and HPU 2 primers were used to amplify a 411bp product of urease A gene. Primers 93089 and 93261 were used to amplify a 400bp product of Cag A gene.

A PCR mixture of 10 mM tris-HCl (pH = 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% (w/v) gelatine, containing 0.2mM of each nucleotide, 0.5 μM of each oligonucleotide primer and 2.5U Taq polymerase also contained 10 μl of DNA from gastric biopsy and dental plaque specimens. A total of 100 μl reaction volume was obtained. Hp 11637 strain was used as the positive control.

PCR cycles were arranged according to the target genes and according to the other investigators' reports and as described before (2,5,7). PCR products were visualized by 2% agarose gel electrophoresis.

Analysis of amplified DNA products : Amplified PCR products were analysed by restriction enzyme digestion. All enzymes and buffers were from MBI, Fermentas (Lithuania). The amplified Ure A products were digested with Hinf I (restriction fragments of 277 and 134bp), and Mlu I (restriction fragments of 300 and 111bp).

Cag A products were digested with Spe I to give 274 and 126bp fragments and Hind III to give 195, 159 and 46bp fragments. Digested samples were analysed by 2% agarose gel electrophoresis and ethidium bromide staining.

Results

50 patients clinically diagnosed as gastric ulcer, duodenal ulcer and gastroesophageal reflux were included in the study. In 23 of the patients (46%) amplified PCR products were observed in gastric biopsy samples with Ure A and Cag A primers. Dental plaque samples did not reveal any PCR products after amplification with both primers.

Ure A gene PCR products revealed 277 and 134bp bands after Hinf I digestion. Mlu I digestion of the same products revealed uncut products in patients 40, 43, 1 and 3 (Fig. 1).

Cag A gene PCR products revealed the expected bands in all samples with both Hind III and Spe I enzymes.

Discussion

In this study, we investigated gastric biopsy and dental plaque specimens of dyspeptic patients. Our aim was to detect different strains (if any) of the bacterium and to detect whether dental plaques and gastric biopsies were infected with the same strain. We didn't detect *H. pylori* in dental plaques including the 23 patients who had positive gastric biopsy specimens.

Oral cavity has been considered as a reservoir for *H. pylori* and different diagnostic methods have been used while investigating *H. pylori* in oral cavity specimens (8,9). Urease genes and 16S rRNA genes have been the base of PCR reactions and a high prevalence of *H. pylori* in oral cavity has been detected by PCR (10,11). However, conflicting results have also been reported by several investigators (12-14). Wahlfors et al. did not detect *H. pylori* by PCR in 29 dental plaque specimens of 29 patients, including 14 patients with positive stomach specimens (12). Hardo et al. studied dental plaque specimens and reported negative culture results. In the same study PCR was positive in only one patient (15). These reports support our negative PCR results in dental plaques. These results suggest that there is not a correlation between dental hygiene and *H. pylori* gastritis, and dental plaques don't seem to play an important role in transmission of the organism.

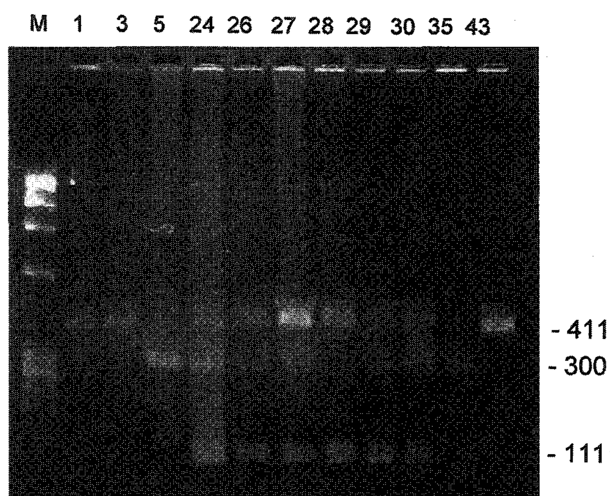

Fig. 1. — Mlu I digested PCR products of patients amplified with urease A primers. M = (× 174 DNA marker digested with Hae III. Numbers refer to patients. Patients 1,3, and 43 did not show digested bands.

On the other hand, it is difficult to isolate *H. pylori* from oral cavity, as there are many different microorganisms in the oral flora (8). *H.pylori* may not be uniformly distributed in the mouth (11), so repeated sampling may increase positive results. In order to overcome these difficulties we obtained dental plaque samples from different parts of the oral cavity. Another point to be careful while studying with *H. pylori* is that, the oral cavity has a flora consisting of various different microorganisms, so the results may be falsely positive or negative. We tried to overcome this by using two different pairs of primers (Ure A and Cag A gene specific primers), hence working with two different genes. Our aim in using Cag A primers was to differentiate *H.pylori* from other urease positive microorganisms.

PCR is a powerful tool for detection of microbial pathogens. It is a rapid, sensitive, specific test, which is used in *H.pylori* detection frequently (16). The negative PCR detection rates could be due to the low number of organisms in dental plaque, PCR inhibitors, the place of the organism in the oral cavity, and the transient existence of the bacteria in the oral cavity (1,3).

Conclusion

Until now, many conflicting results have been reported about the presence of *H. pylori* in the oral cavity. We think it is necessary to standardise sample collection in order to evaluate results.

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