

The detection of *Helicobacter pylori* in paraffin sections using the PCR technique and various primers as compared to histological techniques

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Helicobacter pylori is thought to represent a significant etiopathogenic factor in diseases of the upper gastrointestinal tract. It seems, therefore, important to elaborate effective techniques for its detection. The aim of the present study was to evaluate the effectiveness of *Helicobacter pylori* detection using the PCR technique on paraffin sections with various pairs of primers and to compare the results with those of a histological appraisal. Material for the studies involved 50 paraffin blocks with gastric mucosa biopsies fixed in 4% buffered formalin. In this material 4 tests were performed with the aim of diagnosing *Helicobacter pylori* infection: 1) H+E staining, 2) staining by the Giemsa technique, 3) an immunocytochemical technique with antibodies against *H. pylori* and 4) the PCR technique with various primers. In the present study the most reliable results for *H. pylori* detection as well as the most pronounced correlation were obtained by using the PCR technique with primers for the ureC gene, immunohistochemistry and staining according to Giemsa. Less compatible results were obtained employing the two PCR techniques which utilise various primers. The experiments confirmed the usefulness of the PCR technique in the detection of *Helicobacter pylori* in paraffin sections by using a suitable pair of primers, and also indicated that Giemsa staining and immunohistochemistry should be taken into account.

Key words: PCR technique, *Helicobacter pylori* infection, detection

INTRODUCTION

Infection with *Helicobacter pylori* plays a significant role in the etiopathogenesis of diseases of the stomach. The bacteria used to be defined as a slow bacterial pathogen, indicating that the infection may develop asymptotically and its consequences may appear gradually after a prolonged period [1, 3]. From the moment when the relation between *Helicobacter pylori* infection and the development of gastritis was established, several techniques have

been devised to identify the microbe. Diagnosis of the infection is based on both non-invasive and invasive techniques, which require endoscopy and biopsy. However, it should be stressed that, as no optimal technique has yet become available which would allow the bacteria to be unequivocally identified, only a combination of several techniques is held to yield satisfactory diagnostic results [5]. Studies using molecular biological techniques are of particular significance, since they enable a rapid and

precise diagnosis to be made. Polymerase chain reaction (PCR) is thought to represent one of most precise techniques in the diagnosis of *Helicobacter pylori* in biopsies of the gastric mucosa, dental plaque or in faeces [2]. An increase in the number of amplification cycles and the use of internal primers of the reaction (nested PCR) enhance the sensitivity of the technique, permitting even an individual bacterium to be detected in the material under examination.

In the present study we decided to compare the efficiency of *H. pylori* detection by the PCR technique employing different primers with traditional histological methods.

MATERIAL AND METHODS

The material for the present study consisted of 50 archival paraffin blocks with biopsies of gastric mucosa, endoscopically sampled from patients diagnosed with chronic gastritis confirmed by histopathology. The mucosa samples were first fixed in 4% buffered formalin for 24 hours and then embedded in paraffin. Paraffin sections were then used to perform 4 independent tests in order to diagnose *Helicobacter pylori* infection: 1) staining with haematoxylin and eosin, 2) staining by the Giemsa technique, 3) immunohistochemical staining (labelling) with the use of monoclonal antibodies against *Helicobacter pylori* and 4) molecular studies based on PCR using pairs of various primers for a fragment of the *ureC* gene and 16S rRNA. In order to perform the PCR reactions, DNA was used following its isolation from the paraffin sections.

RESULTS AND DISCUSSION

The studies described above provided different results for *Helicobacter pylori* detection, depending upon the research technique applied. An amplification product of 187 bp was obtained in 23/50 cases (46%) using the PCR technique and primers for the fragment of the *ureC* gene followed by the second PCR reaction (nested PCR). However, when primers for the 16S rRNA gene fragment were used, a positive result following the second PCR and an amplification product (of 109 bp in size; Fig. 1) was obtained in 42/50 cases (84%), which confirms the results of other investigators [6]. By employing the immunohistochemical technique (IHC; Fig. 2),

M 1 2 3 4 5 6 7 8 9 PC NC

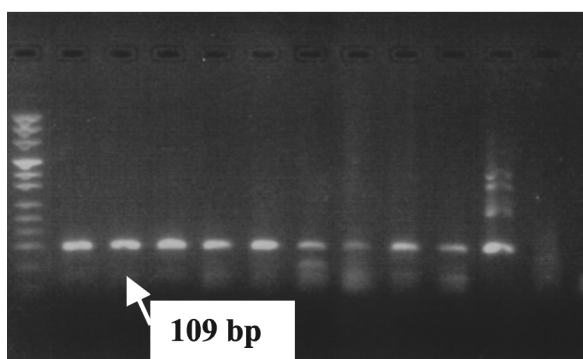


Figure 1. PCR for 16S rRNA gene fragment with positive (PC) and negative control (NC) — 9 samples. Size of amplification product: 109 bp. DNA markers: M.

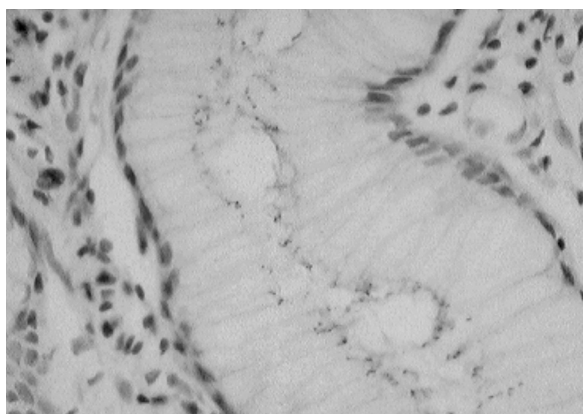


Figure 2. *Helicobacter pylori* on the surface of gastric mucosa visualised by immunohistochemical detection — magnification $\times 400$.

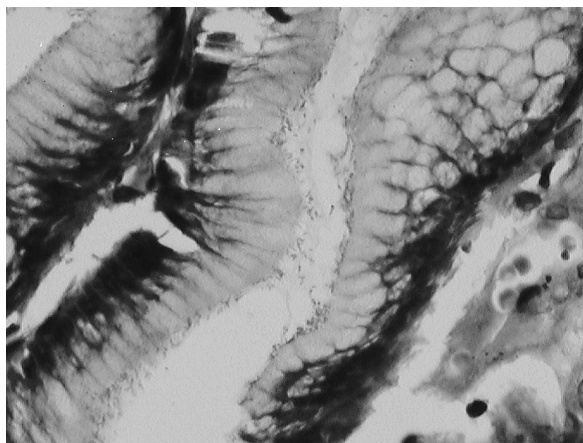


Figure 3. *Helicobacter pylori* on the surface of gastric mucosa. Giemsa staining — magnification $\times 400$.

the infection was detected in only 19/50 cases, representing the lowest percentage of bacteria detection (38%). Closely related results were obtained using histological techniques of detection, such as staining by the Giemsa technique (G; Fig. 3) or with haematoxylin and eosin (H+E). Positive results in 27/50 cases (54%) were obtained by using Giemsa staining, while by employing the H+E technique 29/50 positive results were obtained (58%).

The present study confirms the usefulness of the PCR technique in the detection of *Helicobacter pylori* on paraffin sections. The selection of appropriate primers significantly affects the results obtained. In this study the most reliable technique proved to be PCR with the use of primers for the *ureC* gene. Although the percentage of positive results for amplifying *ureC* was lower than in the case of the 16S rRNA gene, the correlations with two other histological techniques, Giemsa staining and immunocytochemistry, indicate a higher reliability of amplification using primers for the *ureC* gene. PCR using primers for the *ureC* gene is thought to be highly specific, even though less sensitive [4]. The usefulness of Giemsa staining should also be stressed. This relatively simple technique, economic and easy to perform in any histopathological laboratory, may yield satisfactory diagnostic results in the hands of an experienced pathologist. Coupled to another test, in the form of the PCR technique, it may provide

valuable information on *Helicobacter pylori* infection and on the associated pathology of the gastric mucosa. Thus the technique of bacteria detection in paraffin sections also opens a valuable source for retrospective studies on material that has been fixed.

REFERENCES

1. Andersen LP (2001) Pathogenesis and host response in *Helicobacter* infection. *FEMS Immunol Med Microbiol*, 30: 85.
2. Goosen C, Theron J, Ntsala M, Maree FF, Olckers A, Botha SJ, Lastovica AJ, van der Merwe SW (2002) Evaluation of a novel heminested PCR assay based on the phosphoglucosamine mutase gene for detection of *H. pylori* in saliva and dental plaque. *J Clin Microbiol*, 40: 205–209.
3. Gościński G (2002) Presence of *Helicobacter pylori* antigens in stools of children with chronic gastritis (in Polish). *Pol Merk Lek*, 12, 68: 104–107.
4. Lu J-J, Perng Ch-L, Shyu R-Y, Chen Ch-H, Lou Q, Chong SK, Lee CH-H (1999) Comparison of five PCR methods for detection of *Helicobacter pylori* DNA in gastric tissues. *J Clin Microbiol*, 37: 772–774.
5. Megraud F (1997) How should *Helicobacter* infection be diagnosed? *Gastroenterology*, 112: 93–98.
6. Roosendaal R, Kuipers EJ, van der Brule AJC, Pena AS, Uytendaele AM, Walboomers LMM, Meuwissen SGM, de Graaf J (1994) Importance of the fiberoptic endoscope cleaning procedure for detection of *Helicobacter pylori* in gastric biopsy specimens by PCR. *J Clin Microbiol*, 32: 1123–1126.