An investigation of Helicob culture, histopathological examination methods and sensitivities

Asuman Inan, *MD,* ***Serda Gulsun,*** *MD,* ***Hakan Guveli,*** *MD,* ***Jal***

ABSTRACT

Objectives: In this study, the determination of *Helicobacter pylori* (*H. pylori*) by culture, histopathological and serological methods in cases of endoscopically diagnosed as duodenitis and duodenal ulcer (DU), a comparison of their relative advantages, and its antibiotic sensitivities were investigated.

Methods: *Helicobacter pylori* was investigated using 3 methods (culture, histopathological and serological examination) in 50 patients (25 diagnosed with duodenitis and 25 with DU) in the Department of Gastroenterohepatology, Istanbul Haydarpasa Numune Hospital, Turkey between December 2000 and February 2001. An investigation into its antibiotic sensitivities to amoxicillin, clarithromycin, metronidazole and azithromycin by disc diffusion method and to amoxicillin and clarithromycin by E-test were investigated.

Results: *Helicobacter pylori* bacteria were observed in Gram stained preparates prepared from biopsy material in 34 out of 50 patients (68%), and were able to be produced in active culture in all these cases. Histopathological examination revealed the presence of *H. pylori* in 80% cases of DU and 60% cases of duodenitis; anti-CagA(IgG) was positively determined in 88% DU cases and in 60% duodenitis cases. There was a significant difference between the 2 groups in terms of diagnosis by histopathological and serological methods. The difference between the 2 groups produced in active culture in 84% of DU cases ar was statistically significant (p=0.03 and disc diffusion methods, 8.8% reproduced in culture were resistan sensitive to clarithromycin. All stra to be sensitive to amoxicillin: 17.6% determined to be resistant to metr azithromycin.

Conclusion: It was observed that rapid and reliable method of pre-dia that histopathological examination considerable importance in diagn investigation of the positivity of anti- guide in the identification of virulen In addition, it was also concluded t examination does not require invasiv be an advantage. The culture me with the aim of diagnosis in cases id endoscopy, and that in cases resista be applied for the purpose of deter sensitivity. E-test and disc diffusion rather good correlation for whic diffusion method can be used in t antimicrobial sensitivity of *H. pylori*

Saudi Med J 2005; Vol. 26

From the Department of Infectious Diseases (Gulsun), Diyarbakyr State Hospital, Department of Gastroenterology, Division of Endoscopy (Guveli), and the Departm Hospital, Istanbul, *Turkey*.

Received 13th September 2004. Accepted for publication in final form 19th Decemb

Address correspondence and reprint request to: Dr. Asuman Inan, Department o Istanbul, PO Box 34668, *Turkey*. Tel. +90 (532) 4154627. Fax. +90 (216) 3359355

Following the definition of *Helicobacter pylori* (*H. pylori*) as a result of [illegible] peptic ulcer pathogenesis, [illegible] was isolated from stomach mucosa biopsy specimens [illegible] individuals with chronic active gastritis and peptic ulcers.[1,2] In later studies, it was determined [illegible] the eradication of *H. pylori*, [illegible] duodenal ulcer (DU) cases and 70% of gastric ulcer cases healed completely.[3,4] [illegible] endoscopic examination [illegible] examination, bacteria culture, [illegible] probe and polymerase chain reaction (PCR) tests; [illegible] need for invasive intervention [illegible] test, serological tests, stoma[illegible] with PCR and the investigation [illegible] ammonia in urine.[5,6] The eradication of *H. pylori* is rather difficult, and usually [illegible] use of more than one drug, and there is [illegible] that has been shown to be 100% effective. The drugs used in treatment are proton pump inhibitors or ranitidine, bismuth compounds, [illegible] amoxicillin, metronidazole, [illegible] tetracycline. The treatment methods [illegible] for a proton pump inhibitor [illegible] antibiotics (amoxicillin, [illegible] metronidazole). The results [illegible] successful, with an approximately 90% eradication rate.[7,8] This rate has been reported as [illegible] therapy and 18% with single agent treatment. However, the major factor affecting [illegible] variations in antibiotic resistance patterns. From the clinical point of view, resistance [illegible] nitroimidazoles and macrolides [illegible] many treatment regimes is important.[4,9]

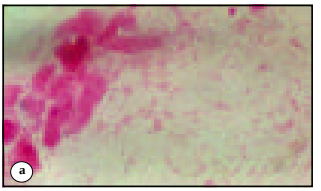
Methods. A total of 50 patients, 25 diagnosed with duodenitis and 25 with [illegible] of Gastroenterohepatology, [illegible] Numune Hospital, Turkey, between December 2000 and February 2001, were included in the study. Cases which had used antibiotics within the preceding month, or had used non-steroid anti-inflammatories before their complaints began, and cases with illnesses which could not tolerate upper gastrointestinal system endoscopy were excluded from the study.

Using sterile biopsy forceps, a total of 4 biopsy specimens: one from the corpus, 2 from the antrum and one from the duodenum [illegible] serum specimens were taken [illegible] biopsy specimen from the antrum [illegible] brain-heart infusion medium [illegible] the Pylori agar (Biomerieux, Marcy l'Etoile, France) within 30 minutes [illegible] microaerophilic environment [illegible] bag from Biomerieux) at 37[illegible] catalase and oxidase tests were performed [illegible] mm diameter transparent colonies [illegible] considered as positive with [illegible]

[illegible]

Statistical analysis was perfor[illegible] exact test.

Results. [illegible]



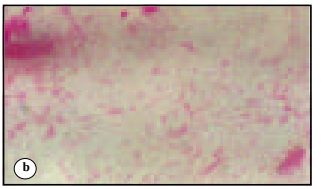


Figure 1 - Gram stained preparate from biopsy material of patients with **a)** duodenal ulcer and **b)** duodenitis.

preparates from biopsy material in 34 (68%) of the 50 patients. Reproduction in culture was determined in all the Gram stain positive cases. *Helicobacter pylori* was positively identified in cultures in 34 (68%) of the 50 patients. Of these, reproduction took place in 21 (84%) of the 25 patients in the DU group and in 13 (52%) of the 25 patients in the duodenitis group. *Helicobacter pylori* positivity in culture is shown in **Figures 1a and 1b** .

Helicobacter pylori was positively identified in histopathological method from biopsy material in 35 of the 50 patients. Of these, reproduction took place in 20 (80%) of the 25 patients in the DU group and in 15 (60%) of the 25 patients in the duodenitis group and although there was a difference between the 2 groups it was not statistically significant (p=0.2165). *Helicobacter pylori* was produced in biopsy material culture in 21 of the DU cases and 13 duodenitis cases, and the difference between the 2 groups was statistically significant (p=0.0322).

Anti-CagA (IgG) was positively identified in serological methods in 37 (74%) of the 50 patients. Of these, serological positiveness was obtained in 22 (88%) of the 25 patients in the DU group and in 15 (60%) of the 25 patients in the duodenitis group. Using the serological method, serological positivity was determined in 22 of the DU cases and 15 of the duodenitis cases, and this difference was significant (p=0.05). It was determined by E-test that 8.8% of the 34 *H. pylori* strains reproducing in culture were resistant to clarithromycin and 91.2% were sensitive to it. The figures were again 8.8% resistant and 91.2% sensitive using the agar disc diffusion method. It was determined using the E-test and agar disc diffusion methods that the 34 *H. pylori* strains were 100% sensitive to amoxicillin. Levels of resistance of 17.6% to metronidazole and 11.7% to azithromycin in *H. pylori* strains were determined using the agar disc diffusion method.

Discussion. In our study, the importance of the culture used in the diagnosis of *H. pylori* and of the histopathological and serological investigations used in diagnosis, the importance of the investigation of CagA (IgG) positivity in the identification of virulent strains, the correlation of the E-test and disc diffusion culture methods and the resistance of *H. pylori* to antimicrobials in our region were all investigated. Reproduction in culture was identified in all Gram positive cases. Furthermore, it was observed that Gram staining is significant since it is easily applied and a rapid and reliable method of pre-diagnosis. Bacteria of *H. pylori* morphology were identified in Gram stained preparates prepared from biopsy material in 34 of the 50 patients. This level was 84% in the duodenitis group and 52% in the DU group. *Helicobacter pylori* was determined by culture in 34 (68%) of the 50 patients in the study. Reproduction in culture occurred in 21 of the 25 DU cases (84%) and in 13 of the 25 duodenitis cases (52%). Mirghani et al[10] determined culture positivity levels of 56% in DU and 60% in duodenitis patients, and Cammarota et al[11] determined levels of culture positivity of 84.2% in DU cases and of 66.6% in duodenitis cases. These differences in the culture findings were explained in terms of the bacterium not being in a whole state in the stomach antrum submucosa and being maintained in patch style in specific regions. In another aspect, it may be said that the application of the culture method will not only be useful in determining the bacterium's antibiotic sensitivity, but also from the point of view of diagnosis.

In addition to its place in pathological diagnosis, the way that histological examination permits the bacterium to be shown by staining has led some researchers to recommend this method as the gold standard. In our study, *H. pylori* was determined by the histopathological method in 35 (70%) of the 50 patients. Biopsy results were identified as positive in 20 (80%) of the 25 DU cases and 15 (60%) of the duodenitis cases. In addition, Anti-CagA (IgG) positivity was compared with histology and culture in order to determine the relationship between DU

and duodenitis and Anti-CagA (IgG) positivity. The fact that serological methods in the diagnosis of *H. pylori* do not require endoscopy, and are cheaper, faster and easier to apply than other methods. They are particularly recommended in initial screening examinations before endoscopy or therapy in dyspeptic patients below the age of 45. Different strains of *H. pylori* exhibit different levels of virulence, and the CagA gene and high molecular weight products of this gene are important in determining this virulence.[4] In this study, Anti-CagA (IgG) was investigated in 50 serum specimens using the ELISA method, and was determined as positive in 37 (74%). Serological positivity was determined in 22 (88%) of the 25 patients in the DU group and in 15 (60%) of the 25 patients in the duodenitis group. This difference was statistically significant (p=0.005). *Helicobacter pylori* infected patients may be seropositive while histology, culture and PCR are negative. This can be accounted for in 2 ways. The serology results may be erroneously positive, although this is unlikely with IgG ELISA; alternatively, the number of micro-organisms may be low for other methods in these patients. Serological tests are faster, easier and cheaper than urea breath test and endoscopic procedures. Serology is non-invasive and does not require the use of radioactive material. With these advantages serology is of considerable value in the diagnosis of *H. pylori* and will be usefull in the investigation of anti-Cag (IgG) positivity in the identification of virulent strains.

We did not used any other method in *H. pylori* diagnosis, which is the PCR method, as various primers have been defined for cagA amplification, as particular primers need to be defined for every geographical region, otherwise false negative results can be obtained, and there are no defined primers for our region. One of the aims of our study was to examine the cagA difference between the DU and duodenitis patient groups, and this was found to be statistically significant. These results indicate that DU table had more virulent *H. pylori* strains than duodenitis.

E-test and disc diffusion methods showed a rather good correlation, for which reason it was concluded that the disc diffusion method could be used in the determination of antimicrobial sensitivity in *H. pylori* strains. It was determined by E-test that 8.8% of the 34 *H. pylori* strains reproducing in culture were resistant to clarithromycin, it was 8.8% resistant using the agar disc diffusion method. It was determined using the E-test and agar disc diffusion methods that the 34 *H. pylori* strains were 100% sensitive to amoxicillin. Franzin et al[12] also determined a sensitivity level of 100% to amoxicillin using both methods, and despite the E-test being more expensive than the disc diffusion method they reported that it was an appropriate and easily applied method for the determination of antimicrobial sensitivity in clinical microbiology laboratories. In this study, levels of resistance of 17.6% to metronidazole and 11.7% to azithromycin in *H. pylori* strains were determined using the agar disc diffusion method. Other study, metronidazole sensitivity in 100 *H. pylori* strains using E-test, disc diffusion and agar dilution methods was investigated. They reported that the E-test and disc diffusion results were compatible and that disc diffusion was a good alternative for determining metronidazole sensitivity.[13]

In conclusion, rising levels of antimicrobial resistance and the gravity of the diseases and complications to which it is linked, the diagnosis and standardization of treatment of *H. pylori* is seen to be acquiring increasing importance. These resistance and multiple resistance levels are sounding a warning in terms of increasing surveillance on the subject of *H. pylori* .

References

1. Athernon JC, Blaser MJ. Helicobacter infections. In: Fauci AS, Braunwald E, Isselbaher KJ, editors. Harrison's Principles of Internal Medicine. 14th ed. New York: The McGraw Hill Companies Inc; 1998. p. 941-943.
2. Blaser MJ. Where does *Helicobacter pylori* come from and why is it going away. ***JAMA*** 1999; 282: 2260-2262.
3. Peterson VL, Graham DY. *Helicobacter pylori.* In: Sleisenger and Fordtran's Gastrointestinal and Liver Disease. Philadelphia: W. B. Saunder's Company; 1997. p. 604-619.
4. Sezgin O, Tunç B. The effect of *Helicobacter pylori's* eradication in peptic ulcer. ***Current Gastroenterology*** 1999; 3: 268-275.
5. Büke Ç, Günhan C, Özütemiz O. Investigation of Helicobacter pylori using urea, culture and histopathological methods in stomach and duodenum diseases. ***Turkish Journal of Infection*** 1998; 12: 61-64.
6. Conn A, Borer M, Snyder C. Current Diagnosis. ***Peptic Ulcer Disease*** 1997: 2: 582-586.
7. Peterson WL, Fendrick AM, Cave DR, Peura DA, Garabedian-Ruffalo SM, Laine L. *Helicobacter pylori* related disease guidelines for testing and treatment. ***Arch Intern Med*** 2000; 160: 1285-1291.
8. Graham DY. Therapy of *Helicobacter pylori*: Current status and issues. ***Gastroenterology*** 2000; 2: 118.
9. Simsek I & Soyturk M. The role of Helicobacter pylori in gastric diseases. ***Current Gastroenterology*** 1999; 3: 381-392.
10. Mirghani YA, Ahmed S, Ahmed M, Ismail M, Fedail S. Detection of Helicobacter pylori in endoscopic biopsies in Sudan. ***Trop Doct*** 1994; 24: 161-163.
11. Commanote G, Tursi A, Montalto T, Papa A, Veneto G, Trua F, et al. Clinical assesment of the relationship of Helicobacter pylori to gastroduodenal pathologies. ***Panminerve Med*** 1995; 37: 178-181.
12. Franzin L, Fennezio M, Cabodi D, Paolo F. Clarithromycin and amoxicillin susceptibility of *Helicobacter pylori* strains isolated from adult patients with gastric or duodenal ulcer in Italy. ***Current Microbiol*** 2000; 40: 96-100.
13. Chaves S, Gadanho M, Tenreiro R, Cabrita J. Assessment of metronidazole susceptibility in *Helicobacter pylori*: statistical validation and error rate analysis of breakpoints determined by disc diffusion test. ***J Clin Microbiol*** 1999; 37: 1628-1631.