Noninvasive Stool Antigen Assay for Screening of *Helicobacter Pylori* Infection and Assessing Success of Eradication Therapy in Patients on Hemodialysis

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Introduction. *Helicobacter pylori* infection can be diagnosed by biopsy-based or noninvasive methods. Our aim was to identify *H pylori*-positive patients on hemodialysis by the noninvasive method of *H pylori* stool antigen (HPSA) and investigate its diagnostic accuracy for assessment of the eradication of infection after treatment in comparison with urea breath test (UBT).

Materials and Methods. Serology, HPSA, and UBT were performed on 87 hemodialysis patients. Infection with *H pylori* was confirmed if at least 2 tests were positive. Patients with *H pylori* infection received a 2-week course of triple therapy. To evaluate success of eradication HPSA and UBT were done after 8 weeks.

Results. Eighty-seven patients were enrolled in the study, of whom 39 (44.8%) were proved to have *H pylori* infection. The HPSA was positive in the stool specimens of 37 patients (42.5%) and the serology test was positive in 39 (44.8%). The HPSA had a 87.1% sensitivity and a 93.7% specificity for detection of *H pylori* infection. Thirty-seven patients completed the treatment period. Success of *H pylori* eradication was documented in 30 of the 37 patients (81.1%) based on UBT. After the treatment, the HPSA was negative in 32 of 37 of the stool specimens (86.4%), showing a 42.8% sensitivity and a 93.3% specificity to detect the failure of eradication of *H pylori*.

Conclusions. *Helicobacter pylori* stool antigen assay is a noninvasive reliable tool to screen *H pylori* infection before therapy and assess the success of eradication in patients on hemodialysis.

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INTRODUCTION

Helicobacter pylori infection is a major global health problem, with an estimated worldwide prevalence of approximately 50%.[1] In underdeveloped countries, up to 66% of individuals harbor the organism.[1] As a gram-negative flagellated spiral bacterium, *H pylori* colonizes the stomach and creates the basis of pathogenesis of numerous gastric diseases, including chronic gastritis, duodenal and gastric ulcers, and some types of gastric cancer and lymphoma.[1-3] Although the immunological mechanisms involved in *H pylori*-associated gastritis are not fully understood, the gastric inflammation is considered to be regulated by many kinds of inflammatory and cytoprotective factors.[4] In addition, some authors have suggested that post-kidney-transplant peptic ulcer and stomach tumors may be more frequent in patients infected with *H pylori*.[5,6] *Helicobacter pylori* infection may also carry an increased risk of cardiovascular disorders.[7] Patients with end-stage renal failure undergoing dialysis have an

increased risk of accelerated arteriosclerosis; related vascular complications are the main cause of death in these patients. For all these reasons, eradication may well become a routine measure in the future management of patients on dialysis or, at least, in the group of patients awaiting kidney transplantation.

Diagnosis of *H pylori* infection can be made with both invasive and noninvasive tests. Invasive tests include histology, culture, and rapid urease test which require endoscopy to obtain biopsies of the gastric mucosa. Noninvasive tests for the diagnosis of *H pylori* infection, which are based on analysis of samples of breath, blood, or stool, have been developed.[8] Serological tests are unable to distinguish active from past infection.[9] All patients over 45 years of age and those with "alarm" symptoms of weight loss or bleeding require an endoscopy to exclude gastric malignancy, and require an invasive test to be performed.[10] Noninvasive tests are useful for primary diagnosis, when a treatment indication already exists, or to monitor treatment success or failure. They are also useful in patients who cannot tolerate endoscopy, children, and epidemiological population studies. In 2000, a consensus report[11] stated that 2 noninvasive tests, urease breath test (UBT) and *H pylori* stool antigen (HPSA), could be used both safely and cost effectively to screen *H pylori*-positive patients (below the age of 45 years) without the alarm symptoms.

Urease breath test has been shown to be excellent in performance, but is expensive, may involve a visit to the hospital, and may be complicated for children to perform. *Helicobacter pylori* stool antigen can be referred from primary care, and the test is technically simple and relatively cheap to perform. The Premier Platinum HPSA (Meridian Diagnostics, Cincinnati, Ohio, USA), a polyclonal antibody-based stool enzyme immunoassay, has been evaluated extensively. In 2001, a meta-analysis of 4769 untreated patients in 43 separate studies using the Premier Platinum HPSA kit showed a weighted mean sensitivity of 92.1% and specificity of 91.9%.[12] The present study aimed to compare the efficacy of noninvasive methods including serology, UBT, and stool tests, as well as to assess the comparative reliability of 2 noninvasive tests (HPSA and UBT) for the detection of the failure of *H pylori* eradication after treatment.

MATERIALS AND METHODS

This study was a cross-sectional assessment of diagnostic tests for *H pylori* infection in hemodialysis patients. From October 2007 to March 2008, a total of 87 consecutive patients with chronic kidney failure undergoing dialysis in two dialysis units were included in this study. All of the participants provided written consent. The exclusion criteria were treatment with proton pump inhibitors, which could not be discontinued at least 2 weeks before the tests were performed; antibiotic treatment 4 weeks before recruitment; a history of gastrointestinal bleeding; advanced systemic disease; and any factor that precluded adequate collection of the blood, breath, or stool samples. Eligible patients were those who had active *H pylori* infection diagnosed by anti-*H pylori* serology, HPSA test, or 13 C-UBT. The participants were diagnosed with *H pylori* infection if at least 2 of the tests were positive.

Blood collection was performed during the dialysis and the patients were asked to take a stool sample for the study. Both sera and feces were immediately frozen until analysis. Sera were tested for *H pylori* antibodies by a commercial enzyme-linked immunosorbent assay in accordance with the manufacturer's instructions. Patients with an antibody titer of 1.8 or more were regarded as positive for *H pylori*. An enzyme-linked immunosorbent assay using polyclonal antibodies Premier Platinum HPSA was performed in accordance with the manufacturer's specifications (Astra SRL, Via Ciro Menotti, Milano, Italy). Readings were made at 450/630 nm. Tests with an absorbance higher than 0.12 were considered positive; those less than 0.1, negative; and those between 0.1 and 0.12, undetermined. The 13C-UBT was also performed using the modified European protocol.

Patients with *H pylori* infection were administered a 2 weeks' course of triple therapy. Patients received a 2-week course of triple-drug eradication therapy with omeprazole, 20 mg twice daily, amoxicillin, 1 g twice daily, and clarithromycin, 250 mg twice daily, followed by a 6-week course of omeprazole, 20 mg twice daily. Eradication of *H pylori* in group 2 was tested by UBT and HPSA 8 weeks after treatment.

Diagnostic accuracy of HPSA and serology tests was assessed by calculation of the sensitivity,

specificity, positive and negative predictive values, and the likelihood ratios. These tests were compared with UBT results as gold standard for detection of *H pylori* infection and for assessment of eradication success of this infection.

RESULTS

Eighty-seven patients on hemodialysis were enrolled in the study, of whom 39 (44.8%) were proved to be infected with *H pylori* (21 men and 18 women; mean age, 59 years) and 48 (55.2%) were not infected (27 men, 21 women; mean age, 51 years). *Helicobacter pylori* stool antigen was positive in the stool specimens of 37 patients (42.5%) and the serology test was positive in 39 (44.8%). Thirty-seven patients completed the treatment period. The HPSA test and UBT were performed 8 weeks after the completion of therapy. Success of *H pylori* eradication was documented in 30 of the 37 patients (81.1%). After the treatment, the HPSA was negative in 32 of 37 of the stool specimens (86.4%) and the UBT was negative in 30 (81.1%).

The HPSA had a 87.1% sensitivity and a 93.7% specificity for detection of *H pylori* infection in our patients. The positive predictive value and negative predictive value for the HPSA were 91.8% and 90.0%, respectively. Positive and negative likelihood ratios were 13.82 and 0.13, respectively. After the treatment, the HPSA had a 42.8% sensitivity and a 93.3% specificity to detect the failure of eradication of *H pylori* with a 60.0% positive predictive value and a 87.5% negative predictive value and positive and negative likelihood ratios of 6.3 and 0.6, respectively.

The use of immunoglobulin G as a screening method for *H pylori* infection proved to be less effective, because it showed a sensitivity of 76.6% and a specificity of 71.9%. Monitoring the success of triple therapy, immunoglobulin G had a specificity of only 21.9% in this population.

DISCUSSION

Concerning *H pylori* infection in patients with end-stage renal disease, many issues has remained to be clarified. In patients on dialysis, the incidence of ulcer is similar to that in patients who do not receive dialysis.[13,14] Also, it has been reported that the prevalence of *H pylori* infection is lower in patients on dialysis.[15-20] Methods for the detection of *H pylori* infection are classically divided into invasive and noninvasive. The former require demonstration of the microorganism in gastric biopsy samples; therefore, an endoscopy has to be performed. The current gold standard for diagnosing *H pylori* infection is endoscopic biopsy of the gastric tissue for rapid urease test, histology, and culture. However, such an invasive procedure has major disadvantages of anesthesia, discomfort, and the possibility to be a source of ethical problems. Noninvasive tests are easy to perform and do not produce significant discomfort. Noninvasive methods can allow a patient to avoid the discomfort and risk of invasive endoscopy. These include serological antibody against *H pylori*, UBT, and HPSA test. The HPSA test is based on polyclonal and more recently developed monoclonal antibodies against the infectious agent. The United States and European authorities approved the stool antigen tests as a reliable tool in the primary diagnosis and also in the monitoring of posttreatment outcome of *H pylori* infection.[21,22] This test reflects patients with active infection and makes possible the differentiation of the subjects with immunity because of previous infections.[23]

Two noninvasive methods of serology test and UBT were the only ones available until recently. Few articles have addressed the reliability of these tests in patients with kidney failure.[20,24] In the present study, HPSA test had sensitivity and specificity of 87.1% and 93.7%, respectively for *H pylori* screening in hemodialysis patients. Although one study has suggested that the UBT is the most reliable noninvasive test in the general population, it may be less efficient in patients with chronic kidney failure.[25,26] In contrast with previously reported studies in this population,[27] the UBT seems to be the most reliable noninvasive test, with sensitivity and specificity values around 95%.[27] Despite the excellent diagnostic accuracy of the UBT, it requires special instrumentation and specialized staff. Antibody tests, although easy, will remain positive for at least 1 to 2 years following successful eradication. Several noninvasive diagnostic methods based on the detection of fecal antigens of *H pylori* have been developed. At present, their diagnostic accuracy is controversial.[21,28] Three different forms of the test have been commercialized, an enzyme-linked immunosorbent assay using polyclonal antibodies, an enzyme-linked immunosorbent assay using a monoclonal antibody, and a rapid

immunochromatographic test using a monoclonal antibody. To date, only the Premier Platinum HPSA has been tested in patients with chronic kidney failure.[28] Theoretically, the monoclonal-antibody-based stool antigen tests would have better diagnostic accuracy because of increased antigenic specificity. Indeed, sensitivity and specificity of those tests were calculated as 94% and 97%, respectively.[29] Wang and colleagues believed noninvasive stool antigen assay could effectively screen *H pylori* infection and assess success of eradication therapy in hemodialysis patients.[28]

The present study aimed to compare the efficacy of 3 noninvasive methods (serology, UBT, and stool test) as well as to assess the comparative reliability of 2 noninvasive tests for the detection of failure of *H pylori* eradication after treatment, HPSA and UBT. In this context, qualitative enzyme-linked immunosorbent assay polyclonal antibodies were done for determination of the bacterial antigen in human stool. According to the detailed information provided within the commercial kit, both the sensitivity and specificity of the test used in our study were 95%.

In the present study, the HPSA test had sensitivity and specificity of 87.1% and 93.7%, respectively, in *H pylori* screening of hemodialysis patients. Conversely, the use of immunoglobulin G as a screening method for *H pylori* infection proved to be less effective as it showed a sensitivity of 76.6% and a specificity of 71.9% in this study. According to the positive likelihood ratios of HPSA (13.82) and serology test (2.72) and negative likelihood ratio of HPSA (0.13) versus serology test (0.32), it seems that HPSA test is a better diagnostic and also screening test for detection of *H pylori* infection in hemodialysis patients. Also, the reliability of HPSA was compared with UBT in evaluation of *H pylori* eradication after treatment. These results suggested that HPSA test was a good test to detect and prove eradication of *H pylori* infection with a specificity of 93.3% and a positive likelihood ratio of 6.38.

CONCLUSIONS

The present study suggests that the noninvasive test of HPSA perform fairly well in patients undergoing dialysis and is reliable for screening and following up the *H pylori* infection in hemodialysis patients. However, we did not have gastric biopsy specimens to confirm our results. We recommend further investigation of the diagnostic value of HPSA in hemodialysis patients using biopsy results as gold standard.

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CONFLICT OF INTEREST

None declared.

REFERENCES

1. Torres J, Leal-Herrera Y, Perez-Perez G, et al. A community-based seroepidemiologic study of Helicobacter pylori infection in Mexico. J Infect Dis. 1998;178:1089-94.
2. [No author listed]. NIH Consensus Conference. Helicobacter pylori in peptic ulcer disease. NIH Consensus Development Panel on Helicobacter pylori in Peptic Ulcer Disease. JAMA. 1994;272:65-9.
3. [No author listed]. Proceedings of the American Digestive Health Foundation International Update Conference on Helicobacter pylori. McLean, Virginia, USA, February 13-16, 1997. Gastroenterology. 1997;113:S1-169.
4. Zevering Y, Jacob L, Meyer TF. Naturally acquired human immune responses against Helicobacter pylori and implications for vaccine development. Gut. 1999;45: 65-74.
5. Munoz de Bustillo E, Sanchez Tomero JA, Sanz JC, et al. Eradication and follow-up of Helicobacter pylori infection in hemodialysis patients. Nephron. 1998;79:55-60.
6. Sarkio S, Rautelin H, Kyllonen L, Honkanen E, Salmela K, Halme L. Should Helicobacter pylori infection be treated before kidney transplantation? Nephrol Dial Transplant. 2001;16:2053-7.
7. Fong IW. Emerging relations between infectious diseases and coronary artery disease and atherosclerosis. CMAJ. 2000;163:49-56.
8. Hoshina S, Kahn SM, Jiang W, et al. Direct detection and amplification of Helicobacter pylori ribosomal 16S gene segments from gastric endoscopic biopsies. Diagn Microbiol Infect Dis. 1990;13:473-9.
9. Vaira D, Holton J, Menegatti M, et al. New immunological assays for the diagnosis of Helicobacter pylori infection. Gut. 1999;45 Suppl 1:I23-7.
10. [No author listed]. Guidelines for clinical trials in Helicobacter pylori infection. Working Party of the European Helicobacter pylori Study Group. Gut. 1997;41 Suppl 2:S1-9.
11. Coupe MO, Anderson JV, Morris JA, Alstead EM, Bloom SR, Hodgson HJ. The effects of the 5-hydroxytryptamine (5HT3) receptor antagonist ICS 205-930 in the carcinoid syndrome. Aliment Pharmacol Ther. 1988;2:167-72.
12. Gisbert JP, Pajares JM. Diagnosis of Helicobacter pylori infection by stool antigen determination: a systematic review. Am J Gastroenterol. 2001;96:2829-38.

13. Sotoudehmanesh R, Ali Asgari A, Ansari R, Nouraie M. Endoscopic findings in end-stage renal disease. Endoscopy. 2003;35:502-5.
14. Prakash J, Agrawal BK. Upper gastrointestinal mucosal lesions in chronic renal failure. Indian J Gastroenterol. 1991;10:131-2.
15. Jaspersen D, Fassbinder W, Heinkele P, et al. Significantly lower prevalence of Helicobacter pylori in uremic patients than in patients with normal renal function. J Gastroenterol. 1995;30:585-8.
16. Loffeld RJ, Peltenburg HG, vd Oever H, Stobberingh E. Prevalence of Helicobacter pylori antibodies in patients on chronic intermittent haemodialysis. Nephron. 1991;59:250-3.
17. Uemura N, Okamoto S, Yamamoto S, et al. Helicobacter pylori infection and the development of gastric cancer. N Engl J Med. 2001;345:784-9.
18. Nakajima F, Sakaguchi M, Amemoto K, et al. Helicobacter pylori in patients receiving long-term dialysis. Am J Nephrol. 2002;22:468-72.
19. Shousha S, Arnaout AH, Abbas SH, Parkins RA. Antral Helicobacter pylori in patients with chronic renal failure. J Clin Pathol. 1990;43:397-9.
20. Misra V, Misra SP, Dwivedi M, et al. Decreased sensitivity of the ultrarapid urease test for diagnosing Helicobacter pylori in patients with chronic renal failure. Pathology. 1999;31:44-6.
21. Vaira D, Vakil N, Menegatti M, et al. The stool antigen test for detection of Helicobacter pylori after eradication therapy. Ann Intern Med. 2002;136:280-7.
22. Dominguez J, Forne M, Blanco S, et al. Comparison of a monoclonal with a polyclonal antibody-based enzyme immunoassay stool test in diagnosing Helicobacter pylori infection before and after eradication therapy. Aliment Pharmacol Ther. 2006;23:1735-40.
23. Vaira D, Malfertheiner P, Megraud F, et al. Diagnosis of Helicobacter pylori infection with a new non-invasive antigen-based assay. HpSA European study group. Lancet. 1999;354:30-3.
24. Rowe PA, el Nujumi AM, Williams C, Dahill S, Briggs JD, McColl KE. The diagnosis of Helicobacter pylori infection in uremic patients. Am J Kidney Dis. 1992;20:574-9.
25. Huang JJ, Huang CJ, Ruaan MK, Chen KW, Yen TS, Sheu BS. Diagnostic efficacy of (13)C-urea breath test for Helicobacter pylori infection in hemodialysis patients. Am J Kidney Dis. 2000;36:124-9.
26. Megraud F, Brassens-Rabbe MP, Denis F, Belbouri A, Hoa DQ. Seroepidemiology of Campylobacter pylori infection in various populations. J Clin Microbiol. 1989;27:1870-3.
27. Lopez T, Quesada M, Almirall J, Sanfeliu I, Segura F, Calvet X. Usefulness of non-invasive tests for diagnosing Helicobacter pylori infection in patients undergoing dialysis for chronic renal failure. Helicobacter. 2004;9:674-80.
28. Wang YL, Sheu BS, Huang JJ, Yang HB. Noninvasive stool antigen assay can effectively screen Helicobacter pylori Infection and assess success of eradication therapy in hemodialysis patients. Am J Kidney Dis. 2001;38: 98-103.
29. Gisbert JP, de la Morena F, Abraira V. Accuracy of monoclonal stool antigen test for the diagnosis of H. pylori infection: a systematic review and meta-analysis. Am J Gastroenterol. 2006;101:1921-30.

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