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Evaluation of two enzyme immunoassays for detecting *Helicobacter pylori* in stool specimens of dyspeptic patients after eradication therapy

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The aim of the current study was to assess the reliability of two enzyme immunoassays in detecting the *Helicobacter pylori* status of stool specimens of Turkish dyspeptic patients in the post-treatment period. Forty-eight patients with non-ulcer dyspepsia who were positive for *H. pylori* underwent a 1 week regimen of triple therapy. Stool samples of patients were obtained 2 and 6 weeks after eradication therapy and a [^{13}C]urea breath test was performed 6 weeks after therapy in order to assess the reliability of mAb-based (Amplified IDEIA HpStAR, DakoCytomation) and polyclonal-antiserum-based (Premier Platinum HpSA, Meridian Diagnostics) stool antigen test kits and to compare their diagnostic accuracies. Using a minimum cutoff OD_{450} value of 0·19 for Amplified IDEIA HpStAR and 0·16 for Premier Platinum HpSA the sensitivity, specificity, positive predictive value, negative predictive value and diagnostic accuracy of the tests were determined 2 and 6 weeks after completion of eradication therapy. At both the second and the sixth week in the post-treatment period the diagnostic accuracy of Amplified IDEIA HpStAR was significantly better than the Premier Platinum's (75 % versus 50 %, $\chi^2 = 6{\cdot}4$; $P = 0{\cdot}011$, and 90 % versus 69 %, $\chi^2 = 6{\cdot}316$; $P = 0{\cdot}012$, respectively). In light of these findings the mAb-based Amplified IDEIA HpStAR has a high diagnostic accuracy for *H. pylori* infection in Turkish dyspeptic patients 6 weeks after completion of eradication therapy.

INTRODUCTION

Colonization of the human gastric mucosa with *Helicobacter pylori* potentially leads to chronic gastritis and peptic ulcer disease. Additionally, this micro-organism has been identified as a risk factor for the development of mucosa-associated lymphoid tissue lymphoma and gastric carcinoma (Parsonnet *et al.*, 1991), which is estimated to be the world's second most common cancer (Parkin *et al.*, 1988). The gold standard for diagnosis is the growth of the bacterium in culture, which requires invasive procedures for biopsy sampling; however, as most dyspeptic patients initially contact a general practitioner non-invasive testing has become more important.

In primary care a test-and-treat approach is recommended for patients under the age of 45 years with persistent dyspepsia, except those who have predominantly gastro-oesophageal reflux disease symptoms or alarm symptoms such as unexplained weight loss, dysphagia, recurrent vomiting, digestive bleeding or anaemia (Malfertheiner *et al.*, 2002). These authors recommend the diagnosis of infection and monitoring of eradication by urea breath test (UBT) or stool antigen tests if endoscopy is clinically not indicated. Testing after the eradication treatment should be performed in all patients after a minimum of 4 weeks. UBT is a very sensitive and specific method with very high diagnostic accuracy but has a number of disadvantages including the high cost, and the need for trained personnel and specialized instrumentation to obtain and to interpret the breath samples.

As Turkey is a developing country and the prevalance of *H. pylori* infection is as high as 85 % (Us & Hascelik, 1998; Selimoglu *et al.*, 2002), local validation of inexpensive, non-invasive tests in the post-treatment check-up period is an important issue. Thus the aim of the present study was to compare the diagnostic accuracy of two enzyme immunoassays (EIAs), namely Amplified IDEIA HpStAR (formerly the FemtoLab *H. pylori* kit) and Premier Platinum HpSA, for detecting *H. pylori* antigens in Turkish dyspeptic patients' stool specimens after eradication therapy.

Abbreviations: EIA, enzyme immunoassay; NPV, negative predictive value; PPV, positive predictive value; ROC, receiver operator characteristics; UBT, urea breath test.

METHODS

A total of 89 patients who were referred to the endoscopy unit of Istanbul University, Cerrahpasa Faculty of Medicine between June 2003 and March 2004 and proved to have normal upper gastrointestinal macroscopic findings were included. From each patient four antrum and three corpus biopsies were collected for histology (two antrum and one corpus biopsy specimen), rapid urease test (one antrum and one corpus biopsy specimen) and culture (one antrum and one corpus biopsy specimen).

A patient was classified as being *H. pylori* positive if the culture and/or both histology and rapid urease test were positive, and as *H. pylori* negative only if all of these tests were negative. Eleven patients with discordant results were excluded and 58 of the remaining 78 patients (75 %) who were *H. pylori* positive were enrolled in the study and received an eradication treatment consisting of lansoprazole 30 mg b.i.d., clarithromycin 500 mg b.i.d. and amoxycillin 1000 mg b.i.d. for 1 week. Forty-eight of 58 patients (83 %) were available for follow-up for 6 weeks in the post-treatment period and completed the entire course of the trial.

The study group only consisted of patients with non-ulcer dyspepsia who were not treated with antibiotics, bismuth-containing compounds, histamine-2 receptor antagonists or proton pump inhibitors during follow-up. Patients were asked to return their stool samples 2 and 6 weeks after the end of eradication therapy. The stool samples were divided into aliquots, frozen (−80 °C) and stored until tested. Stool specimens were analysed for *H. pylori* antigen using the Amplified IDEIA HpStAR (DakoCytomation) and the Premier Platinum HpSA (Meridian Diagnostics) EIAs as described by the manufacturers. The results were analysed by spectrophotometry (EL 312e, Biotek). OD_{450} values of greater than 0·19 for the Amplified IDEIA HpStAR kit and 0·16 for the Premier Platinum kit were recorded as positive.

In all patients, monitoring of eradication was performed by [^{13}C]UBT (Helicobacter Test INFAI) for comparison using a validated protocol (Cadranel *et al.*, 1998) 6 weeks after the therapy was discontinued. The test was performed after an overnight fast of 12 h. Breath samples were collected in duplicate before and 30 min after ingestion of 200 ml of orange juice and 75 mg of [^{13}C]urea dissolved in 30 ml of tap water. Breath samples were analysed by isotope-ratio mass spectrometry (GV 2003) and results were expressed as 'delta over baseline' (DOB). DOB >4 ‰ was considered positive.

The study was approved by the local ethics committee of our faculty and all patients gave their written informed consent to participate in the study.

Standard methods were used to calculate sensitivity, specificity, predictive values of positive and negative results, and 95 % confidence intervals of these values. The χ^2 and Fisher's exact tests were used to compare sensitivities and specificities, and $P < 0{\cdot}05$ was regarded as identifying a significance. Calculations were performed using conventional software (SPSS 12 for Windows).

RESULTS AND DISCUSSION

A total of 48 patients with non-ulcer dyspepsia who were *H. pylori* positive, (32 female; age, mean ± SD, 37·33 ± 11·42 years, range, 18–62 years) were available for follow-up after eradication treatment. UBT disclosed successful *H. pylori* eradication in 33 (69 %) of the 48 patients.

Using a minimum OD_{450} cutoff value of 0·19 for the Amplified IDEIA HpStAR kit and 0·16 for the Premier Platinum HpSA kit the sensitivities, specificities, PPVs, NPVs and diagnostic accuracies of both tests were determined 2 weeks and 6 weeks after eradication therapy (Table 1). After 2 weeks of eradication therapy, the specificity and diagnostic accuracy of the mAb Amplified IDEIA HpStAR were significantly greater than those of the polyclonal Premier Platinum HpSA ($\chi^2 = 7{\cdot}333, P = 0{\cdot}007; \chi^2 = 6{\cdot}4, P = 0{\cdot}011$, respectively), but the sensitivities, PPVs and NPVs were not statistically different ($P = 1$, $\chi^2 = 2{\cdot}095$; $P = 0{\cdot}148$, $P = 0{\cdot}539$, respectively). At the sixth week in the post-treatment period the sensitivities, specificities, PPVs and NPVs of the tests were not significantly different ($P = 0{\cdot}169$, $\chi^2 = 3{\cdot}264$; $P = 0{\cdot}071$, $\chi^2 = 3{\cdot}142$; $P = 0{\cdot}076$, $P = 0{\cdot}097$, respectively), but the diagnostic accuracy of the Amplified IDEIA HpStAR was significantly superior to the Premier Platinum's ($\chi^2 = 6{\cdot}316$; $P = 0{\cdot}012$). The comparisons of the overall diagnostic accuracies of Amplified IDEIA HpStAR at week 2 (36/48, 75 %) and week 6 (43/48, 90 %) ($\chi^2 = 3{\cdot}503$;

Table 1. Sensitivities, specificities, PPVs, NPVs and diagnostic accuracies of the two stool antigen tests at 2 and 6 weeks post-treatment

Data are presented as % (95 % confidence interval), except where indicated otherwise. PPV, positive predictive value; NPV, negative predictive value.

Test kit	Time post-treatment (weeks)	Sensitivity	Specificity	PPV	NPV	Accuracy	False positive (no.)	False negative (no.)
HpStAR mAb kit	2	93 (81–100)	*67 (51–83)	56 (37–75)	96 (87–100)	†75 (63–87)	11	1
HpSA polyclonal kit	2	87 (69–100)	*33 (17–49)	37 (21–53)	85 (65–100)	†50 (36–64)	22	2
HpStAR mAb kit	6	93 (81–100)	88 (77–99)	78 (59–97)	97 (90–100)	‡90 (81–98)	4	1
HpSA polyclonal kit	6	67 (43–91)	70 (54–85)	50 (28–72)	82 (68–96)	‡69 (56–82)	10	5

*,†,‡ $P < 0{\cdot}05$.

$P = 0{\cdot}061$), and of Premier Platinum at week 2 (24/48, 50 %) and week 6 (33/48, 69 %) ($\chi^2 = 3{\cdot}498$; $P = 0{\cdot}061$) did not reach statistical significance.

The receiver operator characteristics (ROC) analyses of both tests at the second and sixth weeks are shown in Fig. 1(a) and Fig. 1(b), respectively.

Several invasive and non-invasive diagnostic methods have been proposed for detecting *H. pylori* since Marshall and Warren (1984) isolated a *Campylobacter*-like organism from the stomachs of patients with gastritis and peptic ulcer disease. One of the attractive novel concepts is based on the detection of *H. pylori* in faeces. Recently stool EIAs detecting *H. pylori* antigens have been introduced. This method has the advantage of being inexpensive, non-invasive and easy to perform. The first stool antigen test to be marketed was the Premier Platinum HpSA, which is based on reactivity of polyclonal antiserum. Subsequently the mAb-based Amplified IDEIA HpStAR kit became available.

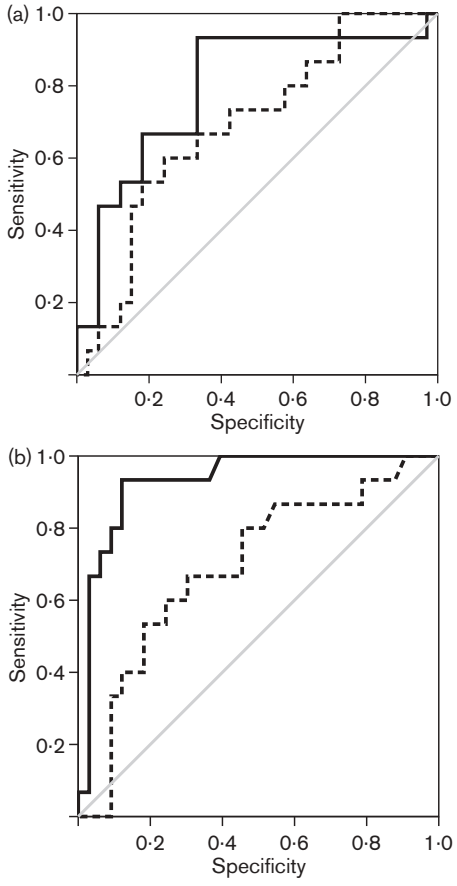


Fig. 1. ROC curve of Amplified IDEIA HpStAR and Premier Platinum HpSA at (a) the second week and (b) the sixth week after eradication treatment for *H. pylori*. Areas under ROC curve: (a) Amplified IDEIA HpStAR, 0·794 (95 % confidence interval; 0·649–0·939); Premier Platinum, 0·689 (95 % confidence interval; 0·531–0·847); (b) Amplified IDEIA HpStAR, 0·93 (95 % confidence interval; 0·855–1); Premier Platinum, 0·693 (95 % confidence interval; 0·53–0·855). Black line, IDEIA HpStAR; dotted line, Premier Platinum; grey line, reference line.

Gisbert & Pajares (2004) carried out a meta-analysis of 39 studies (3147 patients) that evaluated different monoclonal and polyclonal stool antigen tests for the confirmation of *H. pylori* eradication 4–8 weeks after therapy. These authors reported overall accuracies for the monoclonal and polyclonal tests of 86 %, 92 %, 76 % and 93 % for mean sensitivity, specificity, PPV and NPV, respectively. In the same meta-analysis it was mentioned that relatively low accuracy was reported in some post-treatment studies with the polyclonal stool antigen test and, when compared to the polyclonal test, excellent results were achieved in all the six studies evaluating the mAb-based stool antigen tests at 4–8 weeks post-treatment (96 % and 97 % for mean sensitivity and specificity, respectively).

In the current study, 6 weeks after completion of the eradication treatment, the sensitivity and specificity of the mAb-based stool test were better than those of the polyclonal-based test. Despite our small study population (48 patients, 33 of them successfully eradicated thus only 15 *H. pylori*-positive patients) the overall diagnostic accuracy of the Amplified IDEIA HpStAR mAb-based kit at the sixth week was significantly greater than the Premier Platinum polyclonal-antibody-based kit (90 % versus 69 %, $\chi^2 = 6{\cdot}316$; $P = 0{\cdot}012$). This is in keeping with the meta-analysis of Gisbert & Pajares (2004), Makristathis *et al.* (2000), Agha-Amiri *et al.* (2001) and Weingart *et al.* (2004), who all report high sensitivity and specificity of the mAb-based test kit before and after eradication.

Although the Premier Platinum polyclonal-antibody-based stool antigen test is a recommended alternative to the UBT for the primary diagnosis of *H. pylori*, its use in the post-treatment setting is only recommended when the UBT is not available (Malfertheiner *et al.*, 2002) as there are conflicting reports of its efficacy. Several investigators (e.g. Trevisani *et al.*, 1999; Forne *et al.*, 2000; Bilardi *et al.*, 2002) have expressed concern about the specificity of the test after therapy, whereas Vaira *et al.* (2000) and Odaka *et al.* (2002) considered the test useful. The differences in these studies may be attributable to antigenic variation within the samples tested (Makristathis *et al.*, 2000).

The second aim of the present study was to investigate whether the use of stool EIAs was appropriate for the evaluation of eradication outcome as early as 2 weeks after the end of therapy, as a recent study (Odaka *et al.*, 2002) concluded that the polyclonal HpSA test was useful for evaluating eradication success even 2 weeks after the completion of treatment. They found that the sensitivity of the test was 88·9 % versus 88·9 %, and the specificity was 90·9 % versus 97·1 %, 2 and 6 weeks after eradication therapy, respectively. Our results indicate that applying either the mAb- or polyclonal-based test 2 weeks post-therapy results

in a high number of false-positive results and is therefore not an appropriate alternative to the recommended 6 week period (Table 1).

Stool antigen tests are inexpensive, simple and easy to perform, and do not require dedicated equipment; therefore local validation in developing countries is important. Although a major limitation of the present study is the small number of patients studied, in particular as only 15 patients failed eradication, the diagnostic accuracy of the mAb test was significantly better than the Premier Platinum polyclonal-antibody-based kit. We therefore propose that the mAb-based Amplified IDEIA HpStAR should be considered as a useful test for monitoring *H. pylori* status 6 weeks after completion of treatment in Turkish dyspeptic patients.

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