

RESEARCH ARTICLE

Evaluation of the Atlas *Helicobacter pylori* Stool Antigen Test for Diagnosis of Infection in Adult Patients

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Abstract

Background: *Helicobacter pylori* (*H.pylori*) is one of the most important causes of dyspepsia and gastric cancer and diagnosis can be made by invasive or non-invasive methods. The Atlas *Helicobacter pylori* antigen test is a new rapid non-invasive method which is simple to conduct. The aim of this study was to determine its sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy. **Materials and Methods:** This prospective study was conducted between July 2012 and December 2013. Stool samples of 59 dyspeptic patients who underwent upper endoscopy were evaluated for *H. pylori* stool antigen. **Results:** From the 59 patients who participated in this study, there were 36 (61%) males and 23 (39%) females. *H. pylori* was diagnosed in 24 (40.7%) gastric biopsies, 22 (91.7%) of these being positive for the Atlas *H. pylori* antigen test. The sensitivity, specificity, PPV, NPV and accuracy were 91.7%, 100%, 100%, 94.6% and 96.6% respectively. **Conclusions:** The Atlas *H. pylori* antigen test is a new non-invasive method which is simple to perform and avails reliable results in a few minutes. Thus it can be the best option for the diagnosis of *H. pylori* infection due to its high sensitivity and specificity.

Keywords: *Helicobacter pylori*- sensitivity - specificity - Atlas *H. pylori* antigen test

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Introduction

Helicobacter pylori (*H. pylori*) is a Gram-negative microaerophilic bacterium and one of the most common bacterial pathogens of humans that infects more than half of the world's population (Amjad et al., 2010; Zhang et al., 2014). The bacteria has worldwide distribution and the prevalence ranges from 25% in developed countries to more than 90% in developing areas, but not all infected individuals eventually developed the disease (Miernyk et al., 2011; Ghotaslou et al., 2013).

The prevalence of *H. pylori* infection varies widely by geographic area, age, race, and socioeconomic status (Brown et al., 2002). *H. pylori* infection is associated with chronic gastritis, gastric or duodenal ulcer, gastric cancer and MALT-lymphoma (Ben Mansour et al., 2010; Zhao et al., 2012). *H. pylori* was classified as a class I carcinogen in humans by a working group of the World Health Organization International Agency for Research on Cancer (IARC) based on various epidemiological studies (Khalilpour et al., 2013)

There seems to be no firm agreement as to which method should be used as gold standard for the detection of *H. pylori* infection (Redeen et al., 2011). Gastric biopsy based tests which include culture, histology and the rapid urease test (RUT) are considered the standard diagnostic tests (Al-Humayed et al., 2008; Kalem et al., 2010). However, these tests necessitate an upper gastrointestinal endoscopy and are considered invasive tests.

Non-invasive tests include the urea breath tests (UBT) and serology and stool antigen test (Bhewa et al., 2007; Redeen et al., 2011). Urea breath tests and stool antigen test can detect active infection while serology test does not differentiate between active infection and exposure to *H. pylori* (Ricci et al., 2007; Peng et al., 2009).

The choice of a given testing strategy is influenced by sensitivity, specificity, the clinical circumstances and the cost-effectiveness of the test (Peng et al., 2009). In the last years, many studies have focused on noninvasive methods; *H. pylori* stool antigen test provides a simple alternative to the urea breath test and is appropriate for diagnosis and follow-up of infection (Gisbert and Pajares,

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2004; Shimoyama et al., 2009).

The aim of this study was to assess the efficacy of new Atlas *Helicobacter pylori* Antigen Test for the detection of *H. pylori* infection in dyspeptic patients and find its sensitivity, specificity and negative and positive predictive values and accuracy in the diagnosis of *H. pylori* infection.

Materials and Methods

Patients

This is a prospective study conducted at Hospital Universiti Sains Malaysia and Hospital Kuala Lumpur among 59 adult dyspeptic patients between July 2012 to December 2013. The patients were selected from patients who presented with gastrointestinal symptoms at the endoscopy unit of Universiti Sains Hospital, Kubang Kerian, Kelantan and Hospital Kuala Lumpur, Malaysia. After receiving a full explanation of the purpose of the study, each patient gave informed consent and was enrolled into the study.

Gastric antral biopsies were collected for rapid urease test as well as stool sample for the detection of *H. pylori* antigen from stool. The diagnosis of infection was based on the RUT. Patients were considered *H. pylori*-positive when the results of RUT were positive. This test was performed with a homemade solution with 1 mL distilled water, one drop 1% phenol red, and 100 mg urea. One antral sample were placed in the solution and maintained at room temperature. The test was considered positive when the color changed from yellow to red within 24 hours (Pourakbari et al., 2011).

Inclusion and exclusion criteria

Patients were excluded from the study if they had received treatment with antibiotics, proton pump inhibitors, H2 receptor antagonists and bismuth compounds within the last four weeks. Patients with previous gastric surgery, long-term use of corticosteroid and immunosuppressant, a history of bleeding or active gastrointestinal bleeding and diarrhoea were also excluded from the study.

This study was approved by the Human Research Ethics Committee, Universiti Sains Malaysia, Kubang Kerian, Kelantan, Malaysia and National Medical Research Resgistry (NMRR).

Detection of *H. pylori* by Atlas *H. pylori* antigen test (Atlas Medical, UK)

Atlas *Helicobacter pylori* Antigen Test (Atlas medical, UK) is a rapid immunoassay using a monoclonal anti-*H. pylori* antibody on a strip for the detection of *H. pylori* infections in stool specimens. The *Helicobacter pylori* antigen reacts with the conjugate-Pink Red latex particles sensibilized with anti-*H. pylori* monoclonal antibody coated to the membrane of the strip. The formed *H. pylori*-conjugate complex, which migrates upward the membrane by capillarity, binds to the specific antibody molecules fixed to the reaction zone.

The stool is collected in a clean container and the test done as soon as possible or stored at 2-8°C for a longer period of time. The test device and sample are put at room temperature (15-30°C) prior to testing. The

Table 1. Sensitivity, Specificity, Positive and Negative Predictive values, and Accuracy of Atlas *Helicobacter pylori* Antigen test in the Detection of *Helicobacter pylori* Infection Atlas *Helicobacter pylori* Antigen Test (n=59)

Atlas <i>Helicobacter pylori</i> antigen test	
True positive	22
True negative	35
False positive	0
False negative	2
Sensitivity (95% CI)	91.7% (72.9-98.7)
Specificity (95% CI)	100% (89.9-100)
Positive predictive value (PPV) (%)	100% (84.4-100)
Negative predictive value (NPV) (%)	94.6% (81.8-99.2)
Accuracy	96.6

*95%CI=95% confidence interval

test was performed according to the manufacturer's instructions. By using the applicator stick of the provided sample diluent vial, a small portion of stool specimen is transferred into the sample diluent and mixed well by shaking gently. The tip of the vial was broken off and four drops were added to the sample well in the test device.

The test was read after 5 minutes of incubation. A positive test result is indicated by appearance of green band at (control line) and red band in the zone marked T (result line). The sample is considered negative when only one green band (control line) appears in the white central zone of the strip. If no colored bands appear or only one band appears in the T zone the result is regarded as invalid and if an inconclusive result is obtained, the test is repeated with a new strip.

Statistical analysis

The sensitivity, specificity and positive and negative predictive values of the Atlas *Helicobacter pylori* antigen test were calculated against the gold standard for diagnosis of *H. pylori* infection by two by two standard method. Calculations of 95% confidence intervals (CI) were conducted for proportions of these values.

Results

A total of 59 patients, who consisted of 36 (61%) males and 23 (39%) females with a mean age of 51.2±13.3 years and ranging from 26-80 years were recruited into the study.

Out of the total, 24 patients were *H. pylori* positive and 35 were *H. pylori* negative by the gold standard method. Atlas *Helicobacter pylori* antigen test was positive in 22 patients and negative in 35. Thus the sensitivity, specificity, PPV and NPV of Atlas *Helicobacter pylori* Antigen Test were 91.7%, 100%, 100% and 94.6% respectively. The diagnostic accuracy was 96.6% (Table 1).

Discussion

H. pylori is acquired in childhood and survives in the human stomach, the only niche known to date (Tan and Wong, 2011; Valliani et al., 2013). Noninvasive testing for *H. pylori* has been strongly recommended as it is less expensive and more patient-friendly than invasive testing

that requires endoscopy (Manes et al., 2001) and does not need a very complicated laboratory facility.

To the best of our knowledge, this is the first prospective study to find out the efficacy of Atlas *Helicobacter pylori* antigen test in the diagnosis of *H. pylori* infection.

The most important finding of this study was that the new stool antigen test showed high sensitivity (91.7%) and specificity (100%) (Table 1). Our result is similar to other studies done on monoclonal stool antigen test based on immune chromatography, study done In Brazil, found the sensitivity and specificity as 88.0% and 87.5%, respectively (Silva et al., 2010). Similar study done in Turkey showed the sensitivity and specificity of HpSA test as 68.9% and 100% respectively (Ceken et al., 2011). In addition to this, study done in Korea, found the sensitivity and specificity of *Helicobacter pylori* stool antigen immunochromatographic assay (S-ICT test as 84.5% and 96.2% respectively (Jekarl et al., 2013).

The advantages of Atlas *Helicobacter pylori* antigen test over stool antigen test especially HpSA test which has been validated and widely used is that it is easy and takes less than 10 minutes. HpSA test based on enzyme immunoassay tests takes over 1 hour to avail the result, Therefore the new Atlas *Helicobacter pylori* Antigen test is more convenient and saves patient time.

The limitations of this study were small sample size and the fact that we only investigated the performance of the test during initial diagnosis of *H. pylori* infection and not in the post treatment setting.

In conclusion, the Atlas *H. pylori* antigen test is a new non-invasive method which is simple to perform and avails the result in few minutes. Our results have shown that, it has high sensitivity, specificity and diagnostic accuracy and can be used as an alternative method in the diagnosis of *H. pylori* infection in adults. However, there is a need for further studies with a greater number of different patients and to find also its effectiveness in the post treatment setting.

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