

## ORIGINAL ARTICLE

## EVALUATION OF NON-INVASIVE DIAGNOSTIC TESTS FOR *HELICOBACTER PYLORI* INFECTION IN SYMPTOMATIC PATIENTS AND HEALTHY VOLUNTEERS

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**Background:** This study was carried out to determine the *Helicobacter pylori* (*H. pylori*) infection in patients who suffered from symptoms and signs of gastritis and gastric ulcer by using non-invasive techniques and to detect the highly sensitive, specific and accurate diagnostic tests. **Methods:** A total of 100 serum and faecal samples from 70 patients presenting with symptoms of gastritis or peptic ulcer disease and 30 healthy volunteers were tested at King Fahd Hospital, Jazan, Saudi Arabia from September 2010 to March 2011. Samples were cultured for *H. pylori* detection and investigated for presence of *H. pylori* antigen in stool, and stomach, and presence of anti-*H. pylori* antibodies in serum by  $^{14}\text{C}$ -Urea Breath Test (C-UBT) and *H. Pylori* Stool Antigen (HpSA). **Results:** Among the 70 suspected patients, *H. pylori* was isolated from the faecal samples of 60 (85.7%) patients by culture. Results of C-UBT were positive in 64 patients and negative in 29 volunteers. HpSA results were positive in 70 patients and 1 volunteer, and HpSA was negative in 29 volunteers. *H. pylori* (IgG, IgM and IgA) antibodies and the sensitivity, specificity, and accuracy were 98%, 100%, and 99% respectively. **Conclusion:** We present and recommend a technique which has sensitivity and specificity in diagnosing *Helicobacter Pylori*. **Keywords:** *Helicobacter pylori*, C-Urea Breath Test, Stool Antigen Test

### INTRODUCTION

*Helicobacter pylori* (*H. pylori*) bacteria are a 'slow' bacterial pathogens and the name comes from Latin meaning 'spiral rod of the lower part of the stomach'. It was first isolated in 1983 in Australia by Warren and Marshall<sup>1</sup> and was found to be present in patients suffering from type B gastritis. The organisms were originally classified as *Campylobacter* but were subsequently reclassified as a new genus, *Helicobacter*. The *H. pylori* have now been associated with gastritis, peptic ulcers, gastric adenocarcinoma, and gastric mucosa-associated lymphoid type (MALT) B-cell lymphomas. *H. pylori* bacterium is a small microaerophilic, non-sporing, gram-negative, helix-shaped bacterium that is about 3  $\mu\text{m}$  long with a diameter of 0.5  $\mu\text{m}$ . It is a curved rod, with multiple unipolar-sheathed flagella. It has a spiral shape in young cultures and can assume a coccoid form in older cultures. Urease production is a consistent finding in *Helicobacter* species of humans that colonise the stomach but is uncommon in species found in the intestines.<sup>2</sup>

The presence of active *H. pylori* infection can be diagnosed non-invasively with the  $^{14}\text{C}$ -UBT. This test was based on detection of enzyme urease, which is produced by *H. pylori*. Since urease is not present in normal human tissues, and since other urease-producing bacteria do not colonise the stomach, the presence of urease in the stomach can be equated with *H. pylori* infection. In the presence of urease, orally administered  $^{14}\text{C}$ -urea is hydrolysed into ammonia and  $\text{CO}_2$ . This

$^{14}\text{CO}_2$  is absorbed into circulation and exhaled through lungs. The presence of a significant amount of  $^{14}\text{CO}_2$  in the exhaled breath indicates active *H. pylori* infection.

A variety of host factors and bacterial factors contribute to the pathogenesis of gastrointestinal diseases resulting from *H. pylori* infection. It is intensely antigenic and secretes various factors like urease, catalase, mucinase, lipase, haemolysin and alkaline phosphatase that decrease viscosity of mucus. The production of catalase protects the bacteria against the toxic effects of reactive oxygen metabolites formed in neutrophilic medium from hydrogen peroxide. The multiple polar flagella permit them to penetrate the mucus layer. Adherence of *H. pylori* to gastric epithelial cells and vacuolating cytotoxin are the virulence factors as they are associated with degenerative changes in the epithelial cells.<sup>3</sup>

A prospective study carried out in the King Fahd Central Hospital, Gizan reported *H. pylori* in 268 (54.9%) of the gastric biopsies from 488 patients.<sup>4</sup> The purpose of this study was to evaluate non-invasive diagnostic procedure to identify infections by *Helicobacter pylori* in symptomatic patients and asymptomatic healthy volunteers.

### MATERIAL AND METHODS

This study was carried out at King Fahd Hospital, Jazan, Saudi Arabia from September 2010 to March 2011. Seventy patients presenting with symptoms of gastritis or peptic ulcer disease and 30 healthy volunteers who were never had any symptom of peptic ulcer or dyspepsia were included in the study.

**<sup>14</sup>C-Urea Breath Test:**

Detection of <sup>14</sup>C-labeled urea (NH<sub>2</sub> <sup>14</sup>CONH<sub>2</sub>) in breath samples was used to identify the presence of the *H. pylori* bacterium. Testing began with the patient swallowing a gelatin capsule for oral administration containing 1 μCi of <sup>14</sup>C labelled urea with 20 ml lukewarm water. At 3 minutes post-dose, the patient drank 20 ml lukewarm water. At 10 minutes post-dose, the patient was asked to take a deep breath, hold it for approximately 5–10 seconds and then exhale through a straw into a mylar balloon. All timed breath samples, a blank (background) sample (i.e., an identically treated breath sample from a person not receiving <sup>14</sup>C-urea) and a standard (a calibrated <sup>14</sup>C standard added to another blank) were counted for 5–20 minutes in a liquid scintillation counter using a <sup>14</sup>C window.

**Stool antigen test (HpSA):**

A fresh stool sample with approximately the size of a peanut was collected and stored at -20 °C for analysis. *H. pylori* antigen was assessed using an ELISA kit provided by Meridian Diagnostics, Cincinnati, OH, USA. According to the manufacturer’s instructions, the absorbencies were read at 450 nm. Positive and negative controls were included with each batch of samples. A positive test was indicated by a yellow colour, and a negative test was either colourless or faint yellow. This was a qualitative test with a polyclonal rabbit anti-*H. pylori* antibody adsorbed to microwells as capture antibody. OD450<0.140 was considered negative, OD450>0.160 was taken as positive and the value in between was equivocal. Equivocal results were repeated.

**Detection of anti-*H. pylori* IgG, IgM and IgA antibodies by ELISA technique:**

The serum or plasma sample was stored refrigerated at (4–8 °C) for up to 48 hours. For a longer storage they were kept at -20 °C. The samples were not frozen and thawed repeatedly. For the performance of the test, the samples were diluted 1:100 with ready-to-use sample diluent (e.g. 5 μL serum+500 μL sample diluent).

Reaction between the antigen and the antibody results to form an immune-complex. After the incubation time, the wells were washed to separate the unbound components by aspiration and/or decantation. The enzyme linked species-specific antibodies (anti-*H. pylori*-IgG, IgM or IgA) were then added to the microwells. The conjugates were bind to the immune complex that formed. The anti-*H. pylori* -IgG, IgM or IgA enzyme conjugate that binds to the immune complex in a second incubation was separated from un-reacted material by a wash step. The resulting dye was measured using a spectrophotometer at the wavelength of 450 nm. The concentration of the IgG, IgM and IgA antibodies was directly proportional to the intensity of the colour.

**RESULTS**

The suspected bacteria isolated from the faecal samples of 60 from 70 patients with symptoms and signs suggesting gastritis was cultured on chocolate agar plates and incubated for 7 days at 37 °C under microaerophilic conditions. The cultures showed typical morphology of *H. pylori*, they grew slowly, forming grey translucent colonies that looked like spreading fluid droplets. Based on the proposed *H. pylori* status, 70 patients were infected and 30 were not infected. In 71 patients, *H. pylori* antigen was detected in stool samples. On the other hand, 29 were negative by HpSA. There was only one false-negative result, and none of the cases were false positive. None of the stool samples were read as equivocal in the present study. The sensitivity, specificity and accuracy of HpSA were found to be 98%, 100% and 99% respectively (Table-1).

**Table-1: HpSA Compared with *H. pylori* status of the subjects**

	<i>H. pylori</i> Status	Infected (positive)	Non-infected (negative)
HpSA	Symptomatic	70 (A)	0 (B)
	Asymptomatic (Healthy)	1 (C) [false-negative]	29 (D)
	Total	71	29
	Sensitivity (%)		100
	Specificity (%)		96.6
Accuracy (%)		99	

Sensitivity = A/(A+B), Specificity=D/(C+D), Accuracy=(A+D)/(A+B+C+D)

**Table-2: Frequency of anti-*H. pylori* IgG, IgM and IgA antibodies in subjects**

	No.	Anti- <i>H. pylori</i> antibodies					
		IgG		IgM		IgA	
		+ve	-ve	+ve	-ve	+ve	-ve
Symptomatic	70	69	1	66	4	59	11
Asymptomatic	30	2	28	2	28	1	29
Total	100	71	29	68	32	60	40
Sensitivity (%)		98.6		94		84	
Specificity (%)		93		93		96.7	
Accuracy (%)		97		94		88	

**DISCUSSION**

The <sup>14</sup>C-UBT has not been routinely recommended for initial diagnosis but has been recommended to document *H. pylori* eradication following anti- *H. pylori* therapy. In the present study, results of <sup>14</sup>C-UBT were compared with *H. pylori* status. Positive *H. pylori* status and positive <sup>14</sup>C-UBT results were in 64 patients, while negative *H. pylori* status and negative <sup>14</sup>C-UBT results were in 29 individuals; we observed six false-negative <sup>14</sup>C-UBT results, and one false-positive <sup>14</sup>C-UBT result. Therefore, the sensitivity, specificity, and accuracy were 91%, 96.6% and 93% respectively. It was inexpensive and provides rapid results, excellent specificity and very good sensitivity in properly selected patients.

HpSA is suitable to use particularly in developing countries. Detection of *H. pylori* antigens using HpSA showed a high sensitivity and specificity

and might be useful for non-invasive diagnosis of *H. pylori* infection in children and adult patients.<sup>5</sup> HpSA is a newly developed non-invasive EIA. It is not time-consuming and identifies active *H. pylori* infection, excellent positive and negative predictive values regardless of *H. pylori* prevalence. The analytical technique of the immunoassay in stool samples can be performed easily in any laboratory and the faecal specimens can be obtained easily, even in newborn children. Spot samples of the stool were sufficient; homogenisation of the stool is not required.

Non-invasive serological tests have been widely used for the diagnosis of *H. pylori* infection. In adults, this method has proved to be highly accurate to diagnose the infection, but in children, especially younger ones, ELISA appeared to show low sensitivity for the diagnosis of *H. pylori* infection in children aged 2 to 12 years, especially in those without duodenal ulcer. When used in children of different ages, the test presented differences in sensitivity: 44.4% in children 2 to 6 years old; 76.7% in children 7 to 11 years old, and 93.1% in children 12 to 16 years old.<sup>6</sup> Antibodies against *H. pylori* have been detected in serum by bacterial agglutination, complement fixation and by ELISA.<sup>7</sup> Among these methods, the ELISA has proved to be the most sensitive and specific test depending on the nature of the antigen.<sup>8</sup> Several studies using non-commercial assays have indicated that serology for *H. pylori* antibodies were accurate both as a primary diagnostic procedure and in monitoring the success of treatment of *H. pylori* infection.<sup>9</sup> Studies of *H. pylori* associated with gastritis in children by an ELISA or indirect immunofluorescence (IIF) assay found that there were significant decreased in serum IgG and IgA concentrations within 9–12 months after successful therapy.<sup>10</sup> *H. pylori*-specific serum IgG titres were higher than serum specific IgA; the opposite was observed in the saliva samples as anti-*H. pylori* IgA titres were higher than specific IgG titres. In the gastric homogenates, specific IgG and IgA were similar.<sup>11</sup> The present results were in good agreement with the result of other investigators.<sup>12,13</sup>

## CONCLUSION

HpSA is suitable to use particularly in developing countries. Detection of *H. pylori* antigens using HpSA shows a high sensitivity and specificity and might be useful for non-invasive diagnosis of *H. pylori* infection

in children and adult patients. HpSA may be useful particularly in selection of the cases requiring endoscopic examination, in monitoring the response to treatment and in epidemiological studies. We recommend using the stool antigen test as a diagnostic test for *H. pylori* infection.

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