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Validation of ^{13}C-Urea Breath Test for the Diagnosis of *Helicobacter Pylori* Infection in the Singapore Population

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ABSTRACT

Introduction: Several tests are available for determining the presence of *Helicobacter pylori (H. pylori)* infection. These may be invasive or non-invasive. The carbon urea breath test (C-UBT) is generally considered to be a simple, non-invasive and accurate test for the detection of *H. pylori* infection both before and after treatment. Commercially available ^{13}C-UBT kits are generally validated in their country of manufacture and the stated accuracy of their tests may not be applicable to our local population.

Aim: The aim of our study was to determine the accuracy of a commercial ^{13}C-urea breath test kit, *Hp-Plus* (Utandningstester i Sverige AB, Sweden), in the Singapore population.

Patients and methods: One hundred patients for oesophago-gastro-duodenoscopy (OGD) were recruited into this prospective study. Gastric biopsies were obtained for the biopsy urease test and histological examination. Blood samples were obtained for *H. pylori* serology. Breath samples were then obtained at baseline and after consumption of 100 mg of labelled ^{13}C-urea. The presence of *H. pylori* infection was defined by a positive result on any two of the three tests (biopsy urease test, histology, serology) performed for the detection of *H. pylori*. Using this "gold standard", the sensitivity, specificity, and positive and negative predictive values of the ^{13}C-UBT were calculated.

Results: In the Singapore population, the ^{13}C-UBT (*Hp plus*) has a sensitivity and specificity of 94.2% and 100% respectively for the detection of *H. pylori* infection. The positive predictive value and negative predictive value of the ^{13}C-UBT is 100% and 88.6% respectively.

Conclusion: The ^{13}C-UBT is a simple, safe, and accurate non-invasive test for the detection of *H. pylori* infection, making it a valuable tool in local clinical practice.

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INTRODUCTION

The aetiological role of *Helicobacter pylori (H. pylori)* infection in gastroduodenal ulcer disease and gastric malignancies is well recognised. Its role in other gastrointestinal conditions, such as gastro-oesophageal reflux disease and non-ulcer dyspepsia, remains controversial.

Several tests have been developed to diagnose *H. pylori* infection. These range from invasive tests such as histological examination, culture, biopsy urease tests to non-invasive tests such as serology and urea breath tests. No single test is universally accepted as the "gold standard" for the diagnosis of *H. pylori* infection. Problems with invasive tests include the patchy distribution of *H. pylori* in the gastric mucosa, difficulty in culture, and human errors in interpretation. Non-invasive tests such as urea breath tests and serology are global rather than local and more accurately reflect *H. pylori* infection status. The urea breath test is generally regarded as the best non-invasive method for diagnosing *H. pylori* infection[1]. Urea breath tests may utilise either ^{14}C or ^{13}C and there are commercial test kits available for both. The main issue surrounding the use of the ^{14}C-urea breath test is the safety of ^{14}C-urea. Being a radioactive isotope, concerns exist as to its safe handling, administration and disposal as well as the appropriateness of administration to children, pregnant women or women of childbearing age. Regulations governing the use of radioactive material also make its use restricted to selected sites. ^{13}C is a stable, natural, non-radioactive isotope that does not require any special handling. Several ^{13}C-urea breath test kits are commercially available. These ^{13}C-urea breath test kits are generally validated in their country of manufacture and the stated accuracy of their tests may not be applicable to our local population. We studied the accuracy of a commercial ^{13}C-urea breath test kit, *Hp-Plus*

(Utandningstester i Sverige AB, Sweden), in the Singapore population.

PATIENTS AND METHODS

This was a prospective study in which 100 patients for oesophago-gastro-duodenoscopy (OGD) were invited to participate in the study. The study was approved by the Changi General Hospital ethics committee. The patients gave written informed consent. Demographic details, a history of smoking, alcohol consumption and non-steroidal anti-inflammatory drug (NSAID) usage were recorded. Exclusion criteria were recent (within one month) use of antibiotics, bismuth-containing compounds, proton-pump inhibitors and previous resective gastric surgery.

Patients were sedated with intravenous midazolam and endoscopy was performed. Endoscopic findings were recorded. Biopsy specimens were taken from the antrum and corpus of the stomach for histological examination as well as testing with a biopsy urease test kit.

Biopsy Urease Test

Two biopsy specimens, one each from the antrum and corpus, were placed into the yellow CLOtest (Delta West Ltd, Australia) gel. The specimens were examined at three hours and at 24 hours after being kept at the endoscopy room temperature of about 28ºC. Any colour change of the gel from yellow to shades of red was read as a positive CLOtest. The CLOtest has previously been validated in the Singapore population and found to have a sensitivity and specificity of 77% and 96% respectively[2].

Histology

Biopsy specimens were processed routinely, embedded in paraffin wax and stained with haematoxylin and eosin. The specimens were examined by histopathologists who were blinded to the results of the other tests (biopsy urease test, serology and ^{13}C-UBT). *H. pylori* infection was described as present or absent.

Serology

An in-house enzyme-linked immunosorbent assay (ELISA) was used to determine the presence of *H. pylori* antibodies (IgG) quantitatively. This ELISA has previously been validated in the Singapore population[3]. At a threshold for seropositivity at 45 ELISA units, our ELISA had a sensitivity and specificity of 94.2% and 92.3% respectively.

^{13}C-Urea Breath Test (UBT)

A commercial ^{13}C-UBT kit, Hp-Plus (Utandningstester i Sverige AB, Sweden) was used. The ^{13}C-UBT was performed three hours after OGD as it was felt that the clinical effects of any drugs given during OGD would have been negligible by then. Gastrointestinal bleeding can lead to a false positive result and the manufacturer's recommendation was to wait for at least one hour after gastric biopsy before performing the ^{13}C-UBT. The patients were asked to drink a solution containing citric acid as a test meal. Two baseline expired breath samples were then collected. Patients were then asked to drink a second solution containing citric acid and labelled ^{13}C-urea. They were then asked to lie on their left side for 30 minutes. At 30 minutes, two more samples of expired breath were collected.

The samples of expired breath were analysed using the Automated Breath ^{13}Carbon Analyser (ABCA) (Europa Scientific Ltd, UK) which comprises an autosampler, a gas purification module and a mass spectrometer. The ratio of $^{13}CO_2$ to $^{12}CO_2$ in the expired breath samples was measured. Results were expressed as a $\Delta\delta^{13}CO_2$ – value which was defined as the difference in $^{13}CO_2$ in parts per thousand between the baseline pre-^{13}C-urea breath sample and the 30-minute breath sample. The test was considered positive for *H. pylori* infection when the $\Delta\delta^{13}CO_2$ - value was greater or equal to 3.5 parts per thousand.

"Gold standard" definition for the presence of *H. pylori* infection

There is no single test accepted as the "gold standard" for the diagnosis of *H. pylori* infection. Instead, combinations of available tests, both invasive and non-invasive, are used as the "gold standard"[4]. In our study, the presence of *H. pylori* infection was defined by a positive result in any two of the three tests (biopsy urease test, histology, serology) performed for the detection of *H. pylori* infection. Absence of *H. pylori* infection was defined as negative results in two or more of the tests. Using this "gold standard", the sensitivity, specificity, positive predictive value and negative predictive value of the ^{13}C-UBT were calculated.

RESULTS

A total of 100 patients (70 males and 30 females) with a mean age of 45 years (range 21 years to 75 years) were included in the study. Demographic details of the study population are listed in Table I. The most frequent endoscopic finding was that

Table I. Demographic data of patient population.

Gender (male/female)	70/30
Mean age in year (range in year)	45 (21-75)
Race (Chinese/Malay/Indian)	89/5/6
Smoking habit (Yes/No)	69/31
Alcohol consumption (Significant/Insignificant)	89/11
NSAID usage (Regular/Infrequent)	92/8

Table II. Endoscopic diagnoses of patient population.

Duodenal ulcer	34
Gastric ulcer	8
Duodenal ulcer + Gastric ulcer	2
Duodenitis	7
Gastritis	21
Gastroduodenitis	5
Reflux oesophagitis	2
Non-ulcer dyspepsia	12

Table III. Results of tests for diagnosing *H. pylori* infection.

True positive cases

	True positive cases	^{13}C-UBT positive cases
H +, S +, BUT +	62	62
H +, S +, BUT -	6	2
H +, S -, BUT +	0	0
H -, S+, BUT +	1	1
Total	69	65

True negative cases

	True negative cases	^{13}C-UBT negative cases
H -, S -, BUT -	23	23
H +, S -, BUT -	5	5
H -, S +, BUT -	3	3
Total	31	31

Legend: H = Histology, S = Serology, BUT = Biopsy Urease Test, ^{13}C-UBT = ^{13}C-Urea Breath Test

of duodenal ulcers. Table II lists the endoscopic diagnoses of the 100 patients.

The number of true positive cases for *H. pylori* infection was 69. The remainder 31 were considered true negative cases (see Table III). Of the true positives, ^{13}C-UBT was positive in 65 cases (sensitivity = 94.2%) while of the true negative cases, ^{13}C-UBT was negative in all 31 cases (specificity = 100%). The positive predictive value and negative predictive value of the ^{13}C-UBT were 100% and 88.6% respectively.

DISCUSSION

H. pylori infection is a chronic infection that has wide implications for public health, especially in countries such as Japan, Hong Kong and Singapore, where the high incidence of gastric carcinoma contributes significantly to the overall morbidity, mortality and healthcare expenditure of the society. The Asia-Pacific Consensus Conference on the management of *Helicobacter pylori* infection[7] recommended that in countries with a high incidence of gastric cancer, patients with uninvestigated dyspepsia above an age cut-off (depending on the national gastric cancer incidence) should be investigated by endoscopy. In Singapore, this age cut-off is 35 years[6]. It was also recommended that young patients without alarm symptoms should be tested for the presence of *H. pylori* infection using a locally validated non-invasive test. Such a test should have a sensitivity and specificity of 90% or greater. If the patient is found to be positive for *H. pylori* infection, endoscopy should be performed. In those who tested negative for *H. pylori* infection, the probability of missing serious gastroduodenal disease is very low.

The commonly available non-invasive tests for the determination of *H. pylori* infection status are urea breath tests and serological tests. Direct comparisons between UBTs and serology have shown serology to be less accurate in the diagnosis of *H. pylori* infection[7-9]. Serological methods are limited in their usefulness in assessing post-treatment *H. pylori* status as qualitative antibody assays remain positive for up to three years after bacterial eradication. In the follow-up of patients who have received *H. pylori* eradication therapy, serological evaluation is unreliable for up to six months post-treatment[7,10,11]. In order to document cure of *H. pylori* infection serologically, an ELISA-based test of a six-month post-treatment sample and a stored pre-treatment sample would have to be analysed concurrently. This would be impractical. Several studies have demonstrated the efficacy of the ^{13}C-urea breath test in the assessment of *H. pylori* eradication[12,13]. The fact that the ^{13}C-UBT can be used a month after completion of eradication therapy gives it an advantage over serological tests.

The use of labelled carbon urea breath test (C-UBT) was first described in 1987 by Graham DY et al[14]. *H. pylori* produces a powerful urease. In the C-UBT, exhaled breath samples are obtained from the patient before and after the consumption of labelled urea. In the presence of *H. pylori* infection, hydrolysis of the labelled urea by the urease occurs,

liberating labelled carbon dioxide which is then measured. The C-UBT reflects the current bacterial status in patients with *H. pylori* infection. Studies done on Western populations have rated the sensitivity of the ^{13}C-UBT at 90%-92% and its specificity at 96%-100% when compared against biopsy-based tests[4,7,15]. Labelling of the urea can be done with either ^{13}C or ^{14}C. The main disadvantage of ^{14}C is that it is a radioisotope and as with all radioactive material, there are strict regulations relating to the licensing, security, storage, recording and moving of stock. ^{14}C has a half-life of 5.7 years and only 70% of the given dose is excreted, with the remaining 30% being incorporated into the patient's tissue carbon pool[16]. Safety considerations therefore do not permit its use in children or pregnant women and repeated testing should be avoided. ^{13}C is a stable, natural, non-radioactive isotope found in about 1.11% of expired carbon dioxide. Special handling procedures are not required. The small volume of expired breath required for analysis also allows for a test-tube of expired air to be sent by post for commercial laboratory analysis. The non-radioactive nature of ^{13}C allows for the use of the ^{13}C-UBT in children as well as for repeat non-invasive assessments of *H. pylori* status. The main disadvantage of the ^{13}C-UBT is the high cost of the gas chromatograph isotope ratio mass spectrometer (GC-IRMS) which is required to measure $^{13}CO_2$ in expired breath.

In our study, the commercial ^{13}C-UBT kit used (*Hp-Plus*) had a sensitivity and a specificity of 94.2% and 100% respectively in the local Singapore population when validated against a combination of other tests (serology, biopsy urease test and histology). The positive predictive value was 100% while the negative predictive value was 88.6%. This met the standards recommended by the Asia Pacific Consensus on the management of *Helicobacter pylori* infection for a non-invasive test.

CONCLUSION

The sensitivity and specificity of the ^{13}C-UBT for the diagnosis of *H. pylori* infection in the Singapore population is 94.2% and 100% respectively. It is a non-invasive test and the non-radioactive nature of ^{13}C allows for its use in children and pregnant patients. The ^{13}C-UBT is thus a simple, safe and accurate non-invasive test for the determination of *H. pylori* infection status, making it a valuable tool in local clinical practice.

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