VALIDATION OF ^{13}C-UREA BREATH TEST WITH NON DISPERSIVE ISOTOPE SELECTIVE INFRARED SPECTROSCOPY FOR THE DIAGNOSIS OF *HELICOBACTER PYLORI* INFECTION: A SURVEY IN IRANIAN POPULATION

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ABSTRACT

The urea breath test (UBT) which is carried out with ^{13}C or ^{14}C labeled urea is one of the most important non invasive methods for detection of *Helicobacter pylori* infection. Application of ^{13}C-UBT is becoming increasingly popular because of its non radioactive nature which makes it suitable for diagnostic purposes in children and women of child bearing ages. While isotope ratio mass spectrometer (IRMS) is generally used to detect ^{13}C in expired breath, this instrument is expensive and recently non dispersive isotope selective infrared (NDIR) spectroscopy which is a lower cost technique has been employed as a reliable counterpart for IRMS in small clinics. The aim of this study was to assess the validity of NDIR spectroscopy technique in Iranian population in comparison with histological examination, rapid urease test and ^{14}C-urea breath test as gold standard. Seventy six patients with dyspepsia were underwent ^{13}C-UBT for diagnosis of Helicobacter pylori infection. Good agreements were found between the ^{13}C-UBT and gold standard methods. The ^{13}C-UBT showed 100% sensitivity, 97.3% specificity, 97.56% positive predictive value, 100% negative predictive value and 98.65% accuracy. On the basis of these results it could be concluded that ^{13}C-UBT performed with NDIR spectroscopy is a reliable, accurate and non invasive diagnostic tool for detection of *Helicobacter pylori* infection in the Iranian population.

Keywords: *H. pylori*, ^{13}C-urea breath test, Non dispersive isotope selective infrared spectroscopy.

INTRODUCTION

Helicobacter pylori has been recognized as a causative factor in various diseases including gastritis, duodenal ulcer, gastric ulcer, stomach cancer and Malt lymphoma (1-4). Several invasive and non invasive methods have been developed for detection of *Helicobacter pylori* infection. Invasive diagnostic methods require mucosal biopsy during endoscopy, followed by examination of the specimens by culture, rapid urease test or histological tests. Non invasive methods include antibody detection (serology), stool antigen test and urea breath test (5-6). While serology (ELISA) is simple and easy method to perform, it can neither differentiate active from remote infection, nor can it quickly confirm *Helicobacter pylori* eradication after treatment, since it remains positive for a long period despite successful eradication (7-8). Fecal antigen test is a practicable, non invasive alter-native with high sensitivity and specificity (6).

The urea breath test (UBT) is generally regarded as one of the best non invasive methods for diagnosis of *Helicobacter pylori* infection (9). UBT is carried out using ^{13}C or ^{14}C labeled urea. In the presence of *Helicobacter pylori*, the labeled urea is metabolized by urease to yield labeled carbon dioxide which can be detected in the patient's expired breath as a marker of infection. While both isotopes seem to offer similar diagnostic accuracy, ^{14}C-UBT is generally accepted as simpler, faster and cheaper technique. The main issue surrounding the use of ^{14}C-UBT is the safety of ^{14}C-urea. Being a radioactive isotope, concerns exist about its safe handling, administration and disposal as well as appropriateness of its usage in children and pregnant women. In contrast ^{13}C is a non radioactive isotope that can be used safely for detection of *Helicobacter pylori* infection in children and women of child bearing age (10). The most commonly used method for the analysis of $^{13}CO_2$ in expired breath has been application of an isotope ratio mass spectrometer (IRMS) which is an extremely sensitive analytical device that measures $^{13}CO_2$ as the $^{13}CO_2/^{12}CO_2$ ratio (11). However this sophisticated device has the great disadvantage of high cost. The widespread

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demand for ^{13}C-urea breath tests has led to development of cheaper and more practicable analytical systems such as non dispersive isotope selective infrared (NDIR) spectroscopy (10-12, 14-15) and laser assisted ratio analyzer (LARA) (10-13). On the basis of slightly different absorption spectra between $^{13}CO_2$ and $^{12}CO_2$ carbon dioxide molecules the isotope ratio can be determined using non dispersive isotope selective infrared (NDIR) spectrometer. In comparison with IRMS, NDIR spectrometer is a low cost device that preserves the precision of the test, hence there have been a growing number of reports of the ^{13}C-UBT using a NDIR spectroscopy (11). The aim of this study was to assess the validity of NDIR spectroscopy technique in Iranian population in comparison with histological examination, rapid urease test and ^{14}C-urea breath test as gold standard.

MATERIALS AND METHODS

^{13}C-urea was purchased from Campro Scientific, Netherlands. The ^{13}C-UBT was performed using a non dispersive infrared spectrometer (Heli FAN plus, Fischer ANalysen Instrumente GmbH, Germany). Breath samples were collected in aluminum plastic bags (Tecobag) purchased from Tesseraux GmbH, Germany.

Seventy six patients (40 female and 36 male; age of 18-66 years; median age of 39) who were referred for endoscopy because of digestive disorders to Shariati Hospital, Tehran University of Medical Sciences, Tehran, Iran were referred by specialists included in this study. The study was performed in the Research Institute for Nuclear Medicine, Tehran University of Medical Sciences, Tehran, Iran. Females who were pregnant, patients who had a history of gastric surgery and had taken bismuth and antibiotics within the last 4 weeks or proton pump inhibitors within the last 14 days were excluded from the study.

After obtaining written informed consent, all patients underwent upper gastrointestinal endoscopy with antral biopsies as well as ^{14}C-urea breath test within one week (16).

Rapid urease test, histological examination and ^{14}C-UBT were used as gold standard in this study. The microorganism was detected by Haematoxylin and Eosin and modified Giemsa staining. Specimens were examined by expert pathologists who were blinded to all clinical information. Patients were considered to be *Helicobacter pylori* positive (HP+) and negative (HP-) when the results of two of the three tests were in agreement respectively. The ^{13}C-urea breath test was carried out by a previously reported method (12).

After overnight fasting, patients were asked to drink a solution of 75 mg of ^{13}C-urea in 200 ml orange juice. Breath samples were collected before and 30 minutes after drinking of the test solution in aluminum plastic bags with a volume capacity of 300 ml. The bags were immediately connected to the NDIR spectrometer and were analyzed within a few minutes. The results were presented as delta-over-baseline values (DOB) which indicate the change in the $^{13}CO_2$ to $^{12}CO_2$ ratio in the expired breath samples. The test was considered positive for *Helicobacter pylori* infection when the $\Delta\delta^{13}CO_2$ value was greater or equal to 3.5 parts per thousand. The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), accuracy and associated confidence intervals (CI) for ^{13}C-UBT were determined in comparison to the gold standard (17). Statistical analyses were performed with SPSS for windows statistical package (SPSS, Chicago, IL).

RESULTS AND DISCUSSION

A summary of demographic characteristics and endoscopic findings of the patients is presented in Table 1. Based on the gold standard examinations the number of true positive cases for *Helicobacter pylori* infection was 40 in this study and the remainder was considered as true negative cases.

Table 1. Clinical and endoscopic characteristics of the patients from the *Helicobacter pylori* (HP+) and (HP-) groups.

	HP(+)	**(HP-)**
Female	21	19
Male	19	17
Endoscopic Findings		
Normal	10	13
Peptic Ulcer	3	1
Gastritis	8	11
Esophagitis	4	7
Gastritis + Duodenal Ulcer	4	0
Gastritis + Esophagitis	8	3
Gastritis + Duodenal Ulcer + Peptic Ulcer	3	0
Duodenal Ulcer +Peptic Ulcer	0	1

When compared to the gold standard, the ^{13}C-UBT showed only one false positive result, leading to sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy of 100%, 97.3%, 97.56%, 100% and 98.65% (Confidence intervals 95%) respectively (Table 2). In the case of false positive result, the result of ^{14}C-urea breath test was also positive which suggests due to probable sampling bias the invasive methods could have missed the infected patient (18).

Table 2. Diagnostic performance of the ^{13}C-urea breath test for the detection of *Helicobacter pylori* infection.

Sensitivity (CI 95%)	Specificity (CI 95%)	PPV (CI 95%)	NPV (CI 95%)	Accuracy (CI 95%)
100%	97.3%	97.56%	100%	98.65%

PPV= Positive Predictive Value, NPV= Negative Predictive Value, CI= Confidence Interval
The sensitivity, specificity, positive predictive value, negative predictive value and accuracy were calculated according to literature (17).

Different studies have rated the sensitivity and specificity of this test at 90-97.8% and 96-100% respectively (12, 20). Although some studies have demonstrated that NDIR spectrometry is at least as accurate as mass spectrometry (15), some others have rated this technique inferior to IRMS (21), and as a result it is essential to validate this technique prior to extensive clinical application in ethnic population. In the present study, the results of the infrared spectroscopy method were compared with those obtained by a combination of biopsy methods and ^{14}C-UBT in an Iranian population. Our results showed that ^{13}C-UBT with NDIR spectrometry is 100% sensitive and 97.3% specific in comparison with the urease test, histological examination and ^{14}C-UBT grouped together.

CONCLUSION

^{13}C-UBT by NDIR spectrometry was found as a highly sensitive, specific and non invasive method for detection of *Helicobacter pylori* infection in an Iranian population. The low cost and technical simplicity are advantages of this technique, and make the method particularly effective for routine clinical practice.

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