MICRODOSE ^{14}C UREA BREATH TEST FOR THE DIAGNOSIS OF *HELICOBACTER PYLORI*: A SURVEY IN IRANIAN POPULATION

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ABSTRACT

The carbon -14 urea breath test (UBT)is a non-invasive and simple method for the diagnosis of *Helicobacter pylori* infection. Attempts have been made to use lower doses of ^{14}C-urea in the UBT in order to reduce the radiation risk of the test. The aim of this study was to assess the accuracy of a microdose (1 µCi [37 KBq]) ^{14}C-UBT in Iranian population for validation of its diagnostic accuracy against gold standard methods. Eighty and two patients were subjected to upper gastrointestinal endoscopy as well as ^{14}C-UBT in one week. Rapid urease test and histological examinations were used as gold standard. Breath samples were collected 10, 20 and 30 minute after ingestion of 1 µCi of ^{14}C- urea solution and their activities were measured using a scintillation counter and expressed as counts per minute (cpm) and disintegration per minute (dpm). Good agreement was observed between the ^{14}C-UBT and gold standard for samples which were collected 20 minutes after ^{14}C-urea administration. The ^{14}C-UBT showed 100% sensitivity, 95% specificity, 95.45% positive predictive value, 100% negative predictive value and 97.50% accuracy. The results of this study showed good concordance between the ^{14}C-UBT and invasive methods.

Keywords: *Helicobacter pylori*, Urea breath test, Carbon-14, Microdose.

INTRODUCTION

The discovery of the association between *Helicobacter pylori* (HP) and peptic ulcer disease has been considered as a turning point in understanding of upper gastrointestinal (GI) disease (1).

HP is a spiral, gram negative bacterium that has been recognized as a causative factor in gastritis, duodenal and peptic ulcer, gastric adenocarcinoma and MALT lymphoma (2-4). Investigations for simple and accurate methods for diagnosis of HP infection as well as development of various therapeutic regimes for its eradication have been of great concern.

H.pylori has been identified by invasive methods such as histological examination or rapid urease test on biopsy specimens as well as non invasive methods like antibody detection, stool antigen test and urea breath test (UBT). The urea breath test is regarded as one of the best non invasive methods for diagnosis of *H.pylori* infections (5) and is a simple, robust, non invasive, accurate and inexpensive test (6) which is based on the production of high amount of urease by *H.pylori*. When urea labeled with carbon isotope is ingested by a *H.pylori* positive (HP+) subject, it is rapidly broken down in the gastric mucosa into ammonia and labeled carbon dioxide which can be detected in the breath as a marker of infection. UBT is carried out using urea labeled with ^{13}C or ^{14}C. The fundamental difference between ^{13}C and ^{14}C is the non radioactive nature of ^{13}C isotope which makes this stable isotope suitable for use in children and pregnant women but it is much more expensive than ^{14}C-UBT. The main issue regarding the application of ^{14}C urea breath test is the matter of safety. Although the radiation equivalent of a 5 µCi (185 KBq) ^{14}C-UBT is less than that of a chest X-ray and less than %1 of the radiation received during a barium meal (7), attempts have been made to use lower doses of ^{14}C-urea in the UBT (8-9).

The aim of this study was to assess the accuracy of a low dose (1 µCi [37 KBq]) in diagnosing *H.pylori* infection in Iranian population by validating its diagnostic accuracy against biopsy urease test and histological examinations as gold standard methods.

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MATERIALS AND METHODS

Hyamine, liquid scintillation cocktail and thymolphthalein, and ^{14}C-urea were purchased from (Romil,UK), (Sigma, USA) and Iranian atomic energy organization respectively. All the samples were counted on a liquid scintillation counter (Betamatic, Kontron Instruments, UK). Eighty two patients (40 female and 42 male; age of 18-71 years; median age of 41) which referred to the research institute for nuclear medicine of Tehran University of Medical Sciences, Tehran, Iran, were included in this 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.

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 7 days were excluded from the study.

Rapid urease test (CLO test) and histological examination were used as gold standard.

Patients with both tests positive were considered (HP+) and those with both tests negative were considered (HP-). Patients with positive results in either tests were excluded from the study.

After overnight fasting, patients were first asked to brush their teeth and rinse their mouths with tap water, then swallow a solution containing 1 µCi of ^{14}C urea with 50 ml of water and brush their teeth and rinse their mouths again. Breath samples were collected 10, 20 and 30 minutes after ingestion of ^{14}C urea through a disposable drinking straw into a liquid trap connected to the hyamine. This solution was prepared by dissolution of 1 mmol of hyamine in 1 ml of ethanol. Thymolphthalein was added to this solution as a pH indicator at a concentration of %0.1. When saturated with CO_2 the blue hyamine solution became colorless. Ten ml of liquid scintillation cocktail was added to the samples and the mixtures were counted in a liquid scintillation counter. The radioactivity in each vial was expressed as counts per minute (cpm). Based on the measured cpm values and background activity of each sample, the number of disintegrations per minute (dpm) was calculated according to the previously reported method (10). Statistical analyses were performed with SPSS for windows statistical package (SPSS, Chicago, IL). The sensitivity, specificity, positive predictive value, negative predictive value and accuracy were calculated (11).

RESULTS AND DISCUSSION

Based on rapid urease test and histological examination the number of true positive cases for *H.pylori* infection were 45 in this study and the remainder were considered as true negative cases. The endoscopic diagnoses of the patients involved in this study are presented in Table 1 and results of ^{14}C-UBT for samples which were collected 10, 20 and 30 minutes after ^{14}C-urea administration are shown in Table 2.

Scattergrams of dpm values for both (HP+) and (HP-) groups which were obtained at 10, 20 and 30 minutes after ^{14}C-urea ingestion are given in figure 1.

Good agreement was observed between the ^{14}C-UBT and gold standard methods for samples which were collected 20 minutes after ^{14}C-urea administration. Our results were in agreement with previous reports concerning the observation of false positive results for samples which were collected 10 minutes after ingestion of the labeled urea because of contamination of oral flora with *H.pylori* (12) which became negative if samples were collected after 20 minutes (13).

In this study the best time for collection of the expiratory samples was found to be 20 minutes. The cut off value was adjusted at 75 and at this value results of ^{14}C-UBT for samples which were collected 20 minutes after ^{14}C-urea ingestion showed 100% sensitivity, 95% specificity, 95.45% positive predictive value (PPV), 100% negative predictive value (NPV) and 97.50% accuracy.

Reviewing literature regarding the validation of microdose ^{14}C-UBT for detection of *H.pylori* reveals that with exception of one study (20) this test provides very good sensitivity and specificity [90-100% and 76-100% respectively](2, 6, 12-19). While it has been proved that the radiation from ^{14}C-UBT is much lower than that from the widely used diagnostic procedures (7), several attempts have been made for application of microdose ^{14}C-UBT (8-9, 14). One of disadvantages of microdose ^{14}C-UBT is the higher incidences of false positive results in early samples because of the presence of urease producing bacteria in the oropharynx. Late breath sampling may result in false negative results because of emptying urea from the stomach.

In order to overcome this problem several guidelines like brushing the teeth and washing the mouth before and after of ^{14}C urea ingestion (21), co ingestion of a meal to delay gastric emptying (22) and administration of ^{14}C-urea enclosed in a gelatin capsule (9, 13-14, 18, 20) have been proposed. However ingestion of a low dose of ^{14}C-urea enclosed in a gelatin capsule might increase the false negative results and sampling errors because of restriction of the assessment to the lodged area of the capsule in the stomach (16).

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

	HP(+) cases	**(HP-) cases**
Female	21	19
Male	24	18
Endoscopic Findings		
Normal	12	13
Peptic Ulcer	3	1
Gastritis	9	10
Esophagitis	4	7
Gastritis + Duodenal Ulcer	4	0
Gastritis + Esophagitis	9	5
Gastritis + Duodenal Ulcer + Peptic Ulcer	4	0
Duodenal Ulcer + Peptic Ulcer	0	1

Table 2. Diagnostic performance of the ^{14}C- urea breath test for the detection of *Helicobacter pylori* at 10, 20 and 30 minutes after urea ingestion.

Sample Collection Time	Sensitivity	Specificity	PPV	NPV	Accuracy
10 Minutes (%95 CI)	95.24	75	80	93.75	85.12
20 Minutes (%95 CI)	100	95	95.45	100	97.50
30 Minutes (%95 CI)	80.95	90	89.47	81.81	85.45

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 (11).

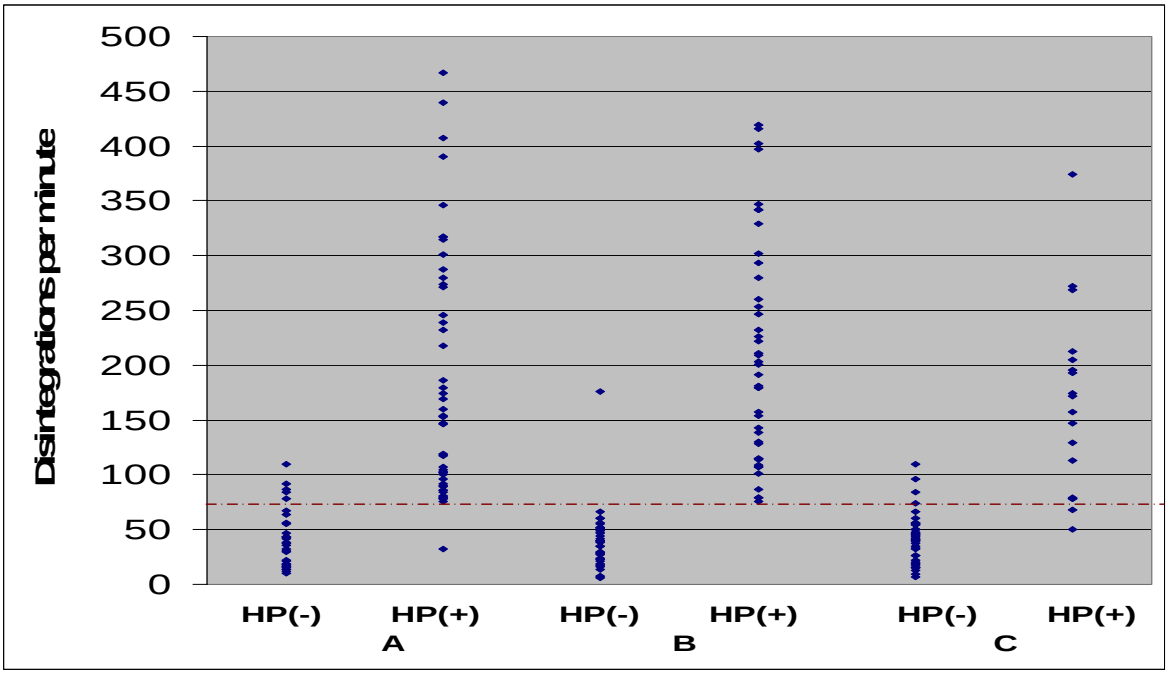

Figure 1. Scattergram of ^{14}C-Urea Breath Test (UBT)counts in 82 patients. A: collected 10 minutes after ^{14}C- uera ingestion, B: collected 20 minutes after ^{14}C-urea ingestion and C: collected 30 minutes after ^{14}C-urea ingestion.

The results of this study show good concordance between the ^{14}C-UBT and gold standard methods. By using appropriate cut off value and sampling time as well as application of suitable technical procedures, ^{14}C- UBT test becomes a rapid, non invasive and accurate method for diagnosis of *H.pylori* infection. Our results are comparable with the studies performed on south East Asian (16), Middle Eastern (21) and Latin American population (17).

CONCLUSION

Microdose 1 µCi (37 KBq) urea breath test is a simple, fast and inexpensive method with negligible radiation burden for diagnosis of *H.pylori* infection. Results of this investigation correlate well with reports of similar studies and indicate the high accuracy of this test.

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