

DIAGNOSTIC ACCURACY OF C-14 UREA BREATH TEST FOR DETECTION OF HELICOBACTER PYLORI IN PATIENTS WITH GASTRITIS

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ABSTRACT

Objective: To evaluate the diagnostic accuracy of C-14 Urea Breath Test for detection of helicobacter pylori infection in patients with gastritis.

Study Design: Cross-sectional validation study.

Place and Duration of Study: Nuclear Medical Centre, Armed Forces Institute of Pathology, from Feb to Aug 2016.

Methodology: After fulfilling inclusion and exclusion criteria, 110 patients of both genders, aged between 18-50 years, were enrolled. Prior written informed consent was obtained from each patient. First, they were assessed by C-14 Urea Breath Test, followed by endoscopic biopsy and histopathology. Results of C-14 Urea Breath Test were compared to histopathology diagnosis which was taken as gold standard.

Results: Patients' ages ranged from 18-50 years with a mean \pm SD of 37.45 ± 10.21 years. Seventy four (67.3%) of them were males and 36 (32.7%) were females. Sixty four (58.2%) patients were suspected of helicobacter pylori on C-14 Urea breath test. However, histopathology of endoscopic biopsy confirmed helicobacter pylori in 66 (60%) patients yielding 64 true positive, 44 true negative and 2 false negative cases. Calculated sensitivity was 96.97%, specificity 100% and accuracy was 98.18% for C-14 Urea Breath Test with negative and positive predictive values of 95.65% and 100% respectively.

Conclusion: C-14 Urea Breath Test is highly accurate, sensitive and specific test for detection of helicobacter pylori infection, irrespective of patient's age and gender.

Keywords: C-14 urea breath test, Endoscopic biopsy, helicobacter pylori, Histopathology.

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INTRODUCTION

Helicobacter pylori (*H. pylori*) is a gram negative spiral-shaped bacterium which thrives in the digestive tract. *H. pylori* infection has a very high prevalence worldwide, and may be found in more than half of the world's population¹. The organism grows in the acidic environment of the gastric mucosa. It has high urease activity which can convert urea present in the gastric mucosa to carbon dioxide and ammonia².

Traditionally *H. pylori* infection is diagnosed with non-invasive as well as invasive techniques. Invasive methods include polymerase chain reaction (PCR), culture and histopathology of the endoscopic biopsy specimen³. However, non-invasive tests are the first line of action for diagnosing *H. pylori* infection. Among these, stool antigen test, serological tests, and urea breath test are the most frequently used⁴.

C-14 labeled urea is administered orally, where it reacts with bacterial Urease enzyme produced by *H. pylori* inside patients' stomach. Radiolabelled urea is hydrolyzed, producing radio labeled CO₂, which is absorbed into the bloodstream and is then exhaled

from the lung. The activity of radiolabeled CO₂ can be measured in the breath sample⁵.

Being highly sensitive and specific as well non-invasive test, Urea Breath Test is very attractive procedure for microbiologists and clinicians for detection of *H. pylori* infections especially in asymptomatic patients where Urea Breath Test is considered a gold standard. Adding to its advantages is the fact that Urea Breath Test is free of sampling errors which are more commonly encountered in case of endoscopic biopsy. This superiority makes it very popular among clinicians, especially in asymptomatic, elderly, and pediatric subjects⁶.

Considering high prevalence of the disease and economic constraint in developing countries like ours, use of C-14 Urea Breath Test is recommended over invasive procedures like endoscopic biopsy and histopathology⁷.

The study objective was to evaluate the diagnostic accuracy of C-14 Urea Breath Test against culture and histopathology of endoscopic biopsy. The rationale behind this study was to evaluate C-14 Urea Breath Test in our setup and to subsequently recommend it as an accurate, non-invasive, sensitive and specific test for *H. pylori* detection in routine management of patients of chronic gastritis.

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METHODOLOGY

This research project was carried out at Armed Forces Institute of Pathology, Rawalpindi's Nuclear Medical Centre, from February to August 2016. Sample size (n=110) was calculated by using WHO sample size calculator with confidence interval 95%, Power of test 80%, level of significance 5%, Anticipation of the population proportion (P0)=0.9 (sensitivity of urea breath test >90%) and prevalence of 50%¹. Through non probability consecutive sampling, 110 patients were included in the study, after fulfilling the inclusion and exclusion criteria. Patients with acid peptic disease, aged 18-50 years, were included in the study that had no history of antibiotic, Proton pump inhibitors or H2 receptor blocker treatment in last 2 weeks. Patients on chronic NSAID, steroid or immunosuppressive therapy were excluded. Patients with any history of gastric procedure in the past were also excluded. Pregnant females were not included in the study.

The study was conducted after approval from hospital ethics committee (Armed Forces Institute of Pathology Ethical Review Committee approval signed 20 Jan 2016). Eligible patients were enrolled in trial after taking informed consent. For C-14 Urea Breath Test, patients were asked to fast overnight. Heliprobe® system was employed for conduction of the test. Patients had to swallow a capsule containing 1 μ CiC-14 labeled urea with 20ml of lukewarm water. After 5 min, patient again had to take 20ml of lukewarm water. At 10 min of radiopharmaceutical administration, the patient was instructed to take a deep breath and hold it for almost 5-10 sec. Patient then exhaled into a Mylar balloon through a straw. Liquid scintillation counter was used to analyze the breath sample in C-14 β particles window. Activity was measured in disintegration per minute (dpm). Patient was declared positive if breath samples had a count rate of 200 dpm and negative when the count rate was <50 dpm. However, the indeterminate cases having count rate of 50-199 dpm were also declared negative for the sake of study. Diagnostic criterion is described in table-I.

Table-I: Diagnostic criteria.

Diagnostic Criteria	
Negative	<50 dpm at 10 min
Indeterminate	<50-200 dpm at 10 min
Positive	>200 dpm at 10 min

Later on gastric endoscopy was performed and 2 gastric biopsy specimen were obtained, one from the antrum and other from corpus. Both of the samples were studied under standard hematoxylin and eosin

and giemsa stains for histopathology. The result was considered positive if *H. pylori* was detected in any one of the stain and declared negative if both specimen showed no growth.

SPSS-20 was used to analyze all the collected data. Numerical variables like age were presented as mean \pm SD. Categorical variables like gender and presence of *H. pylori* on C-14 urea breath test and histopathology were presented as frequency and percentage. A 2 x 2 contingency table was generated to calculate sensitivity, specificity, positive and negative predictive values and accuracy of C-14 Urea Breath Test for diagnosis of *H. pylori* infection keeping the histopathology diagnosis as standard.

RESULTS

A total of 110 patients were included in the study with their ages ranging from 18-50 years having mean \pm SD of 37.45 \pm 10.21 years. Sixty eight (61.8%) patients were aged between 18-34 years while rest of the 42 (38.2%) were aged between 35-50 years. Seventy four (67.3%) of the patients were male and remaining 36 (32.7%) were female.

Sixty four (58.2%) patients diagnosed as positive for *H. pylori* infection on C14 Urea Breath Test were confirmed to be positive on histopathology of the endoscopic biopsy. However, out of 46 (42.8%) patients labeled *H. pylori* negative by C14 Urea Breath Test, 44 were confirmed as negative by histopathology of endoscopic biopsy while 2 were diagnosed as positive for infection. Therefore, 64 cases came out to be true positive, 44 true negative and 2 were false negative. None of the cases was false positive. C14 Urea Breath Test, therefore, yielded 96.97% sensitivity, 100% specificity, 98.18% diagnostic accuracy along with negative and positive predictive values of 95.65% and 100% respectively. The results are shown in table-II & III.

Table-II: Comparison of urea breath test and histopathology.

		Histopathology		Total
		Positive	Negative	
Urea Breath Test	Positive	64 (TP)	0 (FP)	64
	Negative	2 (FN)	44 (TN)	46
Total		66	44	110

Table-III: Diagnostic parameters.

Diagnostic Parameters	Formula	Value
Sensitivity	TP/(TP+FN)	96.97%
Specificity	TN/(TN+FP)	100%
Positive Predictive Value	TP/(TP+FP)	98.18%
Negative Predictive Value	TN/(TN+FN)	95.65%
Diagnostic Accuracy	(TP+TN)/n	100%

DISCUSSION

H. pylori is a gram-negative pathogen living in human gastric mucosa. Without the specific antibiotic treatment, this infection can cause chronic active gastritis which can subsequently leads to peptic ulcer in 15-20% of the patients and gastric malignancy and mucosa-associated lymphoid tissue (MALT) lymphoma in approximately 1-3%. Chronic *H. pylori* infection has been identified as one of the most important etiological factors for gastric cancer which is third leading cause of cancer death globally⁸. Javed *et al*, have reported prevalence of *H. Pylori* infection 92% among patients of gastritis⁹. A recently published systematic review and meta-analysis by Hooi *et al*, classified Pakistan among countries with highest *H. pylori* prevalence (81%)¹⁰.

Presently a lot of methods are employed to detect the presence of *H. pylori*. While all of them have their own limitations, disadvantages and advantages, there is a general division of the available tests into invasive and non-invasive tests. Invasive techniques need gastro-endoscopy and capturing gastric biopsy specimens for histopathology, culture and rapid urease activity. Other category is of noninvasive tests use peripheral samples, like as stools, blood and breath samples to be used in detecting antibodies, urease activity or bacterial antigens¹¹.

Specificity and the sensitivity of histopathology for *H. pylori* diagnosis varies from 53-90%, based upon the density of colonization and pathologist's experience, while that of rapid urease test (RUT) ranges from 95-100% and 85-95% respectively. However, for the detection of *H. pylori*, culture is used as a gold standard method with specificity 100% despite the fact that in various laboratories it has variable sensitivity to a considerable level¹².

For identification of antibodies against *H. pylori*, a huge number of tests have been used. Most of the kits are based on enzyme immunoassay (EIA) and immunoblot (IM) as major immunological techniques. Advantages of serological tests include easy availability, low cost, and easy to perform methodology. They should be given priority in patients with bleeding ulcers, gastric atrophy or those who had recently used antibiotics or proton pump inhibitors¹².

Antigen detection in stool assay is the most recent non-invasive techniques. *H. pylori* antigens that are excreted in stool are detected by ELISA using either monoclonal or polyclonal antibodies¹³. Gisbert *et al*, (2006) evaluated the stool antigen detection technique

and found the specificity and sensitivity of 97% and 94%, respectively¹⁴.

C14 is a β -emitting radioactive isotope of carbon, with maximum β -energy of 156 keV and half-life of 5730 years. It is measured in a liquid scintillation counter. Standard oral dose of 1 μ Cid delivers an estimated radiation dose of <3 μ Sv, which is negligible when compared to routine radiological procedures which, on average produce radiation burden of 5-30 mSv in each study. However, even this meagre radiation dose can be avoided by using newer C-13 based Urea Breath Test. C-13 is a stable isotope of Carbon which is measured by mass spectrometer instead of any scintillation detector¹⁵. Charets *et al*, compared C-13 and C-14 Urea Breath Test and found that sensitivity of C-13 Urea Breath Test ranged from 89-97%, while that of C-14 urea breath test ranged from 89-94%¹⁶. However, other study has shown that C-13 urea breath test provides clearer positive or negative results, with fewer borderline results as compared to C-14 urea breath test¹⁵.

In our study, patients aged from 18-50 years with a mean \pm SD of 37.45 \pm 10.21 years. Aftab *et al*, and Zhou *et al*. have reported similar mean ages, of 34.2 \pm 11.6 years and 36 \pm 11 years, in Bangladeshi and Chinese populations respectively^{17,18}. However, relatively higher mean age has been reported previously by Mehmood *et al*, (45 \pm 6.3 years) and Yakoob *et al*. (41 \pm 13 years) in Pakistan^{19,20}. There were 74 (67.3%) male and 36 (32.7%) female patients in our study with a male to female ratio of 2.1:1. A similar ratio of 2:1 has been reported previously by Yakoob *et al*, in another local study²⁰. Many other authors have also reported male predominance; Mehmood *et al*. (64.9% vs. 35.1%) and Rasheed *et al*. (51.9% vs. 48.1%)^{19,21}.

This study yielded 96.97% sensitivity, 100% specificity and 98.18% accuracy for C-14 Urea Breath Test for the diagnosis of *H. pylori* with negative and positive predictive values of 95.6% and 100% respectively. Pooled results of a recent meta-analysis by Zhou *et al*, comprising 18 studies, indicated that the C-14 Urea Breath Test showed a diagnostic sensitivity of 0.96 (0.95-0.96 at 95% CI) and specificity of 0.93 (0.91-0.94 at 95% CI)²². Özdemir *et al*, also reported 96.6% sensitivity, 100% specificity, 97.7% accuracy, 100% positive predictive value and 93.7% negative predictive values for C-14 Urea Breath Test²³.

Thabit *et al*, reported similar specificity of 100% but with very low sensitivity of 81.6%, as compared to the present study. Rasool *et al*, on the other hand,

reported similar high sensitivity of 98% but very low specificity of 91%²⁴.

CONCLUSION

C-14 Urea Breath test was found to be 96.97% sensitive, 100% specific and 98.18% accurate for the diagnosis of *H. pylori* with negative and positive predictive values of 95.65% and 100% respectively. The diagnostic performance of C-14 Urea Breath Test was unaffected by patient's age and gender. This high sensitivity and specificity of C-14 Urea Breath Test, along with its non-invasive technique and low cost, make it an ideal screening and diagnostic tool.

CONFLICT OF INTEREST

This study has no conflict of interest to be declared by any author.

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