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Original Article

Superiority of non-capsulated ^{14}C-urea breath test over capsule based method for detection of *Helicobacter pylori* infection – a preliminary report

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ABSTRACT

Background: ^{14}C-urea breath test (^{14}C-UBT) is employed as a 'gold standard' technique for the detection of active gastric *Helicobacter pylori* infection and is recommended as the best option for "test-and-treat" strategy in primary health care centers.

Aim: To compare the performance of capsulated and non-capsulated ^{14}C-UBT protocols for the detection of *H. pylori* infection in patients.

Methods: Fifty eight *H. pylori* infected patients underwent routine upper GI endoscopy and biopsies were processed for rapid urease test (RUT) and histopathology examination. Capsulated ^{14}C-UBT was done in a novel way by using 74 kBq of ^{14}C-urea along with 6.0 MBq of ^{99m}Tc-diethylene triamine penta-acetic acid (^{99m}Tc-DTPA) to simultaneously monitor the movement and the fate of ingested capsule after delineating the stomach contour by using 20.0 MBq of ^{99m}Technetium pertechnetate ($^{99m}TcO_4$-) under dual head gamma camera. Non-capsulated ^{14}C-UBT was performed within 2 days of the previous test and the results of these protocols were compared.

Results: In 3 out of 58 *H. pylori* positive cases (5.17%), ^{14}C-UBT results were found to be negative by using the capsulated method. Interestingly, on monitoring the real time images of the capsule in these cases it was found that misdiagnosis of *H. pylori* infection occurred mainly due to either rapid transit of the ^{14}C-urea containing capsule from the upper gastric tract or its incomplete resolution in the stomach during the phase of breath collection.

Conclusion: Use of non-capsulated ^{14}C-UBT protocol appears to be a superior option than the conventional capsule based technique for the detection of *H. pylori* infection.

KEYWORDS: capsulated urea breath test, ^{14}C-UBT, ^{14}C-urea, ^{14}C-urea breath test, *Helicobacter pylori*

Introduction

Helicobacter pylori infection has been incriminated for many diseases of the upper gastrointestinal tract[1] and is also believed to be associated with many other non-gastric diseases.[2] It is estimated that more than half of the adult population in the developed world and more than 80% of that in the developing countries is infected with this bacterium.[3, 4]

Conventionally the ^{14}C-urea breath test (UBT) with its high diagnostic accuracy (>95%) is employed worldwide for the

detection of *H. pylori* infection, for follow-up after eradication of infection and for epidemiological purposes. A large body of data leading to this conclusion has been recently considered by the Maastricht Consensus Report which concurred that UBT is a simple, non-invasive, practical, highly accurate and reproducible test of choice for the confirmation of *H. pylori* infection.[5] The main advantage of this test is that it samples the whole stomach and thus prevents any sampling error because of patchy distribution of these microorganisms in the stomach.[6]

Marshall and Surveyor in 1988[7] introduced a non-capsulated liquid based ^{14}C -UBT for detection of active *H. pylori* infection. To avoid the problem of ^{14}C-urea hydrolysis in the oropharynx owing to the presence of urease producing microorganisms, this test was subsequently modified by Hamlet and his co-workers in 1995.[8] These authors recommended the administration of the tracer (^{14}C-urea) in a gelatin capsule to prevent the release of ^{14}C-urea before reaching the stomach and to avoid overlapping results between infected and non-infected patients. At present majority of diagnostic centers employ the capsulated ^{14}C-UBT protocol[9-11] while others use the non-capsulated protocol.[12,13]

Till date, only two reports have been published in literature which compared the data of capsulated versus non-capsulated ^{14}C-UBT protocols in *H. pylori* infected and non-infected subjects.[8,14] The major limitation of these studies were that the authors did not instruct the subjects to brush their teeth and rinse their mouths before performing the non-capsulated ^{14}C-UBT test, a prerequisite strongly advocated by the earlier workers.[7,15] In addition, the test conditions were not identical to facilitate comparison of these two ^{14}C-UBT protocols.[14] No study so far has been done to monitor the movement of ^{14}C-urea containing capsule after its ingestion and to know the extent of its dissolution in the stomach while collecting the breath samples.

Considering the above lacunae, we developed a novel design for our study by employing a ^{14}C-UBT-cum-scintigraphy technique to simultaneous monitor the real-time movement of the capsule in the upper gastric tract while performing the ^{14}C-UBT. The aim of our preliminary study was to compare the results of capsulated versus non-capsulated ^{14}C-UBT protocols in *H. pylori* infected patients. Our studies demonstrate how sometimes *H. pylori* infection can be misdiagnosed due to either rapid transit of the ^{14}C-urea containing capsule from the upper gastric tract or its incomplete resolution in the stomach during the phase of breath collection after ingestion. In this article we present three cases which were misdiagnosed for *H. pylori* infection using the capsulated protocol but were confirmed on using the non-capsulated method.

Methods

Subjects

Fifty eight patients (41 males; 17 females; mean age 40.05 ± 10.86 years; range 19-71 years) with upper gastrointestinal symptoms referred from Gastroenterology department of Post Graduate Institute of Medical Education and Research, Chandigarh, India were included in this study. Upper GI endoscopy was done and multiple biopsies were obtained from each patient for rapid urease test (RUT) and histopathology examination. None of these patients had taken any drugs that interfered with the UBT results.[16] The Ethics Committee of the Institute cleared the study and each of these patients gave written informed consent.

^{14}C-urea breath test (^{14}C-UBT): Non-capsulated UBT

^{14}C-urea of high specific activity (1.85 GBq/mmol, Amersham, UK) was dissolved in triple distilled autoclaved water, dispensed in small sterilized vials and stored at -20°C till further use.

Non-capsulated ^{14}C-UBT was performed as reported earlier[17] and the patient was prepared for the procedure as described elsewhere.[15] Briefly, overnight fasting patients were asked to thoroughly brush their teeth and rinse their mouths to get rid of oral urease producing commensal flora just before starting the UBT. Each patient was given 74 kBq of ^{14}C-urea (0.4 mL) in 10 mL of water with the instruction to swallow it immediately followed by additional 250 mL of drinking water. One breath sample was obtained before ingestion of ^{14}C-urea (to serve as background) and others at 10, 15 and 20 min directly into a CO_2 trapping solution containing 0.5 mmol ethanolic benzethonium hydroxide in 10 mL toluene-based scintillation fluid along with a drop of alcoholic phenolphthalein as a pH indicator. Breath samples were collected until the color of the trapping solution changed from pink to colorless. Radioactivity in breath samples was measured as disintegration per minute (DPM) mode in liquid scintillation counter (Wallac 1409, Finland). The results were expressed as $^{14}CO_2$/mmol CO_2 exhaled as percent of administered ^{14}C-urea dose and a value

higher than 0.006% at any one time point was considered as an indicator of *H. pylori* infection.[18]

Capsulated UBT

^{14}C-UBT study was repeated within 2 days of the first breath test using 0.5 mL capacity pharmaceutical grade gelatin capsule (M/S Parke Davis, USA). A background breath sample was obtained in trapping solution before starting the ^{14}C-UBT. Each patient was given 74 kBq (0.4 mL) of ^{14}C-urea along with 6.0 MBq (0.1 mL) of ^{99m}Tc-diethylene triamine penta-acetic acid (^{99m}Tc-DTPA) in a capsule. The capsule was prepared in less than 15 sec to maintain its strength. The patient was instructed to swallow the capsule immediately with 250 mL of drinking water. Breath samples were obtained up to 20 min as stated earlier and radioactivity was measured. ^{99m}Tc-DTPA being a non-absorbable marker was used in the capsule to monitor its real time movement in the stomach.[19]

Scintiscanning

Prior to conducting the capsulated ^{14}C-UBT, the stomach contour of each patient was delineated as baseline reference for monitoring the fate of capsule. For this, each overnight fasting patient was initially injected intravenously 20.0 MBq of ^{99m}Technetium pertechnetate ($^{99m}TcO_4^-$). After half an hour static anterior scintiscan images of upper abdomen were obtained for one min on gamma camera (dual head single-photon emission computed tomography; E-Cam, Siemens, Germany) with the patient lying in supine position. Thereafter the patient was administered a gelatin capsule containing ^{14}C-urea and ^{99m}DTPA orally, as stated earlier. Immediately dynamic scintiscanning of anterior aspect of abdomen was done for 23 min and images were acquired at the rate of one frame per min. During acquisition of the images, breath samples were also collected.

Results

Out of 58 *H. pylori* infected cases (confirmed with histological evaluation, RUT and non-capsulated ^{14}C-UBT protocol), 3 (5.17%) were found to be negative for *H. pylori* using capsulated the ^{14}C-UBT protocol. **Table 1** shows discriminate ^{14}C-UBT values with and without encapsulation of ^{14}C-urea in these cases. By employing the non-capsulated protocol, we observed that the breath $^{14}CO_2$ values in these patients were higher than the cut-off value at two or more time points; whereas by using the capsulated method the test values remained low at all the time points.

In this study, we showed real time movement of the ^{14}C-urea containing capsule in the upper gastric tract with scintigraphic imaging using ^{99m}DTPA as a gastric emptying marker. These images revealed that the misdiagnosis of *H. pylori* infection occurred mainly due to either rapid transit of the capsule from the upper gastric tract or its incomplete resolution in the stomach during the phase of breath collection after ingestion.

Figure 1 (A-C) shows the scintiscans of 3 patients in whom discordant ^{14}C-UBT results were obtained by employing the capsulated and non-capsulated protocols. For reference, the anterior contours of the stomach obtained 30 min after iv administration of $^{99m}TcO_4^-$ and before capsule ingestion are shown for each patient in order to exactly monitor the position and status of the ingested capsule. The clinical and ^{14}C-UBT data of each patient is given below:

Case No. 1: BNM, 45 years male, reported with symptoms of dyspepsia. Upper GI endoscopy and antral biopsy revealed hiatus hernia, esophagitis, antral hyperemia, multiple small ulcers in duodenum with presence of *H. pylori* infection. Scintigraphic image frame 3 of **Figure 1A,** shows that the capsule started leaking within 3 min of its ingestion and the tracer (^{14}C-urea) moved out completely from the stomach within 7-8 min. Thus the scintigraphic images clearly demonstrate that there was total absence of ^{14}C-urea in the stomach at the time when breath samples were collected at 10, 15 and 20 min. Hence, the capsulated ^{14}C-UBT results did not show any presence of *H. pylori* infection. On the other hand, non-capsulated ^{14}C-UBT results at 10 and 15 min were clearly indicative of *H. pylori* infection (**Table 1**).

Case No. 2: PK, 46 years male, reported with suspected dyspepsia. Upper GI endoscopy and antral biopsy revealed small gastric ulcer, antral gastritis with *H. pylori* infection. Scintigraphic image frame 2 of **Figure 1B,** shows that the capsule started leaking within 2 min of its ingestion and the tracer (^{14}C-urea) almost moved out completely from the stomach within 3 min which subsequently entered into the intestine leaving the stomach completely free of tracer. The scintigraphic images demonstrate complete absence of ^{14}C-urea in the stomach at 10 min. This resulted in negative ^{14}C-UBT with capsulated UBT protocol; whereas, non-capsulated ^{14}C-UBT results at all time points revealed the presence of *H. pylori* infection (**Table 1**).

Table 1: Discordant ^{14}C-UBT results with and without encapsulation of ^{14}C-urea in three patients

Name	Age/Sex	Endoscopic/biopsy findings	^{14}C-UBT (%$^{14}CO_2$/mmol breath CO_2) (X 10^{-4})					
			Without Capsule			With Capsule		
			10 min	15 min	20 min	10 min	15 min	20 min
BNM	45 M	Hiatus hernia , esophagitis, antral hyperemia, D1- multiple small ulcers, with *H.pylori*	130	140	1	3	4	2
PK	46 M	Small gastric ulcer, antral gastritis with *H. pylori*	140	130	100	14	15	9
DS	59 M	Gastroesophagial reflux disease, inflammation chronic active with *H.pylori*	92	62	53	43	56	43

Cut- off value above 60 X 10^{-4} % $^{14}CO_2$/mmol breath CO_2 was considered as indicative of *H. pylori* infection.

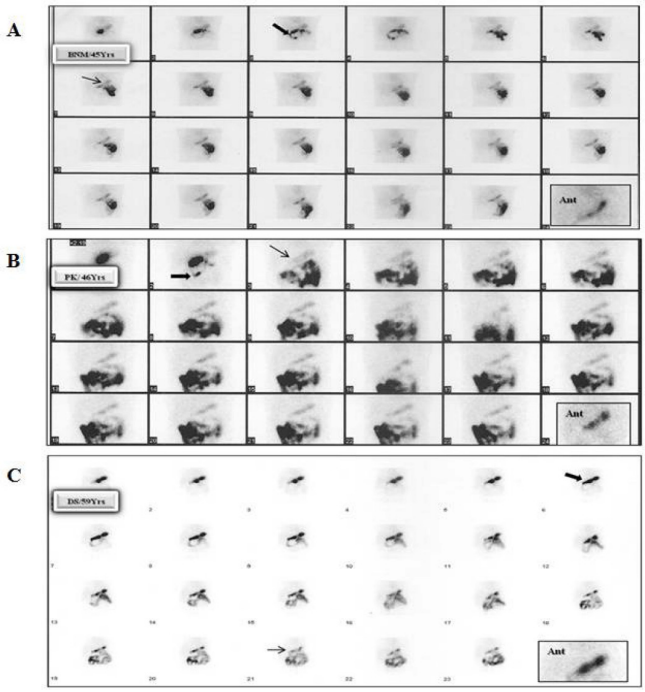

Figure 1: Dynamic scintiscan images (1frame/min) obtained by gamma camera in patients named BNM (A), PK (B) and DS (C) for 23 min after ingestion of capsule containing 74 kBq (0.4 mL) of ^{14}C-urea along with 6.0 MBq (0.1 mL) of ^{99m}Tc-diethylene triamine penta-acetic acid (^{99m}Tc-DTPA). The panels marked Ant in Fig 1 (A-C) shows the static anterior images of stomach contours of the respective patients obtained 30 min after i.v. administration of 20.0 MBq of $^{99m}TcO_4^-$ before capsule ingestion. Block arrow shows the start of leakage of capsular radioactivity from the stomach and the line arrow depicts the empty stomach.

Case No. 3: DS, 59 years male, reported with symptoms of gastro-esophageal reflux disease. Upper GI endoscopy and antral biopsy revealed chronic active inflammation with *H. pylori* infection. Scintigraphic image frame 6 of **Figure 1C** shows that the capsule started leaking at sixth min and its contents started moving towards the duodenum. Subsequently, at the eighth min the entire capsule with its tracer moved out of the stomach leaving only trace amount of the ^{14}C-urea in the stomach, which resulted in generation of low quantity of $^{14}CO_2$ at all time points. Whereas, the non-capsulated ^{14}C-UBT protocol showed exhalation of significant amount of $^{14}CO_2$ especially at 10 and 15 min indicating the presence of *H. pylori* infection (**Table 1**).

Discussion

In this study, we report three *H. pylori* positive cases where we obtained discordant results of ^{14}C-UBT by using the capsulated and non-capsulated protocols and found the non-capsulated method to be a superior option than the capsulated one for the diagnosis of *H. pylori* infection. This is the first study, which provides direct scintigraphy based evidence indicating that the misdiagnosis of *H. pylori* infection can occur mainly due to either rapid transit of ^{14}C-urea containing capsule from the upper gastric tract or its incomplete resolution in the stomach during the phase of breath collection after ingestion.

The capsulated ^{14}C-UBT was introduced to avoid the

problem of ^{14}C-urea hydrolysis in the oropharynx due to presence of urease producing microorganisms and also to prevent the release of ^{14}C-urea before it reaches the stomach.[8] These authors compared the results of capsulated and non-capsulated ^{14}C-UBT protocols in a small number of patients without any consideration of their oral hygiene. They concluded that capsulated ^{14}C-UBT protocol obviates the problem of false positive results in early breath samples and makes it possible to diagnose *H. pylori* infection with 99.8% reliability from a single 10 min breath sample. Further, Lerang et al[14] reported better specificity of capsulated ^{14}C-UBT than the non-capsulated protocol (97% vs. 89%) with equal sensitivity for both (100%). While comparing both the ^{14}C-UBT protocols these authors did not keep the test conditions identical in terms of amount of tracer dose administered, timings of breath collection, expression of results etc. In addition, none of the above mentioned studies monitored the movement and fate of ^{14}C-urea containing capsule after its ingestion.

In the present study, while keeping all the test parameters identical we used a non-absorbable tracer marker (^{99m}DTPA) along with ^{14}C-urea in a capsule that helped us to delineate the exact position and fate of the capsule in the stomach while collecting breath samples for UBT simultaneously.

In 2 of the present 3 discordant cases, the capsule with its contents moved out of the stomach before the first breath sample was obtained at 10 min; whereas in 1 case (DS) traces of ^{14}C-urea leaked from the capsule in stomach which resulted in breath $^{14}CO_2$ level below the cut-off value at all time points despite the fact that the patient had *H. pylori* infection. Thus there was no chance for ^{14}C-urea present in the capsule to come in contact with the *H. pylori* infected site(s) in the stomach yielding false negative tests in these patients. On the other hand, employing the non-capsulated ^{14}C-UBT protocol facilitated ^{14}C-urea to immediately make contact with the entire stomach mucosa thereby initiating prompt hydrolysis of the urea by the *H. pylori* urease enzyme, as has been shown in **Table 1**.

Due to its high accuracy (>95%), the ^{14}C-UBT assay provides a clinical "gold standard" against which the accuracy of other tests for the diagnosis of *H. pylori* infection can be validated.[9,14,20-23] Our preliminary observations showed that for the detection of active *H. pylori* infection, the use of liquid based non-capsulated ^{14}C-UBT protocol appears to be a better option for the patients. However, it is important to validate these findings in a larger cohort of patients before we can draw tangible conclusions for patient management.

Our study concludes that the use of non-capsulated ^{14}C-UBT appears to be a superior option over the conventional capsule based protocol for detection of *H. pylori* infection. Comparative data from a large number of patients needs to be generated further before one can arrive at definitive conclusions.

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