

## Original Research Article

# Use of the ammonia breath test in the diagnosis of *Helicobacter pylori* infection

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### ABSTRACT

**Background:** *Helicobacter pylori* is one of the most common infections in humans with a prevalence of 50% worldwide. Cheap and effective diagnosis is the first step of eradication. The objective of the study was to evaluate the efficacy of the ammonia breath test in the diagnosis of *H. pylori*.

**Methods:** A total of 100 patients with symptoms of gastritis. All patients underwent the ammonia breath test, rapid urease test, and histopathological examination.

**Results:** The ammonia breath test had a sensitivity of 91.04%, a specificity of 87.87%, a positive predictive value (PPV) of 93.84%, and a negative predictive value (NPV) of 82.85%. The rapid urease test demonstrated a sensitivity of 85.07%, a specificity of 93.93%, a PPV of 94.61%, and an NPV of 75.6%.

**Conclusions:** The ammonia breath test is a cheap and viable alternative in the early diagnosis of *H. pylori* infections.

**Keywords:** *Helicobacter pylori*, Rapid urease test, Ammonia breath test

### INTRODUCTION

*Helicobacter pylori* are one of the most common infections in humans, with a prevalence of 50% worldwide and up to 80% in developing countries. It is a gram-negative helical organism that causes gastric inflammation and is one of the most important risk factors for gastritis, gastric ulcers, duodenal ulcers, and gastric carcinoma. Symptomatic (peptic ulcers, gastritis, dyspepsia, mucosa-associated lymphoid tissue (MALT) lymphoma and early gastric cancer).<sup>1,2</sup> Patients should undergo testing, and if positive, documented complete eradication of *H. Pylori* eradication decreases the risk of peptic ulcer disease and gastric cancer testing for *H. pylori* comes in several forms, both invasive and noninvasive.<sup>3,4</sup> We aimed to evaluate the noninvasive ammonia breath test as an alternative to better-established tests, especially in environments sensitive to cost and

with a deficiency of skilled labor. We compare the commonly used rapid urease test and the noninvasive ammonia (pH paper) breath test to the gold standard histopathological examination in our study.

### METHODS

We randomly selected a series of 100 patients who underwent upper gastrointestinal endoscopy at our hospital, Vinayaka Mission's Kirupananda Variyar Medical College and Hospital, Salem between November 2017 and November 2019.

#### *Inclusion criteria*

Ages eligible for study, 18 years to 75 years (adults, seniors), gender women and men symptoms of gastritis (pain in the epigastric region and/or abdomen, burning

chest pain, dysphagia, odynophagia, dyspepsia, regurgitation, bloating, belching, nausea, vomiting) were included.

**Exclusion criteria**

Non-consenting patients; age<18 or>75 years; abnormal coagulation profile; severe anemia (hemoglobin level<6 g/dl); severe systemic disease or advanced chronic liver disease; use of *H. pylori* regimen, proton pump inhibitors, H2-receptor antagonists, or antibiotics within 30 days of presentation to OPD. History of *H. pylori* eradication within 30 days of the test; history of gastric surgery; patients diagnosed as carcinoma stomach were excluded.

**Methodology**

This analytical study was conducted after getting clearance from the institutional review board and informed consent from all patients included in our study. All patients underwent three investigations; the rapid urease test (RUT), ammonia breath test, and histopathological examination. Each patient underwent upper gastrointestinal endoscopy in which multiple tissue biopsies were taken from the antral and prepyloric regions. A sample is placed on the RUT kit and one drop of distilled water is added. The RUT kit is resealed and the result is read 24 hours later. Another sample is sent to the pathologist for histopathological examination. Following the procedure, the patient is asked to drink a supersaturated solution of edible urea (5 mg of urea dissolved in 10-20 ml of water), and wait for 10 minutes. The patient is then asked to gargle chlorhexidine and exhale over wet litmus paper through the mouth. The color change (which signifies a positive result) is read 10 minutes later.

**Statistical analysis**

All the data were entered and analyzed using SPSS version 22. Mean and standard deviation was derived for all the parametric variables and the percentage was derived for frequency variables. Chi-square test and Man-Whitney U test are the non-parametric tests used for deriving the statistical inference.

**RESULTS**

Table 1 shows hundred tested. Before urea ingestion, *H. pylori*-positive subjects had significantly lower breath ammonia levels than negative subjects (mean±SD, 0.04 ppm±0.09 vs 0.49 ppm±0.24, P=0.002) and had a significantly greater increases in breath ammonia after urea ingestion (range 198-1494% vs 6-98%). Total 67% of our study population to be positive for *H. pylori* infection as per histopathological examination

Table 2 shows our study found that the reliability of the RUT and the ammonia breath test were broadly comparable. The RUT demonstrated a sensitivity of

85.07%, a specificity of 93.93%, a PPV of 94.61%, and an NPV of 75.6% while the ammonia breath test had a sensitivity of 91.04%, a specificity of 87.87%, a PPV of 93.84% and an NPV of 82.85%.

**Table 1: Distribution of study participants according to HPE results (n=100).**

HPE	Frequency	Percentage (%)
Positive	67	67
Negative	33	33
Total	100	100

**Table 2: Rapid urease test and ammonia breath.**

Variables	RUT (%)	Ammonia breath test (%)
Sensitivity	85.07	91.04
Specificity	93.93	87.87
PPV	94.61	93.84
NPV	75.60	82.85

Table 3 shows the rapid urease test to have 57 true positives, 2 false positives, 10 false negatives, and 31 true negatives out of study population of 100 patients.

**Table 3: Sensitivity & specificity analysis of RUT.**

RUT	HPE		Total
	Positive	Negative	
Positive	57	2	59
Negative	10	31	41
Total	67	33	100

Table 4 shows the ammonia breath test to have 61 true positives, 4 false positives, 6 false negatives, and 29 true negatives out of study population of 100 patients.

**Table 4: Sensitivity & specificity analysis of ammonia breath test.**

Ammonia breath test	HPE		Total
	Positive	Negative	
Positive	61	4	65
Negative	6	29	35
Total	67	33	100

**Table 5: Cohen's kappa correlation.**

RUT	Ammonia breath test		Cohen's Kappa	P value
	Positive	Negative		
Positive	51 (86.4)	8 (12.6)	0.535	<0.001
Negative	14 (34.1)	27 (65.9)		

Table 5 shows a moderate agreement was found between the RUT and the ammonia breath test. Cohen's kappa

was 0.535 and the Chi-square test showed significant association with a p value of <0.001.

## DISCUSSION

During the past 3 decades, the incidence of colorectal the urea breath test is a rapid diagnostic procedure used to identify infections by *Helicobacter pylori*, a spiral bacterium implicated in gastritis, gastric ulcer, and peptic ulcer disease. It is based upon the ability of *H. pylori* to convert urea to ammonia and carbon dioxide. Conventional means of diagnosis such as endoscopic biopsy, the rapid urease test, and the 13C or 14C labeled urea breath test, while proven to be reliable and accurate, present their challenges in cost and skill sensitive environments and are contraindicated in children or pregnant women.<sup>5</sup> The ammonia breath test is a newer, low-cost noninvasive alternative, and functions by detecting the change in pH that occurs due to the release of ammonia due to the action of urease.<sup>6</sup> This has not received much study in humans.<sup>7,8</sup> Our study shows the Ammonia breath test to be highly sensitive and specific for *H. pylori* infection in the population studied. Furthermore, the results agree broadly with those of the RUT.<sup>9</sup> We propose that the high sensitivity when taken together with the low cost and skill required to administer the test makes the ammonia breath test an ideal candidate for a screening test.<sup>10</sup> Possible challenges include false positives due to other urease producing bacteria, such as those in the oropharynx, and false negatives due to emptying of urea from the stomach.<sup>11,12</sup> Other confounders include pH changes due to mucosal changes, acid secretion, alcohol consumption, NSAID intake, and variance in the time of breath sampling.<sup>13</sup> Procedures to mitigate these potential failure modes such as mouth washing, having a meal to delay gastric emptying, and the use of multiple samples have been suggested and require further investigation.<sup>14</sup> Another potential modification is the use of a urea capsule with an enteric coating to minimize the risk of false positives due to urease producing bacteria in the mouth.<sup>15</sup>

## CONCLUSION

The ammonia breath test, though it is quite simple and has several failure modes, remains one of the cheapest and easiest to administer in a developing country like ours. We believe that the reliability of the test can be improved with further study. Use of modifications such as a urea capsule with an enteric coating, and the establishment of a strict procedure including preparatory steps like a mouthwash and gastric emptying, and procedural steps like the time of measurement and the number of samples have the potential to improve results. We envision the ammonia breath test as a quick and easy screening test, to be used for cheap and rapid diagnosis in resource-sensitive environments, in the field, or as part of *H. pylori* eradication campaigns. The results of the test can be used for early initiation of further diagnostic workup or anti-*H. pylori* therapy. Attempts to find urease

inhibitors to use to cripple the organism and aid in treatment are ongoing. Countrywide *H. pylori* eradication programs are being developed as a strategy to eliminate gastric cancer. Urease inhibitors and/or vaccines which include urease antigens are likely to play an important role in these endeavors.

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