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### DIAGNOSTIC ACCURACY OF RAPID UREASE TEST FOR THE DIAGNOSIS OF *HELICOBACTER PYLORI* IN GASTRIC BIOPSIES IN NIGERIANS WITH DYSPEPSIA

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Running title: DIAGNOSTIC ACCURACY OF RAPID UREASE TEST

#### ABSTRACT

**Background:** The strong association of *Helicobacter pylori* (*H. pylori*) with dyspepsia has caused a major paradigm shift in patients' management. It has been observed that histology is usually employed as the routine test for the diagnosing *H. pylori* in centres where Oesophagogastroduodenoscopy (OGD) is available in Nigeria. Because of the drawbacks attendant to the use of histology, in terms of cost effectiveness and technical expertise, it is necessary to evaluate the diagnostic accuracy of a simpler alternative for ease of management of patients with dyspepsia.

**Objective:** This study evaluated the diagnostic accuracy of rapid urease test (RUT) in the diagnosis of *Helicobacter pylori* (*H. pylori*) in patients with dyspepsia.

**Methods:** Eighty-six consecutive adult patients with dyspeptic symptoms presenting for endoscopy were recruited after giving informed consent. Gastric antral biopsies were collected at endoscopy for RUT and histology. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) of RUT was calculated using histology as the reference standard.

**Results:** Of the 86 subjects, there were 39 (45.3%) males and 47(54.7%) females. The mean age was 49.19±13.75 years. The age range was 23 to 85 years. The sensitivity, specificity, PPV, NPV of RUT was 93.33%, 75.6 %, 80.76 %, and 91.17 % respectively.

**Conclusion:** RUT is accurate for the diagnosis of *H. pylori* infection. Its use will serve as a good alternative to histology in management of patients with dyspepsia in resource poor environments, except in patients who need histology for reasons other than *H. Pylori* diagnosis.

**Key words:** *Dyspepsia, Helicobacter pylori, Rapid Urease test, Histology.*

#### LIST OF ABBREVIATIONS

H&E	Haematoxylin and Eosin	OGD	Oesophagogastroduodenoscopy
<i>H. pylori</i>	<i>Helicobacter pylori</i>	PPV	Positive predictive value
IgG	Immunoglobulin G	RUT	Rapid urease test
NPV	Negative predictive value		

#### INTRODUCTION

*Helicobacter pylori* is a gram negative, spiral, flagellated bacterium with the capability of abundant urease production. *H. pylori* bacterium is usually found under the mucus layer in the gastric pits and in close apposition to gastric epithelial cells (1). Since the discovery of *H. pylori* by Warren and Marshall (2), it has been evidently demonstrated that the organism plays a major role in several upper gastrointestinal diseases which present as dyspepsia (2- 4). *Helicobacter pylori* infection causes chronic active gastritis in the antrum (antral gastritis), the corpus (corpus gastritis) or in both (pangastritis). It is a major aetiological factor in peptic ulcer disease, gastric carcinoma, and gastric mucosal associated lymphoid tissue (MALT) lymphoma (2, 5, 6). Haemorrhage and perforation

are the most frequent complications of peptic ulcer disease and are associated with substantial morbidity, mortality and health care costs (6). Peptic ulcer disease can be cured by eradicating *H. pylori* so that complications no longer occur (1).

There are various diagnostic tests for *H. pylori* which can be broadly classified into invasive and non-invasive tests (7). Invasive tests utilise endoscopic gastroduodenal biopsy samples for histology, culture, rapid urease test (RUT), polymerase chain reaction and fluorescent in-situ hybridization. The non-invasive tests do not require endoscopy; they include urea breath test, immunoglobulin G, A and M serology, stool antigen test, saliva antibody test (8, 9) and urinary antibody test (10). In Nigeria, the non-invasive tests are not generally available with

the exception of IgG serology. Serological screening is of limited value, especially in a hyper-endemic area like Nigeria, because it has low discriminatory the routine test for diagnosing *H. pylori*. There are relatively few histopathologists in Nigeria, and they are usually concentrated in government owned Teaching Hospitals located in urban centres. Histology report on gastric mucosal biopsies for *H. pylori* usually takes two weeks or more to be available while RUT results for *H. pylori* could be read within 5 minutes to 24 hours of test (11). The RUT is also much cheaper compared to histology for *H. pylori* diagnosis, as the test is carried out in the endoscopy suite by the Endoscopist or an assistant. There has been a recent modest increase in the availability of gastrointestinal endoscopic facilities and it is envisaged that more centres with no histopathology services will begin to have such facilities as expertise increases in Nigeria. In view of the foregoing advantages of the RUT, and the generally low socioeconomic status of majority of the Nigerian populace, it is desirable to evaluate RUT diagnostic accuracy using histology as the reference standard.

#### MATERIALS AND METHODS

The study was carried out at the Endoscopy sub-unit of the Gastrointestinal & Liver Unit, Department of Medicine, University College Hospital, Ibadan, Nigeria. Ethical approval was sought and obtained from the Joint University of Ibadan/ University College Hospital Institutional Review Committee. Eighty- six consecutive adult patients with dyspeptic symptoms presenting for OGD were recruited after giving informed consent. Diagnosis of dyspepsia was made clinically according to the Rome working teams' definition (12). Patients' symptoms had persisted for a minimum of 3 months or recurrent in nature for the same period. Those who were previously treated for *H. pylori* infection or who had received antibiotics, proton pump inhibitors or bismuth compounds in the preceding 4 weeks were excluded. Base line bio-data were obtained.

OGD was performed on all the participants using Olympus (GFI-XQ20) or Pentax (FG29W) forward-viewing oesophagogastroduodenoscopes. All patients had pharyngeal spray with 2% xylocaine. Most of the patients had conscious sedation with intravenous diazepam and pethidine or pentazocine. All were monitored with multiparameter pulse oximeter(EDANinstruments).

power between previous and current infection. Centres that have OGD usually employ histology as

Two gastric antral mucosal biopsies were taken for each of RUT and histology.

#### Rapid urease test (RUT)

Two of the four antral biopsies taken from each patient were used immediately for RUT. The RUT consisted of two dry filter paper containing urea, phenol red (a pH indicator) in a sealed plastic slide. If the urease enzyme of *H. pylori* was present in an inserted tissue sample, the resulting decomposition of urea to CO<sub>2</sub> and NH<sub>3</sub> caused the pH to rise and the colour of the dot turned from yellow to a bright magenta. Results were read within 3 hours after sampling according to the manufacturer's specification. The colour change from yellow to magenta was considered a positive result while no colour change was regarded as negative.

#### Histology

The other two antral biopsies were fixed in 10% formaldehyde and transferred to the histopathology laboratory for processing. Four micron thick paraffin sections were stained with routine Haematoxylin and Eosin (H&E) for detection of *H. pylori* and gastritis. Giemsa stain which is a special histochemical stain for *H. pylori* was also used for better yield. Slides were examined microscopically for *H. pylori* by the help of a Gastrointestinal Pathologist. The presence of submucosal helical (*Helicobacter-like*) organisms was regarded as positive while its absence was regarded as negative.

#### Data analysis

Data was analyzed using Statistical Package for Social Sciences, version16.0 (SPSS Inc. Chicago Illinois). Results were presented as means ± standard deviation for quantitative variables and number (percentages) for qualitative variables. Sensitivity, specificity, positive and negative predictive values of RUT was calculated by two by two standard method.

#### RESULTS

- A total of 86 adult patients with dyspepsia participated in the study. The mean age was 49.19 (±13.75) years. There were 39 (45.3%) males and 47(54.7%) females. The minimum age was 23 years and the maximum was 85 years. The results of the diagnostic tests are shown in table 1. The RUT was positive in 52 (60.5 %), while histology was positive in 45(52.35%).

TABLE1: RESULTS OF RAPID UREASE TEST AND HISTOLOGY IN SUBJECTS

Test	Frequency(n=86)	
	Positive	Negative
Rapid urease test	52 (60.5 %)	34(39.5 %)
Histology	45(52.3%)	41(47.7%)

**TABLE 2: COMPARISON BETWEEN RUT AND HISTOLOGY**

	Histology (n=86)		Total
	Positive	Negative	
<b>Rapid urease test</b>			
+ve	42	10	52
-ve	3	31	34
<b>Total</b>	<b>45</b>	<b>41</b>	<b>86</b>

**TABLE 3: DIAGNOSTIC ACCURACY OF RUT**

VARIABLE	VALUE %	95% C. I.*
Sensitivity	93.33	81-98
Specificity	75.6	59-87
PPV	80.76	67-90
NPV	91.17	76-98

\*C. I. = Confidence Interval

Table 2 shows the comparison between results of RUT and histology. The total number of those who were both positive for RUT and histology (true positive) was 42(48.84%), those who were positive for RUT but negative for histology (false positive) were 10(11.62%), those that were both negative for RUT and histology (true negative) were 31(36.04%), while those that were positive for histology but negative for RUT (false negative) were 3 (3.49%).

The sensitivity, specificity, PPV and NPV of RUT were 93.33%, 75.6 %, 80.76 %, and 91.17 % respectively (table 3).

## DISCUSSION

The diagnosis of *H. pylori* by culture, gram stain and histology, which are biopsy based methods, is well established. However, several drawbacks attend to them. Firstly, the delay in the availability of results, and secondly the rarity of good microbiology and histopathology laboratory support especially in developing countries like Nigeria. Other problems associated with histological diagnosis of *H. pylori* arise because the result depend on the competence of the pathologist, the time spent to examine the slides (inter-observer variability) and the variability of staining techniques (11, 13). Special stains for biopsy specimens improve visual detection of the bacteria. To mitigate these problems in our study, the service of a Gastrointestinal Pathologist was employed and Giemsa stain was used in addition to routine H&E. Giemsa stain is the most frequently used stain for *H. pylori* diagnosis in routine clinical

practice, because of its diagnostic performance and lack of technical difficulty in preparation in comparison with the other stains (13, 14). A major advantage of histological examination over other biopsy based methods is that it also provides information about gastric mucosal pathology.

The RUT practically overcomes these drawbacks since it is not dependent on the experience and accuracy of individual laboratories as in the case of other biopsy based methods. As usefully and attractive as RUT is, it has its own draw backs. In theory, patients that salivate or have reflux alkaline bile into the stomach could have a weak positive reaction because the liquid may contaminate a small gastric biopsy specimen such that the resulting surface pH is >6.0 (6). Similarly, chronic use of proton pump inhibitor may lead to achlorhydria and subsequent superficial colonisation of the gastric mucus layer with urease-producing organisms e.g. *Klebsiella* or *Proteus mirabilis* (1, 6). These organisms can give a false-positive urease test after 24 hours of inoculation but generally negative tests remain so when read within the specified time by the manufacturer (6, 15).

It has been shown that the use of proton pump inhibitors increases the numbers of false-negative tests (16). Two possible mechanisms by which this is done have been identified. Firstly, the medication may directly inhibit *H. pylori* urease activity. Secondly, the changing patterns of *H. pylori* colonisation after acid suppression may delay the positivity of RUT. *Helicobacter pylori* often only resides in the corpus during long-term use of proton pump inhibitors and can therefore not be detected in antral biopsies (16). The problem of chronic proton pump inhibitor use was avoided in our study by excluding patients who were on the drug in the preceding four weeks to the test.

The presence of blood may also adversely affect the performance of RUT leading to a false negative result. This is due to the buffering effect of serum albumin on the pH indicator, rather than by a direct inhibition of the urease activity.

Rapid urease tests have specificity and sensitivity of greater than 90%, but false-positive results do occur (17). The RUT had a sensitivity and specificity of 97.4 and 96.1% respectively in an earlier study conducted by van Keeken *et al.* (6) in the Netherlands. The relatively lower values obtained in our study compared to that of van

Keeken *et al.* and other previous similar studies (13,18, 19, 20) could be explained by the fact that they all used more than one diagnostic method as reference standard, as no single presently available test provides the definitive diagnosis of *H. pylori* by itself (13, 21). For instance, van Keeken *et al.* used a combination of histology and culture as the reference standard in their study. The implication of this is that any infection missed by one test due to the patchy distribution of the infection and consequent sampling error could be easily picked by the other tests, thereby increasing the number of positive results by the reference standard. This is further buttressed by the fact that there were less positive results by

histology (52.3%) as compared to RUT (60.5 %) in our study. It is concluded from our study that the RUT with two gastric biopsies is accurate for the diagnosis of *H. pylori* infection. Compared with histology, RUT is simpler, it gives more rapid test result and it is much cheaper. It will serve as a good alternative to histology in managing patients with dyspepsia in resource poor environments like Nigeria, except in patients who require histology for reasons other than *H. Pylori* diagnosis.

#### CONFLICT OF INTEREST

We declare no conflict of interest

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