Sensitivity and Specificity of an in-house Rapid Urease test for Detecting *Helicobacter pylori* Infection on Gastro-duodenal Biopsies in Sudan

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Abstract

Background: *Helicobacter pylori (H. pylori)* infection is commonlyassociated with chronic gastritis, peptic ulcer, and gastric carcinoma. This organism has a strong urease reaction due to a preformed enzyme; a characteristic that can be used for rapid detection of the pathogen.

Objective: To assess the sensitivity and specificity of an in-house rapid urease test(iRUT) for detecting *H. pylori* Infection on gastro-duodenal biopsies in Sudan.

Materials and methods: This was a descriptive, cross-sectional, qualitative study. 120 endoscopic biopsies were collected fromgastro-duodenal inflammation patients attending Fedheil Specialized Hospital (Khartoum, Sudan); during October to December, 2014. All biopsies were investigated by culture and iRUT; while culture results were proposed as gold standard.

Results: Over a period of 3 month study, a total of 120 patients consisting of 59 males (49.2%) and 61 females (50.8%), aged >20 years with upper abdominal dyspeptic complaints referred for endoscopy. *H. pylori* was isolated from 70 patients (58.3%)after the cultivation of biopsy samples. 50 samples (41.7%) were negative. As regard the iRUT, 66 biopsy specimens (55.0%) were found positive for *H. pylori* and 54 specimens (45.0%) were found negative.The results of iRUT compared to culture results showed a sensitivity and specificity of 75.7% and 75.5% respectively.Cross tabulation of the iRUT and culture results showed 53 biopsy specimens (44.2%) were found positive for *H. pylori* by both iRUT and culture techniques; while 37 specimens (30.9%) were found negative by these two techniques.

Conclusion: Our results indicate that the iRUT is a-sensitive, specific, simple, and cost effective test. It can be appropriately applied for detecting H. pylori infection in gastric biopsy specimens.

Key words: *Helicobacter pylori,* Biopsy, Culture, Rapid urease test

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Introduction

Rapid diagnosis and treatment of *H. pylori*presents tremendous challenge due to its high prevalence in developing countries[1]. There are several techniques available for detection of *H. pylori* including bacterial culture, cytological examination, and serological testing[2]. Biopsy urease test is arapid technique, and is widely used for detection of *H. pylori* infection. It's a simple, reliable test and can provide rapid results. Despite the availability of many commercial such preparations in the market, physicians face difficulty in their assessment due to various experimental factors However,the unit cost ofcommercial rapid urease test is relatively high for routine work in developing countries[3] such as Sudan. Hence we had prepared afresh urease test reagent from the locally available ingredients for use in our laboratory. This test had provided easy interpretation after a short incubation period without false positive results[2]. In this study we have assessedthis local rapid urease test in detection of *H. pylori* in gastric biopsy specimens; andcompared its results to those of biopsy cultures as a gold standard technique.

Materials and methods

This is a comparative, cross-sectional, qualitative study. Target populations were patients referred to Fedheil Specialized Hospital (Khartoum, Sudan) during the period from October to December, 2014. 120 consecutive adult male and female patients with dyspeptic symptoms, and underwent upper gastrointestinal endoscopy were enrolled in this study. Patients who had antibiotic or proton pump inhibitor therapy within the previous two weeks were excluded from the study. Verbal consent was obtained from each patient prior to inclusion in the study. The study was approved by the ethical committee in Al Neelain University (Khartoum, Sudan).Data were analyzed statistically using the SPSS software package program (version 16 for windows 7). The chi-square test or Fisher's exact test was used to evaluate variables correlation.Sensitivity was calculated according to the equation: TP/(TP+FN); specificity: according to the equation: TN/(FP+TN); positive predictive value:according to the equation: TP/(P+FP); and negative predictive value:according to the equation: TN/(TN+FN). Where: TP=True positive, FN=False negative, PPV=Positive predictive value, NPV=Negativepredictive value.

Threeendoscopy biopsy specimens were collected from each patient.
Two endoscopy biopsies were immediately placed in sterile bijou bottles containing 20% glycerol in brain heart infusion (BHI) broth and transported in ice to the laboratory within 2 hrs.of collection for culture of *H. pylori*. In the laboratory, biopsies were homogenized under aseptic conditions in 20% glycerol in BHI broth and a loopfull was plated primarily on freshly prepared *Brucella* agar base supplemented with 7% sheep's blood (Oxoid, England) and *H.pylori* supplement (Oxoid, England). The antibiotics amphotericin B (2.5 µg/l), trimethoprim (2.5 µg/l), vancomycin (5 µg/l), and cefsulodin (2.5 µg/l) were added to the medium. All plates were incubated at 37°C for 3-5 days under microaerophilic conditions (5%-10%) according to Anaerocult, Basingstoke, England. Translucid, small-size colonies produced were identified on the basis of colonial morphology and the presence of curved and spiral-shape bacteria on performing Gram staining. Diagnosis was confirmed by oxidase test, urease test, and catalase test.

In-house rapid urease test (iRUT): The rapid urease test reagent was prepared from the ingredients: 1% urea, 0.1% phenol red, and 10 ml sterile distilled water. The third endoscopy biopsy was immediately immersed in a tube containing1ml of the local urease test reagent. On the basis of change in color from yellow to red, at room temperature after 3 hours, the iRUT was considered positive. For quality control freshly-prepared reagents were used for inoculating the biopsy samples (Fig. 1).

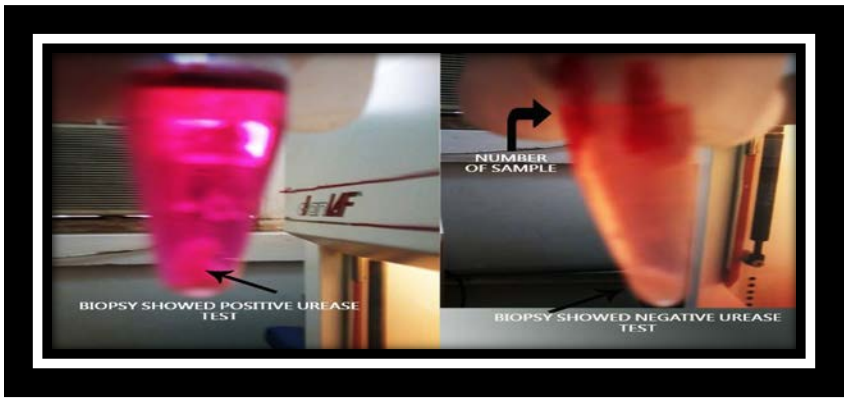

Fig. 1: Positive in-house rapid urease test

Results

Over a period of 3 month study, a total of 120 patients consisting of 59 males (49.2%) and 61 females (50.8%), aged >20 years with upper abdominal dyspeptic complaintsreferred for endoscopy.*H. pylori* was isolated from 70 patients (58.3%)after the cultivation of biopsy samples. 50 samples (41.7%) were negative.

As regard the iRUT, 66 biopsy specimens (55.0%) were found positive for *H.pylori* and 54 specimens (45.0%) were found negative.The results of iRUT compared to culture results showed a sensitivity and specificity of 75.7% and 75.5% respectively; with a PPV and a NPV of 81.5% and 68.5% respectively.A true positive result was that of culture alone or both the iRUT and the histological examination. Cross tabulation of the iRUT and culture results showed 53 biopsy specimens (44.2%) were found positive for *H. pylori* by both iRUT and culture techniques; while 37 specimens (30.9%) were found negative by these two techniques (Table I).

Table I: Cross tabulation results of the iRUT for positive cultures

iRUT results	Culture results		Total
	Negative	Positive	
Negative	37 (30.9%)	17 (14.1%)	54 (45%)
Positive	13 (10.8%)	53 (44.2%)	66 (55%)
Total	50 (41.7%)	70 (58.3%)	120 (100%)

Discussion

H. pylori is responsible for one of the world's most common bacterialinfections. The significant role of *H. pylori* in the etiology of gastric disease is now undisputed. Many factors like low socio-economic conditions such as overcrowding, poor sanitation, close contact with infected persons, food habits, smoking, and other environmental factors may appear to be associated with colonization and infection of *H. pylori* in humans[4].In spite of the current commercial, non-invasive RUT with adequate sensitivity and specificity for reporting the existence or absence of *H. pylori*, an endoscopy along with histopathology conserve as the only method to confirm the presence of *H. pylori* infections. According to most studies, rapid urease tests have been reported to be more sensitive than the histology technique. However, histology is still necessary for detecting pathological manifestations associated with *H. pylori* infections[5].

Culture of *H. pylori*may not be practical in all countries, however, it is highly specific. Its poor sensitivity may occur if adequate transport media are not used. Culture usually requires an experienced and an expertized staff. It is considered expensive and often not available. As regard histology techniques for diagnosis of *H. pylori*it is known have more than 95% sensitivity and specificity. However detection of *H. pylori*may beimproved by the use of special stains (Warthin-Starry silver stainor hematoxylin-eosin stain or Giemsa stain). RUT is a cheap test and its post-treatment sensitivity is reduced[6].

In some studies the sensitivity of RUT was 95.6%. The accuracy of the tests for *H. pylori* diagnosis can be arranged in order as follows: RUT>PCR>histology>stool antigen test>serology. Thus, simultaneous utilization of biopsy-based and RUT is recommended for *H. pylori* infection confirmation[7].It was also reported that the RUT test had a sensitivity of 76.7% and a specificity of 100%. Compared with the gold standard, the RUT test had a sensitivity and specificity of 97.4% and 96.1% respectively; and it appeared to be a good and reliable alternative test[7].

Detection of *H. pylori* infection on gastric biopsy specimens commonly uses rapid urease test (RUT) because its results can be rapidly and easily interpreted.Some researchers developed an iRUT with a sensitivity and specificity comparable to histological examination between 65% and100%[2]. Our study was designed to evaluate the performance of a local iRUT for detection of *H. pylori*, using culture technique as a gold standard.The sensitivity and specificity of our iRUT was found 75.7% and 75.5% respectively; which was similar to some of the above-mentioned reports.

Conclusion

Our results indicate that the iRUT is asensitive, specific, simple, and cost effective test. It can be appropriately applied for detecting H. pylori infection in gastric biopsy specimens.

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