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ACCURACY OF SEROLOGICAL AND STOOL ANTIGEN TESTS (NON-INVASIVE) FOR DETECTION OF *H. pylori*

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ABSTRACT

Background ***Helicobacter pylori*** **is a major bacterial causative pathogen of various gasto-doudenal diseases, including chronic gastritis, peptic ulcer disease (PUD), gastric cancer, and mucosa-associated lymphoid tissue lymphoma. The prevalence of *H. pylori* is about 50% worldwide and could reach more than 70% in developing countries. The aim of the present study was to evaluate the diagnostic accuracy of both serological and stool antigen tests comparing with rapid urease test for detection of *H. pylori*.**

Methods **A 114 patients with gastroduodenal disorders aged 20- 60 years were recruited in this study. Endoscopic biopsy was obtained from each patient at endoscopic unite in the Al Sader Hospital, Basrah/Iraq. All biopsies were used for rapid urease test. Three ml. of venous blood were collected from each patient and used for serological test. Stool samples were collected and used for detection of *H.pylori* Ag. The sensitivity, specificity, positive predictive value, negative predictive value, accuracy, and area of these under receiver operating characteristic (ROC) curve for detection *H.pylori* Ag/antibody tests were determined using rapid urease test as gold standard method.**

Result **The results showed that the sensitivity of *H. pylori* Ag and serological test were (92.6%, 69%), specificity (69.9%, 63.6%), positive predictive value (98.6 %, 82%) negative predictive value (84%, 45.6%) accuracy (93.8%, 67%) and area under ROS (0.9-0.996, 0.664-0.776) respectively.**

Conclusion**: Stool antigen test is more reliable test for detection *H. pylori* in comparison to rapid urease test.**

KEYWORDS: *H. pylori*, *H. pylori* stool Ag, Serological tests, Rapid urease test,

1.INTRODUCTION

Helicobacter pylori was known to be the etiologic cause of gastritis, peptic ulcer disease and associated with gastric cancer development.[1] This pathogen is known to induce several gastric disorders, but may also be associated with extra gastric diseases like anemia, dyspepsia, and immunological disorders. [2]

The epidemiological evidence has shown that *H.pylori* rates ranging from 20- 50% in the adult populations of the developed world but the occurrence is much more in the developing countries with prevalence as high as 90% in some countries.[3] Prevalence of *H. pylori* infection varies from 7.3-92.0 % depending on age, geographic location, and socioeconomic status of the populations. Also, the epidemiology of *H.pylori* infection varies greatly among countries and even between population groups within the same country.[4]

Several studies have shown that the prevalence of *H.pylori* is still high in most countries. In Iraq, Erbil, Kurdistan region, the prevalence of *H.pylori* infection was (39.4 %).[5]

H. pylori secrete urease enzyme, as virulence factor involved in bacteria colonize and induce a strong inflammatory response in the gastric epithelium. HPU(*H.pylori* urease)enzymatic causes hydrolysis of urea into ammonia, thereby neutralizing the acid.[6] By a mechanism not yet fully understood, HPU is also involved in the dysregulation of gastric epithelial tight junctions .Urease is promotes activation of neutrophils.[7]

Serologic tests are available and relatively cost-effective tests which are often used for screening for infection in patients whose other tests yielded borderline results. However, these tests are not suitable to diagnose active infection

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or follow-up of eradication because of its low accuracy.[8] Rapid urease test (RUT) for detection of urease in gastric mucosa have high sensitivity and specificity with many versions have been approved for use in humans.[9] Invasive testing methods involve biopsy during endoscopy combined with RUT, histological examination and microbial culture. All of these methods are time consuming and invasive requiring medical supervision and laboratory equipment. *H. pylori* colonies tend to form in clusters that can often be missed during biopsy resulting in a false negative diagnosis.[10]

The non-invasive method like urease testing is inconvenient requiring theingestion of isotopically labeled urea in addition to specialized instrumentation[11].

Stool antigen tests have been used as the most accurate for diagnosis and for confirmation of presence of *H. pylori*. This method is highly sensitive, specific and useful for diagnosis, therapeutic monitoring, and test of eradication.[12]

The *H. pylori* Ag rapid test specificity and positive predictive value were significantly higher than those of the serological test .[13] The main advantages of this test are its cost compared with C-urea breath test and also the possibility of performing it in any laboratory.[14] Currently, the patients and clinicians prefer non-invasive methods and a debate about the diagnostic test more accurate to be used for diagnosis of *H. pylori*.

Invasive method, including rapid urease test, histology, culture, and molecular methods. using gastric specimens for detecting *H. pylori* infection [2].Rapid urease test is based on the production of urease enzyme by *H. pylori* bacteria and the presence of this enzyme in the gastric mucosa , the presence of H. pylori in biopsy specimen convert the urea reagent in test paper to ammonia[15].

The aim of the present study is to test the accuracy of the non- invasive method in comparison to standard one for diagnosis of *H. pylori* infection.

2. MATERIAL AND METHODS

2.1 patients and subjects: In the present comparative study, one hundred and fourteen patients with age ranged 20-60 years, 50 (43.9%) males and 64 (56.1%), were females were enrolled. They were referred for endoscopy unit at Al-Sader Teaching Hospital, Basra, south of Iraq through the period from Sep, 2019 to Feb, 2020.

2.2 exclusion and inclusion criteria: Receiving antibiotics in the previous four weeks , proton pumps inhibitors in the past two weeks or H2-blocker agents in the past one week were excluded from the study. Excessive GI bleeding and gastrostomy history were excluded as well. The patients were considered as infected with *H. pylori* when RUT gives positive result. RUT was used as a golden standard test.

Stool specimens and venous blood samples were collected from all the participants before endoscopy procedure, and the patients were signed a questionnaire form involved name, age, gender, chief complains, history of the medication and past medical history. Informed consent for their agreement in participation in the study according to the Institutional Ethics Committee of the Basra health department, was taking from each patient.

2.3 Specimens and sample processing

A**. Fecal Antigen Test**: One step rapid fecal antigen test (CTK Biotech, Inc. 13855 Stowe Drive Poway, CA 92064, USA). All specimens and kits (cassettes and reagents) were brought to room temperature (25 C). Small portion of stool sample was taken using sterile applicator stick of the reagent bottle, transferred into the reagent container and shaken for few seconds. The cassette was removed from the foil pouch, reagent bottle was held upright with the tip pointing away from the test performer, and the tip was snapped off, the bottle was held vertically over the sample well of the cassette, 3 drops (120-150µl) of diluted stool samples were added to the sample well. Result was read within 15 minutes. A distinct pink band appearing on the test region in addition to a pink control band indicated a positive result. Negative result was obtained when only one colored band appeared on the control region (no apparent band on the Test region). Absence of color on both regions indicates invalid result.

B. Serological assay for *H. pylori* Antibody rapid test –cassette :(CTK Biotech, Inc. 13855 Stowe Drive Poway, CA 92064, USA). The specimen (blood) and test components (cassette and reagents) were brought to room temperature (25 C) before use, the device (cassette) was removed from its packet, and the test device was placed on a clean and flat surface. The device was labeled with specimen's ID number; the pipette dropper was held vertically and filled

with specimen. 1 drop (about 30-50ul) of specimen was dispensed into sample well and air bubble was avoided, 1 drop of the sample diluent was added immediately, and results were read in 15 minutes. Positive result was obtained when both C and T bands developed; the test indicates the presence of antibodies to H. pylori in the specimen. Negative result was considered when only the C band developed. Absence of C band indicates an invalid result.

C. Rapid Urease Test (RUT): Each patient has taken 1-2 biopsies from antrum and body for RUT. Reading the RUT results within 1 hour by using (Helicotech UT plus strong biotech corporation. Taiwan). Peel back the adhesive label on the test slide. Transfer 2-3 biopsy specimens onto the test paper with the applicator included in the test kit. Re-seal and press the adhesive label over the test paper to squeeze the tissue fluid out of the biopsy specimens. Record the biopsy date, time, and the patient's information on the label. Monitor the test slide at 20-30 sec. and observe any color change over the period of an hour.

2.4 Statistical methods:

Sensitivity and specificity with confidence intervals (ROC), positive and negative predictive values, and accuracy of the serological and *H. pylori* Ag rapid test were calculate against RUT as standard test. Statistical analysis was performed using IBM SPSS (Version 24). P-value ≤ 0.05 was considered as significant.

3. RESULT

The outcome of all three methods used in the current study show in (table 1). The highest positive results for *H. pylori* achieved by RUT, which used as gold stander test.

Table (1): Frequency outcome of three methods among 114 patients

Diagnostic method	Positive	%
RUT	81	71.1
Stool Ag rapid cassette	76	66.7
Serologic test(Ab-detection)	68	59.6

The positive result obtained by combination of RUT with serological test, Ag rapid cassette , or both of them shown in (table 2).

Table (2): Outcome of positive result in each test

Diagnostic method	Positive	%
RUT alone	81	71.1
RUT + serologic test	56	49.1
RUT + Ag rapid cassette	75	65.7
RUT + serologic test + Ag rapid cassette	55	48.2

The diagnostic accuracy of *H. pylori* Ag rapid and serological tests was determined by calculating the sensitivity, specificity, positive predictive value, negative predictive value, accuracy and area under the Receiver Operating Characteristic (ROC) curve.

The Receiver Operating Characteristic (ROC) curve was created for each test by plotting the sensitivity against the (1-specificity). The result from ROC curve analysis are shown in fig(1).According to the rapid urease test , the sensitivity, specificity, positive predictive value, negative predictive value, accuracy and area under the Receiver Operating Characteristic (ROC) curve for each test shown in table (3)

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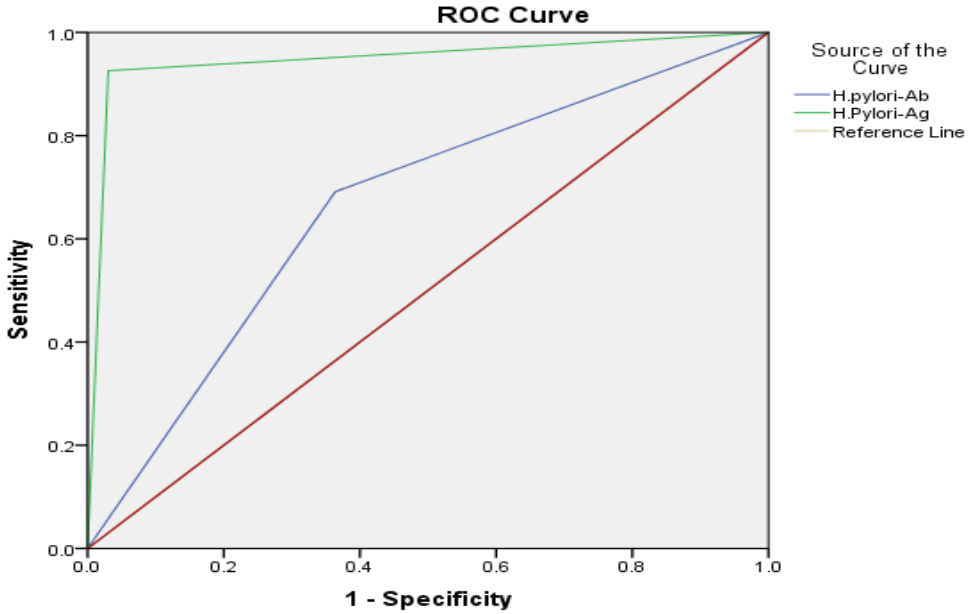

Fig (1) :

ROC curve of serological test and *H. pylori* Ag rapid test for *H. pylori* infection diagnosis

Table (3): Comparison of serological method and *H.pylori* Ag rapid test with rapid urease test detection of *H. pylori*

Test type		Rapid Urease Test Positive	Rapid Urease Test Negative	Total	Statistics*
Serological test	Positive	56(82.4%)	12(17.6%)	68(100%)	P = 0.01 SP= 63.6 % SN= 69 % PPV= 82% NPV= 45.6% Acc= 67% area under ROC=0.664
	Negative	25(54.3%)	21(45.7%)	46(100%)	
	Total	81(71.1%)	33(28.9%)	114(100%)	
***H.pylori* Ag rapid test**	Positive	75(98.7%)	1(1.3%)	76(100%)	P = 0.00 SP= 69.9% SN= 92.6% PPV= 98.6% NPV= 84% Acc=93.8% area under ROC=0.948
	Negative	6(15.8%)	32(84.2%)	38(100%)	
	Total	81(71.1%)	33(28.9%)	114(100%)	

SN: Sensitivity, SP: Specificity, Acc: accuracy , PPV: Positive Predictive Value
NPV: Negative Predictive Value , p value: probability

4. DISCUSSION

H. pylori colonizes the human stomach during the lifespan of the carrier, and lives in the human stomach.[16] About 20 to 30 percent of people infected can develop peptic ulcer disease.[17] Therefore, it is important to early diagnose *H. pylori* using an accurate diagnostic method. In developing countries such as Iraq, as *H. pylori* is a very common [18]. Different methods were used to diagnose of *H. pylori* in our laboratories. In this study, we evaluated the accuracy of two routine methods that commonly used in our country by measuring different qualification parameters. We performed this analysis through measuring the sensitivity, specificity, NPV, PPV and accuracy of two routine *H. pylori* diagnostic tests (stool antigen

and blood antibody test) frequently done in Iraqian laboratories and compared with RUT.

In current results serology test based on Ab detection had the lowest accuracy indicating that it is not alone will be reliable test for primary diagnosis of *H. pylori*. Our data regarding stool antigen test and RUT were similar to other study in Iran Isfahan.[8] Moreover, stool antigen was worthy test for *H. pylori* diagnosis in our study because the sensitivity, specificity, positive predictive value, negative predictive value, accuracy, and area under ROS were much higher than those obtained by serological test and closer to that result of gold standard test. The efficacy of stool tests for detecting *H. pylori* infection depends greatly on the antigen released from pathogen and selected for detection. less antibody detection in our results may due to presence of polyclonal antibody that need different antigenic composition to be integrated in cassettes.[19] For this reason, the findings were much less accurate overall than those of monoclonal antibody integrated in stool tests cassettes. However, not all studies on monoclonal antibody can identify the same bacterial antigen.Genetic differences among *H. Pylori* strains in geographic variations may affect outcomes of diagnostic efficacy and their usefulness, so it would be better tested regionally.

Our study concluded and confirm that stool antigen test could be a reliable test for diagnosing *H. pylori* infection instead of RUT among non-treated patients. It could be considered as a noninvasive first-line routine diagnostic test in our region.

Rapid urease test is the widely useful invasive test for the diagnosis of H. pylori infection because it is rapid , inexpensive, easy to perform, Based on the H. pylori urease enzyme activity.

And its higher accuracy compared with culture .histopathology or urea breath test for the diagnosis of H.pylori infection and very rapid positive reaction time. And many study consider the rapid urease testing is excellent H.pylori diagnostic test [20]

H. pylori Ag rapid test showed a good sensitivity, specificity and accuracy for diagnosing *H. pylori* infection in all age groups. This method may be useful and applicable in clinical practice as an office-based test because it is easy to perform, not require fasting , noninvasive tool and cost effective method to diagnose active infection within symptomatic patients and later follow up the effectiveness of antibiotic treatment.

Conflicts of Interest

No conflicts of interest neither financial Disclosures

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