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Effectiveness of Monoclonal Stool Antigen Immunoassay for Detection of Helicobacter pylori in Iraqi Patients

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Abstract
There are currently several methods to test for *H. pylori* infection, endoscopy tests (Invasive) and non-endoscopic tests (Noninvasive). The aim of current study was to validate stool antigen tests (SAT) and rapid urease test (RUT) findings, used histopathological examination as the gold standard for diagnosis of *H.pylori* and comparison diagnostic specificity and sensitivity between non-invasive test findings. Gastric antral biopsy specimens and stool sample were taken from each patient to confirm infection of *H.pylori* by (histology, SATand RUT). While comparing the results of the tests was, sensitivity and specificity of RUT were 92.7% and 90%, and 96.2% and 81.8% for the SAT. Positive predictive value and negative predictive values for RUT were 96.2% and 90% and 92.7%and 81.8% for SAT. Round off that the rapid stool antigen test can be served as an alternate to RUT for *H. pylori* infection diagnosis in Iraqi patients.
Keywords:*H.pylori*, non-invasive tests*, H. pylori* diagnosis, rapid Stool antigen test.

1. Introduction

Helicobacter pylori (*H. pylori*), is the pathogen found originally in stomach. The microorganism has respectable role in pathogenesis of gastritis, peptic ulcer disease and associated with the development of gastric cancer (Mattar et al., 2007; Mattar et al., 2010) .It spread in the half worldwide, count on the socioeconomic conditions and decontamination status (Ahmed et al., 2007) : in the advanced countries being below 40% but 80% in growing countries (Kusters et al., 2006).

Helicobacter pylori (H. pylori) infection is mostly gained in early infancy and deep-seated *H. pylori* infections may lead to duodenal sore or gastric adenocarcinoma (Kindermann & Lopes,2009; Mourad - Baars et al., 2010; Moya & Crissinger , 2012; Ruggiero, 2012) .Prophylactic, measures during early infancy might reduce the average of *H. pylori* infection and subsequently the risk of malignancy diseases. Thus, careful and soon diagnosis of *H. pylor*i infection is greater prominence for management of *H.pylori* - associated diseases.

Diagnosis of active infection is based on the invasive tests of histopathological examination of the biopsy specimens, rapid urease test (RUT) and direct identification of the microorganism by culture and the noninvasive tests of urea breath test and stool antigen test (SAT) (Oderda et al., 2004; Guarner et al., 2010; Bytzer et al., ,2011;McNulty et al.,2011; Redeen et al.,2011). Recently, a non-endoscopic diagnostic test based on the detection of *H. pylori in* stool has been growing. Some SAT that use polyclonal antibodies to *H. pylori* have shown diverse outcome (Blanco,2008) Additional newly, novel lateral flow stool antigen test which could increase the precision of this test have been developed (Krausse et al.,2008; Yang & Seo, 2008) .

It is important to correctly diagnose *H.pylori* infection. The selection diagnostic mode must be simple and serviceable to all age. Assess the SAT and RUT, lateral flow stool antigen test with rapid urease test and histopathological diagnosis in terms of sensitivity, specificity, positive and negative predictive value to clarify if a SAT is worth to perform in Iraqi patients instead of RUT.

2. Patients and Methods

2.1Patients and samples

Ethical clearance to conduct the research was sought and obtained from the patients and study performs on newly diagnosed patients with peptic ulcer disease, who attended the Endoscopy Unit of Baghdad Teaching Hospital/Medical City from 1st of February to 30st of June 2015. 75 subjects agreed to participate in this study and took their Biopsy specimen for diagnosis of histopathological examination and RUT, and Stool specimens from each patient were collected and kept on -20°C until used for diagnosis of SAT. The patient's age, gender, and complaints were recorded in the patient forms before upper gastrointestinal endoscopic examinations were done. The patients included 40 males and 35 females (range30–70years).

In order to be eligible for this study, no treatment with antibiotics, proton pump inhibitors (ppIs) for the last 2 weeks, H2 receptor blockers and bismuth salts (Hunt et al., 2011) Also, should not have had a gastric surgery and the absence of diarrhea at the time of sampling.

2.2 Rapid Urease Test (RUT)

The presence of urease at the gastric mucosal biopsy specimen taken from the antrum at gastroscopic examination was tested by a pH indicator, RUT. This test was performed with a homemade solution with 1 ml

distilled water, one drop of 1% phenol red and 100 mg urea, prepared just before endoscopy. One antrum sample was placed in the solution and maintained at room temperature. The test was considered positive when the color changed from yellow to red within 24 hours (Pourakbari et al., 2011) .

2.3 Stool Antigen Test (SAT)

Identifies *H.pylori* antigen present in Stool. Fecal specimens were collected; in clean dry dish; from each patient. The test employs monoclonal antibodies specific for *H. pylori* antigens to selectively distinguish *H. pylori* antigen in human fecal specimens, according to the technique of *H. pylori* antigen rapid test device (feces) (Marshall et al., 1985; Soll, 1990) .

2.3.1 Principle

The one step *H. pylori* antigen test device (feces) is a qualitative lateral flow immunoassay for the finding of *H. pylori* antigen in human feces samples, and based on the appearance of colored lines across the central window of the cassette, two lines, C (control) and T (test), indicated positive test, only one line in C indicated negative result. A pale colored line in T was also considered positive.

2.4 Statistical Analysis

Statistical analysis was performed using SPSS version 11.5. For comparison of categorical variables x2. For assessment of the consistency of two diagnostic tests. P- Value of ≤ 0.05 was statistically significant. Histopathological examination was considered as the gold standard.

3. Results and Discussion

Among the 75 patients, 35 (46.6%) were females and 40(53.3%) males with an age range of (30 -70) years. The results revealed that 73.3% of subjects were positive and 26.6% of the subjects were negative for *H. pylori* infection by histopathlogical examination as the gold standard in this study, sensitivity and specificity 100%. In the *H. pylori*-positive, the rapid stool antigen test and RUT detected *H. pylori* in 51of the 53 and 51 of the 55 positive patients (sensitivity 96.2%and 92.7%) , two and four false-negative respectively; and in the *H. pylori*-negative, 18 presented negative results in the both test (specificity 81.8%and 90%) , four and two false-positives respectively. Considering that 51 patients of 53 and 51patients of 55 these had positive SAT and RUT .the positive predictive value (PPV) of the RUT and SAT were (96.2% and 92.7%) respectively, and that 18 presented negative results in the both test, the negative predictive value (NPV) of the SAT and RUT were (81.8% and 90%) respectively, the differences were not statistically significant, as shown in table (1). Thus results obtained with RUT and SAT tests were generally similar to those obtained by histopathological examination.

Table 1: Detection of *H. pylori* infection by rapid urease test and stool antigen

Repaid urease test			
Stool antigen		**Positive**	**Negative**
Total	**positive**	51	4
	Negative	2	18
Male	**positive**	25	5
	Negative	4	6
Female	**positive**	20	5
	Negative	4	6

The results of the RUT and the SAT were positive for 72.5% and 75%, respectively, of 40 men and 68.5% and 71.4%, respectively; of 35 women .The difference in the results was not significant between men in both tests and women also in both tests.

Age distribution of *H. pylori* positive and negative in subjects according to RUT and SAT, no statistical significant difference was found is shown in table (2).

Table2: Age distribution of *H. pylori* positive and negative in subjects according to RUT and SAT

TEST				
Age in year	**SAT**		**RUT**	
	+ve	**-ve**	**+ve**	**-ve**
30-40	2	4	2	4
40-50	6	15	6	16
50 -60	8	23	9	2
60-70	4	13	5	12

Histological detection for *H.pylori* infection highly sensitivity and specificity can reach to 95% under optimal status (Malfertheiner et al., 2012), direct visualization and monitoring of markers of inflammatory (Lehours &Yilmaz,2007) , in another hand costly, uncomfortable for all age group of patients and H. *pylori* were

not evenly distributed throughout the gastric mucosa (Abo-Shadi et al., 2013) .Therefore do not prefer to first chosen.

Non-endoscopic tests (Noninvasive) with high accuracy ,rapid stool antigen test for the detection of *H.pylori* (Malfertheiner et al., 2007) ,this test identification of active infection , simple to apply , serviceable to all age and follow up the patients after eradication (Tuncer et al.,2004) .

In the current study, *H. pylori* infection detection by monoclonal stool antigen test and rapid urase test, found 18 presented negative results in the both test, four and two false-positives results respectively may be expound Coccoid shape of *H. pylori* that is the morphologic manifestation of bacterial cell doom and does not mean an hazard of infection (Blanco et al., 2008) .51of the 53 and 51 of the 55 positive patients by both test , two and four false-negative respectively that due to low concentration of Helicobacter in stool that is not enough For a reaction exam . Usually, rapid stool antigen test high specificity and sensitivity **(**Gisbert et al., 2006; Elwyn et al., 2007***)*** also RUT specificity is lower than that of other detection exams (Elwyn et al., 2007) .Therefore, the discrepancy between the results of the two tests seemed to be caused by false-negative results were obtained by the stool antigen test for rapid urease positive patients.

The gold standard in this study was the histopathological diagnosis of the endoscopic biopsy material, in the light of literature. Specificity of RUT in comparison with histopathological diagnosis was 90%, sensitivity 92.7%, positive predictive value (PPV) 96.2%, and negative predictive value (NPV) was 90%. When compare with the global literature, Specificity of biopsy urease test is reported as 95%, and sensitivity 80–95%, this is closer to results (Suerbaum & Michetti, 2002; Choi et al., 2011) .

H. pylori can detection in feces by classical, immunoassay (EIA) utilizing polyclonal antibodies (Hooton et al., 2006) showing highly sensitivity and specificity can reach to 98.3%, and by SAT immunoassay utilizing monoclonal antibodies (Yang &Seo, 2008) submitting sensitivity, specificity, PPV and NPV (53-95%, 56-99%, 97% and 98%), respectively (Yang &Seo, 2008) rapid stool antigen is easy to apply that do not have tool for carrying the EIA, that work with small specimen and is quicker than the classical immunoassay EIA (Yang &Seo, 2008 ; Blanco,2008) .

The need to diagnosis of active infection as well as follow-up after treatment therefore, there is an importance excess in studies on rapid stool antigen. In differentiation with invasive mode in base detection, the sensitivity and specificity of SAT was reported as 88.9% and 96.4% by (Makrishathis et al., 1998) and 94% and 91% by (Vaira et al., 1999) .Which are closed to this found specificity of SAT in comparison with histopathological examination was 81.8%, sensitivity96.2. %, PPV 92.7% and NPV 81.8%., and also in study done in Turkey found the same results (Ozdemir M, Baykan , 2005) .

4. Conclusion

In this study SAT and biopsy urease tests were both specific tests in comparison with histopathological examination. The sensitivity of SAT higher than biopsy urease tests and it gives positive results in 2/3rd of the patients; it may be used as alternative to endoscopic examination to detect *H. pylor*i particularly in growing countries.

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