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Evaluation of Monoclonal chromatographic immunoassay antigen stool test in the diagnosis of *Helicobacter Pylori*

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Abstract

H. Pylori infection can be detected by invasive (endoscopy based) and non-invasive methods. The aim of study is to evaluate monoclonal chromatographic immunoassay antigen stool test in *H. Pylori* diagnosis. 117 patients with chronic dyspeptic symptoms underwent invasive tests for *H. Pylori* (Rapid urease test and brush cytology) and stool antigen. patient classify as *H. Pylori* positive if he or she tested positive for both invasive tests. The sensitivity and specificity of the CTK Biotech® USA stool antigen when compared with invasive test were 67.24% and 96.61 respectively. We concluded that CTK Biotech® USA stool antigen lacks the sensitivity in diagnosis of *H. Pylori*, uses of new generations of stool antigen with high sensitivity is recommended for diagnosis of *H. Pylori.*

Keywords: H. Pylori; Rapid urease test; CTK Biotech® USA

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Introduction

Helicobacter pylori are gastric microorganisms were observed for the first time 85 years ago but the true association of these microbes with gastritis was not fully understood, until 1982 when Marshall and Warren identified and cultured the gastric bacterium, *Campylobacter pyloridis* was the first name which changed later to *Helicobacter pylori* [1,2]. There is strong association between *H. Pylori* infection and duodenal and gastric ulcer, gastric lymphoma and adenocarcinoma of stomach [3]. It is important to identify the patients with *H. Pylori* infection and to decide whether the treatment should be started or not according to the guidelines [4]. Diagnosis of *H. Pylori* depends on different methods, which are generally classified to invasive (required

endoscopy to obtain biopsies) or non-invasive like antigen stool test, urea breath and serology. The sensitivity of invasive test reaches to 91-94% and high specificity of 94-99% with an advantage to identify pathological changes such as gastric adenocarcinoma unfortunately the good trained personnel and the cost are the major limitations [5]. Owing to high accuracy and simplicity ^{13}C-urea breath test is widely used test in the hospital but the expensive spectrometer which is preferred device for the ^{13}C measurement is the major obstacle behind the limited spreading of this methods in mass screening or primary clinical practice [5]. Enzyme immunoassay (EIA) was the first used test of stool antigen of *H. Pylori*, which depend on detection of polyclonal antibody against *H. Pylori*, this qualitative test result in change in the color which indicate the positive and negative results. Specific cutoff values of certain optical density read by spectrophotometery are set for negative, equivocal and positive results. Monoclonal rather than polyclonal antibody then developed to eliminate the equivocal result and improve the accuracy of the test. Lastly immunochromatographic rather than enzyme EIA used which contain positive line indicate positive result when compare to control line. The aim of the study is to evaluate the chromatographic immunoassay antigen stool test in the diagnosis of *H. Pylori*.

Patients and Method

117 patients (72 males and 45 females) selected from those attending the endoscopy unit in Al- Hussein teaching hospital in Samawah for various dyspeptic symptoms during the period from January 2012 to December 2013 were enrolled in this study. Patient who had recent upper gastrointestinal bleeding, or those who receiving proton-pump inhibitors, antibiotic, or non-steroidal anti-inflammatory drugs were excluded from the study.

Brush cytology was performed using cytology sheathed brush at the endoscopic end reaching to the antrum, mucosal surface of antrum then brushing done with the superior, inferior, and lateral surface, the brush then retracted under the sheath, withdraw from the scope and vigorous to and fro brushing performed on clean laboratory glass slides. Each slide stained by Giemsa stain. *H. pylori* appeared blue/grey with blue nuclei and the background pink/pale blue to be used for cytological examination by the pathologist who was unaware about the result of stool antigen or rapid urease test.

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Urease test. One biopsy specimen from antrum, and one from body were used immediately for urease test detection, the biopsies specimen was putted in tube, each contains 1 milliliter of modified urea broth medium (*the solution composed of Urea: 20 gm/ml, phenol red: 0.04 gm/l, KH2 Po4: 2gm/l Nacl: 5gm/ml*) and labeled for time of taking biopsy. Each tube was maintained at room temperature and followed for change in color within 24 hours. The test was considered positive for *H. Pylori* when the color of urea broth changed from yellow-orange to pink.

***H. pylori* infection was considered positive when both brush cytology and rapid urease tested positive**

Stool Antigen

All patients have been tested for stool antigen, Improper stool sample include delay sample for more than half hour, acute diarrhea in the last two days, or inadequate amount of stool sample were excluded from the study. Using as manufacture prescription the improved rapid test (CTK Biotech® USA) is membrane-based assay using immunochromatographic assay to identify stool antigen for *H. Pylori* infection. the test depends on two monoclonal antibodies against *H. Pylori*. A properly mixed stool of pea size (approximately of 0.8 gram) was transfer into sample vial to homogenized for 30-60 seconds on a mixer, 500 microliters of stool suspension then added to the test strip vial which left at room temperature for 30 minutes. The appearance of controlled line (pink-purple) indicate correct procedure. Visual reading of the result has been performed after 10 minutes.

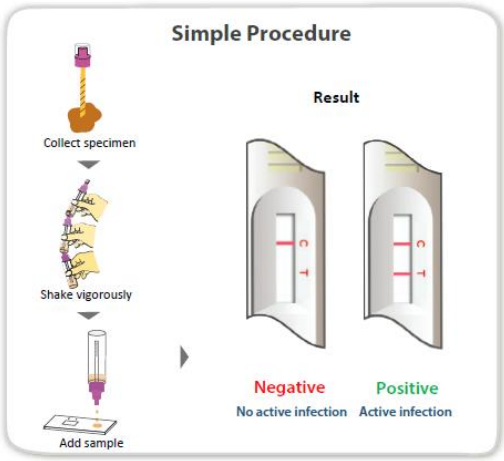
Interpretation of Assay Result

Negative Test: If only the control line (C) is appeared,

Positive Test: If both controlled "C" and test "T" lines are appeared, the test indicates the presence of *H. Pylori* antigen in stool specimen.

Invalid: If No controlled line "C" is appeared, the assay is invalid whether the test line "T" appear or not.

The results of the rapid stool test were read by expert independent laboratory personal who classified the results of the samples as negative, positive or invalid. The laboratory personal was unaware about the other result of *H. Pylori*



Results

117 patients, 72 male (61.5%), and 48 female (38.5%) were enrolled in the study and subjected to various investigations for diagnosis of *H. Pylori* (brush cytology, urease test and stool antigen). The age of the patients ranged from 15- 69 years mean age 43 years).

Table 1: shows age, sex group distribution of patient.

Table (1) Age group distribution

Age group in years	Patients		Total
	Male	Female	
< 20	6	2	8
20-29	9	5	14
30-39	11	6	17
40-49	29	15	44
50-59	8	6	14
60-69	9	11	20
Total	72	45	117

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Among 117 patients, 61 patients were tested positive for brush cytology, 65 patients tested positive by rapid urease test, those who tested positive for both brush cytology and rapid urease test were 58 patients and they regarded as gold standard. Stool antigen were positive in 41/117 patients, among 41 patients 39 were tested positive by gold standard.

Table 2: showed the correlation between stool antigen, rapid urease test and brush cytology

Test	Result	*H. Pylori*		Total
		Positive	Negative	
Stool antigen	Positive	39	2	41
	Negative	19	57	76
	Total	58	59	117
Rapid urease	Positive	58	7	65
	Negative	0	52	52
	Total	58	59	117
Brush cytology	Positive	58	3	6
	Negative	0	56	56
	Total	58	59	117

The sensitivity of stool antigen in the diagnosis of *H. Pylori* was 67.24% and the specificity was 96.61%, the positive and negative predictive value were 95.12% and 75% respectively, and the accuracy rate was 82.05%.

Table 3: shows the sensitivity, specificity, positive and negative predictive value and the accuracy rate of stool antigen.

Stool Antigen	Sensitivity	Specificity	PPV*	NPV§	Accuracy Rate
	67.24%	96.61	95.12	75%	82.05

*PPV: Positive predictive value

§NPV: Negative predictive value

Discussion

Accurate diagnosis of *H. Pylori* in Iraq represents challenging issue to the most physicians owing to lack of facilities of diagnosis. Urea breathing test is unavailable in Iraq, upper endoscopy although it is available in most of hospital but Iraqi patient afraid from it and they believed it cause carcinoma. For that reason, stool antigen is widely used for evaluation of the presence of *H. Pylori*. Only one type of stool antigen is present so far in Iraq which is chromatographic immunoassay stool antigen. According to the manufacturer, CTK Biotech® (USA) *H. Pylori* is regarded as a sensitive test, and a negative result should therefore indicate that the person truly is negative. In the present study only 67.24% of the individuals were found to be positive for *H. Pylori* in comparison to gold standard, this result is similar to J Andrew et Al [9] and Wu DC et al [10] who found differences in result of sensitivity and specificity of stool antigen according to the type and manufacturer of the test. Chromatographic immunoassay stool antigen CTK Biotech® (USA) lack the sensitivity and specificity for diagnosis *H. Pylori*, new generation of stool antigen like *Femtolab Cnx* should be use for evaluation of H Pylori [9, 11].

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