The Detection of Neutrophiles in Gastric Mucosa of Patients Suspected to be Infected with *Helicobacter Pylori* Using Leukostix

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ABSTRACT:

BACKGROUND:
Helicobacter pylori has been detected in many populations and associated with inflammation of gastro duodenal mucosa. Colonization of the stomach by *Helicobacter pylori* occurs in more than half of human population worldwide. It is the principle cause of chronic active gastritis, peptic ulcer and gastric cancer.

OBJCTIVE:
To detect neutrophiles in homogenates biopsied gastric mucosa semiquantitatively using rapid leukocyte strip test (leukostix).

METHODS:
A total of 115 patients (74 males, 41 females) referred to The Gastrointestinal Tract Center and Gastroscopy Department of Baghdad Medical City and subjected to gastroscopy were included in this study during the period from November 2004 to May 2005.

RESULTS:
The sensitivity and specificity of leukostix at the initial examination were 95.8%, 88.23% respectively.

CONCLUSION:
The leukostix test, using biopsied samples of gastric mucosa was excellent for quantitative determination of neutrophils in patients infected with *H pylori.*

KEY WORDS: helicobacter pylori, gastritis, leukostix

INTRODUCTION:

Helicobacter pylori is a spiral shaped gram negative rod, microaerophilic motile and non spore forming ,it is approximately 0.5 x 3.0 micrometers in size. It has multiple flagella at one pole and is actively motile. It is oxidase positive, catalase positive and a strong producer of urease [1].

Morphologically *Helicobacter pylori* can be divided into spiral or coccid form, in tissue it appears spiral and lies close to gastric mucosal epithelial cells and mucous glands. In culture, *Helicobacter pylori* appears longer, and spiral [2], in old culture, it transform to coccal form [3].

Helicobacter pylori is a fastidious bacterium and requires complex basal medium (either solid or liquid) with some of supplementation such as blood ,heme, serum, charcoal, corn starch or egg yolk emulsion. The isolation of these slow growing bacteria can be enhanced by the addition of appropriate antibiotics to make the media selective for its isolation [4].

Appropriate transport conditions of the biopsy are important in order to avoid desiccation of biopsy samples and long exposure to ambient air [5].

Helicobacter pylori causes peptic ulcer by damaging the mucous coating that protects the stomach and duodenum, this allows powerful stomach acid to get through to the sensitive lining beneath, together the stomach acid and *H.pylori* irritate the lining of stomach or duodenum and cause an ulcer [6].

PATIENTS AND METHODS:

The present study was conducted in the period from November 2004 to May 2005 including 115 patients with dyspepsia,74 males and 41 females ,attending for diagnostic upper gastrointestinal endoscopy at The Gastrointestinal Tract Center and the Endoscopy Department in Baghdad Medical City , those with a history of antibiotic treatment were excluded from this study ,compared to 10 apparently healthy individuals,4 females and 6 males, with no history of dyspepsia or peptic ulcer diseases.

A questionnaire including sex, age, chief complaint and drug history were used in this study.

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Endoscopy was done for them and more than one gastric antral biopsy specimen were taken (3-4 cm) from the pyloric ring. Biopsy specimens were transported to the laboratory in 0.5 ml brain heart infusion broth with ice and kept at 4°C not longer than 4 hours before processing.

The biopsy samples were minced with sterile blades in a sterile petridish then subjected to the following tests:

1. Direct detection

❖ Urea agar slant (oxoid ,England), slants were examined for color changes from yellow to pink at 30 minutes. 1 hour , 2 hours , 4 hours ,8 hours and 24 hours after inoculation [7,8].

2. Cultivation on Chocolate agar (blood agar base, mast grp U.K) for primary isolation. Inoculated organisms were incubated for 5-7 days at 37° C under microaerophilic conditions in an aerobic jar with a gas generating envelope. Plates were examined for positive growth at 24 hours interval after 5 days up to 7 days before discarded as negative [9].

❖ Gram stain was done to identify microscopic morphology of the isolated colonies.

❖ Those colonies which were picked up from agar plate with a sterile loop were inoculated on urea agar slant. A change in color from yellow to pink were considered positive urea test.

❖ Picked colonies were subjected to oxidase test by using a sterile wooden stick, subjected to a strip of filter paper soaked with freshly prepared 1% of the oxidase reagent, positive reaction is indicated by an intense deep, positive blue color appearing within 3-10 seconds.

❖ A part of the growth colonies which was picked up from the agar with an inoculating loop and were placed on the surface of a sterile slide. Then a drop of hydrogen peroxide was added to the colonies on the slide, when a production of gas bubbles was observed then the test was considered as positive catalase reaction.

3. Histopathology

One antral biopsy specimen from each patients was fixed and paraffin wax sections of 4µm thick were cut and stained with Hematoxylin and eosin stain (H/E) (BDH,England).

4. 50 µl of homogenates biopsy sample of gastric mucosa was spotted on to leukostix strip (Cy Bow, Korea) in an attempt to detect tissue neutrophiles semiquantitatively, the strips were observed 5 minutes later and scored by five grades (−),(±),(1+),(2+) (3+),samples that were scored (±) or higher were judged to be positive.

The principle of leukostix test:

The test pad contains an idoxyl ester and diazonium salt.It followed by an azo- coupling reaction of the aromatic amine formed by leukocytes esterase with a diazonium salt on the reaction pad. The azo dye produced causes color change from beige to violet.

Statistical analysis:

The efficacy of the tests was determined by calculating the sensitivity and specificity of each test.

Sensetivity was defined as the proportion of number of *Helicobacter pylori* infected who had a positive test and was calculated as [10]:

$$\frac{\text{No. of True positive}}{\text{No. of True positive + No. of False negative}} \times 100$$

Specificity was defined as the proportion of individual free of *Helicobacter pylori* that had a negative test and was calculated as [10]:

$$\frac{\text{No. of True negative}}{\text{No. of False positive + No. of True negative}} \times 100$$

Patients age group was as follows, 70% (22-40 years) , 15% (41-50 years), 5% (51-55 years),10% (below 22 years). The ages of control group was ranging from 26 - 45 years .

RESULTS:

Helicobacter pylori infection was detected in several conditions , in patients with gastritis,duodenal ulcer,gastric ulcer and duodenitis and in individuals with no ulcer dyspepsia, 47/115 patients were found to be infected when at least two tests were positive including histopathological examination.

As shown in Table (1),41 out of 115 were positive in rapid urease test,the percentage (87.23%) means that (41) out of the total positive (47) showed positive rapid urease test and (6) out of the total positve (47) were negative by rapid urease test, 5 out of 115 were positive in culture,43 showed positive result in histopathological examination, 45 out of the 47 patients proved to be infected with Helicobacter pylori gave positive leukostix test result.

Table 1: H.pylori infection in dyspeptic patients

Tests		No. of positive cases	Percentage of positive cases	No. of negative
Rapid urease test	P	41	87.23%	6
	C	1		
Culture of H.pylori	P	5	10.63%	42
	C	0		
Histopathology examination	P	43	91.5%	4
	C	1		
Rapid leukocyte strip test	P	45	95.8%	2
	C	0		

P: Patients
C: Control

Table (2) shows the percentage of sensitivity and specificity of different diagnostic tests which were used in detecting *Helicobacter pylori* in routine gastroscopy patients. The 41 positive urease means that 41 patients out of the total positive(47) were urease positive, 5 out of total positive (47) were positive in culture and 43 were positive by histopathology , while the number 53 referred to all positive leukostix results .

Table 2: Percentage sensitivity and specificity of diagnostic tests in detecting *Helicobacter pylori* in routine gastroscopy patients.

Tests	Positive cases	Sensitivity	Specificity
Urease	41	87.23	100
Culture	5	10.63	100
Histopathology	43	91.5	100
Leukostix	53	95.8	88.23

Total *H.pylori positive* cases =47

Table (3) shows that 53 patients from the total 115 patients examined by different tests were positive by leukostix test ,45 of these 53 patients were positive by other tests and 8 of them were positive only by leukostix test,the percentage (39.13%)referred to the percentage of positive leukostix test from total 115 patients examined.

Table 3: Rapid Leukostix Test, Results of initial Examination

		Leukostix		Total
		Positive	Negative	
Helicobacter pylori positive	No.	45	2	47
	%	39.13	1.73	40.86
Helicobacter pylori negative	No.	8	60	68
	%	6.95	52.17	59.14
Total	No.	53	62	115
	%	46.8	53	100

DISCUSSION:

The rapid leukocyte strip test, leukostix, is a simple and easy detection system for leukocyte using a strip that has the property to bind to neutrophil esterase, since inflammation of gastric mucosa caused by h.pylori is mainly detected as infiltration of neutrophils in homogenates of biopsied gastric mucosa[10].

The results of this study may agree with those obtained by Mistura and his colleagues [11], who found a sensetivity and specificity (leukostix) of 97.9% and 76.9% respectively at the initial examination , another study showed sensetivity of 98% and specificity of 77% [12].

Suhaila et al 2010 [13], showed similar results to those obtained in our study by other laboratory tests , the efficacy of rapid urease tests , culture and histopathology were 31.8% ,13.9% and 30.3%

respectively, with a sensetivity and specificity of urease tests of 91.55% and 93.65% respectively.

CONCLUSION:

The rapid leukocyte strip test is an excellent diagnostic indicator for possible *Helicobacter pylori* infection using biopsied samples of gastric mucosa.

The leukostix test is a good for quantitative determination of neutrophils in biopsied sample of gastric mucosa and useful in early assessment of treatment efficacy.

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