Diagnostic value of rapid Helicobacter pylori stool antigen test

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Abstract: Background: ImmunoCard STAT HpSA test is a noninvasive rapid qualitative test for the detection of *H. pylori* in human stool.It is monoclonal test based on lateral flow chromatography technique. **Aim:** To evaluate the reliability of ImmunoCard STAT HpSA, for detecting *H. pylori* infection. **Patients and methods:** 160 patients with dyspepsia were enrolled in the study. Patients were not on *H. pylori* eradication therapy, PPI, H2 blocker for the last 4 weeks and not in active gastrointestinal bleeding. All patients underwent upper gastrointestinal endoscopy with 4 quadrants antral and 4 quadrants corporeal gastric biopsies taken. Histopathological examination with hematoxylin and eosin staining were done on gastric biopsies. Stool examination with ImmunoCard STAT HpSA was done in the same day as the upper gastrointestinal endoscopy. **Results:** Patients included 124 males (77.5%) and 36 females (22.5%) with their ages ranged between 18-70 years and mean age 39.1 ± 12.1 years. According to histopathology as a gold standard, 112 patients (70%) were positive for *H. pylori* infection and 48 patients (30%) were negative. According to Immunocard testing 106 patients (66.25%) had positive test and 54 patients (33.75%) had negative test. The Immunocard test had sensitivity, specificity, PPV, NPV and total accuracy of 85.7%, 79.2%, 90.6%, 70.4% and 83.8%, respectively. The test was more sensitive in patients below 39 years old (the mean age)(n=98) (88.6% vs 81%) with higher PPV (91.2 % vs 89.5%), NPV (73.3% vs 66.7%) and accuracy (85.7% vs 80.6%) but less specificity than in patients aged 39 years or older (n=62) (78.6 % vs 80%). Immunocard testing in females had higher sensitivity (92.9% vs 83.3%), PPV (92.9% vs 89.7%), NPV (75% vs 69.6%) and accuracy (88.9 % vs 82.25%) but lower specificity than in males (75% vs 80%).**Conclusion:** ImmunoCard STAT HpSA is efficient and rapid noninvasive test, having a diagnostic value comparable to other invasive and noninvasive methods in detecting *H. pylori*. The test had higher sensitivity, PPV, NPV and accuracy but less specificity in young and female patients.
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1. Introduction

The worldwide prevalence of *H. pylori* is more than 50% and it is more prevalent in developing countries as compared to developed countries **(1)**.Although *H. pylori* related chronic inflammation of the gastric mucosa remains often without clinical symptoms, serious diseases as peptic ulcer disease, mucosa associated lymphoid tissue (MALT) lymphoma, and gastric adenocarcinoma develop in 15% of the infected population**(2)**.

There is no single test that can be considered the gold standard for the diagnosis of *H. pylori*. Rather, the most appropriate test for any specific situation will be influenced by the clinical circumstances, the pretest probability of infection, as well as the availability and costs of the individual diagnostic tests**(3)**.

Diagnostic tests currently used for the detection of H. pylori fall into two categories: invasive and non-invasive, the former requiring endoscopy. The invasive methods, which are biopsy-based, include culture, rapid urease test (RUT) and histology. Non-invasive testing for *H. pylori* can be done by the urea breath test (UBT), serology and analysing body materials such as faeces, urine and saliva**(4)**.

In the last years interest has been focused on noninvasive methods. As serological tests do not discriminate between current or previous infection **(5)** and as UBT requires trained staff for air sampling and expensive instruments such as an isotope ratio mass spectrometer or infrared-isotope ratio spectrometer **(6),** interest in *H. pylori* stool antigen testing developed.

As gastric epithelial cells renew once in one to three days, *H. pylori* is shed and thus can be detected in the stools **(7).**Several commercial stool antigen tests are available: enzyme immunoassays (EIAs) based on either polyclonal or monoclonal Antibodies and rapid bed-side tests like ImmunoCard STATHpSA**(8).**

ImmunoCard STATHpSA test, is a qualitative test based on monoclonal antibodies and lateral flow chromatography technique. It is useful for small laboratories that do not have equipment for performing the EIA and that work with few samples, and it is faster than the conventional EIA**(9).**Because it is easy and convenient to perform, the ImmunoCard STAT HpSA could be used at many situations,

especially for children, pregnant women, old people and others who are not suitable for endoscopy**(7)**.

Aim: The aim of this study is to evaluate the reliability of the Helicobacter pylori rapid stool antigen test, ImmunoCard STAT HpSA, for detecting H pylori infection.

2. Patients and Methods:

The study was conducted on 160 adult patients suffering from dyspepsia. Patients on proton pump inhibitors, H2blockers and h pylori eradication therapy for the last 4 weeks and patients with active gastrointestinal bleeding were excluded. Informed consent was obtained from each patient included in the study and the study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a priori approval by the institution's human research committee.

All the patients were subjected to history taking and full clinical examination with special emphasis on gastrointestinal symptoms and examination.

All patients underwent ***upper gastrointestinal endoscopy***. 4 quadrants biopsy specimens from the antrum and 4 quadrants biopsy specimens from the gastric corpus were taken.

Histopathological examination of biopsy specimens was done after Haematoxylin and Eosin (H&E) staining for *H. pylori* bacterium detection.

Rapid Helicobacter pylori stool antigen test (ImmunoCard STAT HpSA): All patients were examined for *H. pylori* using the rapid *H. pylori* stool antigen test ImmunoCard STAT HpSA (Meridian® Diagnostics, Inc., USA). On the same day of endoscopy, patients collected stool into an air tight container. A small portion (5-6 mm diameter) of stool specimen was transferred into the sample diluent vial using the applicator stick, vortexed for 15 seconds, and then 4 drops were dispensed into the round window at the lower end of the device. The results were read 5 minutes later. Positive and negative results were judged as recommended by the manufactures: ***negative***: Only one blue band appears across the central window, ***positive:*** In addition to the blue control band, another red band (test line) also appears, and ***invalid:*** A total absence of the control colored band regardless the appearance or not of the test line. Invalid test results were discarded.

Statistical Methodology:

Data was analyzed using Statistical Package for Social Science (SPSS) software computer program version 17. Data were described as mean ± standard deviation (SD) for quantitative (Numerical) variables and as frequency & percentage for qualitative (Categorical) variables. Sensitivity, specificity, PPV, NPV and accuracy of the kit were calculated according to the histopathology as gold standard.

3. Results

A total of 160 adult patients were enrolled in the study. 124(77.5%) of them were males and 36 (22.5%) females with their ages ranged from 18 to 70 years old and mean age 39.1 ± 12.1 years. 18 patients (11.3%) were smokers,4 patients (2.5%)were analgesic abuser, 14 patients (8.75%) were HCV positive and 10 patients (6.25%) were diabetic (**Table 1)**.

The most prevalent endoscopic findings in the study patients were pangastritis in 124 cases (77.5%) then lower end esophagitis in 72 cases (45%) (**Table 2)**.

Table 1- Characteristics of study population

Parameter	Findings		
Age	Mean 39.09 ± 12.11	Range 18 – 70	*n=160*
Sex	Male	124	77.5%
	Female	36	22.5%
Smoker	18	(11.3%)	
NSAIDs	4	(2.5%)	
HCV	14	(8.75%)	
DM	10	(6.25%)	

Table 2- Endoscopic findings among study patients

Endoscopic findings	**Frequency (n)**	**Percent(%)**
Pan gastritis	124	77.5%
Lower End esophagitis	72	45%
Antral gastritis	24	15%
Duodenal Ulcer	14	8.75%
Bulb duodenitis	22	13.8%
Hiatus Hernia	6	3.75%
Gastric Ulcer	4	2.5%
Pre-pyloric erosions	4	2.5%

As regard h pylori status according to histopathological examination, 112 patients (70%) were positive for *H. pylori* infection and 48 patients (30%) were negative. Testing for *H. pylori* using ImmunoCard STAT HpSA rapid stool antigen test revealed that 106 patients (66.25%) had positive test and 54 patients (33.75%) had negative test (**Tables 3,4).**

Table (3): *H. pylori* status in study patients according to histopathology and immunocard STAT HpSA test.

Test	Result	Frequency (n)	Percent (%)
Histopathology	Negative	48	30%
	Positive	112	70%
Immunocard	Negative	54	33.75%
	Positive	106	66.25%

Table (4): Performance of the ImmunoCard STAT HpSA test according to histopathology.

Immunocard	Histopathology		Total
	Positive	Negative	
Positive	96	10	106
Negative	16	38	54
Total	112	48	160

Compared with histopathology as gold standard, the ImmunoCard STAT HpSA test had sensitivity, specificity, positive predictive value (PPV), negative predictive values (NPV) and total accuracy of 85.7%, 79.2%, 90.6%, 70.4% and 83.8%, respectively. The test was more sensitive in patients below 39 years old(the mean age of the population study) (n=98) (88.6% vs 81%) with higher PPV (91.2 % vs 89.5%), NPV (73.3% vs 66.7%) and accuracy (85.7% vs 80.6%) but less specificity than in patients ≥39 years (n=62) (78.6 % vs 80%). Immunocard testing in females (n=36) had higher sensitivity (92.9% vs 83.3%), PPV (92.9% vs 89.7%), NPV (75% vs 69.6%) and accuracy (88.9 % vs 82.25%) but lower specificity than in males (n=124)(75% vs 80%)**(Table 5).**

Table (5): Sensitivity, specificity, PPV, NPV and accuracy of the ImmunoCard STAT HpSA testin study population.

Parameters	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
Study population(n=160)	85.7	79.2	90.6	70.4	83.75
Male (n=124)	83.3	80.0	89.7	69.6	82.25
Female(n=36)	92.9	75	92.9	75.0	88.9
Below 39 (years) (n=98)	88.6	78.6	91.2	73.3	85.7
39 (years) or older (n=62)	81	80	89.5	66.7	80.6

4. Discussion:

With histopathology as gold standard, the ImmunoCard STAT HpSA in our study was found to have sensitivity, specificity, positive predictive value, negative predictive value and total accuracy of 85.7%, 79.2%, 90.6%, 70.4% and 83.75%, respectively.

Many studies reported higher sensitivity and specificity. Meta-analysis of eleven trials by Hong *et al.,* revealed that ImmunoCard STAT HpSA for the primary diagnosis of *H. pylori* infection had pooled sensitivity and pooled specificity as 0.93 (95% CI: 0.91-0.94) and 0.93 (95% CI: 0.90- 0.95), respectively **(10).** Also Wu *et al.,* reported Sensitivity and specificity of 95.8% and 91.1% respectively. Positive and negative predictive values in their 253 patients, were also higher than in our study as 90.4% and 96.1% respectively (11).

Cheng and Hu studied 80 patients with rapid urease test, *H. pylori* culture and Warthin-Starry silver staining of gastric biopsy used as "gold standard" in patients who underwent upper gastrointestinal endoscopy and urea breath test as the "gold standard" for the patients who did not undergo gastroscopy. The sensitivity, specificity, and accuracy of immunocard STAT HpSA test were 100%, 93.2%, and 96.3%, respectively**(12).** Lu *et al.,* study on one hundred twenty patients showed that the stool immunocard STAT HpSA test relative to the confirmed results had sensitivity, specificity, and accuracy as 96.8%, 82.8% and 90%, respectively**(13).**

Also Li *et al.,* evaluated 53 patients with gold standard being the urease test, Warthin-Starry staining and culture and they reported ImmunoCard STAT HpSA to have Sensitivity, specificity, positive predictive value, negative predictive value and total accuracy of 92.6%, 88.5%, 89.3%, 92% and 90.6% respectively**(7).**

The study by Chisholm *et al.* reported sensitivity of (87.8 %) and specificity of (89.4 %) for immunocard STAT HpSA test, with culture and

histological examination of gastric biopsies as gold standard **(14).**

In children population, Kalach *et al.,* found the overall sensitivity, specificity, positive and negative predictive values to be 86.2%, 92.9%, 78.1%, and 95.8%, respectively, with an accuracy of 91.4% **(15).** Also Kato *et al.,* reported sensitivity, specificity and accuracy of 90.6%, 95.8%, and 94.0% respectively, in their study enrolling one hundred and eighty-two children and adolescents, 3-17 years of age **(16).**

Some other studies reported much lower sensitivity and specificity than our values. Kaklikkaya *et al.,* compared ImmunoCard STAT HpSA with 4 invasive tests (histology, gram staining, rapid urease test, and culture) on sixty five patients. They found that the ImmunoCard STAT HpSA test had sensitivity of 70.6% and specificity of 70.6%**(17).** Also with the concordance of the rapid urease test, histopathology, and urea breath test as gold standard, Calvet *et al.,* found ImmunoCard STAT HpSA to have sensitivity of 69% and specificity of 90%**(18).** This variation of results in the studies may be explained by the sample size and the gold standard variation in every study.

In our study, the test was more sensitive in patients younger than 39 years old (88.6% vs 81%) with higher PPV (91.2% vs 89.5%), NPV(73.3% vs 66.7%) and accuracy (85.7 % vs 80.6%) but was less specific than in patients aged 39 years or older(78.6% vs 80%).

Also we found that Immunocard testing in females had higher sensitivity (92.9% vs 83.3%), PPV (92.9% vs 89.7%), NPV (75% vs 69.6%) and accuracy (88.9% vs 82.25%) but lower specificity than in males (75%vs 80%). But this needs to be confirmed on a lager female population as females in our study were 36 patients (22.5%).

Taking into consideration the reported sensitivity and specificity of invasive tests:[rapid urease test: 80–95% and 90–100%**(19)**, Histopathological examination: 83–95% and 90–100% **(20)** and culture, 80–90% and 95–100%**(21)**, respectively]and the reported sensitivity and specificity for noninvasive tests:[serology measuring IgG antibodies : 80–100% and 69–95%,**(22),** urea breath test: 81–100% and 80–98%, **(23)** and the stool antigen test performed by conventional immunoassay (EIA) using polyclonal antibodies:88.9% - 98.3% and 77.8% - 98.4%**(9)**, respectively], immunoCard STAT HpSA test has a comparable sensitivity and specificity (85.7% and 79.2% respectively), to other well established tests in detecting h pylori.

Conclusion:

ImmunoCard STAT HpSA is efficient and can be used as an alternative noninvasive method to detect *h. pylori* infection in adults and has a diagnostic value comparable to other well established diagnostic tests for detecting *H. pylori*. The test had higher sensitivity, PPV, NPV and accuracy but less specificity in young and female patients.

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