

Original Article

Helicobacter Pylori Antibodies in Hazara Division Population

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Objective: To evaluate the sensitivity and specificity of a rapid one step test for the qualitative detection of *Helicobacter pylori* (H. pylori) antibody and screen an asymptomatic Hazara division population for H. pylori seroprevalence.

Place & Duration of Study: The study conducted at Inam clinical laboratory, Abbottabad, during the period from 2008 to 2011

Study Design: Analytical study

Materials & Methods: It was designed as a blind comparison of a commercial serological test kit for serological diagnosis of *H. pylori* with a standard biopsy related test referred from various hospitals and private clinics located in Hazara division. We included 70 symptomatic male and female patients, as well as 90 randomly collected sera from asymptomatic patients.

Results: The sensitivity of the Acon One step *H. pylori* Test Device was 59% with a specificity of 89%. Screening of the asymptomatic patient showed an age related increase in antibody prevalence.

Conclusion: Because of the limited sensitivity in the investigated population, the use of the Acon One step *H. pylori* test device alone cannot be recommended for the serological diagnosis of *H. pylori* infection in the Hazara division population.

Key Words: H. Pylori Infection, H.pylori antibodies, serodiagnosis

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Introduction

Helicobacter pylori (H. pylori) has been established as an important etiological factor for chronic gastritis and duodenal ulcer.¹ It is also associated with gastric ulcer and gastric cancer.² As the test-and-treat policy for H. pylori infection is gradually being accepted by general practitioners, a simple, reliable, and noninvasive diagnostic test for H. pylori has become essential in clinical practice.³ At least half the world's population are infected by the bacterium, making it the most widespread infection in the world. Actual infection rates vary from nation to nation; the developing world has much higher infection rates than the West (Western Europe, North America, Australasia), where rates are estimated to be around 25%.⁴ Infections are usually acquired in early childhood in all countries.^{5,6} However, the infection rate of children in developing nations is higher than in industrialized nations, probably due to poor sanitary conditions. In developed nations it is currently uncommon to find infected children, but the percentage of infected people increases with age, with

about 50% infected for those over the age of 60 compared with around 10% between 18 and 30 years.⁴ The higher prevalence among the elderly reflects higher infection rates when they were children rather than infection at later ages.⁵ Prevalence appears to be higher in African-American and Hispanic populations, most likely due to socioeconomic factors

It has become increasingly clear that the *H. pylori* infection plays a major role in the pathogenesis of chronic gastritis and peptic ulcer disease and their recurrence. Furthermore, there is increasing evidence of its importance in the pathogenesis of gastric cancer as well. Therefore efforts have been made to develop noninvasive tests with high sensitivity and specificity to predict *H. pylori* infection.⁷⁻¹³ A rapid, inexpensive, and noninvasive serological test would be useful in the diagnosis of *H. pylori* infection in symptomatic patients. Many recently developed serological kits are available including third generation ELISA tests using combination of purified antigens.^{9,10}

In past few years, increasing numbers of *Helicobacter* species have been identified with small extremely variable genomes undergoing natural

transformation.^{14,15} It has therefore been stressed that serological assays need to be standardized and validated in each population.¹⁶⁻¹⁸

A rapid Acon one step *H. pylori* test device, a qualitative membrane strip based immunoassay (chromatographic immunoassay), utilizes a combination of *H. pylori* antigen coated particles and anti-human IgG to qualitatively and selectively detect *H. pylori* antibodies in serum or plasma, (Manufactured by ACON Laboratories, Inc. 4108 Sorrento Valley Boulevard, San Diego, CA 92121, U.S.A) was carried out and compared with the results of histological staining in the diagnosis of *H. pylori* infection.

To evaluate the sensitivity and specificity of a rapid one step test for the qualitative detection of *Helicobacter pylori* (*H. pylori*) antibody and screen an asymptomatic Hazara division population for *H. pylori* seroprevalence.

Materials and Methods

The study conducted at Inam clinical laboratory, Abbottabad, during the period from 2008 to 2011.

In a period of three years, we investigated 70 sera from symptomatic Hazara division patients who had undergone gastroduodenoscopy for dyspeptic symptoms; namely, epigastric pain, fullness, belching, heartburn, nausea or vomiting.

Inclusion Criteria: Patients for dyspeptic symptoms namely, epigastric pain, fullness, belching, heartburn, nausea or vomiting.

Exclusion Criteria: Patients with a history of recent antibiotics or bismuth therapy were excluded from the study.

We evaluated its ability to identify *H. pylori* infection in 70 symptomatic adult Hazara division populations referred for gastroduodenoscopy. 90 sera from asymptomatic patients from Hazara division in varying age group were also screened for *Helicobacter pylori* antibodies using the same one step *H. pylori* test device. Blood samples were taken immediately after gastroscopy and the one step *H. pylori* test was performed immediately after the extraction of the sera. 100 ul of serum sample placed in the specimen well of the test device. After 10 minutes results were read. The presence of red line in the test region was identified as positive for *H. pylori* antibodies by comparison with positive and negative controls. The observer was not aware of the histological results of *H. pylori* staining.

In another group, 90 sera from asymptomatic Hazara division patients in six different age groups (0 to 2 years, 3 to 10 years, 11 to 20 years, 21 to 30 years, 31 to 40 years and above 40 years of age) were screened for *H. pylori* infection as described above.

Upper gastrointestinal endoscopy was performed. Endoscope routinely sterilized in 2% glutaraldehyde before each endoscopy, using sterile biopsy forceps on each patient. Patients had fasted for at least eight hours before the examination. Antral biopsies were immediately placed in 5% formalin and sent for histopathological studies.

Four different stains were used for the paraffin sections: hematoxylin-eosin modified Gram's stain, alcian blue and PAS. Sorensen's phosphate buffer stock solution was prepared using the following protocol: 9.465 g of sodium phosphate dibasic was dissolved in 1 liter of distilled water; 9.08 g of potassium acid phosphate was dissolved in 1 liter of distilled water. 25 ml of each solution was blended and made up to the final pH of 6.8. Toluidine O stain, used to identify *H. pylori*, was prepared by mixing 50 ml of Sorensen's phosphate buffer with 1 ml of 1% stock toluidine blue solution. The staining method was as follows: 1) section at 6 um to water, 2) stain in toluidine blue buffered solution for 10 to 16 minutes, 3) wash well in water, 4) dehydrate, clear and mount. All slides were examined by the same pathologist, who were unaware of the serological results. *Helicobacter* infection was defined as positive when spiral curved bacteria were identified in the toluidine O stained histology sections in at least one of the biopsies.

Results

50 of the patients were male and 20 were female. Their ages ranged from 19 to 78 years, with an average age of 38 years. 61 of 70 symptomatic patients showed *H. pylori* infection, as defined by positive histological staining at antral biopsy (87%). 36 of these were identified by the use of the One step *H. pylori* test device (sensitivity 59%). One patient showed a positive serological test with negative staining result ($P < 0.01$). The One step *H. pylori* test device correctly identified 8 out of 9. *Helicobacter*-negative patients (specificity 89%).

Only 36 of 61 *H. pylori* infected patients proven by histological staining showed a positive one step *H. pylori* test device. There was only one false positive, but 25 false negative results. The test identified 8 of 9 histologically *Helicobacter*-negative patients (Table I). The sensitivity for One step *H. pylori* test device under these circumstances was 59%, with a specificity of 89%. Therefore, the use of this One step *H. pylori* test device alone cannot be recommended for serological diagnosis of *H. pylori* infection or pre-endoscopy screening in the Hazara division population.

Table I: Correlation of Serological Detection with Histopathology (n=70)

Histopathology (Biopsy)		Serological Kit	
Positive (+)	Negative (-)	Positive (+)	Negative (-)
61	09	36/61	08/09
		1 false positive (+) 25 false negative (-)	
Sensitivity:		59%	
Specificity:		89%	
Positive Predictive Value:		97%	
Negative Predictive Value:		24%	
Accuracy:		63%	

Discussion

It has been clearly shown in other studies that qualitative membrane strip based immunoassay to detect circulating IgG *H. pylori* antibodies are less sensitive and specific than testing sera with immunoblotting or ELISA techniques.⁷⁻¹⁰ Previous studies have demonstrated population-based differences in antibody prevalence. In addition, there are significant age related differences in antibody prevalence within a single population using the same serological tests.^{4,5} The study demonstrated a high prevalence of *H. pylori* infection in both symptomatic and asymptomatic patients in Hazara division, confirming results of previous authors.^{19,20} The increasing prevalence of serologically detectable *H. pylori* infection with advancing age observed in other populations was also confirmed for the asymptomatic Hazara division population.^{20,21}

The false positive serology result may be partially explained by the patchy distribution of *H. pylori* infection within the mucosa, resulting in negative histological results at biopsy. Another explanation is that the patient was unaware or did not remember a recent antibiotic therapy when he/she was interviewed to meet the inclusion and exclusion criteria for the study protocol. The specificity reached 89% when additional biopsies were taken from the antrum to prove *H. pylori* infection. Comparable results using the same serological procedure have been reported by other authors, who used two positive biopsy related results as the gold standard to define *H. pylori* infection.^{8,12,21} If we had screened the sera of asymptomatic patients with the Acon One step *H. pylori* test device and applied the specificity and sensitivity given by the manufacturer (75.9% and 99%), the difference between each age group would be highly significant and could have led to the wrong conclusion. It was therefore important to validate the test in the Hazara division population.

The low sensitivity found in the serological test for *H. pylori* antibody confirm similar result found in other populations, especially in younger patients and does not appear to be due to technical error.^{8,11}

The kits used for the tests were new and had undergone the manufacturer's quality control testing. The tester was unaware of histological staining results and the manufacturer's instructions were strictly followed using positive and negative controls for each sample. Westblom reported even lower sensitivity for serological detection of *H. pylori* antibodies (36% and 38%) when used in pediatric patients.^{9,10} Witteman reported a sensitivity of 43% if serological detection of *H. pylori* antibodies is used in adults where *H. pylori* infection was confirmed by histology and standard culture.⁹

We agree with other authors who believe the problem is directly related to the population tested.^{9,10} Diversity of the host's immune response based on inheritance, age, environmental factors and differences in the antigenicity of *H. pylori* strains may cause a less intense immune response resulting in lower IgG antibody titers not detected by the less sensitive One step *H. pylori* test device.¹⁶⁻¹⁸

Therefore, the use of this One step *H. pylori* test device alone cannot be recommended as a screening test for the serological diagnosis of *H. pylori* infection, especially in the younger Hazara division population. When we compared the overall seroprevalence for all patients under 20 years (N=45) with remainder older than 20 years (N=45), the result was significant (8% to 33%), ($P<0.05$). The values for each group can only show trend. The real seroprevalence tested with a sensitive second and third generation ELISA kit would be expected to be higher than our data suggest. Further studies in the Hazara division population using the more sensitive available serological tests should be carried out to choose the most sensitive one.²²

Conclusion

Because of the limited sensitivity in the investigated population, the use of the Acon One step *H. pylori* test device alone cannot be recommended for the serological diagnosis of *H. pylori* infection in the Hazara division population.

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