Original Article

Evaluation of Serological tests for the diagnosis of Helicobacter pylori infection

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Abstract

A total of 58 outpatients referred for endoscopic evaluation of gastroduodenal symptoms were included in this study. Biopsy specimens were taken from the gastric antrum of each patient. Samples were tested for the presence of H. pylori by standard biopsy related tests (urease, histology, and culture) which are considered as gold standard methods for H. pylori detection. Sera from these patients were tested for anti - H. pylori antibodies by enzyme-linked-immunoassay, immuno-chromatogrphy, and latex agglutination test for the evaluation of performance indices of these techniques.

Sensitivity, specificity, positive and negative predictive values and accuracy of each test were calculated relative to one or more of the "gold standard".

A total of 45 patients gave positive results for the presence of H. pylori by two or more of these tests used.

The other 13 samples showed negative results by all three tests used. Serological tests show sensitivities ranging from 95.5% for ELISA technique to 80% for latex agglutination test. Specificity ranges from 76.9% in ELISA technique to 69.2% by latex agglutination method.

Serological tests can provide a reliable non invasive methods for detection of H. pylori infection.

Introduction

H. Pylori is a Gram-negative, spiral shaped, microaerophilic bacillus that resides beneath and within the mucous layer of the gastric mucosa and produce multiple enzymes such as urease and mucolytic proteases that are important for its survival and for its pathogenic effect[1].

Infection is almost acquired in childhood and the main risk factor for infection is poor socioeconomic condition [2].

Infection is almost always associated with non ulcer dyspepsia, histologic chronic (type B) gastritis and a major risk factor for the development of peptic ulceration, atrophic gastritis, gastric cancer and gastric lymphoma[3].

Standard diagnostic test relies on gastric biopsy. Of these tests urease test is very reliable, sensitive, specific, inexpensive and simple. This test is done by transferring one or preferably two biopsies into urea containing test medium that detects the presence of urease by alkalinization that results from cleavage of urea [4,5]. Histological examination of routinely stained gastric biopsy could have similar sensitivity and specificity by experienced pathologist [1,4]. Culture is the most laborious, tedious and expensive detection method. Even under most favorable conditions, the sensitivity of culture is between 70-80%[4]. Culture

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should be conserved for special circumstances as when antibiotic resistance is suspected[1,3,6].

H. pylori does not only lead to a strong inflammatory response of the gastric mucosa but also induces a profound specific humoral immune reaction. The presence of H. pylori infection can thus be reliably diagnosed by detecting IgG and IgA antibodies directed against specific H. pylori antigens.

Many serodiagnostic tests are available based on the detection of IgG class antibodies versus this organism. Some of these tests are claimed to be almost equivalent to those of histology and biopsy urease testing [7,8,9,10,11]. Others show poor correlation between the presence of H. pylori infection and the antibody response [12].

Materials and Methods

Fifty eight patients attending the endoscopy unit of Al-Kademia teaching hospital with different types of gastric complaints were enrolled in this study.

Blood samples were collected before endoscopy. Gastric antral biopsy specimens were taken.

Patients aged less than 18 years; patients who had taken antibiotics or proton pump inhibitors or bismuth preparations in the previous four weeks were excluded from the study.

The blood specimens collected were allowed to clot and the sera were separated. The sera were frozen and stored at - 20° C until required.

Antral biopsy specimens were collected for culture of H. pylori, histology and urease production.

Culture

Biopsy specimens for culture were transported to bacteriological laboratory in sterile brain heart infusion broth and were kept in a cool bag or 4°C until cultured. The specimens were processed within a limited time of not more than four hours. Antral biopsies were crushed on sterile glass slides, homogenized with sterile needles and then cultured on brain heart infusion agar containing 7% horse blood, 0.25% yeast extract and Campylobacter selective supplement (skirrow-Oxoid SR 69) containing vancomycin, polymyxin and trimethoprim. The pH was adjusted to 6.8-6.9. Plates were incubated in microaerophilic environment generated by gas pack (Generbag Microaer, BioMerieux 45531) at 37°C for up to seven days. Suspected colonies of H. pylori were identified by Grams staining, catalase and oxidase test. Confirmation of the isolate was done by API campy system (Bio Merieux). Subculturing was done in brain heart infusion broth-filled containers, incubated for 3 days under microaerophilic conditions [13,14].

Histology

Hematoxylin and Eosin stain was used by pathologists for identification of the bacteria in the biopsy specimens [13,14].

Urease test

Presumptive evidence of the presence of H. pylori in biopsy material was obtained by placing a portion of the crushed tissue biopsy material directly into urea containing agar which was prepared as follows: 4.6 gm of the urea agar base suspended in 190 mL distilled water, autoclaved at 115°C for 20 minutes, then cooled to 50°C before aseptically adding 10 mL of 40% w/v urea solution, mixed well, distributed into sterile containers and allowed to set at slopes.

A positive test manifested by color changes (yellow to pink) due to alkalinization of media is considered indicative of the organism presence[13,14].

Serology

Serum specimens were tested for anti-H. pylori antibodies using commercially available kits. Techniques included were latex agglutination, immunochromatography, and enzyme linked immunosorbent assay. The sensitivities, specificities, positive and negative pridictive values of those kits were evaluated. The detection of H. pylori in antral biopsy specimens by culture, histology, urease production or any combination of those tests were considered as the "gold standard".

Latex test: the Pylori Dry Latex test (Orion Diagnostics) contains latex particles sensitized with H. pylori antigen. H. pylori antibodies if present in the serum samples will react with the sensitized latex resulting in visually detectable clumps.

Immunochromatography (Bio sign H. pylori WB) is a one step immunochromatographic test for the detection of antibodies to H. pylori in human serum. The method employs a combination of anti-human immunoglobulin dye conjugate (colloidal gold) and highly purified H. pylori proteins. As the sample flows through the absorbent device, the anti-human immunoglobulin dyed conjugate bind to the human IgG antibodies forming an antigen antibody complex. This complex binds to H. pylori proteins fixed in the zone (B) and produces a colored band In the absence of

(Colloidal gold) and highly purified H. pylori proteins. As the sample flows through the absorbent device, the anti-human immunoglobulin dyed conjugate bind to the human IgG antibodies forming an antigen antibody complex. This complex binds to H. pylori proteins fixed in the zone (B) and produces a colored band In the absence of anti- H. pylori antibodies, there is no colored band in the test zone (B). The reaction mixture continues flowing through the absorbent device to the control zone (C). Unbound conjugate binds to the reagent fixed in the control zone (C), producing a colored band, indicating the proper performance of the test.

Enzyme linked immunosorbent assay (Bio-Hit, Finland) The test is based on sandwich enzyme immunoassay technique with purified H. pylori bacterial antigen adsorbed on microwell plate and detection antibody labeled with horse radish peroxidase.

Results

Fifty eight patients participated in this study. Their ages ranged from 18-62 years with an average of 34.7 years.

Forty five of the 58 patients were positive for H. pylori by one or more of the "gold standard" tests (culture, histology and direct urease test). The remaining 13 were negative for H. pylori by all the three tests. The pattern of these results are shown in (table 1).

Immunodiganostic test were done on sera of three tests. The pattern of these results are shown in (table 1).

Immunodiganostic tests were done on sera of those patients. Rapid latex test could confirm the infection in 36 cases of those who were positive by the standard invasive techniques. It missed the diagnosis in 9 cases and gave a false positive reaction in 4 cases thus giving a sensitivity of 80% and a specificity of 69.2%.

Immunochromatographic technique could detect 40 cases of the proved cases. Other indices are shown in table 2.

Value of the ELISA system was calculated as Enzyme immuno units (EIU).

EIU was calculated as the absorption at 450 divided by the absorption of a positive control. Values exceeding 30 EIU were considered as positive (Cut-off value of 30 was used and according to manufacturer instructions). With this cut-off value 43 cases out of the 45 positive cases by the invasive technique gave a positive reaction. On the other hand sera of three of the patients who showed negative results by the standard procedures yielded positive serological test. Thus the serum IgG ELISA had a sensitivity of 95.5% and a specificity of 76.9%.

Details of performance indices of the different techniques are shown in table 2.

Discussion

Ulcer disease is an infectious disease [15,16]. If the infection is diagnosed and treated, ulcer disease can be cured. And as the pathogenic role of H. pylori in ulcer

UREASE	HISTOLOGY	CULTURE	NO. OF PATIENTS
+	+	+	3
+	+	-	35
+	-	+	5
-	+	+	2
-	-	-	13
43(95.5%)	40(88.8%)	10(22.2%)	58

Table 1:The results of biopsy related tests for the detection of H. pylori infection in gastric antral biopsy.

Table 2: Evaluation of the performance of serological tests in comparison with gold standard biopsy related tests.

Serological tests		Gold standard +	Gold standard -	Sensitivity %	Specificity %	PPV %	NPV %	Overall accuracy %
(ELISA)	+	43	3	95.5	76.9	93.5	83.3	91.3
	-	2	10					
Immunochrmotography	+	40	4					
	-	5	9	88.8	69.2	91	64.3	84.4
Latex test	+	36	4	80	69.2	90	50	77.5
	-	9	9					

Table 3: Changes in performance characteristics of Elisa with different cut-off values.

Cut-off value	**Sensitivity %**	**Specificity %**	**PPV %**	**NPV %**	**Overall accuracy %**
10	**100**	**15**	**80.3**	**100**	**81**
20	**95.5**	**53.8**	**87.7**	**77.7**	**86.2**
30	**95.5**	**76.9**	**93.5**	**83.3**	**91.3**
40	**77.7**	**84.6**	**94.5**	**52.3**	**79.3**
50	**62.2**	**92.3**	**96.5**	**41.3**	**689**
60	**55.5**	**100**	**100**	**39.3**	**65.5**

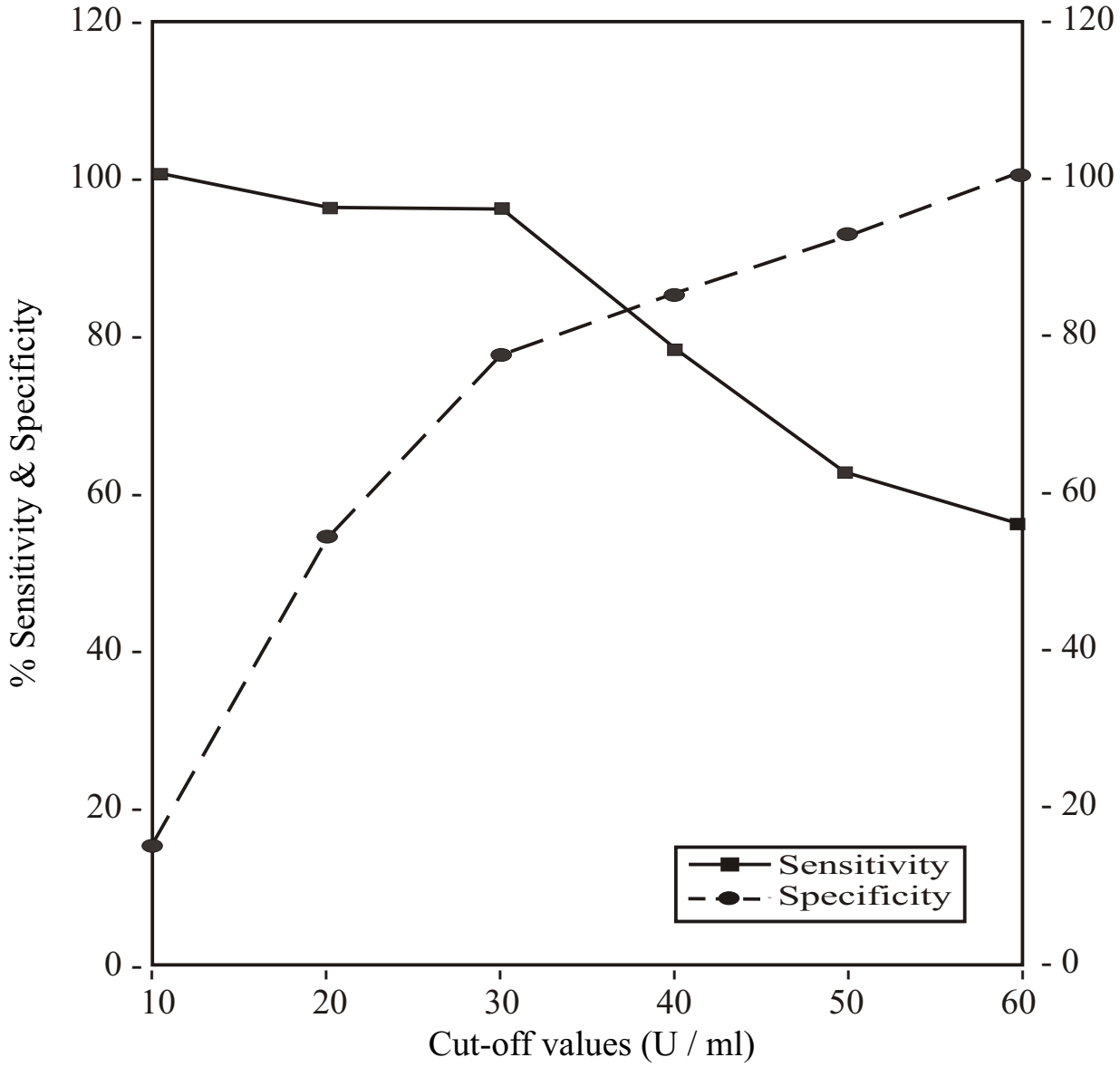

Fig.l
Changes in the sensitivity and specificity with different cut-off values for the ELISA assay.

Discussion

Ulcer disease is an infectious disease [15,16]. If the infection is diagnosed and treated, ulcer disease can be cured. And as the pathogenic role of H. pylori in ulcer disease and other upper gastro-intestinal conditions is established, testing of the organism gains wider acceptance.

Though endoscopy provides means of obtaining the organism for culture, screening for reflux eosophigitis and possible stomach cancer, it is a costy and unpleasant for the patient. Moreover, culture of the organism is difficult to perform and was not even evaluated in many large studies.

In this study, out of the 45 total positive biopsies, only 10 were positive by culture, i.e 22.2%. It should be kept in mind that negative results does no exclude the presence of H. pylori infection, although isolation by of the microorganism by culture certainly indicates it's presence. Many factors could have contributed to this reduced sensitivity of this method in our study.

this reduced sensitivity of this method in our study.

1. Only one biopsy from each patient was taken for culture. Using more than one biopsy from different gastric sites could have raised the number of positive results.
2. It is well known that the bacterium is slow growing and fastidious and it is possible that some H. pylori strains will not form colonies on some currently available media, like the one we have used[17].
3. Patients' ingestion of topical anesthetic, semithicone, prior treatment with antibiotics, H2-receptor antagonists or proton pump inhibitors can reduce the viability of bacteria [18].
4. The use of abundant amounts of gluteraldehyde in sterilization of the endoscope might have deleterious effect on the bacterium.

Histological examination gave acceptable results. This is possibly due to examination of antral biopsies whereby the antrum is more affected than the body[19].

Unlike biopsy related tests, serological tests can detect systemic immunological response to H. pylori infection which effectively sample the whole stomach.

Many serological tests were introduced as non invasive alternative. These studies gave conflicting results regarding different serological tests available for H. pylori diagnosis.

We attempted to evaluate three commercially available kits with different techniques, namely latex test, immunochromato-graphic test and enzyme-linked immunosorbent assay.

Non invasive serological tests are as accurate indicators of H. pylori status as the invasive test. However, latex agglutination technique and immunochromatography are less accurate and less specific than the ELISA test but its ease of use, convenience, lower cost, more rapid results and availability in primary care make it useful for patient screening.

Sera of dyspeptic patients with negative reaction by the gold standard criteria showed positive reaction by serological test in variable percentages. 6%, 10%, 11 % were positive by ELISA, immunochromatography and latex test respectively. This means that serological evidence of H. pylori was greater than the prevalence of infection by biopsy related tests. Such an observation could be due to the following possible causes.

1.H. pylori infection in the stomach may be patchy possibly due to metaplasia or regrowth after failed

of H. pylori was greater than the prevalence of infection by biopsy related tests. Such an observation could be due to the following possible causes:-

1.H. pylori infection in the stomach may be patchy possibly due to metaplasia or regrowth after failed eradication. Such conditions could be detected by serological tests more properly[2].
2. Biopsy specimen sample only a very small part of the stomach whereas antibody detection methods effectively sample the whole stomach.
3. Antibody against H. pylori remain detectable for many months after eradication.

So false positive results by immunological tests may be false negative results by the gold standard criteria.

Latex agglutination test showed a performance characteristics that were lower than the other two tests which could be attributed to the detection limit of this test (0.006-0.06 ug/ml)[20]. Though immunochromatography had better performance than latex test, it is still less than that of the ELISA technique and this is possible due to the lacking of amplification effect of enzyme immunoassay (detection limit of ELISA is <0.0001-0.01 ug/ml)[21].

One of the problems we faced was the calculation of the cut-off value, since the latter must be determined for each assay based on the prevalence of the microorganism in the population. Till now there are no epidemiological studies concerning the seroprevalence of H. pylori antibodies among Iraqi people, therefore the cut-off value suggested by the manufacturer was used. Moreover, changes in performance characteristics of ELISA with different cut-off values was studied. Maximum accuracy was obtained at a cut-off value of 30. Table (3) and figure (1) show the relation of cut-off value and performance indices.

Positive predictive value and negative predictive value are dependant on the prevalence of the organism within a particular population [22].

Conclusions

From this study, one can conclude that non-invasive serological tests are convenient for diagnosis of H. pylori infection due to its good performance characteristics and simplicity of the techniques. These tests vary in performance indices, with ELISA technique having the best over
all accuracy, followed by immunochromatography and latex test.

References

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