Microbiology

Research

PERFORMANCE EVALUATION OF A HELICOBACTER PYLORI IgG ELISA KIT IN A TERTIARY CARE CENTRE IN SOUTH KERALA

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Abstract

Background: Helicobacter pylori is a bacteria present on the surface of gastric mucosa and mucus of man and several other mammals. H. pylori is an established pathogen responsible for different types of malignancies of stomach and peptic ulcers. The invasive method of endoscopic gastric biopsy although very useful, is embarrassingly discomforting for many of those who undergo it. The non-invasive tests like IgG serology are very cost effective though less sensitive and specific compared to urea breath tests and faecal antigen test. A method to increase sensitivity and specificity at the same time will improve the accuracy of the test in this population and reduce false positive and false negative values by use of ROC curve. The endoscopy may thus be restricted to those IgG positive above 50 years, where malignancy is more likely. The aim is to establish and validate a most accurate cut off point for IgG ELISA kit for Indian population to get maximum sensitivity and specificity for the kit provided by a foreign manufacturer validated for their population. **Materials and Methods:** A cross-sectional study by simple random sampling of dyspeptic patients attending medical gastro-enterology OP who satisfies the inclusion criteria were selected to undergo endoscopy and collected blood at the same time. Two samples were collected from gastric antrum used for urease test and histopathology examination using eosin -haematoxylin and Giemsa stain to detect H pylori. Gold standard is selected by the combination of either urease test or/and histopathology. Standard calibrators provided by the manufacturers are used for finding the IgG values for the optical density measured. Patient details and demographic factors are also collected along with the results of the tests in the proforma, entered in Excel and analysed. Sensitivity, specificity, and other parameters were found out by both manufacturer's cut off and ROC cut off. **Result:** The ROC cut off was found to be 0.938 U/ml for IgG compared to manufacturer's cut off at 1.0 U/ml. They have diagnostic accuracy of 88.1% to 90.8% respectively. The diagnostic odds ratio has improved from 48.7 to 73.4%. **Conclusion:** The new cut off value is more suitable for population in this part of India to give more accuracy and detect more true positive cases. Histopathology helps in gauging the stage of disease and help to detect early malignancy also. IgG serology alone may be used in dyspeptic patients below 50 years and eradication therapy may be started in them without endoscopic biopsy.

INTRODUCTION

Stomach cancer is the 6th common cause of cancer as per WHO, and 5th leading cause of deaths due to cancer worldwide.[1] Studies have shown that H. Pylori infection of the stomach causes chronic inflammation of the mucosa of the stomach and is strongly associated with gastric ulcer disease and gastric cancer.[2] Helicobacter pylori is a microaerophilic, fastidious, spiral shaped Gram-negative organism inhabiting in the gastric mucosa or its overlying mucus of infected persons.[3] The bacterium is associated with peptic ulcers, mucosa associated lymphoid tissue (MALT) lymphoma, adenocarcinoma of the body and antrum of the stomach.[4] International agency for research on cancer(IARC) has declared H. pylori as a Class I carcinogen for stomach cancer.[5] Treatment if started early can prevent gastric malignancy, arrest

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progression or reverse the outcome of MALT lymphoma. Thus, the detection of H. pylori infection in high-risk individuals is of utmost importance.

Both invasive and non- invasive tests are available for direct and indirect detection of H. pylori infection. The invasive method needs an endoscopic biopsy, while non-invasive techniques involve antigen or antibody detection in blood, saliva or stool; or by urea breath test. In invasive methods, one has to suffer higher level of discomfort which might prevent one from undergoing the procedure compared to those who are undergoing a non- invasive test. Therefore, a non-invasive screening test with sufficiently high sensitivity and specificity will help to rule out an infection and thus avoid proceeding to endoscopy. The endoscopy may then be restricted to those in whom the non-invasive test is positive, where risk of malignancy high and those above 50 years.[6] Testing for IgG antibody from blood is one of the tests which is a cost-effective indirect method of detection of H. pylori infection.

There are a wealth of studies evaluating the accuracy of H pylori IgG test kits in Western countries, but these cannot be applied to our Indian population. Besides, there is a dearth of such studies from India. Thus, this study was planned to validate the serological test in detecting H. pylori infection by comparing it with combined tests, i.e., urease and histopathology from antral biopsy as gold standard; and also, to find an optimum cut-off value for the particular commercial serology kit for antibody detection in India.

MATERIALS AND METHODS

A cross sectional study was conducted among patients who underwent endoscopic biopsies for dyspepsia in Medical Gastroenterology department of Government Medical College Hospital, Thiruvananthapuram during August 2009 to February, 2010. Patients on proton pump inhibitors and H2 receptor antagonists were advised to stop the treatment for two weeks before collecting biopsies. Pregnant women, those with chronic renal diseases, and gastric malignancies were excluded. Institutional Ethical Committee approval was obtained for the study (IEC no.05/21/2009 MCT). Relevant data were collected using a semi structured interview schedule after getting informed consent. After getting consent, collection of the blood samples for IgG antibody testing and two bits of gastric biopsy samples were taken from antrum of stomach, on the same day. One biopsy bit used for urease test the other bit for histopathology examination by hematoxylin eosin and Giemsa staining; blood sample was used for serum IgG antibody test for H. pylori (by commercial Microwell Helicobacter pylori IgG ELISA kit, Syndron Bioresearch, In Vitro Diagnostics, Carlsbad, CA 92010, USA). Urease test (reagent prepared in house after validation) was done by putting the one bit of endoscopy biopsy in 0.5 ml freshly prepared (prepared on the same day of the test) 10%(w/v) urease broth at pH 6.8 with 2 drops of phenol red as indicator. Change of color from red to yellow within 6 hours indicated positive test. In Histopathology Examination Helicobacter pylori is seen as spiral shaped slender bacteria seen adhering to gastric mucosa or the lumen of gastric glands or embedded in the mucus by Giemsa stain.

Serum sample was diluted 1/20 for antibody detection by the ELISA kit, and conducted the test as per manufacturer's instructions. The specimen for histopathology and urease test were coded differently and without patient's name and identifiable details so that the pathologist and the person doing the urease test was not aware of the identity of the patient. Pathologists independently read the slides and inter observer variation was resolved by consensus. Objectivity was assured by predefined criteria based on modified Sydney system.[7] Gold standard for positive result was taken as, either the Histopathology positive for H. pylori, or Urease test positive, or both. Gold standard negative as, both Histopathology and Urease test negative.

The data collected was appropriately coded and entered in excel sheet. Further analysis was done using SPSS 16.0 version. The sensitivity, specificity, positive and negative predictive values were calculated. Likelihood ratios and diagnostic accuracy and odds were also calculated. An ROC curve (receiver operated characteristic curve) with (1-specificity) on the X-axis and Sensitivity on Y-axis was also constructed. The cut-off value is taken as that value where the sum of sensitivity and specificity is maximized. This curve plays a central role in evaluating diagnostic ability of tests to discriminate the true state of subjects.[8]

RESULTS

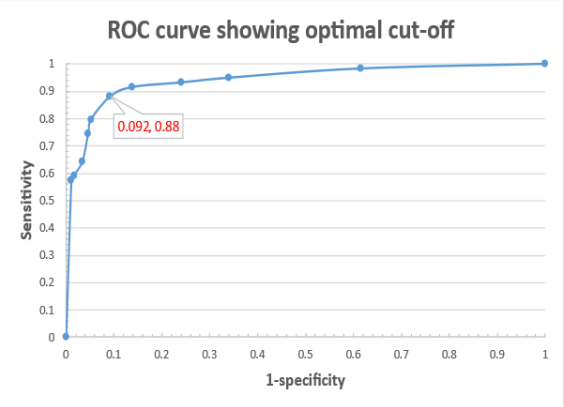

Figure 1: ROC cut off value showing maximum accuracy

The current study conducted to find out the diagnostic accuracy of a serum IgG test in diagnosing H. pylori infection compared to the gold standard (Histopathology/Urease test) among 233 patients who presented with dyspepsia in a tertiary care center gave the following results. Majority of patients

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(47.6%) among the study participants belonged to 41-60 years age group.

As far as the histopathology of the patients are concerned majority of the patients (n=89) were having chronic gastritis followed by mild gastritis (n=56) and 44 patients showed evidence of chronic superficial gastritis. Among the patients whose histopathology was done 50% of patients chronic atrophic gastritis (CAG) with intestinal metaplasia, 46.7% of patients with chronic atrophic gastritis and 41.4% of those with chronic superficial gastritis were positive for H. Pylori [Table 2].

Out of the 59, who showed positivity for the gold standard test, 46 were also positive for the serological test which amounts to a sensitivity of 78% for the test. 174 patients were negative for the gold standard of which 162 were also negative serologically implying a test specificity of 93.1% when the manufacturer's cutoff of 1.0 U/ml was applied. [Table 3].

A Receiver operated characteristic curve was also plotted to find the ideal cut-off of serological test which would bring out the discriminative power of the test [Figure 1]. It can be seen form figure -1 that the best discriminative power of the test i.e., when the sensitivity and specificity are the maximum is not at the manufacturer's cutoff of 1.0 U/ml but at a cutoff of 0.938 U/ml. At this point of 0.938 U/ml the test had a sensitivity of 88.1% and specificity of 90.8%.

When the ROC cutoff of 0.938 U/ml was applied it was seen that out of the 59 who showed positivity for the gold standard 52 were also positive for the serological test which amounts to a sensitivity of 88.1% for the serological test.174 patients were negative for the gold standard of which 158 were also negative serologically implying a test specificity of 90.8%. [Table 4].

As it can be seen form table 5 the overall sensitivity increased from 78% to 88.1% when the cutoff for serological test was taken as 0.938 U/ml instead of the manufacturers cut-off of 1.0 U/ml. The specificity remains almost the same (93.1 and 90.8%). The positive predictive value shows a slight decrease with new cutoff (79.3 and 76.4) whereas negative predictive value increases slightly (92.6 to 95.8%).

Table 1: Distribution of baseline characteristics of study population (n=233)

Characteristic	Age group	Male	Female	Total (+ve)
Age	<20	11(8.1%)	2 (2.1%)	13 (5.6%)
	21-40	55(40.1%)	30 (31.3%)	85 (36.5%)
	41-60	56 (40.9%)	55 (57.3%)	111(47.6%)
	>61	15(10.9%	9 (9.3%)	24 (10.4%)
	Total	137 (100%)	96 (100%)	233(100%)

Table 2: Distribution of types of histopathological lesions and H. pylori positivity in the study population

Histopathology (H&E/Giemsa)	Number	Number Positive for H. pylori (Gold standard)
Normal histology	25	1(0.04%)
Mild gastritis	56	4(7.2%)
Chronic superficial gastritis	44	14(41.4%)
Chronic gastritis	89	31(34.8%)
Chronic atrophic gastritis (CAG)	15	7(46.7%)
CAG with intestinal metaplasia	4	2(50%)
Total	233	59(25.3%)

Table 3: Diagnostic efficacy of H pylori IgG ELISA (Microwell) against combined gold standard of Histopathology and Urease test at 1.0 U/ml cut off value (Manufacturer prescribed)

IgG ELISA test ROC cut off at 40IU/ml	Gold standard +ve (H pylori +ve)	Gold standard -ve	Total
Positive	46	12	58
Negative	13	162	175
Total	59	174	233

Table 4: Different cut off values of IgG antibody with corresponding sensitivity, specificity and (1-specificity) values for ROC curve preparation

Sl. No	Cut off value at U/ml	Sensitivity (%)	Specificity (%)	(1-Specificity)
1	0.513	0.98 (98)	0.385 (38.5)	0.615
2	0.678	0.95 (95)	0.661 (66.1)	0.339
3	0.763	0.93 (93)	0.759 (75.9)	0.241
4	0.888	0.92 (92)	0.86.2 (86.2)	0.138
5	**0.938**	**0.88 (88)**	**0.908 (90.8)**	0.092
6	1.013	0.80 (80)	0.948 (94.8)	0.052
7	1.138	0.75 (75)	0.954 (95.4)	0.046
8	1.238	0.64 (64)	0.966 (96.6)	0.034
9	1.400	0.59 (59)	0.983 (98.3)	0.017
10	1.550	0.58 (58)	0.989 (98.9)	0.011

The bold letters represent the optimum cut off value and corresponding sensitivity and specificity for maximum accuracy for this sample population at this tertiary care centre.

Table 5: Diagnostic efficacy of H pylori IgG ELISA (Microwell) against combined gold standard of Histopathology and Urease test at 0.938 U/ml cut off value

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IgG ELISA test ROC cut off at 0.938 U/ml	Gold standard +ve (H pylori +ve)	Gold standard -ve	Total
Positive	52	16	68
Negative	07	158	165
Total	59	174	233

Table 6: Comparison of Manufacturer's cut off value and optimum cut off for Microwell ELISA test for H pylori IgG

Test characteristic	Manufacturer's cut off 1.00 U/ml (95% CI)	ROC cut off at 0.938 U/ml (95% CI)
Sensitivity	78.0% (65.27% to 87.71%)	88.1% (77.07%to 95.09%)
Specificity	93.1% (88.26% to 96.39%)	90.8% (85.50% to 94.65%)
Positive predictive value	79.3% (68.57% to 87.05%)	76.4% (66.85% to 83.94%)
Negative predictive value	92.6% (88.51% to 95.28%)	95.8% (91.84% to 97.84%)
Positive likelihood ratio	11.3(6.44 to 19.84)	9.58(5.95 to 15.43)
Negative likelihood ratio	0.236(0.15 to 0.38)	0.13(0.07 to 0.26)
Diagnostic accuracy	89.3% (84.57% to 92.94%)	90.1% (85.56%to 93.64%)
Diagnostic odds ratio	47.8	73.4
Prevalence	25.3%	25.3%

DISCUSSION

The antibody response to H. Pylori infection consists of IgG, IgM and IgA. Studies have shown that the sensitivity of IgM is very low and out of the IgA and IgG the higher validity is for IgG.[9,10] The current study which evaluated the diagnostic efficacy of IgG in diagnosing H. Pylori infection showed sensitivity of 88.1% and specificity of 90.8% at a ROC cut-off of 0.938 U/ml. As far a screening test is concerned both sensitivity and specificity should be high if the test must be applied for screening in community. In this perspective, this test showed high levels of both sensitivity and specificity. A study conducted by Himani et.al in Gujarat on validity of IgA and IgG in diagnosing H. pylori infection showed a sensitivity of 100% and specificity of 48.6% and a diagnostic accuracy of 52.6%.[11] In another study conducted in Russia by MINNA MÄKI et al. among 101 patients who attended the gastroenterology OPD the sensitivity of IgG was 92.3% and specificity of 88.6% which is comparable to the current study.[12] In a community based study done in democratic republic of Congo the prevalence of H. pylori was found to be 53.8% and the sensitivity and specificity of the Hp Afr-ELISA was found to be 97.6% and 90.5% at a cut-off of 20.2U/ml.[13] A Swedish study showed that the serological tests for H. pylori had a sensitivity of 99% and specificity of 82% when IgG was used.[14] This added to the fact that histopathology or urease test require invasive procedures and is costly whereas IgG antibody detection is non-invasive. Histopathology is based on a biopsy from the antrum, only single sample is taken for Histopathology. It is known that the distribution of H pylori infection is patchy, there could be a possibility of a sampling error.[15] As far as urease test is concerned it was positive in only 44 patients out of a total of 59 who were either urease positive or histopathology positive. The probable reasons are the use of proton pump inhibitors (PPI) either self-medication or by physician prescription has got anti urease activity producing lower positives. It also reduces the bacterial load in the stomach leading to less chance of detection.[16] A high bacterial load is necessary for the urease test to be positive. Although, two weeks of stopping PPI was advised, some patients would have taken it for symptoms.

This study had a negative likelihood ratio of 0.13. This means that when a person test negative with this diagnostic test one has a high probability of being negative for the disease and hence rules out the disease. Higher the positive likelihood ratio, the test is more indicative of the disease being present.[17] Values around 10 indicates a very good diagnostic test. The positive likelihood ratio of the current test was 9.58 and the negative likelihood ratio was 0.13.

The diagnostic odds ratio (DOR) is a better indicator of discriminative power of the test compared to sensitivity and specificity. Thus, this single measure includes information about both sensitivity and specificity and tends to be reasonably constant despite diagnostic threshold. Higher the value means that higher is the discriminative power of the test.[18] In the current study where the manufactures cut-off of 1.0 U/ml was replaced with ROC cut-off of 0.938 U/ml, DOR increased drastically from 47.8 to 73.4.

In symptomatic individuals, if H pylori test is positive, treatment improves the outcome of disease. So, a high sensitivity means more cases are detected and can be treated. A sensitive test is best for a screening for patients for H pylori. Hence a cut off 0.938 U/ml has not reduced the specificity much. This cut off may be used for hospital patients. According to Guidelines issued by European Helicobacter pylori study group a kit with sensitivity and specificity less than 90% should not be used for diagnostic or screening purposes.[19] However, Helicobacter pylori being highly heterogeneous, sensitivities and specificities vary widely when an externally validated kit is used in an entirely different population.[20] Such externally evaluated kits will have lower sensitivity and specificities when used in other unrelated population. Hence the current study finds it important to validate the cut-off used for positivity in Indian populations before new kits are used here which were validated in different settings.

CONCLUSION

Many diagnostic kits manufactured and evaluated in Western countries are not directly applicable to

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Indian population especially with diagnostic tests for Helicobacter pylori. Diagnostic accuracy has improved in this study from 88.1% to 90.8% using the new cut off value obtained from ROC curve.

Limitations of the Study

This study has taken only two biopsy samples, one for histopathology and the other for urease test. Two samples each for the test would have been better. Since the distribution of H pylori in the stomach is patchy, some positive cases would have missed causing reduced detection of positive cases and it would have affected both gold standard tests (Urease and Histopathology).

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