ORIGINAL ARTICLE

Agreement Between Serology and Histology for Detection of *Helicobacter pylori* Infection

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ABSTRACT

Objective: To determine the percentage agreement between serology and histology for detection of *Helicobacter (H.) pylori* infection.

Study Design: Cross-sectional analytical study.

Place and Duration of Study: Department of Pathology and Microbiology, The Aga Khan University and Hospital, Karachi, from January to December 2009.

Methodology: Fifty subjects were selected by non-probability purposive sampling from laboratory data who had serological testing of *H. pylori* IgG antibody, prior to histological evaluation of endoscopic gastric or/and duodenal biopsies. Serological Quantification of *H. pylori* IgG was carried out with HpG screen ELISA kit (Genesis Diagnostics, UK), using an enzyme linked immunosorbent assay for detection of IgG antibodies against *H. pylori*. Manufacturer's recommended cut-off value was used and results were considered positive when greater than 7 U/ml. For histological diagnosis, an expert histopathologist characterized the presence of spiral bacteria in the mucosal layer or the surface of epithelial cells on microscopic examination, as a positive test.

Results: An agreement of 0.72 was found by Kappa statistics between serology and histopathology results and a good diagnostic accuracy (86%) of serological testing was observed for the diagnosis of *H. pylori* infection.

Conclusion: A substantial agreement was found between serology and histopathology results to detect the *H. pylori* infection. Laboratory-based serologic testing using ELISA technology to detect IgG antibodies is inexpensive, noninvasive and convenient method to detect the *H. pylori* infection in primary care setting.

Key Words: *Helicobacter pylori. Serology. Histology. Agreement.*

INTRODUCTION

Helicobacter pylori (H. pylori) infection is recognized as one of the most important causes or prerequisites of peptic ulcer disease.[1,2] In Pakistan, *H. pylori* exposure rate increases with advancement of age and lowering of socioeconomic status.[3] Peptic ulcer prevalence in Pakistan is high and reported to be 51 – 78% due to *H. pylori* infection.[4]

Direct techniques, for determination of *H. pylori* infection including culture and microscopic demonstration of the organism, require invasive methods like endoscopy for a gastric biopsy specimen while non-invasive methods include urea breath test and serological test for *H. pylori* IgG as a specific disease marker.[5-7] The choice of test depends upon issues such as cost, availability, clinical situation, population, prevalence of infection, pretest probability of infection, and factors such as the use of proton pump inhibitors and antibiotics that may influence diagnostic or screening test results.[8,9]

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A reliable test to detect this infection is crucial, but none of the tests available is suitable for all situations, each having its own drawbacks and pitfalls.[10-12] Invasive tests have been considered as gold standard. However, invasive techniques may suffer from the sampling errors hence, compromising the test sensitivity. *H. pylori* serology is superior in comparison with other diagnostic methods because it is simple, inexpensive, and less cumbersome for the patient.[6,13] Timely diagnosis of *H. pylori* infection is crucial for eradication of infection to prevent atrophic gastritis and gastric cancer which are consequences of peptic ulcer.[14] A good agreement between serology and histopathology is important for improving the diagnostic accuracy of serological testing.[9,12]

The aim of this study was to determine the agreement between serological measurements of *H. pylori* antibody and histological findings of endoscopic biopsies.

METHODOLOGY

A cross-sectional study was conducted at the Department of Pathology at the Aga Khan University and Hospital, Karachi, from January to December 2009. According to Cohan's Kappa statistics, at least 35 subjects were required to achieve the observed and expected agreement of 0.77 and 0.50, confidence interval of 95% and error tau of 0.20. However, to

increase the statistical significance; sample size was increased to 50 subjects to measure rate of agreement within ± 20%.

Fifty subjects were included by non-probability purposive sampling from laboratory data who had serological testing of *H. pylori* IgG antibody, prior to histological evaluation of endoscopic gastric or/and duodenal biopsies. A review of medical records of selected subjects admitted to gastroenterology ward or day care unit for upper GI endoscopy procedure was performed. A preformed proforma of clinical details of the subjects were recorded by reviewing the medical records of patients; including clinical information such as nausea or vomiting, upper abdominal pain, burning and bloating.

Serological Quantification of *H. pylori* IgG was carried out with HpG screen ELISA kit (Genesis Diagnostics, UK), using an enzyme linked immunosorbant assay for detection of IgG antibodies against *H. pylori*. Manufacturer's recommended cut-off value was used and results were considered positive when greater than 7 U/ml. For histological diagnosis, an expert Histopathologist characterized the presence of curved bacteria in the mucosal layer or the surface of epithelial cells on microscopic examination, as a positive test.

Data was analyzed using Statistical Package for Social Sciences (SPSS) version 19. Mean value and standard deviation were computed for quantitative variable for age, whereas frequency and percentage were calculated for gender, age group distribution, clinical symptoms and rate of categorical agreement between the procedures. Percentage of discrepancies for positive and negative results was also calculated. The procedures were considered to be in categorical agreement when they resulted in the same (e.g. positive or negative). Cohen's Kappa statistics was used to find percent agreement between test and conventional method. The agreement between two raters was evaluated by Kappa statistics for both groups separately. Kappa values ranged from -1 to 1, where 1 was considered representing perfect agreement between data sets. Kappa value range from 0.81 to 1.00, 0.61 to 0.80, 0.41 to 0.60, 0.21 to 0.40 and 0.00 to 0.20 were considered representing the strength of agreement as perfect, substantial, moderate, fair and slight respectively.

Sensitivity, specificity, negative predictive value, positive predictive value and accuracy were calculated using the standard formula. Only those cases were taken as true positive which were positive by histopathology. Effect modifiers/confounders were controlled through different variable like age and gender through chi-square test and if frequency was less than five we used Fisher exact test for stratification. P-value was set as < 0.05 for significance.

RESULTS

Among the 50 study subjects, 31 (62%) were males and 19 (38%) were females. The age of study subjects ranged from 6-81 years, however, the mean and median age were found 40.59 ± 15.63 years and 39 years, respectively. Thirty (60%) subjects were found positive for *H. pylori* infection with serological testing. However, 25 (50%) subjects were identified positive for *H. pylori* infection with histopathological diagnosis. Among 30 seropositive subjects, 5 subjects found no evidence of *H. pylori* infection with histological evalu-ation. Concentration of IgG antibodies ranged from 1.0-74.6 U/ml with the mean of 14.7 U/ml on serology. Main symptoms observed were nausea, vomiting, epigastric pain, burning and abdominal bloating.

Agreement between the results of serology and histopathological findings of endoscopic biopsies by Cohen's Kappa coefficient was found substantial (0.72, Table I). Based on histological diagnosis, the sensitivity, specificity, negative predictive value, positive predictive value and accuracy of serology was found 95%, 80%, 96%, 76% and 86% respectively.

Table I: Agreement between serology and histopathology findings for the diagnosis of *Helicobacter pylori* infection (n = 50).

Serological findings	Histopathological findings		Cohen's Kappa agreement	p-value
	Negative	Positive		
Negative	19 (38%)	1 (2%)	0.72	<0.01*
Positive	6 (12%)	24 (48%)		

**p-value < 0.05 significant.*

DISCUSSION

Laboratory-based serologic testing using ELISA technology to detect IgG antibodies is inexpensive, non-invasive, and well suited in primary care practice. The diagnostic accuracy and agreement between non-invasive and invasive techniques is crucial for diagnostic improvement and treatment success of *H. pylori* infection. Positive serology is a possible indication for future biopsy and combination of serology with invasive techniques improves the diagnostic accuracy. There was substantial Cohen's Kappa agreement for serology and histopathology; consistent with the finding of Redeen *et al*., where they found the sensitivity, specificity and accuracy of serology, 99%, 82% and 86% respectively to diagnose *H. pylori* infection.[15] The agreement reported in this study is more strong (0.72) than the results of Alarcon *et al*. where they found the agreement between serology and histopathology 0.45.[16]

In literature, serology testing for *H. pylori* detection is found to be more sensitive for the detection of *H. pylori* infection in subjects with atrophic gastritis, where the sensitivity of biopsy based tests is found decreased.[17,18] In this study, 5 cases with positive serology without any positive finding on histology may be explained with same reason.

In this study, a good sensitivity (95%) and specificity (80%) of serology was found to detect the *H. pylori* infection. Serological sensitivity and specificity reported in this study is superior to findings of local study by Taj *et al*.,[19] but not different with the findings of Redeen *et al*.[15]

For serological testing of *H. pylori,* large studies have found uniformly high sensitivity (90 – 100%), but variable specificity (76 – 96%); the accuracy has ranged from 83 to 98%.[5,20] However, differences between studies results may in some instances be explained by differences in methodology and the choice of gold standard.

CONCLUSION

A substantial agreement was found between serology and histological assessment of endoscopic biopsies for the evaluation of *H. pylori* infection. In comparison with biopsy-based invasive techniques, serology assesses the presence of *H. pylori* in the stomach even when the bacteria are irregularly distributed on the gastric mucosa and may be missed on taking biopsy. Laboratory-based serologic testing using ELISA technology to detect IgG antibodies is inexpensive, non-invasive and convenient method to detect the *H. pylori* infection in primary care setting.

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