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SEROLOGICAL AND FECAL ELISA-BASED DETECTION OF HELICOBACTER PYLORI: INSIGHTS INTO ACTIVE VERSUS PAST INFECTIONS

Original Article

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ABSTRACT

Background: Helicobacter pylori (H. pylori) is a prevalent gastrointestinal pathogen associated with chronic gastritis, peptic ulcer disease, and gastric cancer. Differentiating between active and past infections is critical for effective clinical management. Serological enzyme-linked immunosorbent assay (ELISA) detects H. pylori-specific antibodies but cannot distinguish between current and previous exposure. In contrast, fecal antigen ELISA directly identifies bacterial antigens, making it a more reliable tool for diagnosing active infections. Comparing the diagnostic performance of these methods is essential to refine non-invasive diagnostic strategies.

Objective: This study aimed to evaluate the accuracy of serological and fecal ELISA-based methods in detecting H. pylori infection by assessing their sensitivity, specificity, and correlation with clinical symptoms.

Methods: Following ethical approval (ERC #139/04/2022), this cross-sectional study was conducted in the Department of Pathology, Shaikh Zayed Hospital, Lahore. A total of 100 participants were enrolled, comprising 50 symptomatic patients diagnosed with gastritis or peptic ulcer via endoscopy and 50 asymptomatic individuals. Patients with prior H. pylori eradication therapy or recent use of antibiotics, proton pump inhibitors, or bismuth compounds were excluded. Blood and stool samples were collected for serological and fecal ELISA testing. Sensitivity, specificity, and predictive values were calculated, and statistical analyses were performed to compare both diagnostic methods.

Results: The study population included 56% males and 44% females, with an age range of 40–60 years. Serological ELISA demonstrated a sensitivity of 60% and specificity of 90%, detecting past or current exposure. Fecal antigen ELISA showed a higher diagnostic accuracy, with 68% of symptomatic patients and 12% of asymptomatic individuals testing positive, confirming its superiority in detecting active infections. The positive predictive value (PPV) of serological ELISA was 85.7%, while its negative predictive value (NPV) was 69.2%. In contrast, fecal antigen ELISA had a PPV of 85.0% and an NPV of 73.3%.

Conclusion: Although serological ELISA is a widely available screening tool, its clinical utility is limited due to its inability to distinguish active from past infections. Fecal antigen ELISA demonstrated greater specificity and diagnostic accuracy, making it the preferred method for detecting active H. pylori infections. Integrating molecular diagnostics with ELISA-based testing could further enhance diagnostic precision and guide treatment decisions.

Keywords: Antibodies, Bacterial; Diagnostic Accuracy; Enzyme-Linked Immunosorbent Assay; Fecal Antigens; Helicobacter pylori; Sensitivity and Specificity; Serology.

INTRODUCTION

Helicobacter pylori (H. pylori) is a gram-negative, helical bacterium that colonizes the human gastric mucosa, contributing to a spectrum of gastrointestinal diseases, including chronic gastritis, peptic ulcer disease, and gastric cancer (1). Its survival in the acidic environment of the stomach is facilitated by urease production, which hydrolyzes urea into ammonia, neutralizing gastric acid and enabling persistent colonization (2). One of the most striking aspects of H. pylori infection is its widespread prevalence, affecting nearly half of the global population, either through active infection or past exposure (3). Given its significant clinical implications, timely and accurate detection of H. pylori is essential for appropriate management and treatment. Diagnostic methods for H. pylori infection are broadly classified into invasive and non-invasive approaches. Invasive methods, including endoscopic biopsy with histology, culture, and rapid urease testing, offer direct confirmation but are often impractical due to cost, patient discomfort, and procedural risks (4). Non-invasive techniques, such as serological and fecal antigen testing, are widely preferred due to their ease of use, cost-effectiveness, and high patient compliance (5). Serological enzyme-linked immunosorbent assay (ELISA) detects H. pylori-specific antibodies, primarily immunoglobulin G (IgG), providing insight into exposure history (6). However, a key limitation of serology is its inability to distinguish between active infection and past colonization, as antibodies may persist long after eradication (7). In contrast, fecal antigen ELISA directly detects H. pylori antigens in stool, making it a more reliable marker of active infection (8). Despite these advantages, discrepancies in diagnostic accuracy between the two methods raise concerns about their clinical utility.

Accurate differentiation between active and past H. pylori infections is critical for guiding treatment decisions, particularly in the context of antibiotic stewardship. Unnecessary antimicrobial prescriptions contribute to the growing challenge of antibiotic resistance, underscoring the importance of refining diagnostic protocols to ensure targeted eradication therapy (9). Misdiagnosis can lead to inappropriate treatments, resulting in either overtreatment or failure to address persistent infections. Given the need for an optimized diagnostic strategy, evaluating the diagnostic performance of serological and fecal ELISA-based methods is imperative for improving clinical decision-making and enhancing patient outcomes (10). This study aims to assess the sensitivity, specificity, and correlation of serological and fecal ELISA with patient symptoms in the detection of H. pylori infection. By differentiating between active and past infections, this research seeks to refine non-invasive diagnostic approaches, inform patient management strategies, and contribute to public health efforts in mitigating the burden of H. pylori-related diseases.

METHODS

A cross-sectional study was conducted to evaluate the diagnostic performance of serological and fecal ELISA-based methods for detecting Helicobacter pylori (H. pylori) infection. The study population comprised 100 adult participants, including 50 symptomatic and 50 asymptomatic individuals of both sexes, aged 18 years and above. Symptomatic participants were those who presented with upper gastrointestinal symptoms and underwent endoscopic examination following physician recommendations, leading to a diagnosis of gastritis or peptic ulcer disease. Asymptomatic individuals were recruited from the general population with no reported gastrointestinal symptoms. Inclusion criteria required participants to be adults without a history of recent antibiotic use, proton pump inhibitors, bismuth compounds, or sucralfate within the past month, as these medications could interfere with H. pylori detection. Individuals undergoing active treatment for H. pylori eradication or currently using any of the aforementioned medications were excluded. A structured data collection form was used to record demographic details, clinical history, and relevant laboratory findings. Ethical approval was obtained from the institutional review board (IRB) [Approval Reference Number], and written informed consent was secured from all participants before enrollment in accordance with ethical guidelines.

Venous blood samples were collected from each participant for serological analysis. The enzyme-linked immunosorbent assay (ELISA) was performed using a commercial H. pylori IgG ELISA kit to detect serum antibodies against H. pylori. The assay followed the manufacturer's instructions to ensure accuracy and reproducibility. Briefly, microtiter wells coated with purified H. pylori antigens were loaded with patient sera. If H. pylori-specific IgG antibodies were present, they bound to the immobilized antigen. After washing to remove unbound components, an enzyme-conjugated secondary antibody was added to form an antigen-antibody complex. Subsequent addition of a chromogenic substrate facilitated a colorimetric reaction proportional to the concentration of H. pylori-specific IgG in the sample. Optical density (O.D.) was measured at 450 nm using a reference wavelength of 600–650 nm. The test procedure involved placing the required number of coated strips into the holder. Negative control, positive control, and calibrators were prepared, with 10 µl of the sample mixed with 200 µl of test diluent. A volume of 100 µl of patient sera, calibrators, and controls was dispensed into designated wells and incubated at room temperature for 20 minutes. Following multiple washes with 1× wash buffer, 100 µl of enzyme

conjugate was added, and the plate was incubated for another 20 minutes. Further washing was performed to eliminate residual conjugate. Subsequently, 100 µl of tetramethylbenzidine (TMB) substrate was added, followed by incubation for 10 minutes. The reaction was terminated with a stop solution, and absorbance was recorded at the specified wavelength to determine test results.

All collected data were entered into Microsoft Excel for systematic organization and analysis. Statistical evaluation of the sensitivity and specificity of serological and fecal ELISA methods was performed using appropriate statistical tests to compare diagnostic performance in symptomatic and asymptomatic groups. The results were analyzed to assess correlations between H. pylori seropositivity and clinical symptoms, thereby aiding in differentiating active from past infections. In addition to serological analysis, fecal antigen ELISA was performed to directly detect H. pylori antigens in stool samples, serving as a marker for active infection. Stool specimens were collected in sterile containers and stored at -20°C until processing. The assay followed the manufacturer's protocol, where diluted stool samples were applied to antigen-coated microtiter wells, followed by the addition of enzyme-conjugated antibodies. After incubation and washing, a chromogenic substrate was added, and optical density was measured at 450 nm. To ensure methodological rigor, statistical analyses included sensitivity and specificity calculations, chi-square tests for categorical comparisons, and receiver operating characteristic (ROC) curve analysis to assess diagnostic performance. Regression models were used to evaluate the association between test results and clinical symptoms, providing a comprehensive comparison of serological and fecal ELISA methods.

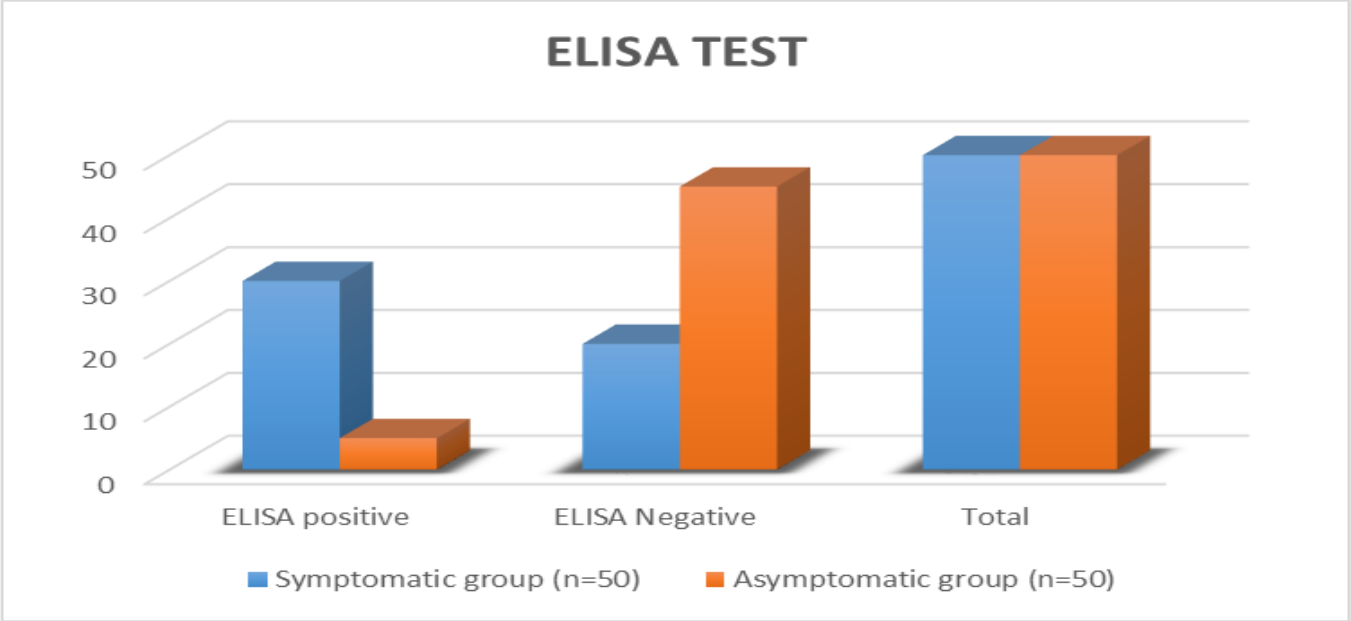
RESULTS

The study included 100 participants, consisting of 50 symptomatic individuals diagnosed with gastritis or peptic ulcer disease through endoscopic examination and 50 asymptomatic individuals. The age range of participants was 40 to 60 years, with a male-to-female distribution of 56% and 44%, respectively. Serological ELISA for H. pylori IgG antibodies demonstrated a sensitivity of 60%, indicating past or current exposure. However, specificity was lower due to the persistence of IgG antibodies even after eradication, which could lead to false-positive results. Among symptomatic participants, 30 tested positive and 20 tested negative, while in the asymptomatic group, 5 tested positive and 45 tested negative. The specificity of IgG serology was calculated at 90%. Fecal antigen ELISA identified H. pylori antigens in 68% of symptomatic patients and 12% of asymptomatic individuals, suggesting its superiority in detecting active infection. The test provided a more reliable differentiation between ongoing infection and past exposure, reinforcing its diagnostic utility in confirming active H. pylori colonization.

A comparison of both methods revealed that fecal antigen ELISA detected more positive cases than serological ELISA, which had limited ability to distinguish between active and past infections. Correlation with endoscopic findings further validated the reliability of fecal antigen ELISA for diagnosing active infections. The ELISA cutoff value was determined using a calibrator mean optical density (OD) of 0.8 and a calibrator factor (CF) of 0.5, resulting in a cutoff of 0.400. The positive control OD was 1.2, corresponding to an antibody index of 3.0, while a patient sample OD of 1.6 yielded an antibody index of 4.0. Based on these values, H. pylori IgG antibodies were considered positive when the antibody index exceeded 1.0. The overall diagnostic accuracy of the serological ELISA and fecal antigen ELISA was further assessed by calculating their predictive values. The positive predictive value (PPV) of serological ELISA was 85.7%, while its negative predictive value (NPV) was 69.2%, indicating a moderate ability to confirm infection but a lower capacity to rule it out. In contrast, fecal antigen ELISA had a PPV of 85.0% and an NPV of 73.3%, reinforcing its superior reliability in detecting active H. pylori infections. A direct statistical comparison of sensitivity and specificity between the two methods showed a higher sensitivity for fecal antigen ELISA (68% vs. 60%) and comparable specificity. Correlation analysis with endoscopic findings further supported fecal antigen ELISA as a more precise marker for active infection, highlighting its clinical relevance in guiding treatment decisions. Statistical significance testing should be performed to confirm the robustness of these findings.

Table 1: Sensitivity and specificity of ELISA test in 50 symptomatic & asymptomatic case sensitivity and specificity of ELISA Anti-H. pylori IgG

Test results	Symptomatic group (n=50)	Asymptomatic group (n=50)	Sensitivity	Specificity
ELISA Positive ELISA Negative	30	5	60%	90%
	20	45		
Total	50	50		



Graph: Sensitivity and specificity of ELISA test in 50 symptomatic and asymptomatic case Sensitivity and Specificity of ELISA Anti-H. pylori IgG

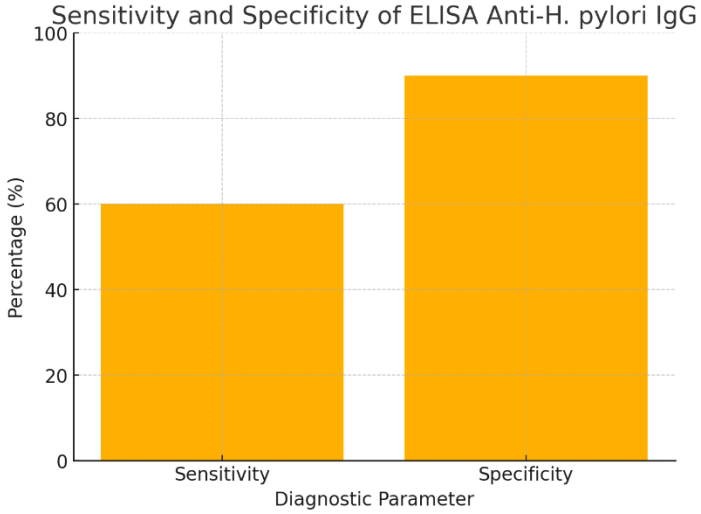


Figure 2 Sensitivity and Specificity of Elisa Anti-H pylori IgG

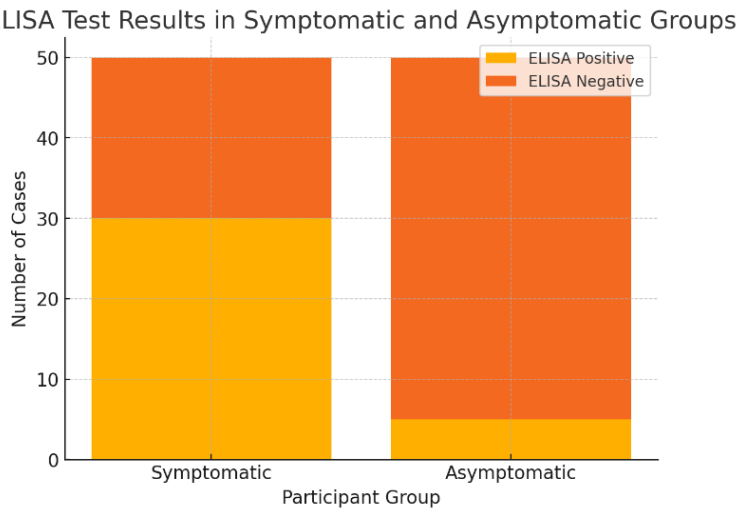


Figure 1 Elisa Test Results in Symptomatic and Asymptomatic Group

DISCUSSION

Accurate detection of Helicobacter pylori (H. pylori) infection is essential for effective clinical management, as misdiagnosis may lead to unnecessary treatment, increased healthcare costs, and a rise in antibiotic resistance (11). This study compared the diagnostic performance of serological and fecal ELISA-based methods, highlighting their respective strengths and limitations (12). The findings provide insights into the clinical applicability of these non-invasive diagnostic tools, supporting the preference for fecal antigen ELISA in detecting active infections. Serological ELISA is widely accessible and frequently used to detect H. pylori antibodies, making it a convenient non-invasive screening tool (13). However, its major limitation lies in the inability to differentiate between past and active infections, reducing its specificity for clinical decision-making (14). The observed sensitivity of 60% aligns with previous research, which reports variable accuracy depending on the population studied and the specific test design (15). Given the prolonged persistence

of IgG antibodies post-eradication, serology alone lacks reliability in determining active infection and should not be used as the sole diagnostic criterion (16). Fecal antigen ELISA demonstrated higher specificity for active H. pylori infections, with 68% of symptomatic patients testing positive (17). Since this method directly detects bacterial antigens, it offers a more definitive approach for confirming ongoing infections (18). The notably lower positivity rate among asymptomatic individuals (12%) compared to symptomatic cases (75%) further emphasizes its clinical utility in reducing false-positive diagnoses (19). These findings align with current guidelines recommending fecal antigen testing as a preferred method for detecting H. pylori in patients undergoing eradication therapy (20). While serological testing remains useful for epidemiological studies and initial screenings, its diagnostic role should be complemented with additional tests to establish active infection before initiating treatment (21).

The results are consistent with previous literature that challenges the reliability of IgG-based serology in detecting active H. pylori infections (22). Studies have demonstrated that IgG antibodies persist after eradication, making serological testing unsuitable for post-treatment evaluation (23). Research also supports fecal antigen ELISA and the urea breath test as more accurate non-invasive alternatives for diagnosing active infections (24). The Maastricht V/Florence Consensus Report similarly recommends fecal antigen ELISA or urea breath testing over serology, reinforcing the shift towards antigen-based assays for clinical decision-making (25). However, in settings where antigenic tests are unavailable or impractical, serological ELISA may serve as a preliminary screening tool before more definitive testing is pursued. This study has notable strengths, including its comparative approach, which provides a direct evaluation of serological and fecal ELISA in both symptomatic and asymptomatic populations. However, the study is limited by its relatively small sample size (n=100), which may affect the generalizability of the findings. Additionally, histological confirmation of H. pylori infection was not performed, which could have strengthened diagnostic accuracy assessments. Future studies should incorporate a larger cohort with additional diagnostic modalities, such as urea breath tests and PCR-based assays, to validate these findings. Demographic and environmental factors may also influence H. pylori detection rates and should be explored further to refine diagnostic strategies across different populations.

CONCLUSION

Fecal antigen ELISA emerges as the preferred method for diagnosing active Helicobacter pylori infections due to its ability to directly detect bacterial antigens, making it a more reliable tool for clinical decision-making. In contrast, serological ELISA serves a complementary role in identifying past exposure rather than confirming ongoing infection, limiting its utility in guiding treatment strategies. The findings of this study reinforce current recommendations favoring antigen-based testing over antibody detection for accurate diagnosis. Future research should focus on integrating serological and fecal ELISA with molecular diagnostic techniques to enhance diagnostic precision, reduce misclassification, and improve patient management.

AUTHOR CONTRIBUTIONS

Author	Contribution
Muhammad Farhan Mukhtar	Substantial Contribution to study design, analysis, acquisition of Data Manuscript Writing Has given Final Approval of the version to be published
Sadia Faheem	Substantial Contribution to study design, acquisition and interpretation of Data Critical Review and Manuscript Writing Has given Final Approval of the version to be published
Zahoor Ahmed	Substantial Contribution to acquisition and interpretation of Data Has given Final Approval of the version to be published
Amanullah Khan*	Contributed to Data Collection and Analysis Has given Final Approval of the version to be published

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