RESEARCH ARTICLE

COMPARISON OF RAPID CASSETTE TEST AND MICRO ELISA METHOD FOR HELICOBACTER PYLORI DIAGNOSIS IN PATIENTS WITH GASTRIC COMPLAINTS

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ABSTRACT

Background: *Helicobacter pylori* (*H. pylori*) is a significant contributor of various gastrointestinal disorders and cancers all around the world. Its diagnosis is dependent on several qualitative and quantitative methods. The present study aims to compare the results of rapid cassette and micro ELISA test methods for diagnosis of *H. pylori* and determining associations with patient endoscopy reports. **Methods:** The study was performed using blood samples collected from 224 patients (142 (63%) females and 82 (37%) males) in various clinics between January 2018 and August 2019, which were sent to the Clinical Microbiology Laboratory of Training Hospital. Serum samples obtained after centrifugation of the blood samples were initially tested with rapid *H. pylori* IgG cassette method, and afterwards in the auto analyzer using ELISA assays specific for *H. pylori*. **Results:** Upper gastrointestinal system endoscopy was performed in 88 of these patients, and biopsy results confirmed definitive diagnosis of *H. pylori* infection in 63 of the patients. Rapid *H. pylori* cassette test results of the 224 patients were negative for 158 (70.5%) patients and positive for 66 (29.5%) patients, whereas micro ELISA IgA test results were negative for 110 (49.1%) patients and positive for 114 (50.9%) patients. Micro ELISA IgG test results were negative for 85 (37.9%) patients and positive for 139 (62.1%) patients. **Conclusion:** Invasive diagnostic methods for *H. pylori* infection may sometimes be inconvenient, and therefore the diagnosis may have to rely on non-invasive tests. Bases on the study results, we believe micro ELISA test results are more reliable with regard to avoidance of missed diagnosis.

INTRODUCTION

Helicobacter pylori (H. pylori) is a Gram negative, spiral shaped, bacilliform bacteria that is able to live and colonize in human gastric environment (1-3). *H. pylori* has a high prevalence globally, and causes chronic infection in nearly half of the world population. It contributes to gastrointestinal system (GIS) complaints such as gastritis, gastric or duodenal ulcer and dyspepsia, and several cancers including adenocarcinoma and lymphoma as well (4-6). Prevalence rates are higher among people living in socioeconomically poor environments and developing countries. Africa currently has the highest prevalence rates, which exceed 70 percent. The causes that lead to high prevalence in these countries are excessive population, insufficient housing, poor hygiene, and polluted water (7,8). Infection is typically acquired during childhood; however, it stays latent for a long time and continues into adulthood.

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Therefore, infected individuals are usually unaware of the infection and tend to spread it to other people. Only a small portion of infected individuals develops disease in their adulthood (9). In 1994, *H. pylori* was defined as a Group 1 carcinogenic agent by International Agency for Research on Cancer (IARC), and was associated with various other systemic disorders including hematological, skin, cardiovascular and respiratory diseases. Given various diagnostic methods and patient clinical conditions, high sensitivity methods should be preferred for diagnosis of the infection (10,11). *H. pylori* infection can be detected with invasive or non-invasive methods. However, due to complications associated with the invasive tests, routine laboratory studies often employ non-invasive immunological assays for diagnosis. Commonly used non-invasive tests are urea breath test (UBT) and serological tests in serum, urine, or other body secretions. These include rapid cassette test (RCT), *H. pylori* stool antigen test (HpSA), and *H. pylori* ELISA assays (8-12). Currently in the USA, serological test methods are the most commonly used diagnostic tools for *H. pylori* infections (13).

The present study aims to compare the results of *H. pylori* IgG cassette test with *H. pylori* IgG and IgA ELISA tests from blood samples of patients thought to have H. pylori infection.

MATERIALS AND METHODS

Patient population: Patient samples were obtained from 224 patients, 142 (63.4%) females and 82 (36.6%) males, who presented to various clinics of Training Hospital due to gastric complaints and provided blood samples between January 2018 and August 2019 for testing *H. pylori* infection.

Specimen collection: Approximately 4 ml of blood samples were collected in serum separator tubes and centrifuged at 5,000 rpm for 10 minutes to obtain a serum sample. Serum samples were stored at -20°C until the time of analysis. Prior to analysis, the samples were brought to room temperature.

H. pylori IgG Cassette Test: The test was a double antigen chromatographic lateral flow immunoassay. The kit package (*H. pylori IgG* Cassette Test, Biocare Diagnostic, PDI GmbH, Essen, Germany) included a cassette, which contains a nitrocellulose diaphragm, with control (C) and test (T) lines marked on the cassette. The T line was coated with *H. pylori* antigens, and the C line was coated with goat antibodies against *H. pylori*. The kit package was stored at room temperature until analysis. After bringing the patient samples to room temperature, 0.2 ml serum (approximately 4 drops) was pipetted to the sample chamber on the cassette. If the patient sample has specific antibodies against *H. pylori*, they combine with the target antigens and cause a red band to appear at the T line. A red band appears at the C line regardless of the presence of antibodies in the patient sample. The result was obtained approximately 5-8 minutes after pipetting the sample. As recommended by the manufacturer, presence of red coloration at the C line, which indicates the validity of the test result, and lack of red coloration at the T line was interpreted as "negative" result, while presence of red coloration at both lines was interpreted as a "positive" test result (Figure 1).

H. pylori ELISA IgG / IgA: These tests are quantitative indirect immunoassays that measures specific IgG or IgA type antibodies against *H. pylori* in human serum or plasma, and it is based on the reaction between antigens adsorbed to the polystyrene surface and antibodies present in the tested sample. In this method, after removing unbound antibodies through a washing step, the antigen-antibody complex was bound to anti-human globulin marked with an enzyme. After a second washing step, an acid stop solution was added, and bound conjugate was added with the help of a substrate to obtain blue colored product that turns to yellow. The kit package (*H. pylori* ELISA IgG or IgA, Vircell MICROBIOLOGISTS, Granada, Spain) contained a 96 well plate coated with *H. pylori* 26695 strain antigens that were soluble in detergent, 25 ml serum diluent, positive and negative controls, conjugate, substrate, and washing solution.

The kit was stored at 4 C until time of analysis. As recommended by the manufacturer, reagents and solutions supplied with the kit were prepared and loaded to an automated device (Triturus ELISA Instrument, Grifols, Barcelona, Spain), and all the steps described above were performed automatically. Antibody index was calculated using the formula: sample optical densities (O.D.)/cut off serum mean O.D.) X 10.

Accordingly, the results were interpreted as "negative" if the index was <9, "borderline" if index was between 9-11, and "positive" if the index was >11. *H. pylori* ELISA IgG and IgA results were reported separately following an analysis time of approximately 4 hours.

Upper GIS Endoscopy report: A total of 88 patients were examined with endoscopy. Endoscopic examination was performed following premedication procedure, using an appropriate gastroscope (Fujinon EG-600 WR, Fujifilm EU, Germany) and a digital image transfer system (Medgate 2000, Aort, Turkey). Esophagus, stomach and duodenum were examined. In case of observation of suspicious lesions associated with *H. pylori* infection, two biopsies were obtained from each of gastric corpus and antrum regions and were sent to pathology laboratory for histological examination. Pathology results were categorized as either positive or negative for *H. pylori*.

Data analysis: *H. pylori IgG* cassette test results were obtained qualitatively either as positive or negative. *H. pylori* ELISA IgG and IgA results were obtained quantitatively, and categorized as either positive or negative. Endoscopic examination and biopsy results were also categorized as positive or negative for *H. pylori*. For comparison of the non-invasive serological test results, endoscopic examination and biopsy results were accepted as the gold standard.

Statistical analysis: Statistical analyses were performed using a software program (SPSS v15, IBM, USA). The results of continuous data analyses were given as minimum, maximum, median, and mean values, and the results of categorical variables as frequency and percentage.

RESULTS

Of the 224 patients included in the study, 142 (63.4%) were female and 82 (36.6%) were male. The mean patient age was 41.2 years (age range: 16-86 years). The results of rapid IgG cassette test for 224 patients were negative in 158 (70.5%) patients and positive in 66 (29.5%) patients. Micro ELISA IgG test results for 224 patients were negative for 85 (37.9%) patients and positive for 139 (62.1%) patients, and micro ELISA IgA results were negative for 110 (49.1%) patients and positive for 114 (50.9%) patients (Table 1).

Table 1. Distribution of the patient serum results for rapid *H. pylori* IgG cassette test and the micro ELISA method.

| Test	*H. pylori* IgG Cassette Test n (%)	*H. pylori* ELISA IgG n (%)	*H. pylori* ELISA IgA n (%)
Negative	158(70.5)	85(37.9)	110(49.1)
Positive	66(29.5)	139(62.1)	114(50.9)
Total	224		

n: Sample count

Endoscopy and biopsy procedure was performed in 88 of the 224 patients. According to the biopsy results, *H. pylori* positivity was detected in 63 (71.6%) out of 88 patients, while the remaining 25 (28.4%) patients had negative results. Of the 63 patients who had definitive diagnosis based on endoscopy report, 40 (63.5%) were female and 23 (36.5%) were male. Among the 88 patients who underwent endoscopic examination, number of positive and negative serological test results were 54 (61.4%) and 34 (38.6%) for the IgG cassette test, 66 (75.0%) and 22 (25.0%) for the ELISA IgA test, and 68 (77.3%) and 20 (22.7%) for the ELISA IgG test,

respectively (Table 2). Of the 63 patients who were determined to have *H. pylori* infection after endoscopy and biopsy procedures, 48 (76%) also had positive test results from the other three test methods (IgG cassette test, ELISA IgA and ELISA IgG), while 6 (9.5%) patients had negative results from all these three tests.

Table 2. Distribution of serological test results of 88 patients who underwent upper gastro intestinal endoscopic examination and biopsy procedures

	Endoscopy results	*Rapid H. pylori* IgG casette test	Micro ELISA	
			H. pylori IgA	*H. pylori* IgG
Positive n (%)	63 (71.6)	54 (61.4)	66 (75.0)	68 (77.3)
Negative n (%)	25 (28.4)	34 (38.6)	22 (25.0)	20 (22.7)
Total	88			

n: Sample count
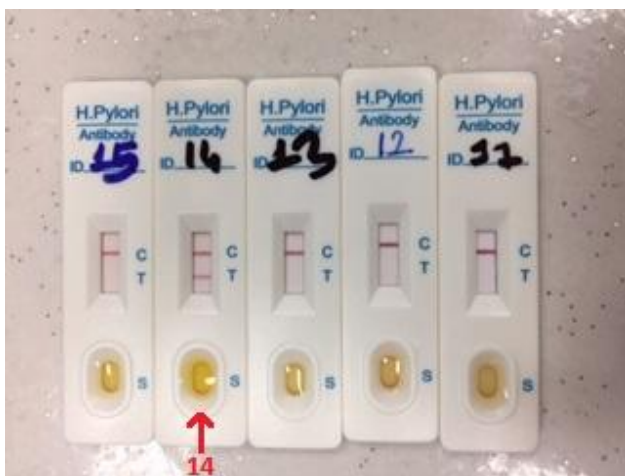
Figure 1. Evaluation of *H. pylori* IgG test results using rapid diagnostic tests in a group of patients. The sample numbered as 14 appears to have a clearly positive test result.

In addition, one patient had positive test results from all three tests, despite a negative endoscopy result. Accepting the endoscopy and biopsy results as the gold standard, the results of the three serological test methods were compared to the endoscopy result. Accordingly, the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy values of the three serological test methods were found as follows: 77.4%, 76.9%, 88.9%, 58.8% and 77.3%, respectively, for IgG cassette test; 85.7%, 52.0%, 81.8%, 59.1% and 76.1%, respectively, for ELISA IgA; 85.7%, 44.0%, 79.4%, 55.0% and 73.9%, respectively, for ELISA IgG (Table 3). Prevalence rates calculated based on the results of these test methods were as follows: 29.5% (66/224) for IgG cassette test, 50.9% (114/224) for ELISA IgA test, and 62.1% (139/224) for ELISA IgG test.

DISCUSSION

Due to their high cost and time-consuming nature, invasive diagnostic methods are obviously not convenient for every patient presenting to various clinics with gastro-intestinal complaints. Instead, non-invasive methods are more commonly preferred as they are faster and more economical alternatives. In the present study, we aimed to compare two serological test methods, rapid cassette test and micro ELISA method, using patient serum samples. Several authors have examined non-invasive methods for diagnosis of *H. pylori* infections using various body secretions (saliva, stool, etc.). For example, Felz et al. (14) applied invasive (upper gastrointestinal endoscopy and biopsy) and non-invasive methods (rapid urease test and [14C] urea breath test) for diagnosing of *H. pylori* in 26 patients with chronic gastritis. All (100%) of the 20 patients that had histologically confirmed diagnosis of *H. pylori* infection were found to have strong positive results from urea breath test. Rahman et al. (15) evaluated the performance of immunoblot test method and immunochromatographic (ICT) tests including CIM and ELISA. They measured anti-*H pylori* IgG antibodies using ELISA, ICT (*H. pylori* rapid test), and immunoblot methods in totally 82 serum samples, of which 61 were obtained from patients with confirmed H.pylori infection using endoscopy. They calculated sensitivity, specificity, positive predictive value, and negative predictive value for these methods, and found that ELISA method had a sensitivity (96.7%) that was close to immunoblot test method (98.3%), and was less expensive than the latter as well. Demiray et al. (16) measured anti-*Helicobacter pylori* IgG antibodies using URINELISA, RAPIRUN, and anti-*Helicobacter pylori* ELISA methods in urine and serum samples of 124 patients that had dyspeptic complaints and underwent upper gastrointestinal system endoscopic examination. Of these patients, 69 patients had positive results from both URINELISA and RAPIRUN, while 109 patients had positive results from anti-*H. pylori* IgG ELISA. The sensitivities of these tests were found as 74.4%, 73.2% and 100%, respectively, and specificities were 81.0%, 78.6% and 35.7%, respectively. In our study, we examined serum samples of 224 patients that had gastrointestinal complaints, using three non-invasive serological test methods (rapid diagnostic test and micro ELISA IgA and IgG). Among these methods, micro ELISA IgG had the highest positivity rate and yielded positive results in 139 (62.1%) patients. In comparison to endoscopy results, IgG cassette test, ELISA IgA test, and ELISA IgG tests had sensitivity rates as 77.4%, 85.7% and 85.7%, and specificity rates as 76.9%, 52.0% and 44.0%, respectively. In one similar study, Tsongo et al. (17) measured serum samples from 174 patients with gastroduodenal ulcer symptoms, using rapid diagnostic test, and stool samples of the same patients using ELISA method, and they compared the two methods. Accordingly, the prevalence of *H. pylori* was found as 29.9% (52/174) with ELISA method and 37.4% (65/174) with the rapid test, respectively. In addition, they applied a questionnaire to assess the socioeconomic levels and life styles of their patients. Accordingly, smoking, poor sanitation and lack of formal education were found as predisposing factors to infection (p<0.05).

The two methods yielded the same results in 87.9% of the patients. In our study, we rather measured serum samples with three serological methods, and we found prevalence rates as 29.5% (66/224) with IgG cassette test, 50.9% (114/224) with ELISA IgA test and 62.1% (139/224) with ELISA IgG test. As observed, prevalence rates were somewhat higher with ELISA methods. This may be related to the selection of the patient group that we included in this study. In a 2013-14 study with 160 students, Yo et al. (11) obtained 0.5 ml of saliva samples and analyzed them within 5 minutes using a *H. pylori* Saliva Test Cassette. They found positive results in 82 of the subjects and negative results in 78, and calculated the oral *H. pylori* infection rate as 51%. In addition, the subjects were questioned

in terms of smoking, dietary and dental care habits and family history. Of the 82 subjects with positive test results, 74 had poor dental care. We did not apply the questionnaire to our patients; however, the results we obtained from the serological tests (positive results for IgG cassette, ELISA IgA, and ELISA IgG were 54 (61.4%), 66 (75.0%) and 68 (77.3%), respectively) were consistent with the findings of the aforementioned study.

Agbor et al. (18) conducted a prevalence analysis to determine the epidemiological profile of *H. pylori* infection by obtaining blood and stool samples from 500 patients with gastric complaints between 2013 and 2015. They used a one-step *H. pylori* antibody device for serum analysis. Three drops of serum was put into a well in the device and the result was obtained after 10 minutes. For stool analysis, a one-step *H. pylori* antigen test device was also used; 50 mg solid or two drops of watery stool sample was transferred to a tube and mixed with buffer.
Two drops of this mixture was put into a well in the device and the result was obtained after 10 minutes. Of the 500 stool samples examined, 237 (43%) were positive for the *H. pylori* antibody test. Seropositivity rates were found to be similar between females and males (42% and 45%, respectively). They calculated the sensitivity and specificity of the antibody test (90% and 98%, respectively) in reference to the antigen test, which yielded higher positive rates. Twenty four samples were positive with the antigen test but negative with the antibody test. In contrast, 4 samples were positive with the antibody test but negative with the antigen test. Both tests yielded positive results for 213 of the samples. As a result, the authors noted that there was no significant difference between the two test methods (p=0.204). In our study, we did not measure antigens in the stool; however, we used three different antibody tests and compared the results to the endoscopy results. The positivity rates that we obtained from the antibody tests were close to the results of the study mentioned above: 29.5%, 50.9%, and 62.1% for IgG cassette test, ELISA IgA test and ELISA IgG test, respectively.

However, females had a little higher positivity rate in our study (63.5% in females vs. 36.5% in males, according to the endoscopy reports). She et al. (19) retrospectively reviewed 4,722 samples (58% female and 42% male) measured between the years 1998-2009, and compared the results of serum *H. pylori* IgG, IgA and/or IgM with the results of stool *H. pylori* antigen test (HpSA). The positivity rate of HpSA (12.1%) was significantly lower compared to IgG (35.6%) and IgA (32.7%) tests (p<0.001), whereas the positivity rate of IgM (4.3%) test was lower than the other three tests (p=0.001). When HpSA was taken as the gold standard, sensitivity, specificity, positive predictive value (PPV)m and negative predictive value (NPV) in all age groups were 87.6%, 61.0%, 22.8% and 97.4% for IgG test, 63.4%, 67.6%, 17.6% and 94.4% for IgA test, and 6.8%, 95.8%, 13.6% and 91.2% for IgM test, respectively. IgG test was found to have better correlation with HpSA than IgA and IgM. Additionally, IgM was reported to have little diagnostic value in *H. pylori* infections. In our study, although the number of samples was lower, our rates were similar to that study. On the other hand, we compared serological test results with endoscopy instead of the HpSA results.

Kazemi et al. (20) examined 94 patients with dyspeptic complaints who underwent endoscopic examination and evaluated rapid urease test, C-urea breath test, histological

examination reports, serum antibody and stool antigen tests. The authors concluded that the stool antigen test was more appropriate than UBT for diagnosing *H. pylori* infection in untreated patients. Similar to that study, we compared the results of serological tests with the gold standard endoscopy reports, and our sensitivity, specificity, PPV, NPV and accuracy values were 77.4%, 76.9%, 88.9%, 58.8%, and 77.3% for IgG cassette test, respectively. In another study, Veijola et al. (21) recruited their study participants via a newspaper advertisement, and included 1,574 adult subjects that did not receive antibiotic treatment for the last 2 months, or H2-receptor antagonists or bismuth or proton pump inhibitors for the last 2 weeks, or receive *H. pylori* eradication treatment for the last 5 years, or have history of gastric operation, chronic GIS disease, pregnancy or lactation. The subjects were tested with rapid whole blood antibody (IgG) test for diagnosis of *H. pylori* infection, and the 300 subjects with positive test results were confirmed with UBT and an in-house EIA-based serological assay (IgG and IgA).
Of the 300 subjects, 196 were confirmed positive with both methods; however, since 11 subjects did not meet the inclusion criteria, 185 positive subjects were left. With the addition of 97 subjects who had positive results from confirmatory tests despite having negative screening test results, the total number of subjects enrolled in the study was 282 (186 females and 96 males). One hundred eighty five subjects who had positive results from all three methods were enrolled in the eradication program, and they were retested after 4 months with serological methods. The success criterion for eradication therapy was defined as at least 40% reduction in IgG antibody level. The performance of the three stool antigen tests, HpSA (polyclonal antibody-based), HpStAR (mAb-based Amplified IDEIA) and ImmunoCard (based on monoclonal H. pylori antibody and a lateral flow chromatography technique), which were applied to the subjects both before and after the eradication treatment, was evaluated in comparison to UBT and serology. Accordingly, pre-eradication sensitivity, specificity, PPV and NPV values of the three stool antigen tests were found as 91.9%, 95.9%, 97.7% and 87.7% for HpSA, 96.2%, 95.9%, 97.8% and 93.0% for HpStAR, and 93.0%, 88.7%, 94.0% and 86.9% for ImmunoCard, respectively. Post-eradication values were 81.3%, 97.0%, 76.5% and 98.2% for HpSA, 100%, 97.6%, 80.0% and 100% for HpStAR, and 93.8%, 97.0%, 75.0% and 99.4% for ImmunoCard, respectively. In our study, we included patients presenting to various clinics. Our female to male ratio was similarly high (63.5%), and another interesting finding was that we found lower sensitivity, specificity, PPV, NPV and accuracy values (85.7%, 44.0%, 79.4%, 55.0% and 73.9%, respectively, for ELISA IgG). This difference might be related to the patient selection. In the study of Abu Shady (22), 100 pediatric patients with an age range of 4-10 years, who were referred to endoscopic examination due to upper GIS complaints were tested with rapid urease test (RUT) and biopsied for histological examination. For RUT, the result was obtained after adding biopsy sample to the urea solution (NaCl, KH2PO4 and NaOH). A change in the color of the urea solution from yellow to red, due to increase in pH induced by *H. pylori*, was accepted as a positive test result. Histological examination was performed after staining with hematoxylin and eosin. Additionally, a microplate enzyme immunoassay (EIA) and an antibody detection kit were used to detect antibodies against *H. pylori* (IgG) in patient serum samples. The analysis was performed per manufacturer's instructions and cutoff threshold was 10U/mL. The gold standard for

diagnosis of *H. pylori* infection was accepted as positive results from both histological examination and the rapid urease test. Accordingly, while standard test result was positive in 57% and negative in 43% of patients, serological test was positive in 60% and negative in 40% of patients. In addition, sensitivity and specificity values of anti-*H. pylori* IgG antibody test were found as 96.5% and 93%, respectively. The authors concluded that IgG antibody test was a good alternative to invasive diagnostic tests such as urea breath test, for diagnosis of *H. pylori* infection, and that IgG type of antibodies produced against *H.pylori* had higher diagnostic value. In contrast to that study, our study included adult patients. Although the study designs were similar, we had lower sensitivity and specificity values. This was due to the fact that our study sample did not include children.

Author's contributions: SA, SB and YK planned and designed the review. SC, AT, SKE and YK carried out data collection. Data analysis was performed by SC, AT and SKE under the supervision of SA. SA and SB supervised the writing of the manuscript. All authors reviewed and approved the final draft of the manuscript.

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