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Serodiagnosis of *Helicobacter pylori* Infection: Comparison and Correlation between Enzyme-Linked Immunosorbent Assay and Rapid Serological Test Results

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CLOser is a new, one-step, qualitative anti-*Helicobacter pylori* immunoglobulin G test having the advantage of convenience and simplicity. We aimed to evaluate its diagnostic accuracy and to compare it with a quantitative enzyme-linked immunosorbent assay (ELISA) (HEL-pTEST II) in a study of 86 adult dyspeptic patients by using the results from histology and urease testing of gastric biopsies as a "gold standard." Forty-six patients were *H. pylori* positive. The sensitivities, specificities, and positive and negative predictive values were 95.7, 72.5, 80.0, and 93.5%, respectively, for CLOser and 93.5, 92.5, 93.5, and 92.5%, respectively, for HEL-pTEST II. The grade of the colored test bands in CLOser was correlated with antibody titers in HEL-pTEST II ($r = 0.71$; $p < 0.001$). The mean antibody titers were 13, 74, 186, and 328 U/ml for the negative, faint, thin, and thick bands, respectively, of CLOser. We concluded that the CLOser rapid serological test yielded sensitivity similar to that of the conventional ELISA. Although CLOser is not suitable for epidemiologic screening for *H. pylori* infection on account of lower specificity, it is particularly convenient and very easy to perform. Therefore, it may eventually become widely used in the office-based care of patients and lead to more cost-effective patient management decisions.

Helicobacter pylori is established as an important etiologic factor for chronic gastritis (3, 7) and peptic ulcer disease (2, 8, 13). The diagnosis of *H. pylori* infection can be made by using several invasive or noninvasive techniques. Invasive diagnostic methods such as culture, histological stains, and urease test require an endoscopic biopsy of gastric mucosa. Serology and the urea breath test (UBT) are current noninvasive tests (9).

Although the ^{14}C-UBT is simple and accurate, protection from radioactivity pollution is not simple (1). It is also not suitable for infants and pregnant women. ^{13}C-UBT does not have the disadvantage of radioactivity but requires mass spectrometry, which is expensive (1).

The easiest way to diagnose *H. pylori* infection in a patient who is not undergoing endoscopy is to test for antibodies to the infection. The enzyme-linked immunosorbent assay (ELISA) is the most commonly used serological test because it is a simple, quick, and low-cost technique suitable for screening large populations (11, 12). However, the ELISA format requires some basic equipment, such as a plate reader and washing machine. Also, for economic reasons, the ELISA is usually not done until enough samples are accumulated.

CLOser (Medical Instruments Corporation, Solothurn, Switzerland) is a rapid *H. pylori* antibody test. It has the advantage of convenience and simplicity. Only one step is needed. Its sensitivity and specificity, however, had not formerly been evaluated. Therefore, we conducted this study to compare the diagnostic values of the qualitative CLOser and the quantitative ELISA (HEL-pTEST II; AMRAD, Kew, Australia) by using the results from histology and urease testing of gastric biopsies as the "gold standard."

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MATERIALS AND METHODS

A total of 86 dyspeptic patients who had undergone endoscopy were selected. Among them, 46 were *H. pylori* positive, including 40 duodenal ulceration patients and 6 gastritis patients (mean age, 60 years; 41 men, 5 women); 40 patients were *H. pylori* negative, all with bile reflux gastropathy (mean age, 47 years; 15 men, 25 women). *H. pylori* status was determined by rapid urease testing and histology. During endoscopy, biopsies were taken from the lesser curvature and greater curvature of the antrum and placed in 10% formalin. Subsequently, these samples were stained with hematoxylin and eosin and modified Giemsa stain to allow observation of the presence of curved rod-shaped bacteria on the mucosal surface. Another two biopsy specimens, one from the greater curvature side of the antrum and another from the greater curvature side of the body, were obtained for rapid urease testing. The reagent for the urease test was prepared according to the method of Hazell et al. (5), and 100 μl of urease test reagent was put into each micro test tube (capacity, 1.5 ml, with tapering tip and safety lid lock; Eppendorf, Hamburg, Germany). Biopsy specimens were inserted into these micro test tubes and incubated at 37°C for 24 h. A color change from orange to pinkish red was read as positive.

A patient was classified as *H. pylori* positive if either urease testing was positive or curved rod-shaped bacteria were seen by histological staining. A patient was classified as *H. pylori* negative if both the urease test was negative and histology failed to detect *H. pylori*.

Serology. For serology studies, blood was drawn immediately after endoscopy and sera were collected and stored at −70°C until assayed.

Quantitative ELISA. Serum specimens were tested for the presence of immunoglobulin G (IgG) antibodies against *H. pylori* by using a quantitative ELISA (HEL-pTEST II). The optical densities of four reference standards were used to plot a standard curve by which the *H. pylori* antibody levels in patient samples were quantitated. The results were expressed in arbitrary units per milliliter. Reference standards 1 to 4 represent 30, 50, 200, and 800 U/ml, respectively (AMRAD). The antigen was an inactive native antigen of *H. pylori*. On the day of testing, we added 100-μl volumes of diluted specimens, diluted positive and negative controls, and duplicates of reference standards 1 to 4 to the appropriate wells of the microtiter plate. The plate was incubated for 15 min at room temperature and then washed six times with wash buffer. After washing, a 100-μl volume of sheep anti-human IgG conjugated to horseradish peroxidase (AMRAD) was added to each well. After a further 15 min of incubation, 100 μl of substrate reagent was added to each well, the plate was incubated in the dark for 15 min at room temperature, and then stopping solution (100 μl of 0.5 M H_2SO_4) was added to each well to terminate the enzymatic reaction. The absorbance was read within 30 min using the 450-nm filter with the 620-nm filter as a reference.

A specimen was considered positive if it contained >50 U/ml. A specimen was considered negative if it contained <30 U/ml. Samples giving a reading between 30 and 50 U/ml were retested. If the result is still between 30 and 50 U/ml, it is considered indeterminate and should be examined by an alternative procedure.

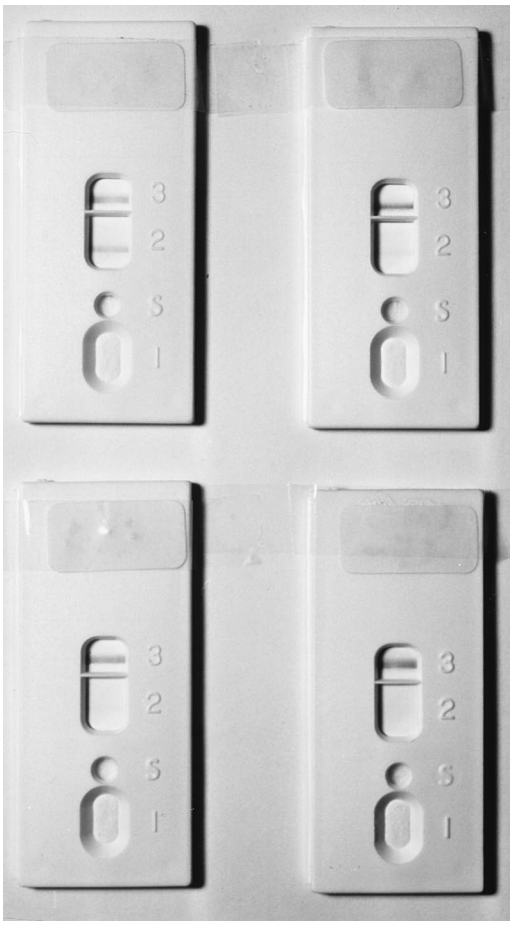

FIG. 1. Photo series of representative negative (lower right), faint (lower left), thin (upper right), and thick (upper left) band results of CLOser. 1, developer well; S, sample well; 2, test window; 3, control window. The grades of the colored band in the test window were defined as follows: thick band, line apparent and usually wider than 1 mm; thin band, line less apparent but confidently visible and usually ≤1 mm wide; and faint band, only a shade of purple seen without a distinct colored band.

Rapid serological test. CLOser was developed by Medical Instruments Corporation. It is a one-step anti-*H. pylori* IgG test using an indirect solid-phase immunoassay technology for the qualitative detection of *H. pylori* antibodies in human serum or plasma. The test procedure is to add 10 μl of serum or plasma to a sample well and to add 3 or 4 drops of developer to a developer well. Results in the test and control windows are read after 3 to 8 min. One colored band (purple) both in the test window and in the control window indicates that IgG antibodies against *H. pylori* have been detected. Only one band in the control window with no distinct colored band in the test window indicates that IgG antibodies against *H. pylori* have not been detected. The grades of the colored bands in the test window were defined as follows: thick band, line apparent and usually wider than 1 mm; thin band, line less apparent but confidently visible, usually ≤1 mm wide; and faint band, only a shade of purple seen without a distinct colored band (Fig. 1).

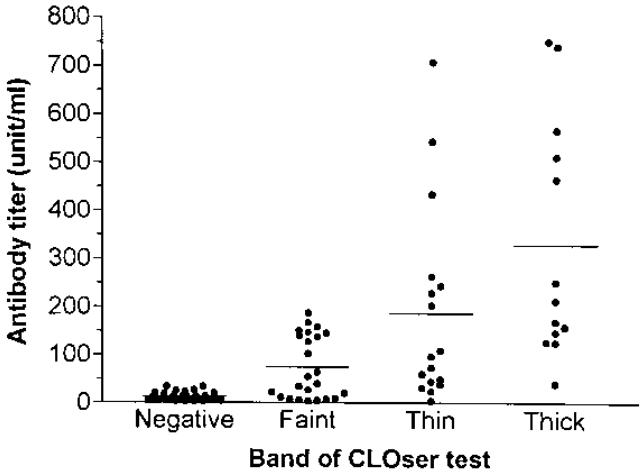

FIG. 2. Comparison of the antibody titers determined by HEL-pTEST II and the grades of the bands in the test window of CLOser. See the legend to Fig. 1 for the definition of the grades of the colored band in the test window.

Statistical analysis. Sensitivity, specificity, and predictive values were calculated for the ELISA (HEL-pTEST II) and the rapid serological test (CLOser) by using the gastric biopsy results as the gold standard. The association between the grades of CLOser bands and the antibody titers of HEL-pTEST II was analyzed by using Spearman's rank correlation (6). Negative, faint, thin, and thick bands were assigned rankings of 1 to 4, respectively. *P* values of <0.05 were considered significant.

RESULTS

The HEL-pTEST II and CLOser results were compared to gastric biopsy findings to assess diagnostic value (Table 1). The sensitivity and negative predictive value of CLOser results dropped from 95.7 to 60.9% and 93.5 to 67.3%, respectively, while the specificity and positive predictive value increased from 72.5 to 92.5% and 80.0 to 90.3%, respectively, when a faint band result was considered seronegative.

Regarding the positive CLOser results, all subjects ($n = 13$) whose tests showed a thick band were *H. pylori* positive. Eighty-two percent of subjects (14 of 17) with a thin band were *H. pylori* positive, and 68% of subjects (17 of 25) with a faint band were *H. pylori* positive. On the other hand, negative CLOser results ($n = 31$) correctly identified 29 of 40 (72.5%) *H. pylori*-negative patients; only two (6%) were false negatives.

A comparison of the quantitative results obtained with HEL-pTEST II with CLOser results is presented in Fig. 2. The grades of the colored band in the test window of CLOser were correlated with antibody titers measured by HEL-pTEST II ($r = 0.71$, $P < 0.001$, Spearman's rank correlation). The mean titers were 13, 74, 186, and 328 U/ml for negative, faint, thin, and thick bands in CLOser, respectively.

TABLE 1. Diagnostic value of two serological tests for *H. pylori*

Test	No. of results				Sensitivity (%)	Specificity (%)	PPV[a] (%)	NPV[b] (%)
	True positive	False positive	True negative	False negative				
HEL-pTEST II	43	3	37	3	93.5	92.5	93.5	92.5
CLOser	44	2	29	11	95.7	72.5	80.0	93.5

[a] PPV, positive predictive value.
[b] NPV, negative predictive value.

DISCUSSION

The present study demonstrates that the HEL-pTEST II ELISA format and the CLOser rapid serological test both had good sensitivities for detection of the presence of *H. pylori* antibodies. However, CLOser was not as specific as HEL-pTEST II. These findings are similar to reports concerning two other commercially available rapid tests, Pyloriset and QuickVue (16, 17).

Compared with HEL-pTEST II, CLOser is easier to perform, there is no need to dilute samples, and results are available in 10 min. However, the simplicity and rapid results of CLOser are of less value when one considers CLOser's specificity compared to that of HEL-pTEST II. In addition, CLOser is more expensive on a per-sample basis. For these reasons, ELISA testing, as performed with HEL-pTEST II, is more appropriate than CLOser rapid serological testing for epidemiologic use.

Our study demonstrated that the grade of the band in the test window of CLOser was significantly correlated with antibody titers determined by ELISAs. The higher the antibody titer, the more intense the band. Based on our experience, when the band in the test window of CLOser was faint, it was difficult to determine the result. Therefore, the accuracy of CLOser was influenced by observer experience and the level of antibody titers. If a faint band was considered seronegative, the sensitivity was decreased while the specificity was increased.

It has been proposed that patients with dyspepsia could be screened for *H. pylori* status before endoscopy is recommended (4). A study by Sobala et al. has found that performing endoscopy for only patients who are seropositive, are over 45 years of age, or are taking nonsteroidal antiinflammatory drugs could reduce endoscopy workloads by 23.3%, while as few as 3% of ulcers would be missed (14). Their data showed that screening young dyspeptic patients for *H. pylori* by serology is more sensitive than symptom-based screening strategies (13). Recent studies in the United Kingdom also demonstrate that serology is a useful adjunct in screening subjects under 45 years of age to reduce endoscopy workloads, although serology is less adequate for patients with esophagitis (10, 15).

For screening of dyspeptic patients, the higher the sensitivity for *H. pylori*, the lower the risk of missing patients with this infection. In this case, both CLOser and HEL-pTEST II could be used as a screening test. However, CLOser had a lower specificity than HEL-pTEST II. This will increase false-positive results, thus increasing the number of unnecessary endoscopies.

In summary, our results showed that HEL-pTEST II is a very sensitive method for detecting the presence of *H. pylori* antibodies and can be used for research and clinical purposes. CLOser yielded sensitivities similar to those of ELISA testing. Although CLOser is not suitable for epidemiologic screening for *H. pylori* infection on account of lower specificity, it is particularly convenient and very easy to perform. Therefore, it may eventually become widely used in the office-based care of patients and lead to more cost-effective patient management decisions.

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