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Evaluation of Urinary Rapid Test for *Helicobacter pylori* in General Practice

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There is increasing interest in noninvasive tests for the diagnosis of *Helicobacter pylori* (*H. pylori*) infection. One such test, a urine-based rapid test kit (RAPIRUN H. pylori Antibody, Otsuka Pharmaceutical Co., Ltd.) for detection of antibody to *H. pylori*, has been developed and is considered ideal. In addition to its noninvasiveness and safe handling—due to use of urine as a sample—the assay procedure used for the urinary rapid test is very simple. Only 10–20 minutes are required to complete an assay, and no instruments are needed. The aim of this study was to examine the clinical usefulness of this urine-based rapid test. A total of 189 patients, including 76 patients with gastroduodenal disease, were recruited. A pair of random single-void urine and serum samples was collected from each of the 189 patients, and antibody to *H. pylori* in the urine and serum samples was measured using the urine-based rapid test kit and three commercially available serum-based ELISA kits. For the patients with gastroduodenal disease, invasive diagnostic methods using endoscopic biopsy specimens such as culture, histology, and rapid urease test were also performed. The sensitivity, specificity, and accuracy of the urinary rapid test were evaluated on the basis of the three serum ELISA results or the invasive diagnostic results. In addition, various urinalyses were performed, and the effects of substances existing in urine on the urinary rapid test results were examined. Of the 189 patients, the urinary rapid test was positive for 110 (58.2%), negative for 78 (41.3%), and invalid for only one patient (0.5%). Based on the three serum-based ELISA results, the sensitivity, specificity, and accuracy of the urinary rapid test were 93.7, 88.9, and 92.2%, respectively. On the basis of the biopsy-based test results, the sensitivity of the urinary rapid test was 100% and its accuracy (95.2%) was equivalent or superior to that of each serum-based ELISA. In addition, no significant differences were observed between groups positive and negative on urinary rapid testing in any urinalysis parameter examined. The novel urinary rapid test kit evaluated in this study enables simple, rapid, and accurate diagnosis of *H. pylori* infection, and is an ideal test method for point-of-care testing. J. Clin. Lab. Anal. 15:154–159, 2001.

Key words: *Helicobacter pylori*; diagnosis; urine

INTRODUCTION

Helicobacter pylori (*H. pylori*) infection plays an important role in the pathogenesis of gastritis, peptic ulcer, gastric adenocarcinoma, and low-grade mucosa-associated lymphoid tissue (MALT) lymphoma (1–4). It is now generally accepted that *H. pylori* eradication therapy may be effective in preventing recurrence of gastric and duodenal ulcer. As eradication therapy has become widespread, easy and noninvasive methods for screening of *H. pylori* infection have become necessary. The serum enzyme-linked immmunosorbent assay (ELISA) method has been widely used for detection of *H. pylori* infection, and has been validated by comparison with reference methods such as histology, culture, and the ^{13}C-urea breath test (^{13}C-UBT). In our previous study based on histological examination using Carnoy's solution for stain-

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ing, the sensitivity and specificity of the serum ELISA tested were 94.0 and 96.7%, respectively (5).

The presence of antibody to *H. pylori* in body fluids other than serum including saliva (6–9) and urine (10,11) has also been demonstrated. A recent study reported that a urine-based ELISA was very accurate and would be useful for screening *H. pylori* infection as an alternative to serum ELISA, based on results of ^{13}C-UBT (12) . On the other hand, several serum- (plasma-) or whole blood-based rapid test kits have been developed and have been widely used as point-of-care testing for screening of *H. pylori* infection.

More recently, a urine-based rapid test kit, RAPIRUN H. pylori Antibody (RAPIRUN), was developed by Otsuka Pharmaceutical Co., Ltd. (Tokyo, Japan) for detection of antibody to *H. pylori* in urine. This urine-based antibody rapid test is the first product in the world to detect specific antibody in urine using a rapid format. The procedure used in the urinary rapid test kit is very simple and requires neither skill to operate nor instruments for measurement. Moreover, only 10–20 minutes are required to complete an assay.

In this study, the diagnostic accuracy of this urinary rapid test was compared with those of three commercially available serum ELISA kits and biopsy-based diagnostic methods using endoscopic tissue specimens such as culture, histology, and rapid urease tests.

MATERIALS AND METHODS

Subjects

This study was performed with patients at screening and during a follow-up period at Shinshu University School of Medicine, from October 1997 to March 1998. The study was approved by the Institutional Review Board of Shinshu University, and informed consent was obtained from each patient. A total of 189 patients aged 24–70 years (107 men and 82 women; median age 51.9 ± 14.0 years) were recruited. The subjects included 76 patients with gastroduodenal disease who had not undergone *H. pylori* eradication therapy, 47 patients with urogenital disease, 10 patients with autoimmune disease, 30 patients with chronic hepatitis, and 26 patients with diabetes mellitus. The 76 patients with gastroduodenal disease underwent standard endoscopy by practicing gastroenterologists, and biopsy diagnostic methods were performed for 24 of them.

Samples

A pair of urine and serum samples was obtained from each patient. Sodium azide (0.1 w/v % at final concentration) was added to the urine samples, which were then stored at 2 to 8°C. The serum samples were stored at –20°C until use.

Urinary Antibody Assay

Anti-*H. pylori* IgG antibodies in urine were tested by using the urinary rapid test kit (RAPIRUN) based on immunochromatographic technology. This kit is composed of ten test devices, ten tubes of sample diluent, and ten disposable syringes. On the membrane in the test device, *H. pylori* antigen extracted from a H. pylori strain (OHPC-040), which was isolated from a Japanese patient with chronic active gastritis, is immobilized on the test zone (T) to detect anti-*H. pylori* antibody, and an anti-human IgG antibody is immobilized on the control zone (C) to detect other urinary IgG antibodies. The assay protocol is very simple, as shown in Figure 1. Using a disposable syringe enclosed in the kit, a urine sample (approximately 0.5 mL) is drawn to the level indicated on the syringe, and transferred to a tube containing 0.5 mL of sample diluent, and then mixed well using the syringe. Using the same syringe, 0.2 to 0.3 mL of the mixture is dropped into the sample well (S) of a test device. After 20 minutes, observation is made for the appearance of red-colored bands on the test (T) and control (C) zones. When a red-colored band is observed only in the control zone, the test result is negative, but when a red-colored band is observed in the test zone (S) in addition to the control zone, the test result is positive. If there is no distinct red band visible in the control zone, the test result is considered invalid. The urinary rapid assay was performed at Shinshu University.

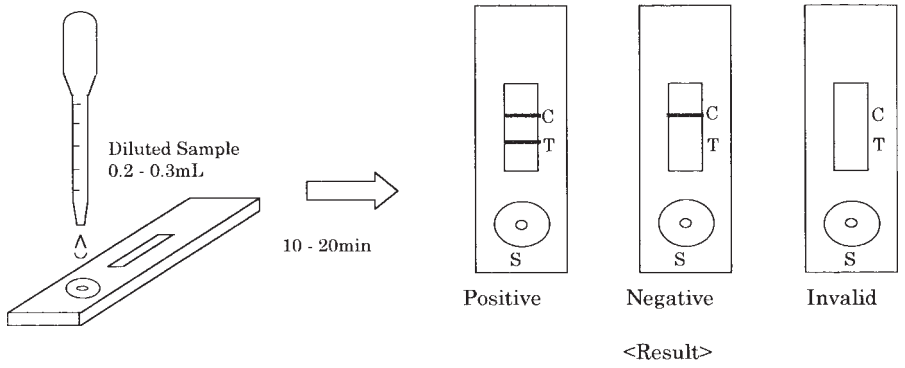

Fig. 1. Schematic assay procedure of newly developed urine-based rapid test kit (RAPIRUN H. pylori Antibody). C, control zone; T, test zone; S, sample window.

Serum Antibody Assay

Serum anti-*H. pylori* antibodies were measured using three commercially available ELISA kits: (1) HM-CAP kit (Enteric Products, West Burg, NY); (2) HEL-p TES kit (AMRAD Biotech, Victoria, Australia); and (3) GAP-G kit (Biomerica, Newport Beach, CA). Each assay was performed according to the manufacturer's instructions. The assay results for each serum ELISA were compared with those of the urinary rapid test. All subjects were classified into three groups: an overall serum antibody-positive group (positive for all three serum ELISA), an overall serum antibody-negative group (negative for all the three serum ELISA), and an overall serum antibody-indeterminable group (other cases). Using the overall positive and negative groups, the sensitivity, specificity, and accuracy of the urinary rapid test was evaluated for each disease. The serum assay was performed at Otsuka Pharmaceutical Co., Ltd.

Urinalyses

In order to examine the relationship between urinary rapid test results and each urinalysis parameter, qualitative urinalyses (glucose, protein, occult blood, white blood cells, specific gravity, pH, and urobilinogen) were performed using urinary test papers (Uro-hema Combi atix SG-L, Miles-Sankyo, Tokyo, Japan) at Shinshu University. In addition to the qualitative assay, some quantitative urinary assays (the protein concentration, creatinine concentration, and osmotic pressure) were performed at a clinical reference laboratory (Otsuka Assay Laboratory, Tokushima, Japan).

Biopsy-Based Diagnosis

Bacteriological (culture) and histological (histology) examinations and rapid urease tests (RUT) were performed using endoscopic biopsy specimens obtained from 24 of the patients with gastroduodenal disease. Culture and histology were performed according to our previously described methods (5,13,14). For histology, Carnoy's solution has been used in our institute as one of the best means for obtaining fixation of biopsy specimens with preservation of the mucous layer in tissue preparations. RUT was performed using a RUT kit (Helicocheck, Otsuka Pharmaceutical Co., Ltd.) according to the manufacturer's instructions. Six gastric mucosal biopsy specimens were obtained from the antrum and corpus (three each) for histology, two biopsy specimens were obtained from the antrum and corpus (one each) for culture, and one biopsy specimen was obtained from the antrum for RUT from each patient.

The patients were classified into three groups: an overall biopsy test-positive group (n = 18), an overall biopsy test-negative group (n = 3), and an overall biopsy test-indeternable group (n = 3), referring to the guidelines provided by the Food and Drug Administration (FDA) in 1995 (15). Based on the classification by the overall assessment, the sensitivity, specificity, and accuracy of the urinary rapid test were compared with those of three serum ELISA.

RESULTS

Assay Results for Each Antibody Test

Of the 189 patients participated in this study, urinary rapid test results were positive for 110 (58.2%), negative for 78 (41.3%), and invalid for only one patient. A similar distribution was observed for the results of HM-CAP assay. On the other hand, HEL-p TES yielded more positive and fewer negative results, and GAP-G yielded fewer positive and fewer negative results. In addition, many indeterminate results were obtained with HEL-p (n = 20, 10.8%) and GAP-G (n = 51, 27.0%) (Table 1).

Comparison with Serum-Based ELISA

The overall agreements (coincidence ratios) between the results of testing with RAPIRUN and those of HM-CAP, HEL-P TES, and GAP-G were 83.1, 76.2, and 64.0%, respectively (Table 2). The test results with RAPIRUN were thus most strongly correlated with those of HM-CAP. The coincidence ratios among the test results for the three serum ELISA were 81.5% (HM-CAP vs. HEL-p TES), 67.7% (HM-CAP vs. GAP-G), and 63.0% (HEL-p vs. GAP-G). The ratios among the three serum ELISA were equivalent to those between RAPIRUN results and each serum ELISA result, and the ratios between GAP-G results and other test results were clearly lower than those for other combinations.

Because the serum test results varied between kits, all patients—except the one whose results were invalid on the urinary rapid test—were classified into overall serum antibody-positive, -negative, and -indeterminable groups according to the overall assessment criteria for the serum antibody assay. With the assumption that this classification was 100% accurate, the sensitivity, specificity, and accuracy of

TABLE 1. Summary of antibody assay results

	Sample	Assay method	Incubation time (min.)	Number of cases		
				Positive	Negative	Invalid
RAPIRUN	Urine	ICT	10–20	110 (58.2%)	78 (41.3%)	1 (0.5%)
HM-CAP	Serum	ELISA	50	107 (56.7%)	74 (39.2%)	8 (4.2%)
HEL-p TES	Serum	ELISA	45	117 (61.9%)	52 (27.5%)	20 (10.8%)
GAP-G	Serum	ELISA	100	84 (44.4%)	54 (28.6%)	51 (27.0%)

TABLE 2. Comparison of urinary rapid test results and serum ELISA results

		RAPIRUN			Coincidence ratio	
		Pos.	Ind.	Neg.	All subjects	Subjects excl. Ind. cases
HM-CAP	Pos.	95	1	11	83.1%	87.2%
	Ind.	3	0	5	(157/189)	(157/180)
	Neg.	12	0	62		
HEL-p TES	Pos.	98	1	18	76.2%	85.7%
	Ind.	6	0	14	(144/189)	(144/168)
	Neg.	6	0	46		
GAP-G	Pos.	75	1	8	64.0%	88.3%
	Ind.	27	0	24	(121/189)	(121/`37)
	Neg.	8	0	46		

Pos., positive; Ind., indeterminante; Neg., negative.

the urinary rapid test were 94.9% (74/78), 88.9% (32/36), and 93.0% (106/114), respectively (Table 3). The sensitivity and accuracy for patients with each disease except autoimmune disease were excellent (90% or higher). Regarding specificity, one false-positive result was observed for each disease except chronic hepatitis (no false-positives). The best accuracy (95.7%) was observed for the patients with gastroduodenal disease.

Comparison With Biopsy Diagnostic Methods

In accordance with the FDA guidelines, 21 positive patients and three negative patients were extracted on the basis of the three biopsy-based diagnostic results. The urinary rapid test results and serum ELISA results for them were compared with the overall biopsy test results (Table 4). RAPIRUN exhibited perfect sensitivity (100%) and the best accuracy (95.2%), and only one false-positive result was observed in the two antibody tests. The one false-positive patient was negative for culture and histology, but did not undergo RUT. The serum ELISA results for this patient were positive on HM-CAP and HEL-p TES and indeterminate on GAP-G, and no negative results were observed in the four antibody assays including RAPIRUN.

TABLE 3. Sensitivity, specificity, and accuracy of urine-based rapid test for each disease determined on the basis of overall assessment of three serum ELISA results

Disease condition	Sensitivity	Specificity	Accuracy
Gastroduodenal disease	96.9% (31/32)	92.9% (13/14)	95.7% (44/46)
Urogenital disease	95.7% (22/23)	85.7% (6/7)	93.3% (28/30)
Diabetes	100.0% (11/11)	66.7% (2/3)	92.9% (13/14)
Autoimmune disease	50.0% (1/2)	75.0% (3/4)	66.7% (4/6)
Chronic hepatitis	90.0% (9/10)	100.0% (8/8)	94.4% (17/18)
Total	94.9% (74/78)	88.9% (32/36)	93.0% (106/114)

TABLE 4. Sensitivity, specificity, and accuracy of urine-based rapid test and serum ELISAs determined on the basis of overall assessment of biopsy-based diagnostic tests

Kit	Sensitivity	Specificity	Accuracy
RAPIRUN	100.0% (18/18)	66.7% (2/3)	95.2% (20/21)
HM-CAP	100.0% (18/18)	66.7% (2/3)	95.2% (20/21)
HEL-p TES	94.4% (17/18)	66.7% (2/3)	90.5% (19/21)
GAP-G	66.7% (12/18)	33.3% (1/3)	61.9% (13/21)

The sensitivity and accuracy of HM-CAP and HEL-p TES were the same as or slightly inferior to those of RAPIRUN, but those of GAP-G were clearly inferior to those of the others. The urinary rapid test kit thus achieved a sensitivity and specificity similar to or even better than those of several widely used quantitative serological tests.

Relationship Between Results of the Urinary Rapid Test and Several Urinalyses

Qualitative (glucose, protein, occult blood, white blood cells, specific gravity, pH, and urobilinogen) and quantitative urinalyses (protein concentration, creatinine concentration, and osmotic pressure) were performed for all 189 patients. The difference in distribution of the results for each parameter was examined for the urinary rapid test-positive and -negative groups after digitization of qualitative urinalysis data for glucose, protein, occult blood, and white cells as follows. Results of (–), (±), (+), (2+), (3+), and (4+) were assigned values of –1, 0, 1, 2, 3, and 4, respectively. No significant difference between the positive and negative groups was observed for any of these parameters (Table 5).

DISCUSSION

Several international groups have recommended *H. pylori* eradication therapy in numerous clinical situations including not only gastric and duodenal ulcer but non-ulcerative dyspepsia, as well as for patients undergoing long-term proton pump inhibitor therapy (16,17). As the indications for *H. pylori* eradication therapy have expanded, simple and rapid tests for *H. pylori* have become necessary. At present, there are several invasive and noninvasive methods for detecting *H. pylori* infection. The biopsy-based methods such as culture, histology, and rapid urease test are too invasive for screening. The ^{13}C-urea breath test is rapid, noninvasive, and the most accurate method, but is expensive as a screening method. Therefore, serological ELISA tests have been widely used for screening because they are noninvasive and convenient. However, it has been reported that the sensitivity and specificity of ELISA kits are likely to differ. In fact, quite different sensitivities and specificities were observed for the three serum ELISA kits tested in this study. In addition, patients who undergo ELISA tests must return to the hospital to obtain test results, because ELISA require 1 to 2 hours for completion of one assay. Recently, whole blood-based rapid test kits have

TABLE 5. Differences in results of urinalysis between groups positive and negative on urinary rapid test

Analyte	Urinary rapid test: Positive (N = 110) Mean	Positive (N = 110) SD	Negative (N = 78) Mean	Negative (N = 78) SD	Significance of difference (*P* value)
Qualitative test					
Glucose	–0.782	0.682	–0.808	0.685	N.S. ($P = 0.798$)
Protein	–0.591	0.849	–0.769	0.643	N.S. ($P = 0.120$)
Occult blood	–0.600	0.890	–0.667	0.848	N.S. ($P = 0.607$)
White cells	–0.773	0.645	–0.923	0.387	N.S. ($P = 0.678$)
Specific gravity	1.019	0.006	1.017	0.006	N.S. ($P = 0.101$)
pH	6.695	0.761	6.737	0.728	N.S. ($P = 0.707$)
Urobilinogen	0.009	0.095	0.000	0.000	N.S. ($P = 0.401$)
Quantitative test					
Protein	10.271	30.940	4.103	10.384	N.S. ($P = 0.092$)
Osmotic pressure	643.845	219.278	639.577	260.104	N.S. ($P = 0.903$)
Creatinine	97.302	70.584	101.223	82.802	N.S. ($P = 0.727$)

become available for the detection of antibody to *H. pylori*. These tests are ideal for screening, because results can be obtained in 10 to 20 minutes. Therefore, patients can quickly proceed to the next step in medical examination, such as confirmation testing or eradication therapy, on the same day as testing.

In this study, a newly developed urine-based rapid test kit was evaluated. Since this urine-based test is rapid (assay period: 10 to 20 minutes), completely noninvasive (with urine as sample) and easy to handle, it is "friendly" to both patients and operators. In addition to these advantages, this study indicated that its sensitivity and specificity are equivalent to or even better than those of widely used serum ELISA tests. The urinary rapid test yielded only one invalid result, while several indeterminate results (n = 8 to 51) were obtained with each serum ELISA. Extremely thin urine samples are rarely observed probably because of too much water intake before sample collection. The protein (IgG) concentrations in these urine samples are extremely low. To avoid false-negative results caused by extremely low IgG concentrations, the urinary rapid test is designed to detect total IgG antibodies on the control zone, and the red-colored band does not appear in the control zone when extremely thin urine samples are measured. The one patient whose RAPIRUN result was invalid in this study was positive for all three serum ELISA, assuming that the patient was actually positive. The protein concentration in urine from this patient was undetectable, and the specific gravity of the urine sample was at the lowest level (1.010) (data not shown). It thus appeared that this urine was extremely thin, and that the invalid result resulted from an extremely low IgG concentration. Therefore, the designation of the control line to avoid false-negative results caused by very low sample concentrations was proven to be useful.

The sensitivity and accuracy for patients with autoimmune disease was lower than those for patients with other diseases in this study. However, the number of patients with autoimmune disease was too small for accurate statistical analysis (n = 6). Therefore, further evaluation is necessary to confirm this finding. In an analysis of differences between the groups testing positive and negative, respectively, on urinary rapid tests, no significant difference was observed for any parameter of urinalysis, indicating that the results of the urinary rapid test are not affected by abnormal urine sample conditions.

In conclusion, the urine-based rapid test kit (RAPIRUN H. pylori Antibody) is a novel, simple, rapid, and completely noninvasive kit for the diagnosis of *H. pylori* infection. In addition, its sensitivity, specificity, and accuracy were equivalent to or even better than those of three widely used serum ELISA kits, as determined on the basis of biopsy-based tests. The urinary rapid test should thus be useful for screening for *H. pylori* infection, especially as a point-of-care test.

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