Rapid Urinary Trypsinogen-2 Test Strip in the Diagnosis of Pancreatitis After Endoscopic Retrograde Cholangiopancreatography

Chih-Wei Tseng, MD,† Chun-Chia Chen, MD,†‡ Shan-Zu Lin, MD,‡ Full-Young Chang, MD,†‡ Han-Chieh Lin, MD,†‡ and Shou-Dong Lee, MD†‡*

Objectives: The aim of this prospective study was to evaluate the diagnostic value of the rapid urinary trypsinogen-2 test strip in post-endoscopic retrograde cholangiopancreatography (ERCP) pancreatitis.

Methods: A total of 150 patients were tested with the urinary trypsinogen-2 test strip and serum levels of amylase and lipase before ERCP and 3 hours after ERCP. The diagnostic value of urinary trypsinogen-2 strip test compared with that of serum amylase and lipase was analyzed.

Results: Post-ERCP pancreatitis was diagnosed in 13 (8.7%) of 150 patients. The sensitivity, specificity, positive predictive value, negative predictive value, and accuracy of urinary trypsinogen-2 dipstick test at 3 hours after ERCP are 84.6%, 97.1%, 73.3%, 98.5%, and 96%, respectively. At the cutoff level of 3 times the upper reference limit, the negative predictive values of amylase and lipase were comparable to that urinary trypsinogen-2 strip test; however, their positive predictive values (42.9% and 36.4%, respectively) were markedly lower than that of urinary trypsinogen-2 test (73.3%).

Conclusions: The urinary trypsinogen-2 dipstick test is a useful test for early diagnosis of post-ERCP pancreatitis. A negative urinary dipstick test at 3 hours after the procedure rules out post-ERCP pancreatitis with a high probability and allows of early discharge plan.

Key Words: endoscopic retrograde cholangiopancreatography, pancreatitis, strip test, urinary trypsinogen-2

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Although magnetic resonance cholangiopancreatography provides excellent anatomic detail of the biliary and pancreatic ducts and has markedly decreased the application of diagnostic endoscopic retrograde cholangiopancreatography (ERCP) in recent years, therapeutic ERCP remains a cardinal intervention for biliary and pancreatic diseases.[1] In a recent systematic survey of 21 studies involving 16,855 patients, the incidences of ERCP-associated complications and mortality were 6.85% and 0.33%, respectively.[2] Acute pancreatitis is a major complication of ERCP. Although most episodes of post-ERCP pancreatitis were mild to moderate, some patients may develop severe or even fatal pancreatitis. Post-ERCP pancreatitis occurs in approximately 1% to 10% of patients undergoing ERCP and may cause mortality of 0.11%.[2] Endoscopic retrograde cholangiopancreatography is still perceived as the most worrisome procedure in the clinical setting of gastroenterology, especially when one is confronting a fatal episode of severe post-ERCP pancreatitis.

Early diagnosis of post-ERCP pancreatitis makes clinical physicians provide intensive care and possible medical treatment. Measurement of serum amylase and lipase levels after the procedure may have a possible role for early recognition of post-ERCP pancreatitis.[3–5] However, asymptomatic elevation in serum amylase and lipase activities after ERCP is common, occurring in approximately 25% to 75% of all patients.[6] Owing to the lack of specificity of pancreatic enzymes, the early diagnosis of post-ERCP pancreatitis is still based on clinical presentations, laboratory features, and radiological imaging.

Trypsinogen, the inactive precursor of trypsin, is secreted from acinar cells into pancreatic juice. The 2 major isoenzymes of trypsinogen are trypsinogen-1 (cationic) and trypsinogen-2 (anionic).[7] Intrapancreatic activation of trypsinogen to trypsin is considered to play a crucial role in the pathogenesis of acute pancreatitis.[8] The inflammatory process in acute pancreatitis leads to leakage of pancreatic enzymes into circulation, and trypsinogen-2 levels increase rapidly both in serum and urine.[9] Serum and urinary trypsinogen-2 levels have been shown to be a useful marker of acute pancreatitis.[7,9–11] Urinary trypsinogen-2 has also been valuable in the early prediction of disease severity.[12] There are few studies investigating the clinical values of urinary trypsinogen-2 dipstick test for early diagnosis of post-ERCP pancreatitis. The aims of this study were to evaluate the diagnostic value of urinary trypsinogen-2 test strip for early diagnosis of post-ERCP pancreatitis and to compare with serum amylase and lipase levels.

MATERIALS AND METHODS

Patients of all ages, ethnicity, and sex who had undergone ERCP at the Division of Gastroenterology of Taipei Veterans General Hospital from Mach 2006 to July 2008 were eligible for this study. We excluded patients with end-stage renal disease, known acute pancreatitis, a history of pancreatic/biliary surgery, or positive pre-ERCP urinary trypsinogen-2 dipstick test. The study protocol was approved by the Hospital Ethics Committee of Taipei Veterans General Hospital. Informed consent was obtained from all patients before collecting urine and serum samples.

Two hundred five patients were assessed for eligibility to enter this study before ERCP. Fifty-five patients including 14 patients with positive pre-ERCP dipstick test, 4 patients with renal insufficiency, 22 patients with known acute pancreatitis, 5 patients with procedure failure, and 10 patients with lost follow-up were all excluded. A total of 150 patients (114 men and 36 women) with a mean age of 69.8 years (range, 29–97 years) were finally recruited in this study. Endoscopic retrograde cholangiopancreatography was performed by experienced gastroenterologists with an Olympus JF-240 electronic duodenovideoscope (Olympus Optical, Tokyo, Japan) after premedication with local pharyngeal

From the *Division of Gastroenterology, Department of Medicine, Buddhist Dalin Tzu Chi General Hospital, Chiayi; †National Yang-Ming University School of Medicine, Taipei; and ‡Division of Gastroenterology, Department of Medicine, Taipei Veterans General Hospital, Taipei, Taiwan.
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Reprints: Chun-Chia Chen, MD, Division of Gastroenterology, Department of Medicine, Taipei Veterans General Hospital, 201, Sec. 2, Shih-Pai Rd, Taipei 112, Taiwan (e-mail: chencc@vghtpe.gov.tw).
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TABLE 1. Diagnoses of 150 Patients Made After ERCP and Number of Patients With Post-ERCP Pancreatitis

Diagnosis	No. Patients (%)	No. Patients With Post-ERCP Pancreatitis
Choledocholithiasis	94 (62.7)	3
Malignancy	22 (14.7)	6
Dilated biliary tract	10 (6.7)	3
Sphincter of Oddi dysfunction	2 (1.3)	1
Others	22 (14.7)	0
Total	150	13

TABLE 2. Diagnostic Value of Urinary Trypsinogen-2 Test at 3 Hours After ERCP for Post-ERCP Pancreatitis

Diagnosis	No. Patients	No. Positive Test	No. True-Positive Test	No. False-Negative Test
Choledocholithiasis	94	4	2	1
Malignancy	22	8	6	0
Dilated biliary tract	10	2	2	1
Sphincter of Oddi dysfunction	2	1	1	0
Others	22	0	0	0

10% lidocaine spray and an intramuscular injection of 40 mg of hyoscine-*N*-butylbromide. Therapeutic ERCP including endoscopic sphincterotomy, balloon dilation of the papilla, stone extraction, or stent placement was performed as indicated.

All patients were tested with urinary trypsinogen-2 test strip and serum amylase and lipase levels before ERCP and 3 hours after ERCP. We followed up the clinical course of all patients until patients discharged from our hospital or death. Further laboratory tests, imaging studies and medical treatment were performed as indicated. The clinical findings and final diagnosis were obtained from the charts. The data of these parameters were compared and analyzed.

Post-ERCP pancreatitis was diagnosed when new-onset or worsened abdominal pain lasted for more than 24 hours and was associated with an increase in serum amylase or lipase levels at least 3 times greater than the upper reference limit at 24 hours after the procedure. The characteristic abdominal pain of acute pancreatitis is located generally in the epigastrium with or without radiation to the back. The abdominal pain might be sudden onset and frequently unbearable, reaching maximal intensity within 30 minutes, and characteristically persisted for more than 24 hours. The severity of the pancreatitis was graded mild when hospitalization was prolonged by 2 to 3 days, moderate by 4 to 10 days, and severe by more than 10 days. The severity was also graded as severe when a pancreatic necrosis, abscess, or pseudocyst occurred or when there was a need for percutaneous drainage or surgery.[13] The complications of ERCP and clinical information were all recorded.

The urinary trypsinogen-2 dipstick test (Actim Pancreatitis; Medix Biochemica, Kauniainen, Finland) is based on the immunochromatography principle. The test was performed by dipping the tip of the strip into a urine sample. Trypsinogen-2 in the sample bound to monoclonal antibody–labeled blue latex particles. The sample fluid with the latex antibody–trypsinogen-2 complex migrated across the nitrocellulose membrane with a catching zone containing another antibody specific for another epitope on trypsinogen-2. A positive result was demonstrated when a blue line developed within 5 minutes in the catching zone. A control line was used to indicate proper function of the strip. If the control line was undetectable, the test was repeated. The detection limit of the test was approximately 50 mg/L.[10] Serum amylase was determined by an enzymatic color test (Boehringer-Mannheim GmbH Diagnostica, Mannheim, Germany). The Kodak Ekatchem clinical chemistry slide (Eastman Kodak Company, Rochester, NY) was used to estimate the level of serum lipase. The reference values of amylase and lipase were less than 190 U/L.

Data were expressed as mean ± SEM. The Student *t* test was used for statistical analysis. A $P < 0.05$ was defined as statistically significant. Sensitivity, specificity, positive predictive value, negative predictive value, and accuracy of urinary trypsinogen-2 strip test in the diagnosis of post-ERCP pancreatitis were defined according to Ransohoff and Feinstein.[14]

RESULTS

The diagnoses of 150 patients after ERCP examination are shown in Table 1. Choledocholithiasis was the most common diagnosis. One hundred twenty-nine patients (86%) underwent therapeutic ERCP, including 80 patients with endoscopic papillary balloon dilatation, 25 patients with biliary stent insertion, and 12 patients with endoscopic papillotomy. Post-ERCP pancreatitis developed in 13 (8.7%) of 150 patients. No fatal case was reported. Pancreatitis developed in 12 (33.3%) of 36 patients with abdominal symptoms at 3 hours after ERCP. One patient with post-ERCP pancreatitis did not have abdominal pain until 12 hours after ERCP. One patient with post-ERCP pancreatitis only experienced mild abdominal fullness at 3 hours after ERCP.

Positive results for urinary trypsinogen-2 dip test were found in 15 patients (10%) at 3 hours after ERCP. Of 13 patients with post-ERCP pancreatitis, 11 (84.6%) had positive urine dipstick test at 3 hours after ERCP. All 13 patients with post-ERCP pancreatitis showed positive urine dipstick test at 24 hours after ERCP. The diagnostic value of urinary trypsinogen-2 test at 3 hours after ERCP for post-ERCP pancreatitis is shown in Table 2. Two patients with choledocholithiasis and 2 patients with biliary tract cancer showed false-positive urinary trypsinogen-2 test result. The sensitivity, specificity, positive predictive value, negative predictive value, and accuracy of the urinary trypsinogen-2 stick test at 3 hours after ERCP diagnosing post-ERCP pancreatitis were 84.6%, 97.1%. 73.3%, 98.5%, and 96%, respectively (Table 3).

The serum amylase and lipase levels at 3 hours after ERCP in patients with post-ERCP pancreatitis were significantly higher than those in patients without post-ERCP pancreatitis (amylase

TABLE 3. Comparison of the Urinary Trypsinogen-2 Test, Serum Amylase, and Lipase Levels for the Detection of Post-ERCP Pancreatitis

	Urinary Trypsinogen-2	Amylase >3× URL	Lipase >3× URL	Lipase >5× URL
Sensitivity, %	84.6	46.1	92.3	84.6
Specificity, %	97.1	94.2	84.7	89.0
PPV, %	73.3	42.9	36.4	42.3
NPV, %	98.5	94.8	99.1	98.4
Accuracy, %	96.0	85.3	85.3	88.7

NPV indicates negative predictive value; PPV, positive predictive value; URL, upper reference limit.

741 ± 802 vs 270 ± 953 U/L, $P = 0.02$; lipase 3378 ± 3608 vs 415 ± 777 U/L, $P = 0.006$). The diagnostic values of serum amylase and lipase levels for post-ERCP pancreatitis at 3 hours after the procedure are shown in Table 3. At the cutoff level of 3 times the upper reference limit, the sensitivity and specificity of serum amylase at 3 hours post-ERCP were 46.1% and 94.2%, respectively. At the cutoff level of 3 and 5 times the upper reference limit, the sensitivity of serum lipase at 3 hours post-ERCP were 92.3% and 84.6%, respectively, with the specificity of 84.7% and 89%, respectively. Asymptomatic elevation of serum amylase or lipase (>190 U/L) was found in 73 patients (48.7%) at 3 hours after the ERCP examination.

DISCUSSION

Acute pancreatitis, one of the major complications of ERCP, is reported to be complicated in 5% of diagnostic ERCP, 7% of therapeutic ERCP, and up to 25% of patients with sphincter of Oddi dysfunction, postendoscopic balloon dilatation or previous post-ERCP pancreatitis.[15–17] In the present study, most of our patients (86%) underwent therapeutic ERCP. The incidence of post-ERCP pancreatitis was 8.7% in our study that is compatible with other reports in the literature. We demonstrated that urinary trypsinogen-2 dipstick test is useful for early diagnosis of post-ERCP pancreatitis as compared with current pancreatic enzymes. Negative urinary trypsinogen-2 test at 3 hours after ERCP was highly reliable for excluding post-ERCP pancreatitis.

Early diagnosis of post-ERCP pancreatitis and identification of high-risk patients are important in clinical practice. The main benefit is to guide decisions regarding hospital admission and provision of optimal care. In addition, it can allow the use of targeted therapies that have the potential to decrease pancreatic inflammation.[18] The early markers of post-ERCP pancreatitis can be calcified into 3 categories: (1) markers of pancreatic injury—serum or urine amlyase/lipse; (2) markers of proteolytic activation—trypsinogen and trypsinogen activation peptide; and (3) markers of systemic inflammation—inflammatory cytokines.[6,18,19] In acute pancreatitis, both serum and urinary trypsinogen-2 levels rise to high levels within a few hours and generally decline within 3 days.[20] In ERCP-induced pancreatitis, serum trypsinogen-2 concentrations may rise within an hour of the insult.[21] Median serum trypsinogen-2 levels in post-ERCP pancreatitis were reported to increase from 53 μg/L at baseline to 485 μg/L 1 hour after ERCP, 1401 μg/L at 6 hours, and 1008 μg/L at 24 hours. Using 3-fold increase as a cutoff value, serum trypsinogen-2 level is a good predictor of post-ERCP pancreatitis with sensitivity and specificity of 93% and 91%, respectively, at 6 hours and of 74% and 87%, respectively, at 1 hour.

There are only 2 studies in the literature evaluating the usefulness of urinary trypsinogen-2 dipstick test for early diagnosis of post-ERCP pancreatitis. Kemppainen et al[22] tested 106 patients undergoing ERCP with a urinary trypsinogen-2 test strip 6 hours after ERCP, in whom 11 patients (10.4%) developed post-ERPC pancreatitis. They showed that the sensitivity and specificity of urinary trypsinogen-2 strip test in diagnosing post-ERCP pancreatitis were 81% and 97%, respectively. Their high negative predictive value (98%) supported the clinical value in excluding the development of post-ERCP pancreatitis. However, waiting for 6 hours after the procedure seems a long time, especially for outpatients. In the other small-scale study, Sankaralingam et al[23] tested 30 patients undergoing ERCP with urinary trypsinogen-2 test strip at 1 and 4 hours after ERCP, in whom 5 patients (17.2%) developed post-ERCP pancreatitis. They demonstrated that urinary trypsinogen-2 test had 100% of sensitivity and negative predictive value at 1 and 4 hours after ERCP. The specificities at 1 and 4 hours after ERCP were 91% and 96%, respectively. In our study, we examined 150 patients undergoing ERCP with urinary trypsinogen-2 test strip before and 3 hours after ERCP, in whom 13 (8.7%) patients developed post-ERPC pancreatitis. We found that urinary trypsinogen-2 strip test at 3 hours after ERCP had high sensitivity (84.6%), specificity (97.1%),and negative predictive value (98.5%) that are compatible with the report of Kemppainen et al.[22] Early recognition of postprocedure pancreatitis is important for the discharge management of outpatients undergoing ERCP. Our results clearly demonstrated that urinary trypsinogen-2 dipstick test is useful for early diagnosis of post-ERCP pancreatitis and highly reliable for excluding post-ERCP pancreatitis.

Serum amylase and lipase values were shown to significantly increase from 1 to 24 hours after ERCP in patients with post-ERCP pancreatitis as compared with those without pancreatitis.[6] However, serum amylase and lipase concentrations are commonly elevated after ERCP in patients without clinical pancreatitis. In the patients without post-ERCP pancreatitis, serum amylase levels peaked at 1.5 to 4 hours after the procedure and returned to normal within 48 hours.[20] The degree and rapidity of pancreatic enzyme elevation were reported to provide a way to identify patients with post-ERCP pancreatitis.[3,4,25] Gottlieb et al[3] showed that serum amylase and lipase values at 2 hours after procedure less than 276 and 1000 U/L, respectively, were useful in ruling out pancreatitis with negative predictive values of 97% and 98%. Testoni et al[4] reported that serum amylase level at 4 hours after the procedure was useful for predicting subsequent pancreatitis in 409 patients undergoing endoscopic sphincterotomy. Two-thirds of patients who developed postprocedure pancreatitis had a serum amylase concentration higher than 5 times the upper limit of reference. In another study recording 1185 ERCP procedures, post-ERCP pancreatitis occurred only among patients with pancreatic-type pain at 24 hours and amylase levels higher than 5 times normal.[24] Serum levels of amylase and lipase were examined at 3 hours after ERCP procedure in our study. We found that the elevations of serum amylase or lipase (>3 or 5 times normal) have high negative predictive values (94.8%–99.1%); however, their positive predictive values (36.4%–42.9%) were markedly lower than that of urinary trypsinogen-2 strip test (73.3%).

Some limitations are present in our study. First, the definition of post-ERCP pancreatitis is not consistent in the literature. We applied the definition from a consensus reported in 1991.[13] Second, patients with positive urinary trypsinogen-2 dipstick test before ERCP were excluded. Increased trypsinogen-2 levels have been reported in gastrointestinal, pancreatic, and biliary tract malignancies, and trypsinogen-2 is also expressed in the epithelium of bile ducts and peribiliary glands.[22,25,26] Third, the case number of post-ERCP pancreatitis in our study is somewhat limited.

In conclusion, the positive predictive value of rapid urinary trypsinogen-2 strip test is markedly superior to that of amylase and lipase for diagnosing pancreatitis at 3 hours after ERCP. Urinary trypsinogen-2 strip test is particularly useful to rationalize the discharge plan of outpatients undergoing ERCP. A negative test result at 3 hours after the procedure rules out post-ERCP pancreatitis with a high probability, and a positive test identifies patients in need of further management.

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