



Use of the urinary trypsinogen-2 dip stick test in early diagnosis of pancreatitis after endoscopic retrograde cholangiopancreatography

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Abstract

Background: This study aimed to prove that the urinary trypsinogen-2 dip stick test can be used for early diagnosis of pancreatitis after endoscopic retrograde cholangiopancreatography (ERCP).

Methods: For this prospective, blinded, pilot study, urine samples were collected before ERCP, 1 h after ERCP, and 4 h after ERCP. The urine dipstick test was used to detect trypsinogen-2 on the basis of immunochromatography. The dipstick results were compared with those of current methods used to diagnose post-ERCP pancreatitis. Once the patient disposition was finalized, pancreatic enzymes, clinical findings, and final diagnosis were obtained from the chart and compared with the urine trypsinogen-2 test findings. The sensitivity, specificity, and positive and negative predictive values were calculated.

Results: The urine trypsinogen dip stick test was performed for 30 patients (15 men and 15 women). Post-ERCP pancreatitis was diagnosed in 5 of 29 patients by clinician assessment, serum pancreatic enzyme levels, or both. The amylase and lipase levels for post-ERCP patients with and without pancreatitis were 650 ± 145 vs 134 ± 26 ($p = 0.023$) and $1,658 \pm 594$ vs 84 ± 17 ($p = 0.057$), respectively. This statement proves that patients who developed post ERCP pancreatitis had significant elevation of amylase and lipase compared to patients who did not have pancreatitis. For the dip stick test, 6 of 28 patients had positive results in 1 h and 6 of 29 patients had positive results in 4 h. The sensitivity of the 1-h test was 1.0, and the specificity was 0.91. The positive predictive value (PPV) was 0.66, and the negative predictive value (NPV) was 1.0. The sensitivity of the 4-h test was 1.0, and the specificity was 0.96. The PPV was 0.8, and NPV value was 1.0.

Conclusion: The urinary trypsinogen-2 dip stick test is useful for early diagnosis of post-ERCP pancreatitis and

allows the testing physicians to begin management early in its course.

Key words: ERCP — Pancreatic — Pancreatitis

One of the most serious complications of endoscopic retrograde cholangiopancreatography (ERCP) is acute pancreatitis. The reported incidence varies from 1.3% to 24.4% [10]. In a large, multicenter study, Freeman et al. [1] reported an incidence of 6.7% for post-ERCP pancreatitis. Early diagnosis of acute pancreatitis is very important because severe disease will develop in approximately 10% of patients. Physical examination is unreliable for patients who have had an ERCP. Therefore, evaluation of serial pancreatic enzymes with clinical findings are used to diagnose acute post-ERCP pancreatitis [8]. However, the optimal time to check the pancreatic enzymes for a diagnosis of pancreatitis is controversial [9].

Trypsinogen-1 and trypsinogen-2 have been studied as markers for pancreatitis. Trypsinogen-2 is excreted by the kidneys, as evidenced by the steep rise in urinary trypsinogen-2 after acute pancreatitis. It should be possible to use urinary trypsinogen-2 to detect acute pancreatitis in post-ERCP patients.

Methods and materials

A prospective, blinded, pilot study was used evaluate the urinary trypsinogen-2 dip stick test as a means for diagnosing post-ERCP pancreatitis among patients who had undergone ERCP. This study was approved by the institutional review board. We obtained informed consent from the patients before collecting urine samples.

Patients of all ages, ethnicity, and sex who had undergone ERCP at our institution 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 dipstick test results indicating an already elevated trypsinogen-2 level. The treating physician was blinded to the urinary trypsinogen-2 results.

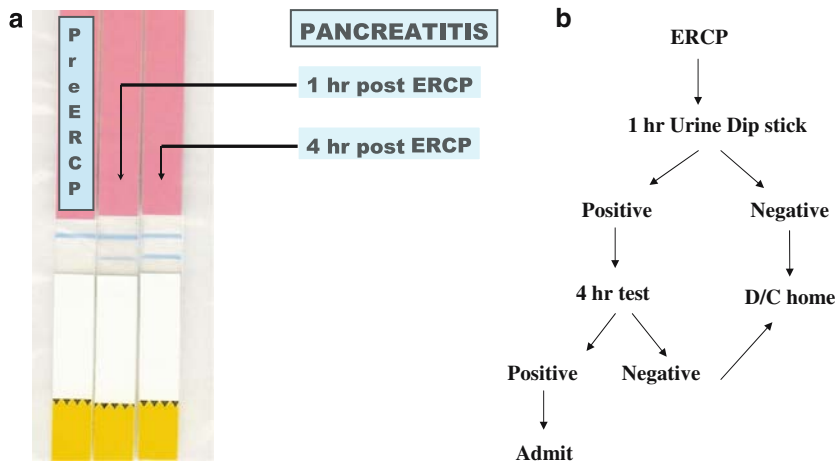


Fig. 1. **a** The test strip from patient who developed post ERCP pancreatitis. Pre ERCP test shows one blue line which is a negative test. Post ERCP test 1 and 4 h are positive as indicated by two blue lines. **b** Algorithm to use urine dip stick test in determining the disposition of post ERCP patients.

All ERCPs were performed by a single gastroenterologist using the same preoperative preparation and the same techniques during the procedures. Interventions such as stent placement, sphincterotomy, sphincter of oddi manometry were performed as indicated. The diagnosis and treatment of post-ERCP pancreatitis was based on clinical symptoms and/or elevated serum amylase and lipase levels without the knowledge of urinary trypsinogen-2 results. The patient's records were accessed after the disposition was determined. If the patients had been treated for post-ERCP pancreatitis as diagnosed by the gastroenterologist, then they were considered positive for pancreatitis.

We collected urine samples before ERCP, then 1 h and 4 h after ERCP. We used the urine dipstick (Actim Pancreatitis Medix Biomedica OY AB, Kauniainen, Finland) to detect trypsinogen-2 on the basis of immunochromatography. The test was conducted by dipping the test strip into urine collected from patients. We waited at least 5 min after the sample was obtained as recommended by the manufacturers. There are two antibodies in the test strips. The first antibody, bound to latex particles, binds to the trypsinogen-2 in urine as the complex migrates up the test strip. The second antibody then reacts with this migrating complex. A blue line appears if the urine trypsinogen-2 concentration is detectable but less than 50 mg/l. This is the control line. Another blue line appears if the concentration is more than 50 mg/l. The test is positive (Fig. 1) if two blue lines appear and negative if one blue line appears. We performed the urine dipstick test before ERCP, then 1 h and 4 h after ERCP. Also, we checked the amylase and lipase levels at 4 h and recorded physical examination findings such as abdominal tenderness, nausea, and vomiting.

Fisher's exact test was used to evaluate the success of urine dipstick results in predicting pancreatitis. The sensitivity, specificity, and positive and negative predictive values were calculated.

Results

Evaluation of the urinary trypsinogen-2 dip stick test was performed for 30 patients (15 men and 15 women) with an average age of 55 years (range, 18–86 years). The age difference between the patients with and those without pancreatitis was not statistically significant ($p = 0.27$). One patient with a history of pancreatic adenocarcinoma was excluded because the pre-ERCP test results were positive. The diagnoses made after ERCP are shown in Table 1.

Post-ERCP pancreatitis developed in 5 of the remaining 29 patients, as diagnosed by the gastroenterologist. Positive results for the urine dip stick test were found for 6 of 28 patients in 1 h. One patient with a later diagnosis of pancreatitis was unable to give a urine sample for the 1-h test. Of the six patients with positive urine dip stick test results in 1 h, four were given a

Table 1. The diagnosis made post ERCP and patients who developed pancreatitis as diagnosed and managed by the physician taking care of the patients

Diagnosis	No. of patients	No. of patients with pancreatitis
Sphincter of oddi dysfunction	10	2
Cholelithiasis	6	1
Dilated pancreatic duct	2	0
Normal	7	0
Pancreatic divisum	1	0
Pancreatic mass	2	1
Primary sclerosing cholangitis	1	1

Table 2. The sensitivity, specificity, PPV and NPV for the 1 and 4 h urine dip stick tests

	Sensitivity	Specificity	PPV	NPV
1-h test	1.0	0.91	0.66	1.0
4-h test	1.0	0.96	0.8	1.0

PPV, positive predictive value; NPV, negative predictive value

diagnosis of clinical pancreatitis by the physician caring for them. At 4 h, 6 of 29 patients had positive urine dip stick test results. Clinical pancreatitis also was diagnosed for five of these six patients. The sensitivity, specificity, positive predictive value, and negative predictive value are shown in Table 2. For the 1-h urine dip stick test, the sensitivity was 1 and the specificity was 0.91. For the 4-h urine dip stick test, the sensitivity was 1.0 and the specificity was 0.96.

The pre-ERCP amylase level was 53.9 ± 11 U/l, as compared with post-ERCP amylase level of 109 ± 20 U/l ($p = 0.011$). For all the patients, the pre-ERCP lipase level was 18 ± 4 U/l, as compared with a post-ERCP lipase level of 73 ± 14 U/l ($p = 0.001$). The post-ERCP amylase level was 134 ± 26 U/l for the patients without pancreatitis, as compared with 650 ± 146 U/l for the patients with pancreatitis ($p = 0.02$). The post-ERCP lipase level for the patients without pancreatitis was 85 ± 18 U/l, as compared with $1,658 \pm 594$ U/l for the patients with pancreatitis ($p = 0.05$) (Table 3). Four of the five patients with

Table 3. The post ERCP amylase and lipase levels for patients with and without pancreatitis as diagnosed by the physician taking care of the patient

	Patients without pancreatitis	Patients with pancreatitis	<i>p</i> Value
Post-ERCP amylase	134 ± 26 U/l	650 ± 146 U/l	0.02
Post-ERCP lipase	85 ± 18 U/l	1658 ± 594 U/l	0.05

ERCP, endoscopic retrograde cholangiopancreatography

clinical pancreatitis at 1 h had abdominal findings including pain, nausea, and vomiting. The fifth patient had only elevated amylase and lipase. Pancreatitis developed in four of the five patients with abdominal symptoms. One patient who had no pain at 4 h experienced the development of pancreatitis.

Discussion

Pancreatitis is the most feared complication after ERCP since 1 out of 10 patients may develop severe pancreatitis, leading to intensive care unit monitoring and multi organ failure. Controversy exists regarding the most reliable method for diagnosing pancreatitis in post-ERCP patients. Clinical assessment after ERCP can be unreliable, as described by Gottlieb et al. [2]. In this study, pancreatitis developed in one-third of the patients who had no pain 2 h after ERCP, whereas another one-third of the patients who had pain did not experience pancreatitis.

For a better diagnosis of pancreatitis, clinical assessment combined with chemical markers was used to diagnose post-ERCP pancreatitis. Different levels of serum pancreatic enzymes such as amylase and lipase have been used to diagnose post-ERCP pancreatitis. Thomas and Sengupta [11] showed that the 4-h amylase level with a 1.5-fold higher than normal cutoff can reliably exclude pancreatitis. Gottlieb et al. [2] showed that serum amylase values six times the upper reference limit 2 h after ERCP can predict the development of pancreatitis with 90% probability. Serum amylase and lipase levels are not reliable predictors of the severity of acute pancreatitis, which limits the usefulness of these markers.

Trypsinogen, a protease released by pancreatic acinar cells, is converted to trypsin, a proteolytic enzyme, by trypsinogen activation peptide. Trypsinogen-1 and trypsinogen-2 are the two isoenzymes of trypsinogen that can be detected even in healthy individuals. Both of these enzymes are detected at higher levels in serum after an acute insult to the pancreas. Trypsinogen-2 rises to higher levels than trypsinogen-1. The kidneys appear to excrete trypsinogen-2 because there is a steep rise in urinary trypsinogen-2 after acute pancreatitis.

Trypsinogen-2 has been studied as a potential marker for diagnosing pancreatitis. Published studies evaluating the use of trypsinogen-2 for the diagnosis of acute pancreatitis in Finland have shown good sensitivity and specificity [5–7]. Kemppainen et al. [7] reported that elevated urinary trypsinogen-2 had a 94% sensitivity for

diagnosing acute pancreatitis in emergency room patients. Hedstrom et al. [4] also showed that the level of serum trypsinogen-2 correlated with the severity of acute pancreatitis. Kemppainen et al. [7] measured the serum levels of trypsinogen-2 after ERCP and showed that a threefold increase in serum trypsinogen-2 was an accurate indicator of ERCP-induced pancreatitis [5]. They showed that the median serum trypsinogen-2 concentration for patients without pancreatitis 6 h after ERCP was 3.6 µg/l [6]. However, for patients with post-ERCP pancreatitis, the serum trypsinogen-2 levels started to rise as early as 1 h and peaked at 6 h with a median concentration of 1,780 µg/l (range, 29–10, 700 µg/l) [6].

The Actim Pancreatitis Medix Biomedica urine test strip measures concentrations of serum trypsinogen-2 as low as 50 µg/l. It has been shown that the results from a urinary trypsinogen-2 test strip agrees well with the rise in serum trypsinogen-2 for a diagnosis of post-ERCP pancreatitis [6]. But the urine dip stick cannot be used to assess the severity of pancreatitis because it is not a quantitative measurement.

In our study, we evaluated the use of the urine dip stick for diagnosing post-ERCP pancreatitis early during the course of the disease. The diagnosis and management of pancreatitis were completed by the gastroenterologist caring for the patient using clinical assessment and/or serum amylase and lipase levels. Although the CT scan is the diagnostic study for acute pancreatitis, it is not practical or cost effective to perform this study on a routine basis. Many patients had a certain level of discomfort immediately after the ERCP procedure. Therefore, only patients with significant abdominal pain and tenderness were considered to have positive abdominal signs. The serum amylase and lipase levels in patients with a clinical diagnosis of pancreatitis were significantly higher, correlating well with previous studies showing the elevated pancreatic enzymes used to diagnose pancreatitis.

One patient, with a later diagnosis of pancreatic adenocarcinoma, had positive pre-ERCP urine dip stick test results. It has been shown [3] that the use trypsinogen-2 levels to diagnose pancreatitis lacks specificity because this enzyme can be released secondarily to inflammatory changes or malignancies in the pancreatic and biliary system. It is a consideration in the future to conduct a study using the urine dip stick test, to follow up pancreatic cancer patients after resection, and to diagnose recurrence. Also, one patient in whom pancreatitis developed was unable to give a urine sample during the 1-h test, which may have been related to dehydration combined with third spacing secondary to an acute inflammatory process. This is a potential hindrance in doing the 1-h study.

As shown in previous studies, there is a steep rise in serum trypsinogen-2 levels starting 1-h after ERCP and peaking in 6 h. This explains the decreased specificity of the 1-h compared with the 4-h urine dip stick test. But with the high sensitivity and negative predictive value of both the 1-h and the 4-h tests, we can reliably rule out pancreatitis if the test result is negative. Unlike serum trypsinogen-2 levels, the urine dip stick test is not helpful in predicting the severity of the pancreatitis. Early dis-

charge of patients with a negative urine dip stick test result is feasible. The urine dipstick test is rapid and avoids the need for blood draws. The dip stick is approximately one-fifth the cost of tests for serum amylase and lipase levels.

In conclusion, this pilot study to evaluate the use of urinary trypsinogen-2 in the diagnosis of post ERCP pancreatitis suggests that the test is rapid and sensitive. This results in potential cost savings from early discharge and decreased laboratory expenses. Further studies should be conducted to define the magnitude of benefit.

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