Ulus Travma Acil Cerrahi Derg 2010;16 (2):125-129
Klinik Çalışma

The role of urine trypsinogen-2 test in the differential diagnosis of acute pancreatitis in the Emergency Department

Acil serviste akut pankreatit ayırıcı tanısında idrar tripsinojen-2 testinin yeri

Yunsur ÇEVİK,[1] Cemil KAVALCI,[2] Mehmet ÖZER,[3] Murat DAŞ,[1] Gülten KIYAK,[3] Mehmet ÖZDOĞAN[3]

BACKGROUND
The aim of the study was to investigate the role and importance of the urine trypsinogen-2 dipstick test in the differential diagnosis of acute pancreatitis in the Emergency Department and to compare results with those of conventional tests.

METHODS
The study was performed prospectively in the patients admitting to the Emergency Department due to upper abdominal pain. Thirty-two of the 87 patients included in the study had acute pancreatitis diagnosis. Serum amylase, lipase, C-reactive protein (CRP) and urine trypsinogen-2 using Actim pancreatitis dipstick were studied in all patients. The statistical analysis was performed using SPSS 11.5 package program.

RESULTS
Urine trypsinogen-2 was found positive in 21 (65.6%) of 32 patients. The sensitivity of the test for pancreatitis was identified as 64%, specificity as 85%, positive predictive value as 72%, and negative predictive value as 81%. These values were statistically significant compared to the control group ($p<0.01$).

CONCLUSION
Although it has lower sensitivity and specificity compared to amylase and lipase, we suggest that urine trypsinogen-2 test may be an important diagnostic tool in excluding the diagnosis of acute pancreatitis, since it provides results within 5 minutes in the Emergency Department, is cheaper, has a higher negative predictive value, and is easy to use.

Key Words: Abdominal pain; emergency department; pancreatitis; urine trypsinogen-2.

AMAÇ
Çalışmanın amacı, idrar tripsinojen-2 dipstik testinin acil serviste akut pankreatit ayırıcı tanısındaki yeri ve önemini araştırmak ve sonuçları konvasiyonel yöntemlerle karşılaştırmaktır.

GEREÇ VE YÖNTEM
Çalışma üst abdominal ağrıyla acil servise başvuran hastalarda prospektif olarak yapıldı. Çalışmaya alınan toplam 87 hastanın 32'si akut pankreatit tanısı alan hastalardan oluşmaktaydı. Tüm hastalarda amilaz, lipaz, C-reaktif protein (CRP) ve *Actim pancreatit dipstick* kullanılarak, idrar tripsinojen-2 bakıldı. İstatistiksel analiz SPSS 11.5 paket programı kullanılarak yapıldı.

BULGULAR
Akut pankreatit tanısı alan 32 hastanın 21'inde (%65,6) idrar tripsinojen-2 pozitif bulundu. Testin pankreatit açısından duyarlılığı yaklaşık %64, özgüllüğü %85, pozitif prediktif değeri %72, negatif prediktif değeri %81 olarak belirlendi. Bu değerler kontrol grubuna göre istatistiksel olarak anlamlıydı ($p<0,01$).

SONUÇ
İdrar tripsinojen-2 testinin duyarlılığı ve özgüllüğü amilaz ve lipaza kıyasla düşük olsa da, acil servislerde 5 dakika gibi kısa bir sürede sonuç vermesi, ucuz olması, kullanım kolaylığına sahip olması ve negatif belirleyicilik değerinin yüksek olması nedeniyle akut pankreatit tanısının dışlanmasında önemli bir tanı aracı olacağını düşünmekteyiz.

Anahtar Sözcükler: Karın ağrısı; acil servis; pankreatit; idrar tripsinojen-2.

Departments of [1]Emergency Medicine, [3]General Surgery, Ataturk Training and Research Hospital, Ankara; [2]Department of Emergency Medicine, Trakya University Faculty of Medicine, Edirne, Turkey.

Atatürk Eğitim ve Araştırma Hastanesi, [1]Acil Kliniği, [3]Genel Cerrahi Kliniği, Ankara; [2]Trakya Üniversitesi Tıp Fakültesi, Acil Tıp Anabilim Dalı, Edirne.

Correspondence *(İletişim):* Yunsur Çevik, M.D. Ankara Atatürk Eğitim ve Araştırma Hastanesi Acil Servis, Bilkent, Ankara, Turkey.
Tel: +90 - 312 - 291 25 25 / 3261 Fax *(Faks)*: +90 - 312 - 291 27 05 e-mail *(e-posta):* yunsurcevik@yahoo.com

Pancreatitis is one of the common causes of abdominal pain, yet it has no pathognomonic clinical signs and no diagnostic method is accepted as the gold standard.[1] The patients admit with complaints such as severe pain in the upper abdominal region radiating to the back, nausea and vomiting.[2] In many patients, acute pancreatitis has a mild course and resolves spontaneously, but approximately 20% of the cases are severe and can be fatal.[3] In clinical practice, amylase originating from pancreatic acinar cells is the most common diagnostic test. A three-fold increase in the upper limit of the amylase value supports the pancreatitis diagnosis. However, it has been reported that amylase has been in the normal range in one-fourth of the patients admitting to the hospital.[2] Furthermore, amylase increases in many intra-abdominal inflammatory cases and also in salivary gland pathologies. This can cause delays and can be misleading in the diagnosis. Lipase also originates from acinar cells. A two-fold increase in the upper limit of the lipase value supports the pancreatitis diagnosis. Even though its accuracy rate is higher compared to amylase, lipase is also not specific to the pancreas and can increase in intra-abdominal inflammation and in renal failure.[4,5]

Trypsinogen is a pancreatic proteinase and has two subforms as trypsinogen-1 and trypsinogen-2. In the earlier phase of pancreatitis, it can be activated by zymogens, and trypsinogen-2 can be detected in higher quantities in the urine due to the insufficient reabsorption.[6-8]

The aim of the study was to investigate the role and importance of the trypsinogen-2 dipstick test in the differential diagnosis of acute pancreatitis in the Emergency Department and to compare results with those of the conventional methods (amylase, lipase).

MATERIALS AND METHODS

Patients

The study was conducted prospectively in the patients admitted to the Emergency Department of the Ankara Atatürk Research and Training Hospital with the complaint of upper abdominal pain between January 1, 2007 and August 1, 2007. The diagnosis of pancreatitis was established based on the clinical findings, ultrasonography, computed abdominal tomography, and amylase values. The study was approved by the local ethical committee, and informed consents were collected from the patients. The control group comprised patients admitted to the Emergency Department due to upper abdominal pain who were diagnosed as acute cholecystitis, ileus, peptic ulcer activation, and mesenteric ischemia.

Laboratory and Imaging Methods

Blood and urine samples were collected from both groups; amylase and lipase were examined in the blood samples and trypsinogen-2 was examined in the urine samples. Amylase and lipase tests were performed with enzymatic technique (Dade Behring Dimension RxL MaX Instrument), and C-reactive protein (CRP) was performed by immunonephelometric techniques (Dade Behring BNII Nephelometry Instrument). Reference range for serum amylase concentration is 25-115 U/L, for lipase 114-286 U/L, and for CRP 0-4.99 mg/L.

Trypsinogen-2 in the urine was examined using Actim© pancreatitis dipstick (Medix Biochemica, Kauniainen, Finland). In this test functioning by immunochromatography method, two types of antibody against human trypsinogen-2 are present. One of these antibodies binds to the latex particles on the stick, and is highlighted in blue. The second antibody will bind to the mobile region, which will give the result of the test having a structure of nitrocellulose membrane, and will bind to a different epitope of the trypsinogen molecule. If the urine sample contains trypsinogen-2 in normal quantities, only one blue strip is seen (detection limit 50 micrograms/L). If the sample contains trypsinogen-2 at a level >50 mcg/L, a second blue strip will appear. The result is obtained five minutes later. If two blue strips appear at the result section, the test is considered positive, while only one blue strip is considered negative. If no blue strip occurs, the test is considered invalid and should be repeated.[9,10]

Pancreatitis severity was graded by Ranson criteria. Efficiency of CRP and urine tripsinogen-2 tests in determining the severity was assessed.

Statistical Analysis

Statistical analysis was performed using SPSS 11.5 for Windows package software. The demographic and clinical characteristics of the patients are expressed as mean ± standard deviation and as the percentage. The normal distribution test of the quantitative variables was done with One-Sample Kolmogorov-Smirnov test. Nonparametric approaches were used when the data did not exhibit normal distribution. Continuous data were compared by the Mann-Whitney U test. Test-strip results were compared by McNemar test. All P values were two-tailed, and those with $p<0.05$ were defined as statistically significant. The sensitivity, specificity, negative predictive values and positive predictive values were detected for amylase, lipase, CRP, and urine tripsinogen-2 tests in the patients with a diagnosis of acute pancreatitis. The validity of the tests was evaluated by an analysis of receiver operating characteristic (ROC) curves. The area under the ROC curve (AUC) indicates the accuracy of the test, with a value of 1.0 representing 100% sensitivity and specificity, and a value of 0.5 indicating no discriminatory power.

RESULTS

Thirty-two of 87 patients included in the study had acute pancreatitis diagnosis. Five (15.6%) of the patients with diagnosis of acute pancreatitis were male and 27 (84.4%) were female and the mean age was 53.81±16.81 years. The control group included 55 patients. Twenty-three (41.8%) of 55 patients in the control group were male and 32 (58.2%) were female, and the mean age in this group was 47.11±19.16 years.

Blood amylase, lipase, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and gamma glutamyl transferase (GGT) levels of the patients with acute pancreatitis were found significantly higher compared to the control group ($p<0.01$). No statistically significant difference was found in CRP levels between the groups ($p=0.084$).

When the patients with the diagnosis of acute pancreatitis were compared to the control group in terms of trypsinogen-2 test positivity, a statistically significant difference was found in favor of the acute pancreatitis group ($p<0.01$).

While urine trypsinogen-2 was found positive in 21 (65.6%) of 32 patients with the diagnosis of acute pancreatitis, it was detected as negative in 11 (34.4%). The sensitivity of the test was 64%, specificity was 85%, positive predictive value was 72%, and negative predictive value was 81% for the diagnosis of acute pancreatitis. These figures were statistically significant compared to the control group ($p<0.01$).

When the times to hospital admission after the onset of the symptoms were considered, it is noticeable that urine trypsinogen-2 values exhibit false-negativities in patients with acute pancreatitis diagnosis for admissions after 24 hours (Table 1). When only the admissions within the first 24 hours were considered, the sensitivity of the urine trypsinogen-2 test in the diagnosis of pancreatitis was 83%, specificity was 87%, positive predictive value was 75%, and negative predictive value was 92%.

Table 1. The relationship between pain duration and urine trypsinogen-2 in patients with acute pancreatitis diagnosis

Pain duration	Urine trypsinogen-2		Total
	Positive	Negative	
0-2 hours	1	2	3
2-6 hours	4	0	4
6-12 hours	5	0	5
12-24 hours	5	1	6
> 24 hours	6	8	14
Total	21	11	32

As calculations were made for amylase in patients with the diagnosis of pancreatitis, values increased more than three-fold (>345 U/L) were primarily considered. In these cases, the sensitivity of amylase for acute pancreatitis was 84%, specificity was 95%, positive predictive value was 90%, and negative predictive value was 91%. While calculating these figures for the lipase test, more than two-fold increases (>575 U/L) were primarily considered. In these cases, the sensitivity of lipase for acute pancreatitis was 100%, specificity was 96%, positive predictive value was 94%, and negative predictive value was 100%. The sensitivity of CRP for acute pancreatitis was 78%, specificity was 35%,

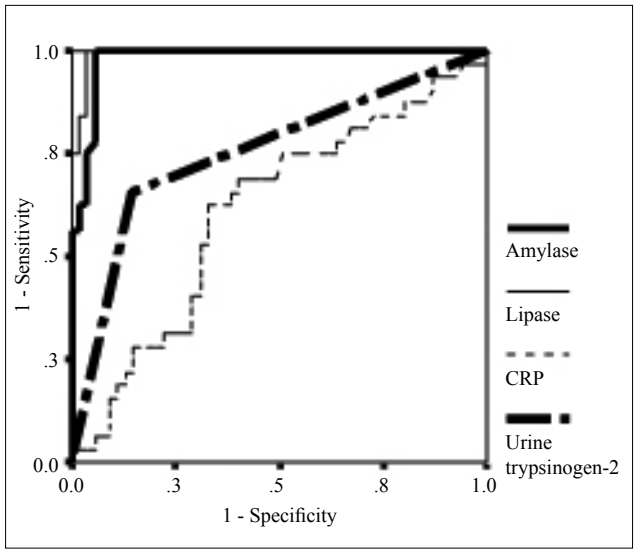

Fig. 1. Receiver operating characteristic curves for the urine trypsinogen-2 dipstick test, serum amylase, lipase, and CRP.

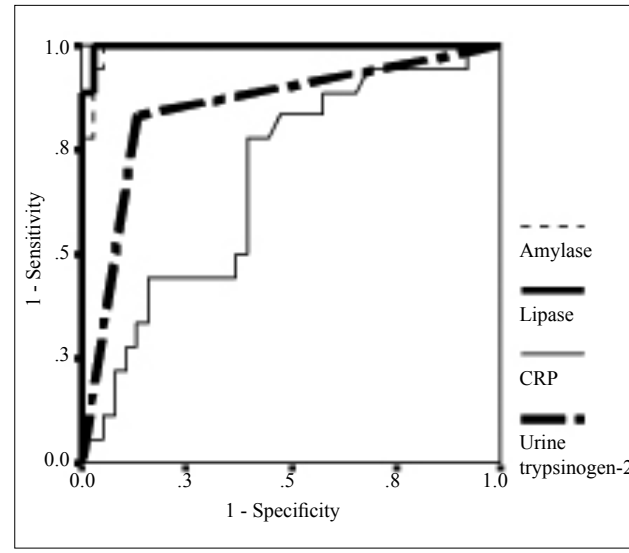

Fig. 2. Receiver operating characteristic curves for the urine trypsinogen-2 dipstick test, serum amylase, lipase, and CRP when only the admissions within the first 24 hours were considered.

Table 2. The diagnostic values of the urine trypsinogen-2 test, serum amylase, serum lipase and CRP in acute pancreatitis

	Sensitivity (%)	Specificity (%)	Positive Predictive Value (%)	Negative Predictive Value (%)
Urine trypsinogen-2	64	85	72	81
Serum amylase	84	95	90	91
Serum lipase	100	96	94	100
CRP	78	35	41	73

positive predictive value was 41%, and negative predictive value was 73%. In 4 of 5 patients whose pancreatitis was confirmed by a more than two-fold increase in lipase but who had less than three-fold increase in amylase levels, urine trypsinogen-2 test was found positive.

Urine trypsinogen-2 test and amylase, lipase and CRP tests were compared by ROC analysis (Fig. 1). The AUC was 0.755 ($p<0.01$) for trypsinogen-2, 0.981 ($p<0.01$) for serum amylase, 0.993 ($p<0.01$) for serum lipase, and 0.611 ($p=0.084$) for CRP. When only the admissions within the first 24 hours were considered, AUCs of 0.851 ($p<0.01$) for trypsinogen-2, 0.993 ($p<0.01$) for serum amylase, 0.997 ($p<0.01$) for serum lipase, and 0.681 for CRP ($p=0.03$) were determined (Fig. 2). The diagnostic values of the urinary trypsinogen-2 test, serum amylase, serum lipase, and CRP for acute pancreatitis are compared in Table 2.

While 9 of 32 patients (28.1%) were graded as severe, 23 (71.9%) were graded as mild pancreatitis according to the Ranson criteria. While all patients with severe pancreatitis (100%) had a positive urine trypsinogen-2 test, only 12 of 23 patients with mild pancreatitis (52.2%) had a positive test. Also, CRP was found positive in all of the severe pancreatitis and in 69.6% of the mild pancreatitis patients. While urine trypsinogen-2 test was found to be significant ($p=0.012$) in grading the severity of acute pancreatitis, CRP was not significant ($p=0.065$).

DISCUSSION

Acute pancreatitis is one of the relatively frequent abdominal emergency cases seen in the Emergency Department. Earlier diagnosis is mandatory to achieve a better prognosis. The diagnosis for acute pancreatitis is achieved by considering the clinical picture and amylase and lipase values.[11] Although epigastric pain, nausea and vomiting are the most common clinical signs, they are nonspecific. Furthermore, the typical clinical scenario is not observed in 10% of the patients with acute pancreatitis.[12] Amylase and lipase are also not specific to pancreatitis and their sensitivities are lower in late admissions, in hypertriglyceridemia and in chronic alcohol intake.[13] Computed abdominal tomography with contrast has a high accuracy rate, and is useful in determining the severity of the pancreatitis. However, the ionizing radiation that it possesses and its high cost restrict its routine use for the diagnosis of pancreatitis in the Emergency Department.[9] For this reason, researches have been focused on reliable tests with high accuracy rates, which are cheaper and can provide results rapidly.

The most important advantages of the urine trypsinogen-2 test are its availability and ease of use, in addition to the short test result time of 5 minutes. The sensitivity of the urine trypsinogen-2 test for pancreatitis has been reported as 86% to 100% in many studies.[9,10,12] However, Pezzilli et al.[14] found this figure as 53% in their study. In our study as well, the sensitivity of the urine trypsinogen-2 test was found as 64%. When 18 patients with pancreatitis, in whom urine trypsinogen-2 was examined during the first 24 hours after the onset of the symptoms, were taken into account, sensitivity was calculated as 83%, which is in accordance with the literature. In the study performed by Pezilli et al., the admissions on the second and even third day were also included into the study, which probably accounts for the lower sensitivity determined in their study.

The specificity of the trypsinogen-2 test in terms of pancreatitis has been reported between 72% and 95% in the literature.[9,10,12,15] This figure was calculated as 85% in the present study, and this is consistent with the literature. This ratio was found as 87% when only admissions in the first 24 hours were considered. Thus, the sensitivity and specificity of the urine trypsinogen-2 test decrease in admissions after 24 hours. Despite its low sensitivity and specificity compared to amylase and lipase, trypsinogen-2 dipstick test can be useful in Emergency Departments due to the quick result time (in 5 minutes).

Although the negative predictive value of the trypsinogen-2 test was 81% when all admissions are assessed, it increased to 92% when only the admissions during the first 24 hours were taken into account. This result is within the range of the negative predictive values of amylase and lipase (91% and 100%, respectively). Lempinen et al.[16] reported this ratio as 85% and Kylänpää-Bäck et al.[10] reported it as 99%.

A higher negative predictive value is very important in excluding the diagnosis of pancreatitis, when the test is used together with other clinical indicators. Positive predictive value of the urine trypsinogen-2 test was found as 72% in our study. When the admissions done during the first 24 hours were considered, this ratio was calculated as 75%. Chen et al.[12] reported the positive predictive value of the test as 81.1%, and Kamer et al.[15] reported this value as 96.6%. In our study, positive predictive value of this test was found lower compared to amylase and lipase (90%, 94% respectively). Since the trypsinogen-2 level might increase in many diseases such as hepatobiliary, pancreatic and colonic malignancies, lower positive predictive value can be found. Thus, we suggest that this test should absolutely be supported with other diagnostic methods in the diagnosis of acute pancreatitis.

In this study, severity of pancreatitis was graded by Ranson criteria. It has been known that CRP is correlated with the severity of acute pancreatitis and can be used as an alternative to computed tomography.[17] In our study, we did not find any statistically significant correlation between CRP levels and severity of pancreatitis. This might be due to the low number of our study group. However, we found a significant association between urine tripsinogen-2 positivity and the severity of acute pancreatitis. There are a few conflicting studies on the value of the trypsinogen-2 dipstick test as a predictive test for the severity of pancreatitis.[15,16] Lempinen et al.[16] compared urine trypsinogen-2 with serum CRP for early differentiation between severe and mild acute pancreatitis and concluded that urine trypsinogen-2 is superior to serum CRP for the early prediction of disease severity in the first 24 hours of admission for pancreatitis. Kamer et al.,[15] however, compared urine trypsinogen-2 between severe and mild acute pancreatitis and did not find any statistically significant correlation between urine trypsinogen-2 levels and severity of pancreatitis.

In conclusion, although urine trypsinogen-2 dipstick test has lower sensitivity and specificity compared to amylase and lipase, since it provides results rapidly in the Emergency Department, is cheap and easy to use, and has high negative predictive value, it provides important advantages in excluding the diagnosis of pancreatitis, especially for admissions within the first 24 hours. We suggest that since the sensitivity of the test gradually decreases after 24 hours, its reliability is lower for delayed admissions. The urine tripsinogen-2 test might be useful in grading the severity of acute pancreatitis, especially during the earlier period of the disease.

REFERENCES

1. Robert J Vissers, Riyad B Abu-Laban. Acute and chronic pancreatitis. In: Tintinalli JE, Kelen GD, Stapczynski JS, editors. Emergency medicine. A comprehensive study guide. 5th ed. McGraw-Hill; New York: 2000. p. 573-7.
2. Matull WR, Pereira SP, O'Donohue JW. Biochemical markers of acute pancreatitis. J Clin Pathol 2006;59:340-4.
3. Dervenis C, Johnson CD, Bassi C, Bradley E, Imrie CW, McMahon MJ, et al. Diagnosis, objective assessment of severity, and management of acute pancreatitis. Santorini consensus conference. Int J Pancreatol 1999;25:195-210.
4. Tietz NW. Support of the diagnosis of pancreatitis by enzyme tests--old problems, new techniques. Clin Chim Acta 1997;257:85-98.
5. Vissers RJ, Abu-Laban RB, McHugh DF. Amylase and lipase in the emergency department evaluation of acute pancreatitis. J Emerg Med 1999;17:1027-37.
6. Hedström J, Sainio V, Kemppainen E, Puolakkainen P, Haapiainen R, Kivilaakso E, et al. Urine trypsinogen-2 as marker of acute pancreatitis. Clin Chem 1996;42:685-90.
7. Bulut A, Hoca O, Öztürk F. Akut pankreatitin erken tanısında idrar tripsinojen-2 testinin yeri. İnönü Üniversitesi Tıp Fakültesi Dergisi 2007;14:13-6.
8. Sáez J, Martínez J, Trigo C, Sánchez-Payá J, Compañy L, Laveda R, et al. Clinical value of rapid urine trypsinogen-2 test strip, urinary trypsinogen activation peptide, and serum and urinary activation peptide of carboxypeptidase B in acute pancreatitis. World J Gastroenterol 2005;11:7261-5.
9. Kemppainen EA, Hedström JI, Puolakkainen PA, Sainio VS, Haapiainen RK, Perhoniemi V, et al. Rapid measurement of urinary trypsinogen-2 as a screening test for acute pancreatitis. N Engl J Med 1997;336:1788-93.
10. Kylänpää-Bäck M, Kemppainen E, Puolakkainen P, Hedström J, Haapiainen R, Perhoniemi V, et al. Reliable screening for acute pancreatitis with rapid urine trypsinogen-2 test strip. Br J Surg 2000;87:49-52.
11. Steinberg W, Tenner S. Acute pancreatitis. N Engl J Med 1994;330:1198-210.
12. Chen YT, Chen CC, Wang SS, Chang FY, Lee SD. Rapid urinary trypsinogen-2 test strip in the diagnosis of acute pancreatitis. Pancreas 2005;30:243-7.
13. Skipworth JR, Pereira SP. Acute pancreatitis. Curr Opin Crit Care 2008;14:172-8.
14. Pezzilli R, Morselli-Labate AM, d'Alessandro A, Barakat B. Time-course and clinical value of the urine trypsinogen-2 dipstick test in acute pancreatitis. Eur J Gastroenterol Hepatol 2001;13:269-74.
15. Kamer E, Unalp HR, Derici H, Tansug T, Onal MA. Early diagnosis and prediction of severity in acute pancreatitis using the urine trypsinogen-2 dipstick test: a prospective study. World J Gastroenterol 2007;13:6208-12.
16. Lempinen M, Kylänpää-Bäck ML, Stenman UH, Puolakkainen P, Haapiainen R, Finne P, et al. Predicting the severity of acute pancreatitis by rapid measurement of trypsinogen-2 in urine. Clin Chem 2001;47:2103-7.
17. Kim YS, Lee BS, Kim SH, Seong JK, Jeong HY, Lee HY. Is there correlation between pancreatic enzyme and radiological severity in acute pancreatitis? World J Gastroenterol 2008;14:2401-5.