

## Original Research Article

# A comparative analysis of urine trypsinogen-2 test strip with serum lipase in diagnosis of acute pancreatitis in emergency set-up

Jagmohan Mishra, Biplab Mishra\*, Afroza Firodous

Department of General Surgery, S.C.B Medical College, Cuttack, Odisha, India

**Received:** 06 November 2018

**Accepted:** 01 December 2018

### \*Correspondence:

Dr. Biplab Mishra,

E-mail: [biplab14111988@gmail.com](mailto:biplab14111988@gmail.com)

**Copyright:** © the author(s), publisher and licensee Medip Academy. This is an open-access article distributed under the terms of the Creative Commons Attribution Non-Commercial License, which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

## ABSTRACT

**Background:** Acute pancreatitis possess difficulty in diagnosis in its emergency presentation. Hence to segregate this disease is important from other specific or non-specific causes of acute abdomen. Hence in suspected cases in majority of patients the urinary trypsinogen-2 test strip (Actim pancreatitis) can be used to detect this disease, especially in emergency set-up. The result of the strip test is then corroborated with findings of serum lipase in the blood.

**Methods:** Author prospectively compared 205 consecutive patients with acute abdominal pain admitted to the casualty, SCB Medical College and Hospital. The patients were tested on admission with the Actim pancreatitis test strip. Serum amylase, serum lipase, and urine trypsinogen-2 concentrations were also determined quantitatively.

**Results:** The Actim pancreatitis test strip was sensitive in 93% cases and specific in 92% cases. This was superior to that of serum lipase (sensitivity 77% and specificity 87%). With a cut-off  $>3\times$  the upper reference limit, the sensitivity of serum lipase was only 52% while the specificity was 98%. The high sensitivity for the Actim pancreatitis test strip resulted in every high negative predictive value of 99%.

**Conclusions:** In patients with acute abdominal pain seen in emergency department, a negative dipstick for urinary trypsinogen-2 rules out acute pancreatitis with high degree of probability and therefore appears to be more suitable for screening of acute pancreatitis. With its high specificity with a cut-off  $>3\times$  the upper reference limit, serum lipase is suitable as a confirmatory test for pancreatitis when a positive dipstick result is obtained.

**Keywords:** Acute pancreatitis, Acute abdomen, Actim pancreatitis strip, Serum lipase, Trypsinogen-2

## INTRODUCTION

For diagnosis of acute pancreatitis there are no absolute physical signs.<sup>1</sup> Atypical presentation is so frequent that the diagnosis is often delayed and a marked proportion of the cases may be undiagnosed until autopsy.<sup>2</sup> Serum amylase and lipase are commonly used laboratory methods in the evaluation of patients with abdominal pain although neither of them is specific for AP.<sup>3,4</sup> Several other laboratory tests (i.e., urine amylase, amylase creatinine clearance rate, serum elastase-I) have been investigated but have not shown any advantage over amylase or lipase in the diagnosis of AP.<sup>5-7</sup> In AP, serum

amylase levels increase within 2-12 hours and return to normal in 3-5 days.<sup>8</sup> Because the concentration of serum amylase may normalize soon after the onset of symptoms it is normal in up to 19% of the cases at presentation, the assay is not considered suitable for screening of AP.<sup>9</sup> Pancreatic lipase is a pancreatic enzyme synthesized in the exocrine acinar cells. It catalyzes the hydrolysis of triglycerides into diglycerides and fatty acids.<sup>10</sup> In pancreatitis, serum lipase rises within 48 hours and remains elevated longer than serum amylase (8-14 days).<sup>8</sup> The sensitivity and specificity of serum lipase in the diagnosis of AP vary considerably in different studies. This may partly be due to different assay methods.<sup>11</sup>

Trypsinogen is 25 kD pancreatic proteinase with the two main isoenzymes, trypsinogen-1 (cationic) and trypsinogen-2 (anionic).<sup>12,13</sup> AP is most commonly triggered because of extra pancreatic origin but irrespective of the etiology, premature activation of trypsin within the pancreas is considered a common feature at the acinar cell level.<sup>14,15</sup> In AP trypsinogen-2 levels increase rapidly both in serum and urine.<sup>13,16,17</sup> Thus, trypsinogen-2 and also the trypsin-2-a1-antitrypsin complex are accurate diagnostic markers of AP and show a marked correlation with the disease severity.<sup>18</sup> Earlier studies have shown that trypsinogen-2 is a more reliable marker for AP than amylase. However, comparisons with amylase are biased by the fact that amylase is routinely used as a major diagnostic criterium for AP.<sup>17</sup>

The dipstick test based on immunochromatographic measurement of trypsinogen-2 uses two new monoclonal antibodies, now commercially available (Medix Biochemica, Kaunianen, Finland) and has proven to be highly sensitive (96%) and specific.<sup>19</sup> The present prospective study was designed to compare the Actim pancreatitis test strip with serum lipase for screening of AP by analyzing a consecutive series of patients with acute abdominal pain.

## METHODS

Two hundred five consecutive patients with acute abdominal pain admitted to the emergency unit at SCB Medical College and Hospital between December 2017 and April 2018 were investigated prospectively. In 27 patients, a diagnosis of AP could be established. The etiology of AP was alcohol in 17 patients, biliary in six, pancreas divisum in one, and unknown in three. There were 9 female and 18 male patients (mean age: 48years, range: 30-80years).

In 4 patients the diagnosis was based on consistent clinical findings (epigastric pain, nausea and vomiting) in combination with an elevated amylase level (above 300IU/L in serum) and diagnostic findings on contrast enhanced computed tomography. In 23 patients, the diagnosis with highly elevated amylase levels (serum amylase over 900IU/L). The severity of AP was categorized by the clinically based classification of the 1992 Atlanta Symposium.<sup>20</sup> Exclusion of AP in patients with acute abdominal pain (178 patients) was based on clinical, radiographic, endoscopic and surgical findings.

The actim pancreatitis test strip is based on the immunochromatography principle. The test was carried out by briefly dipping the tip of the test strip into urine. Trypsinogen-2 in the sample migrated through the strip binding 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 a different epitope on trypsinogen-2. The test was considered positive when a clear blue line

was detected within 5minutes in the catching zone. A control line was used to indicate proper function of the strip. If the control line was undetectable the assay was repeated. The detection limit of the test was approximately 50g/L. Urine samples from all patients were obtained in the emergency unit on admission and tested immediately with the urinary dipstick. The concentration of trypsinogen-2 in the urine samples was also measured by a quantitative immuno-enzymometric assay (IEMA) (inhouse assay, MedixBiochemica, Kaunianen, Finland, reference value, <50mg/L). The samples were stored at -20°C until the quantitative measurements were performed. The concentration of serum lipase was measured by a turbidimetric assay based on the degradation of a triolein emulsion (Boehringer-Mannheim, Germany, reference value, <200IU/L). Serum samples for determination of lipase were taken on admission and stored at -70°C until analyzed. Comparison of continuous data was performed by the Mann-Whitney U test.

## RESULTS

The study population consisted of 80 female and 125 male patients aged 17 to 89years with a mean of 50years. Of the 205 patients with abdominal pain 27 had AP. The Actim pancreatitis test strip showed a positive result in 25 of them giving a sensitivity of 93%. Two AP patients with false negative results due to relatively low trypsinogen-2 levels (16.2g/L and 42g/L), as measured by the quantitative trypsinogen-2 assay had a mild attack of AP. The etiology was alcohol in one and unknown in the other.

The test results were also positive in 14 of the 178 control patients with abdominal pain but no evidence of AP. Thus, specificity was 92% (Table 1). The actim pancreatitis test strip had a negative predictive value (NPV) of 99% and a positive predictive value (PPV) of 64%. The diagnoses of the patients with abdominal pain other than AP including the 14 false positive cases are shown in Table 2.

All six patients in whom severe AP developed had a positive Actim pancreatitis test result (sensitivity 100%). The median urinary trypsinogen-2 concentration was significantly higher in patients with AP (920 g/L, range: 11.5-47700g/L) than in controls with abdominal pain (1.4g/L, range: 0.0-4500g/L) ( $P<0.001$ ). The patients with severe AP had significantly higher urinary trypsinogen-2 concentration (median 7160g/L, range 1010-47700g/L) than those with mild disease (median: 530g/L, range: 11.5-44300g/L) ( $P<0.01$ ). In the 14 patients with a false positive dipstick result, the median urinary trypsinogen-2 concentration was 134g/L (range: 36.5-440g/L). The median concentration of serum lipase in patients with AP was 710IU/L (range: 30-2060IU/L) and in patients with abdominal pain from causes other than AP 81IU/L (range: 9-2111IU/L). The difference was highly significant ( $P<0.001$ ). The sensitivity and

specificity of the serum lipase with two cut-off values are presented in Table 1. The serum lipase concentration was not significantly higher in patients with severe AP (median: 689IU/L, range: 256-1072IU/L) than in those with mild disease (median: 710IU/L, range: 30-2060IU/L). Two patients with a severe AP had only

moderately elevated serum lipase concentrations (255 and 388IU/L) and would have remained undetected with the higher cut-off, 600IU/L. A combination of the Actim pancreatitis test strip and serum lipase with a cut-off 600IU/L improved specificity of the dipstick alone from 92% to 99.5% and PPV from 64% to 93% (Table 1).

**Table 1: Diagnostic accuracy of urinary trypsinogen-2 dipstick test and serum lipase activity for detection of acute pancreatitis in 205 patients with acute abdominal pain.**

Test	Cut off value	Acute pancreatitis (N=27)		Other abdominal disorders (N=178)			
		Positive test	Sensitivity%	Positive test	Specificity%	PPV	NPV
Kit test	50g/L	25	93	14	92	64	99
Serum lipase	200IU/L	21	77	23	87	48	96
Serum lipase	600IU/L	14	52	2	98	87.5	93
Kit and serum Lipase	50g/L 600IU/L	14	52	1	99.5	93	93

**Table 2: Diagnoses in 178 patients with abdominal disorders other than acute pancreatitis.**

Diagnosis	Total no. of patients	N with false positive results	Diagnosis	Total no. of patients	N with false positive results
Acute appendicitis	13		Functional disorder of colon	4	
Acute gastritis or dyspepsia	9	3	Hepatic disease	4	1
Acute gastroenteritis	5	1	Infection	7	2
Biliary stones	20				
Blunt trauma	4		Intestinal obstruction	7	1
Cardiac disorder or chest pain	2		Intestinal perforation	5	2
Chronic pancreatitis	3		Malignancy	10	
Colonic diverticulosis	7				
Crohn's disease	1	1			
Diabetes with abdominal pain	1				
Drug or alcohol intoxication	5				
Duodenal or gastric ulcer	1		Unknown	53	3
Esophagitis	5		Urinary infection, colic or retention	12	
			Total	178	14



Green arrow-test, orange arrow-control

**Figure 2: Actim pancreatitis kit test showing positive result.**

## DISCUSSION

The Actim Pancreatitis test strip detected AP more accurately than the quantitative serum lipase determination. The high sensitivity (93%) of the dipstick resulted in a very NPV of 99%.<sup>26,27</sup> Thus, AP could be excluded with a very high probability. A characteristic feature of urinary trypsinogen-2 is the strong, rapid and long-lasting elevation in AP.<sup>13</sup> This was seen also in the present study, the median concentration of urinary trypsinogen-2 in AP being nearly 700-fold that in patients with extra-pancreatic abdominal disorders. In comparison, the sensitivity of serum lipase (55-79%) was low and inadequate for screening purposes. The levels of serum amylase or lipase bear no correlation with the prognosis in AP.<sup>3,10,21</sup> In the present study, the median

concentration of serum lipase was not higher in patients with severe AP than in those with mild disease. Thus, some patients with severe AP may be missed both by serum amylase and lipase.<sup>2,22</sup> This was the case in two patients in the present study, if the higher cut-off value for lipase 600IU/L (3x the upper normal limit) was used. In contrast to amylase and lipase a strong correlation between the concentration of trypsinogen-2 and the severity of AP has been observed and this was confirmed in the present study.<sup>13</sup> The actim pancreatitis test strip detected all patients with severe AP, which author consider highly important in clinical practice.

The median urinary concentration of trypsinogen-2 in patients with abdominal pain from causes other than AP was about 1/30<sup>th</sup> the detection limit of the urinary test strip (50g/L). Thus, the specificity of the dipstick was acceptable and higher than that of serum lipase with cut-off 200IU/L (92% vs 87%, respectively), with the cut-off 600IU/L serum lipase showed very high specificity 98% but sensitivity was unacceptable (52%). The false positive test results in 14 patients with a variety of diagnoses resulted in a relatively low PPV (64 %), which was, however, higher than the PPV of lipase with a cut-off 200IU/L (48%). This result indicate that the diagnosis of AP cannot be established by the test strip alone but hat additional enzyme measurements or radiological examinations are needed. When serum lipase >600IU/L was combined with a positive Actim pancreatitis result, the specificity and PPV were high. Thus, author recommend the use of serum lipase as a confirmatory test for AP in cases with a positive dipstick result. The cause of the relatively low PPV for the Actim pancreatitis test strip and lipase was that the study population included all patients with abdominal pain.

The sensitively of the Actim pancreatitis test strip was higher than that of the quantitative assay for trypsinogen-2 at a cut-off of 50g/L (93% vs 83%, respectively). The better performance of the test strip was probably due to the fact that it was used immediately with fresh urine whereas the quantitative assay for trypsinogen-2 was performed on samples that had been stored at -200°C for several weeks, during which some trypsinogen-2 immunoreactivity was lost.<sup>28</sup>

AP still represented a major diagnostic and therapeutic challenge. The diagnosis was often unsatisfactory in an emergency setting. Computed tomography scan was the most accurate diagnostic methods, but it was not always available and because of its high costs, cannot be performed in all instances when AP is suspected clinically.<sup>24,25</sup> The Actim pancreatitis test can be performed by medical or nursing staff at the point of care. Cases with negative results can safely await further investigations, routine laboratory tests and radiological examinations.

An early diagnosis of AP was essential to provide the patient with adequate treatment and clinical follow-up

and thus reduce the risk of complications and prolonged hospitalization. The non-invasive and simple urinary screening test Actim Pancreatitis was more sensitive and specific than serum lipase, which was useful as a confirmatory test in patients with positive dipstick result.

## ACKNOWLEDGEMENTS

Authors would like to thank all their colleagues, juniors for their kind help and cooperation and acknowledged the help rendered by Bharat serum for providing 100 Actim pancreatitis test strips during the study.

*Funding: Partly funded by Bharat serum*

*Conflict of interest: None declared*

*Ethical approval: The study was approved by the Institutional Ethics Committee*

## REFERENCES

1. Büchler M. Objectification of the severity of acute pancreatitis. *Hepatogastroenterol.* 1991 Apr;38(2):101-8.
2. Wilson C, Imrie CW, Carter DC. Fatal acute pancreatitis. *Gut.* 1988;29(6):782-8.
3. Elman R, Arneson N, Graham EA. Value of blood amylase estimations in the diagnosis of pancreatic disease: a clinical study. *Arch Surg.* 1929;19(6):943-67.
4. Lott JA, Patel ST, Sawhney AK, Kazmierczak SC, Love JE. Assays of serum lipase: analytical and clinical considerations. *Clinical chemistry.* 1986;32(7):1290-302.
5. Werner M, Steinberg WM, Pauley C. Strategic use of individual and combined enzyme indicators for acute pancreatitis analyzed by receiver-operator characteristics. *Clin Chem.* 1989;35(6):967-71.
6. Flamion B, Delhay M, Horanyi Z, Delange A, Demanet H, Quenon M, et al. Comparison of elastase-1 with amylase, lipase, and trypsin-like immunoreactivity in the diagnosis of acute pancreatitis. *Am J Gastroenterol.* 1987;82(6).
7. Millson CE, Charles K, Poon P, Macfie J, Mitchell CJ. A prospective study of serum pancreatic elastase-1 in the diagnosis and assessment of acute pancreatitis. *Scandinavian J Gastroenterol.* 1998;33(6):664-8.
8. Tietz NW, Shuey DF. Lipase in serum-the elusive enzyme: an overview. *Clin Chem.* 1993;39:746-56.
9. Clavien PA, Robert J, Meyer P, Borst F, Hauser H, Herrmann F, et al. Acute pancreatitis and normo-amylasemia: not an uncommon combination. *Ann Surg.* 1989;210(5):614.
10. Clavien PA, Burgan S, Moossa AR. Serum enzymes and other laboratory tests in acute pancreatitis. *Brit J Surg.* 1989;76(12):1234-43.
11. Wong EC, Butch AW, Rosenblum JL. The clinical chemistry laboratory and acute pancreatitis. *Clin Chem.* 1993;39(2):234-43.



12. Robinson LA, Kim WJ, White TT, Hadorn B. Trypsins In: Human pancreatic juice-their distribution as found in 34 specimens: two human pancreatic trypsinogens. *Scandinavian J Gastroenterol.* 1972;7(1):43-5.
13. 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(5):685-90.
14. Steer ML. Frank brooks memorial lecture: the early intraacinar cell events which occur during acute pancreatitis. *Pancreas.* 1998;17(1):31-7.
15. Figarella C, Amouric M, Guy-Crotte O. Enzyme activation and liberation: intracellular/extracellular events. In: *Acute pancreatitis.* Springer; Berlin; Heidelberg: 1987:53-60.
16. Hedström J, Stenman UH, Korvuo A, Kenkimäki P, Tikanoja S, Haapiainen R, et al. Urinary trypsinogen-2 test strip for acute pancreatitis. *Lancet.* 1996;347(9003):729-31.
17. 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. *New Eng J Med.* 1997;336(25):1788-93.
18. Hedstrom J, Sainio V, Kemppainen E, Haapiainen R, Kivilaakso E, Schroder T, et al. Serum complex of trypsin 2 and (alpha) (sub 1) antitrypsin as diagnostic and prognostic marker of acute pancreatitis: clinical study in consecutive patients. *BMJ.* 1996;313(7053):333-7.
19. Kylänpää-Bäck ML, Kemppainen E, Puolakkainen P, Hedström J, Haapiainen R, Perhoniemi V, et al. Reliable screening for acute pancreatitis with rapid urine trypsinogen-2 test strip. *Brit J Surg.* 2000;87(1):49-52.
20. Bradley EL. A clinically based classification system for acute pancreatitis: summary of the international symposium on acute pancreatitis, Atlanta, 1992. *Arch Surg.* 1993;128(5):586-90.
21. Kazmierczak SC, Van Lente F, Hodges ED. Diagnostic and prognostic utility of phospholipase A activity in patients with acute pancreatitis: comparison with amylase and lipase. *Clin Chem.* 1991;37(3):356-60.
22. Lankisch PG, Burchard-Reckert S, Lehnick D. Underestimation of acute pancreatitis: patients with only a small increase in amylase/lipase levels can also have or develop severe acute pancreatitis. *Gut.* 1999;44(4):542-4.
23. Büchler M, eds. *Acute pancreatitis: novel concepts in biology and therapy.* Blackwell; 1999.
24. Kivisaari L, Somer K, Standertskjöld-Nordenstam CG, Schröder T, Kivilaakso E, Lempinen M. Early detection of acute fulminant pancreatitis by contrast-enhanced computed tomography. *Scandinavian J Gastroenterol.* 1983;18(1):39-41.
25. London NJ, Neoptolemos JP, Lavelle J, Bailey I, James D. Contrast-enhanced abdominal computed tomography scanning and prediction of severity of acute pancreatitis: a prospective study. *Brit J Surg.* 1989;76(3):268-72.
26. Abraham P. Point-of-care urine trypsinogen-2 test for diagnosis of acute pancreatitis. *J Assoc Physicians India.* 2011;59:231-2.
27. Chien NH, Hung CS, Lee CL, Wu CH. Rapid urine trypsinogen-2 test for the early detection of pancreatitis in endoscopic sphincterotomy patients. *JOP. J Pancreas.* 2016;17(3):284-8.
28. Nittala R, Basheer OT, Sasi MP. Acute pancreatitis: a study of urine trypsinogen-2 measurement as a screening test. *Inter J Res Med Sci.* 2017;2:897-902.

**Cite this article as:** Mishra J, Mishra B, Firodous A. A comparative analysis of urine trypsinogen-2 test strip with serum lipase in diagnosis of acute pancreatitis in emergency set-up. *Int Surg J* 2019;6:252-6.