

Research Article

Acute pancreatitis: a study of urine trypsinogen-2 measurement as a screening test

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Received: 30 April 2014

Revised: 4 May 2014

Accepted: 4 May 2014

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ABSTRACT

Background: Usefulness of urine trypsinogen-2 as screening test for acute pancreatitis in Indian population.

Methods: We prospectively compared the urinary trypsinogen-2 dipstick test and serum amylase assays in 100 consecutive patients with acute abdominal pain at the emergency department. Urine samples were obtained on admission and tested for the dipstick. The diagnosis of acute pancreatitis was made on the basis of a typical clinical picture and serum amylase at least more than threefold the upper reference limit (300 IU) and/or radiological evidence (Abdominal X ray/ultrasonography/contrast enhanced CT).

Results: 1. Urine trypsinogen-2 dipstick test was having 100% sensitivity and 85.71% specificity in diagnosing acute pancreatitis in Indian population. 2. Serum amylase was having 61.36% sensitivity and 78.57% specificity in diagnosing acute pancreatitis. 3. Sensitivity and specificity of urine trypsinogen-2 dipstick test is more than that of serum amylase in diagnosing acute pancreatitis which is statistically proved to be significant ($P < 0.05$).

Conclusion: Detection of trypsinogen-2 in urine is a simple office test in diagnosing acute pancreatitis in emergency department. As the sensitivity and specificity are high, detection of urine trypsinogen-2 in urine can be used as a screening test, which will definitely help in early diagnosis and prompt treatment of acute pancreatitis.

Keywords: Pancreatitis, Trypsinogen-2, Rapid, Cheap, Urine

INTRODUCTION

Acute pancreatitis is an inflammatory process which occurs in a normal organ and characterised by acute abdominal pain, nausea and vomiting.¹ Diagnosis is reached from the history, clinical features and investigations. Several scoring systems are available to assess the outcome and severity of acute pancreatitis. But none of these scoring systems are useful in diagnosing pancreatitis. Furthermore, the 48 hours delay to collect the standard scores has prompted everybody to investigate new markers, one of them being measurement

of urine trypsinogen-2 within 24 hours of an attack of acute pancreatitis.^{2,4}

Trypsinogen is a 25-kd pancreatic proteinase. The two main isoenzymes, (cationic) trypsinogen-1 and (anionic) trypsinogen-2, are secreted at high concentrations into pancreatic fluid. For unknown reasons, the tubular reabsorption of trypsinogen-2 is lower than that of trypsinogen-1, and consequently, the urinary concentration of trypsinogen-2 is higher.⁵⁻⁷

Measurement of trypsinogen is considered useful in diagnosing acute pancreatitis and assessing its severity.

At a cut-off of 50 ng/mL, trypsinogen-2 measured by a rapid urinary dipstick is a sensitive and specific diagnostic test in acute pancreatitis. The trypsinogen-2 concentration correlates with the severity of the disease.^{8,9}

The question arises, what is the need of early diagnosis of acute pancreatitis. The answer to that is, even though most patients have mild and self-limited form of the disease, around 20% of the attacks are severe, consisting of pancreatic necrosis, sepsis and fulminant multiorgan failure with life threatening morbidity and mortality.^{10,11} Typical clinical features along with measurement of serum amylase and/or lipase is the method most commonly employed for diagnosing acute pancreatitis. But it has also been found that elevation of these enzymes is unsatisfactory in diagnosing acute pancreatitis because of their low sensitivity and specificity.¹²

Then came the need for contrast enhanced computed tomography which is the most accurate method for diagnosing and assessing the severity of acute pancreatitis.¹³ But CT scan cannot be always performed because of its limited availability, high cost and potential side effects.¹⁴ Hence the need arises for a simple, quick, reliable and inexpensive screening test which diagnoses an acute attack of pancreatitis.

METHODS

Study design

Prospective study

Duration of study

2 years

Period of study

February 2011 to January 2013

Number of subjects

200

Setting

Government medical college hospital, Kozhikode, Kerala, India

Inclusion criteria

Subjects presenting with suspected acute pancreatitis and within 24 hours of acute symptoms, admitted to the Casualty. The diagnosis of acute pancreatitis will be made on the basis of typical clinical picture and serum amylase at least more than three fold the upper reference limit (300 IU/l) and typical findings on abdominal X ray/ultrasound/CECT.

Exclusion criteria

Known case of chronic calcific pancreatitis, previous pancreatic/gastrointestinal bypass surgery

Procedure

Urine samples will be obtained on admission in the emergency department and tested with the dipstick. We prospectively compared the urinary trypsinogen-2 dipstick test serum and serum amylase assays in 200 consecutive patients with acute abdominal pain at the emergency department. Acute pancreatitis was classified as severe if one or several local or systemic complications were present (e.g., shock, renal failure, respiratory insufficiency, disseminated intravascular coagulation, pancreatic necrosis, an abscess, a pseudocyst or bleeding).

Diagnosis of acute pancreatitis was based on a history of prolonged upper abdominal pain, serum amylase at least three times the upper limit of normal and the presence of edema or necrosis on abdominal ultrasonography/CECT.

Trypsinogen-2 measurements

The dipstick test for urinary trypsinogen-2 is an immunochromatographic test. After the test strip has been dipped into the urine sample, trypsinogen-2 is bound to monoclonal-antibody-labeled blue latex particles, which migrate across a nitrocellulose membrane with a zone containing another antibody specific for another epitope on trypsinogen-2.

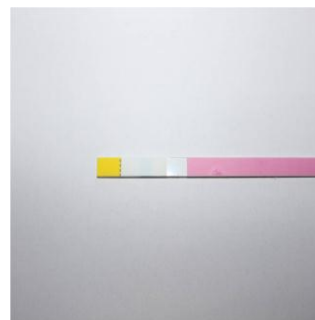


Figure 1: Unused dipstick.

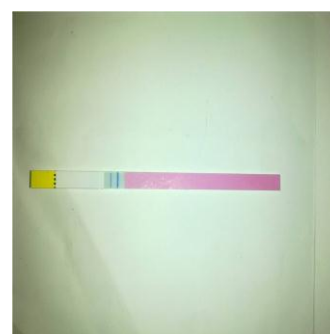


Figure 2: Positive dipstick.

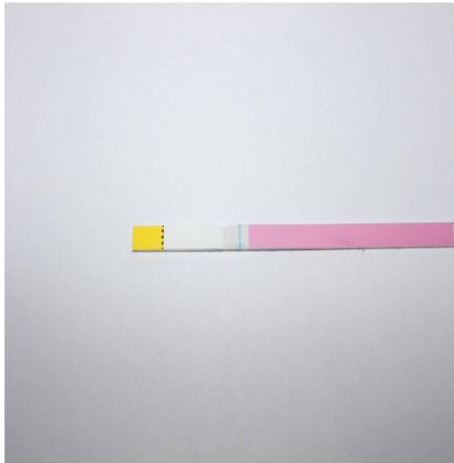


Figure 3: Negative dipstick.

The Actim Pancreatitis test strip (Medix Biochemica, Kauniainen, Finland), an immunochromatographic test, was used for urine trypsinogen-2 determination (detection limit 50 µg/L). The tip of the strip was immersed into a urine-containing vial and was held for 20 s before being completely taken out of the vial. The strip was then kept at room temperature for 5 min to observe whether urine reacted with blue latex particles covered by monoclonal anti trypsinogen-2 antibodies. Excess (>50 µg/L) urinary trypsinogen-2 caused the occurrence of 2 blue stripes, while only one stripe (referred to as the control stripe) was observed when urinary trypsinogen-2 concentration was within the normal range.

The appearance of the control stripe confirmed the accuracy of the assay, while no blue stripes on the test strip suggested an erroneous test, in which case the test was repeated.

Amylase measurements

Serum amylase concentrations were measured using enzymatic assay (Architect C8000; Abbott, Abbott Park, IL, USA; reference interval, 26-100 U/L).

The results were tabulated and processed using SPSS 17.0.

RESULTS

The results of the study are as follows,

The study population

A total of 200 patients were enrolled into the study out of which 44% (88 of 200) were diagnosed as acute pancreatitis and other 56 % (112 of 200) were acute abdominal conditions like bowel perforations, intestinal obstruction, acute cholangitis, gastritis, acute appendicitis, mesenteric lymphadenitis.

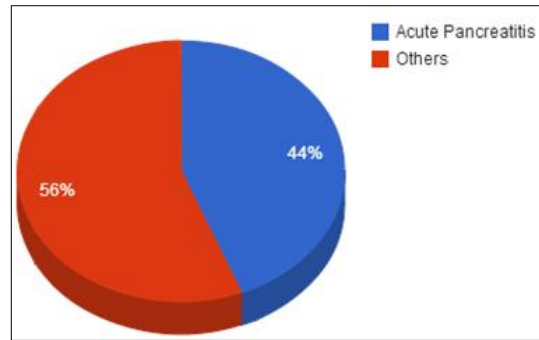


Figure 4: Study population.

Sensitivity and specificity of serum amylase in diagnosing acute pancreatitis

Table 1: Serum amylase in acute pancreatitis and other acute abdomen cases.

Serum amylase (IU/l)	Acute pancreatitis	Others
>300	54	24
<300	34	88

A cut off of more than 300IU/l of serum amylase was taken as diagnostic of acute pancreatitis. In this study the sensitivity and specificity of serum amylase came as 61.38% and 78.57% respectively.

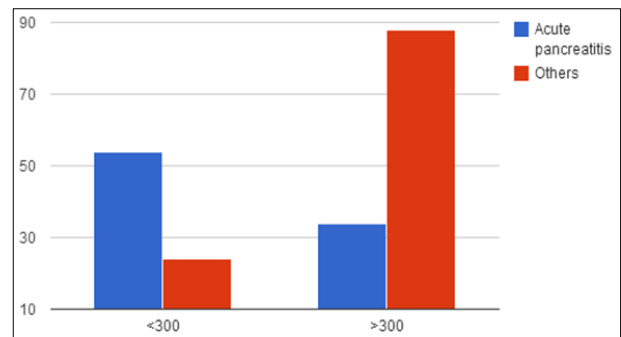


Figure 5: Serum amylase in acute pancreatitis.

The Pearson Chi square value came as 16.518 which is significant at P <0.05.

Sensitivity and specificity of urine dipstick for trypsinogen-2 in diagnosing acute pancreatitis

Table 2: Urine dipstick for trypsinogen-2 in acute pancreatitis and other acute abdomen.

Dipstick	Acute pancreatitis	Others
Positive	88	16
Negative	0	96

The urine dipstick for trypsinogen-2 was false positive in 16 patients. Out of these, 4 patients were diagnosed as having Chronic Kidney Disease. These patients were also having raised amylase values. The sensitivity and specificity of this dipstick came as 100% and 85.71% respectively.

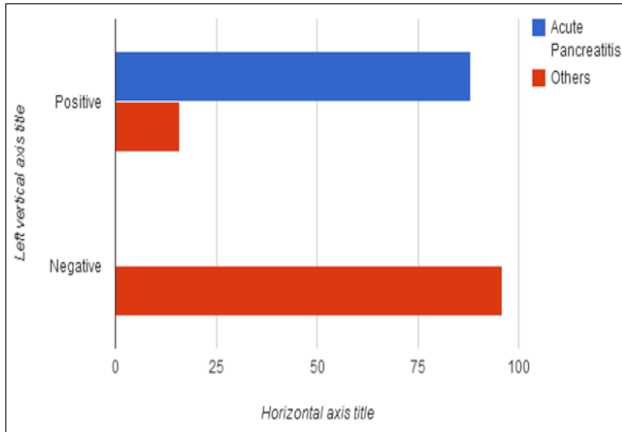


Figure 6: Urine trypsinogen-2 dipstick in acute pancreatitis.

The Pearson Chi square value came as 72.527 which is statistically proved to be significant at $P < 0.05$.

Ultrasound of abdomen

Ultrasound of abdomen when reported as acute pancreatitis, bulky pancreatic head, edematous pancreatitis were taken for the diagnosis and labelled as acute pancreatitis. 34 of the 88 patients (39%) were having unremarkable abdominal ultrasound findings.

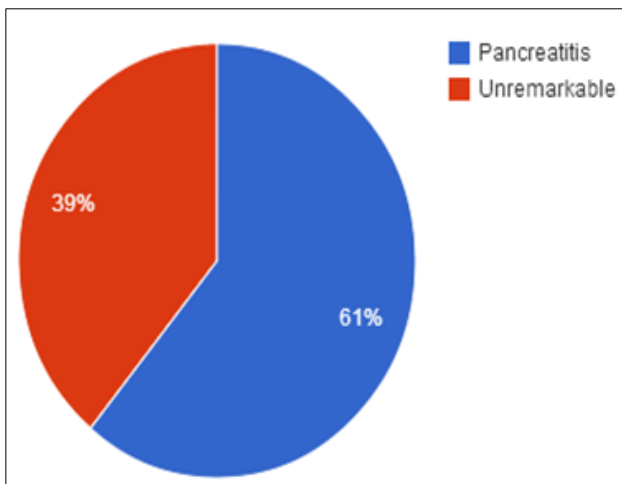


Figure 7: Ultrasound in acute pancreatitis.

Alcohol

68.18 % of the patients diagnosed with acute pancreatitis (60 of 88) were found to have history of alcohol consumption.

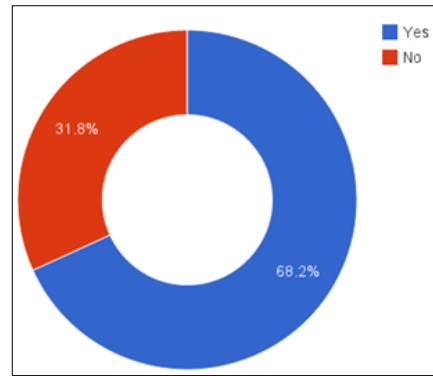


Figure 8: Alcohol consumption among patients with acute pancreatitis.

Mean age

Our study showed that mean age of the patients was 38.91 years. This suggests that we are losing our workforce to this debilitating disease and about 68 % of these were alcoholic. Our study hence suggests to avoid alcohol as a primary prevention of acute pancreatitis.

DISCUSSION

In our study we had a population of 200 patients that included 6 females. A total of 88 were diagnosed to be having acute pancreatitis, all being males. The rest of the patients were acute abdominal cases who were diagnosed to have acute appendicitis, gastritis, cholangitis, acute cholecystitis, intestinal obstruction, mesenteric lymphadenitis and bowel perforation.

In our study, 68% of the acute pancreatitis cases (60 of 88) were having history of recent alcohol intake. This shows high prevalence of alcohol intake in the study population. Another important derivative from our study was the mean age of the study group, which came to be 39 years.

Sticking to our inclusion criteria of typical clinical picture, raised amylase and radiological evidence, we diagnosed 88 patients with acute pancreatitis. Serum amylase cut off in our study was 300 IU/l. Patients suspected to have acute pancreatitis, but <300 IU/l of serum amylase, underwent radiological investigations such as, USG and CECT abdomen. Being non-invasive, cheap and fast, these patients underwent USG abdomen first.

Ultrasound of abdomen was taken as positive for pancreatitis with features such as bulky pancreas, edematous pancreas and peripancreatic collection. Few patients with serum amylase levels <300 IU/l and inconclusive USG abdomen findings were later taken for CECT abdomen, in view of strong clinical suspicion. Ultrasound evaluation of the abdomen in our study has thrown some interesting observations. 34 of the 88 patients were having unremarkable abdominal ultrasound

findings. The advantages of USG abdomen are detection of gallbladder stones or sludge, dilation of the common bile duct and detection of other causes of severe abdominal pain. The demerit being it is highly operator dependent.

Out of the 88 diagnosed cases of acute pancreatitis, 34 patients had serum amylase values of <300 IU/l. These 34 patients underwent ultrasound abdomen and 16 were diagnosed as acute pancreatitis. The rest 18 had to undergo CECT abdomen and were confirmed as acute pancreatitis. Various disadvantages of the CECT scan being its cost, availability and high radiation exposure. The advantage is its ability to grade the severity of acute pancreatitis.

The sensitivity of serum amylase in diagnosing acute pancreatitis in our study came as 61.36% and the specificity came as 78.57%.

All acute abdomen patients who came to Surgery division at the emergency department underwent spot testing of their urine sample for trypsinogen-2 using the dipstick. In our study all the 88 diagnosed cases of acute pancreatitis were positive for urine trypsinogen-2 dipstick. In the rest of the 112 acute abdomen cases 16 were falsely detected as positive by the dipstick. 4 of them were diagnosed to have chronic kidney disease.

Hedstrom et al., in 1996 reported that urinary trypsinogen-2 concentration may be increased in other diseases such as hepatobiliary and pancreatic malignancies, colon cancers and chronic pancreatitis.⁸ Kemppainen et al. in 1997 reported false positive results in patients with abdominal pain who may in some instances have reflected subclinical pancreatic irritation or tumor derived trypsinogen-2.⁴ Trypsinogen-2 has been reported to be a tumor marker for gastrointestinal and ovarian cancers as mentioned above in the study done by Hedstrom et al. It is also expressed in the epithelium of bile ducts and peribiliary glands, reported by Terada et al. in 1991. This explains the rise in cases of cholangitis.

The sensitivity of urine trypsinogen-2 in diagnosing acute pancreatitis in our study comprising Indian population was 100% as all the cases were positive by the dipstick method. The specificity for the dipstick was 85.71%.

Table 3: Previous studies comparing sensitivity and specificity of urine dipstick for trypsinogen-2 in acute pancreatitis.

Previous studies	Sensitivity	Specificity
Kemppainen et al. 1997 ⁴	94	95
Kylanpaa-Backet et al. 2000 ¹⁵	96	97
Pezzilli et al. 2001 ¹⁶	53.3	-
Chen et al. 2005 ⁹	89.6	85.7
Abraham, 2011 ¹⁷	73.9	94.6
Present study	100	85.71

Comparing the sensitivity and specificity of serum amylase and urine trypsinogen-2 dipstick, the latter definitely fares better. Our aim was never to compare dipstick with CECT abdomen, because CECT abdomen was not done in all patients within 24 hours of presentation for diagnosis. CECT abdomen within first 24 hours for diagnostic purpose was done only in patients with unremarkable ultrasound finding. Later all diagnosed patients were graded for severity by CECT abdomen.

Hence the dipstick for detecting urine trypsinogen-2 clearly stands apart in diagnosing acute pancreatitis without the aid of any other investigation.

There are various advantages of the proposed dipstick. Important one being its non-invasive nature. We just ask for a urine sample from the patient. The test can be immediately performed in the casualty/emergency department/outpatients. Department and results will be obtained within 5 minutes. These results are objective, reproducible and hence reliable.

As mentioned before the mean age of the diseased population was 39 years. This shows that the peak, productive population; the breadwinners of their family are succumbing to the morbidity of acute pancreatitis. And this is a productivity loss to the society and to the nation.

At the secondary level of prevention which focuses on early diagnosis and prompt treatment, we have a quickness and reliability in the dipstick for urine trypsinogen-2 to identify the problem cases and go for an aggressive management.

Funding: No funding sources

Conflict of interest: None declared

Ethical approval: The study was approved by the institutional ethics committee

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DOI: 10.5455/2320-6012.ijrms20140829

Cite this article as: Nittala R, Basheer OTM, Sasi MP. Acute pancreatitis: a study of urine trypsinogen-2 measurement as a screening test. *Int J Res Med Sci* 2014;2:897-902.