Original Research Article

Urinary trypsinogen-2 dipstick test for point-of-care screening of acute pancreatitis

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Received: 07 March 2019
Accepted: 30 March 2019

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ABSTRACT

Background: Acute pancreatitis (AP) is associated with high mortality in its severe form. Conventional laboratory tests used in its diagnosis are fraught with multiple shortcomings. Early institution of intravenous fluid resuscitation can reduce morbidity and mortality. Measurement of urinary trypsinogen-2 using a bedside urine dipstick test may prove useful in early identification of AP.
Methods: Patients with symptoms consistent with AP, attending the emergency department, at a tertiary care hospital in southern India, between November 2014 and November 2016, were included in a prospective observational study. The patients underwent routine investigations and additionally were tested with a urinary trypsinogen-2 dipstick test (UTT). The diagnostic performance and the time to reporting of the different investigations were compared with those of UTT. Final diagnosis of AP, made by clinicians, served as the standard.
Results: The sensitivities of serum amylase, serum lipase, UTT, ultrasonography (USG) and contrast-enhanced computed tomography (CECT) were 97.1%, 94.1%, 92.7%, 98.3% and 100%, respectively. The respective specificities were 92.4%, 98.5%, 98.5%, 100% and 100%. The average time required to obtain the test report was about half hour from admission in case of UTT, compared to about 3 hours for serum amylase/lipase, 4 hours for USG and 6 hours for CECT.
Conclusions: The results indicate that UTT test, due to its high performance indices, simplicity and faster availability of reports, can serve as an ideal screening test for AP and help in early institution of treatment.

Keywords: Bed-side, Diagnostic, Pancreatitis, Trypsinogen-2

INTRODUCTION

Acute pancreatitis (AP) is a sudden inflammatory process of the pancreas that evolves over a short period of time, usually hours. The severity of AP may range from mild disease to a severe, life-threatening illness. In its severe form, which is seen in 20% of the patients, there is progressive inflammation and necrosis of the pancreas with associated local or systemic complications and/or organ failures, with significant mortality.[1] Severe forms are recognized by a two-phase systemic disease. In the first phase, which corresponds to host response to local pancreatic injury, there is extensive pancreatic inflammation and/or necrosis followed by systemic inflammatory response syndrome (SIRS) that may lead to multiple organ dysfunction syndrome (MODS) within the first week and accounts for 50% of the deaths. Unless the first phase is arrested by early intervention, the second phase ensues, usually in the second week of illness, including the development of infected pancreatic necrosis to overt sepsis, MODS and death.[2-4]

As the clinical presentation of AP is similar to a number of other acute illnesses, it is difficult to make a diagnosis only on the basis of symptoms and signs. The standard criteria for diagnosing AP includes (1) abdominal pain consistent with pancreatitis, (2) serum lipase and/or amylase ≥3 times the upper limit of normal, and (3) characteristic findings from abdominal imaging.[3,4] These tests are either non-specific, inconsistent or expensive and not universally available.[5] Several prognostic scoring systems based on clinical, laboratorial and radiologic evaluations have been created or adapted to predict outcome-Ransons, APACHE II, bedside index for severity in acute pancreatitis (BISAP), computed tomography severity index, C-reactive protein (CRP), trypsinogen activation peptide (TAP), but most have unsatisfactory accuracy. These scoring systems are either time-consuming, complex and cumbersome or are expensive and not widely available.[6,7]

Early aggressive intravenous hydration with isotonic crystalloid solution in the first 12-24 hours of presentation is considered essential in the management of AP.[8-10] This strategy helps in reducing the risk and extent of pancreatic necrosis, leading to improved clinical outcomes, possibly by improvement in pancreatic perfusion. A rapid and accurate method to identify AP is necessary to achieve this.

Measurement of trypsinogen-2 in the urine may be useful as a bedside test for AP and help in initiating early aggressive goal-directed treatment. There have been multiple reports favouring the use of a 5-minute point-of-care urinary trypsinogen-2 dipstick test (UTT), for early and accurate diagnosis of AP.[11-17] We have evaluated the performance of this test vis-à-vis the routine tests and the advantages it offers.

METHODS

The study was conducted in the 24-bedded emergency department (ED) of a tertiary care hospital, located in southern India. The study was conducted only after approval from the institutional ethics committee.

Population

All adult, non-pregnant, normorenal patients presenting to emergency department, from November 2014 to November 2016, with acute upper abdominal pain with suspected pancreatitis were eligible to be included in the study. Those with a history of pancreatic/biliary surgery or endoscopic retrograde cholangiopancreatography (ERCP) within the previous 60 days were excluded from the study. The patients who were willing to provide written informed consent and a urine sample in addition to routine investigations for diagnosis of AP were included in the study.

This was a prospective observational study, wherein, the laboratory investigations - serum amylase and lipase, aimed at diagnosing AP, were assessed and compared specifically against UTT, with respect to their diagnostic accuracy. Serum amylase/lipase was considered positive for AP if the value was ≥3 times the upper normal limit (ULN). Final diagnosis of AP made by clinicians, based on clinical course, elevated enzyme levels and imaging findings, served as the standard.

Methodology

The patients included in the study underwent all assessments as per the routine hospital practice which included clinical examination, laboratory assessment of haematological parameters, liver function tests, kidney function tests, serum amylase and serum lipase. In addition to these, a urine sample was collected from each patient to test for trypsinogen-2, using the UTT (Actim Pancreatitis; Medix Biochemica) test strip, which works on the principle of immunochromatographic measurement of trypsinogen-2. The test strip is dipped into the patient's urine for 20 seconds within 15 minutes of collection and read after keeping for 5 minutes at room temperature. The sample and blue latex–labelled anti-trypsinogen 2 monoclonal antibodies migrate up the strip and in the presence of excess urinary trypsinogen (>50µg/L) result in the appearance of 2 blue stripes, indicating a positive result; a negative result is demonstrated by only one blue stripe.

Outcomes

The outcomes of interest were, primarily, the sensitivity and specificity of the UTT in the screening of AP and the time advantage it offers over other routine tests. The performance of UTT with regard to its sensitivity, specificity, positive predictive value and negative predictive value, in comparison to serum amylase, serum lipase and imaging modalities have also been evaluated.

Statistical analysis

Based on the prospective observational design of the study, using the single proportion - relative precision formula, a sample size of 134 was calculated, to achieve a precision of 15% with 95% confidence interval (CI). Categorical variables are reported using frequency and percentage. Continuous variables are reported using mean and standard deviation for normally distributed variables otherwise median and inter quartile range are used. The sensitivity, specificity, positive predictive value and negative predictive values of the tests are reported. All the analysis was done using SPSS version 18.0.

RESULTS

Patients

A total of 134 patients with suspected AP were included in the study. The mean age of the patients was 42.83±15.4 years with roughly even distribution in

different age-groups. Majority (78%) of the patients were males. Among the included subjects, 68 had a final diagnosis of AP. Alcohol (in 64.7%) and cholelithiasis (30.9%) were the commonest causes identified.

Test results

The number of positive and negative cases identified by UTT, along with its diagnostic performance are summarized in Table 1.

Table 1: Diagnostic performance of urinary trypsinogen-2 test (actim pancreatitis).

Urinary Trypsinogen-2 test result	Diagnosis of acute pancreatitis		Total
	Positive	**Negative**	
Positive	66	5	71
Negative	2	61	63
Total	68	66	134
Test performance			
Parameter	**Value**	**95% CI**	
Sensitivity	97.06%	89.78% to 99.64%	
Specificity	92.42 %	83.20% to 97.49%	
Positive likelihood ratio	12.81	5.51 to 29.79	
Negative likelihood ratio	0.03	0.01 to 0.12	
PPV	92.96%	85.02% to 96.84%	
NPV	96.83 %	88.60% to 99.17%	

Table 2: Comparison of diagnostic accuracy of different tests.

Test	Sensitivity	Specificity	PPV	NPV
UTT (n = 134)	97.1%	92.4%	93.0%	96.8%
Serum amylase (n = 134)*	94.1%	98.5%	98.5%	94.2%
Serum lipase (n = 134)*	92.7%	98.5%	98.4%	92.9%
USG (n = 123)	98.3%	100%	100%	98.4%
CECT (n = 43)	100%	100%	100%	100%

* For serum amylase (normal limits: 25 - 115 U/L) and lipase (normal limits 73 - 393 U/L), 3 times the upper limit of normal was considered as cut-off.

There were 5 false positive cases, which were ultimately diagnosed as one case each of gastro-intestinal bleeding, peptic ulcer disease, alcohol intoxication, chronic pancreatitis and alpha 1-antitrypsin deficiency.

The corresponding values for sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) for the different tests performed in the study are provided in Table 2.

Time required for testing from presentation to ED

A summary of the time required for collecting and/or sending out the samples for laboratory evaluation or for preparation and taking the patient in for imaging followed by the availability of test report, from the time the patients presented to the ED is provided in Figure 1. The average time taken to collect and/or send the samples for serum amylase and lipase was 142 minutes (2.36 hours). For UTT the time required was only 29 minutes. The average time taken to send laboratory tests depend on various factors like patient affordability, sample collection, processing and transport. Similarly, the average time required to obtain the report (and thereby the diagnosis of AP) was 178 minutes for both serum amylase and lipase and only 34 minutes for UTT. The average time for confirmation of AP using USG and CECT was 242 minutes and 370 minutes, respectively.
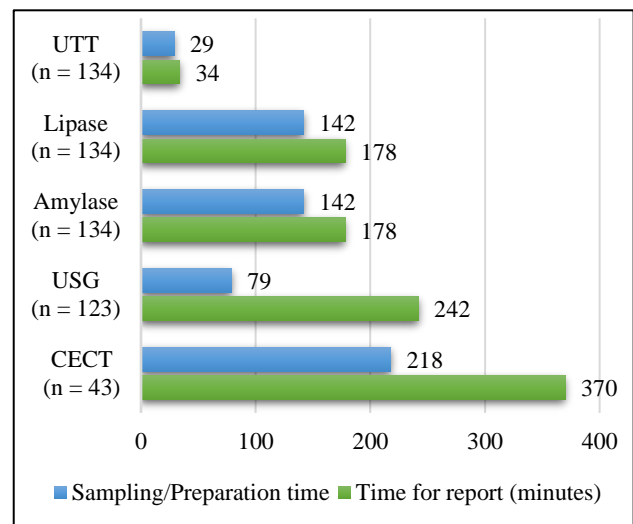

Figure 1: Average time required for obtaining different test reports in acute pancreatitis.

DISCUSSION

AP continues to be a diagnostic and therapeutic challenge due to lack of a single pathognomonic laboratory or clinical sign and lack of interventions targeting the underlying pathology. It is expected that early diagnosis and therapy will improve outcomes.[2,18-20] Early (within 24 hours) aggressive fluid resuscitation is recommended by clinical practice guidelines and is a long-established cornerstone of the initial management of AP.[8-10] By providing micro- and microcirculatory support, fluid resuscitation is associated with reduced SIRS, organ failure and in-hospital mortality.[8,9] However, for instituting this early fluid therapy, the diagnosis of AP needs to be confirmed at the earliest.

Trypsinogen, a pancreatic proteinase, has two major isoenzymes-trypsinogen-1 and trypsinogen-2. Both these isoenzymes are release in limited quantities into circulation and are readily filtered through the glomeruli. Healthy people have higher concentrations of trypsinogen-1; those with AP have a preferential elevation of trypsinogen-2. It is greatly elevated in the early stages of AP and, importantly, remains elevated for several days or even weeks.[11,12] Serum amylase and lipase are the most common laboratory markers used to establish the diagnosis of AP. But they can be non-specific, depending on the time since onset of pain, other intra-abdominal processes, and concomitant chronic diseases such as renal insufficiency.[19] In AP, serum amylase levels increase within 2-12 hours and return to normal within a week, while serum lipase levels increase within 4-8 hours and remain elevated for 8-14 days; urinary trypsinogen-2 levels can remain elevated up to 30 days.[5,21] CECT is the most accurate method for diagnosing and assessing the severity of AP, but cannot always be performed due to its cost, limited availability and potential side effects of the contrast material. In such a situation, a rapid, simple, inexpensive and accurate diagnostic test is highly desirable.

Table 3: Studies that have evaluated urinary trypsinogen-2 (UTT) performance.

Study[12,13,15-17,22-27]	Serum amylase (%)		Serum lipase (%)		UTT (%)	
	Sensitivity	Specificity	Sensitivity	Specificity	Sensitivity	Specificity
Kemppainen et al	85	91	-	-	94	95
Kylänpää-Bäck et al	-	-	-	-	96	92
Lempinen et al	-	-	-	-	62	87
Pezzilli et al	-	-	-	-	53.3	-
Lempinen et al	-	-	-	-	72	81
Saez et al	74	86.4	84	85.7	68	86.4
Chen et al	-	-	-	-	89.6	85.7
Jang et al	41	95	53	99	100	96
Kamer et al	78	87.3	86.2	89.4	91	72
Abraham P et al	75	87.8	64	90.2	73.9	94.6
Mayumi et al	-	-	-	-	68.6	87.1
This study	94.1	98.5	92.7	98.5	97.1	92.4

Present study revealed high sensitivity and specificity of UTT, which was comparable to serum amylase and lipase measurements. The sensitivity and specificity of the different laboratory tests observed in different studies which evaluated the utility of UTT in diagnosing AP is summarized in Table 3. The observations from these studies show that UTT is similar in sensitivity and specificity to serum amylase and lipase measurements. A recent meta-analysis of studies, which evaluated the use of urinary trypsinogen-2 for diagnosing AP, reported a pooled sensitivity, specificity and AUC of 80%, 92% and 0.96, respectively.[11] Kemppainen et al, reported that the sensitivity of the UTT dipstick test was superior to that of serum amylase and a negative UTT result rules out AP with a high probability, while a positive result usually identifies patients in need of further evaluation.[12] Sáez et al, also reported that UTT showed a clinical value similar to amylase and lipase.[13] Kylänpää-Bäck et al, suggested that UTT is better suited for screening of AP owing to its higher probability to exclude those without the disease compared to serum lipase.[14] Abraham et al, showed that the sensitivity and NPV of serum amylase and serum lipase tests were comparable to that of the UT but the specificity and PPV of the UTT test were better.[15] They also had reported a higher specificity of UTT compared to the imaging modalities - USG and CECT. Our study, however, revealed 100% sensitivity and specificity of CECT and 98.33% and 100% sensitivity and specificity of USG, respectively, in diagnosing AP.

Table 4: Characteristics of laboratory tests for acute pancreatitis.

Parameter	Serum amylase/lipase	UTT
Accuracy	High	High
Sample	Blood	Urine
Infrastructure	Laboratory facility	Dipstick (strip)
Time required	Long (minutes to hours) (> 2.5 hours in this study)	Very short (as less as 5 minutes) (30 minutes in this study)

It is evidently clear that UTT is comparable to the conventional laboratory tests used for diagnosing AP. But, importantly, it offers other advantages over them which make it the most attractive screening test for AP (Table 4). UTT does not require laboratory facilities and can be performed almost instantaneously with results

available within 5-6 minutes; serum amylase and lipase estimations would require much more time (more than 2.5 hours in present study) to become available to the treating physician. When compared to the imaging tests, UTT offers to be inexpensive, faster, simpler and universally utilizable. Early initiation of fluid resuscitation based on UTT report has the potential to improve outcomes in AP patients.

The limitations of the study were its single-centre design and small number of patients with AP. Being an observational study, the investigations being compared in the study were not controlled with respect to their timing and imaging studies were not conducted in all patients. Since, there is no single definitive criterion for diagnosing AP, the final hospital discharge diagnosis of AP was considered for evaluating test performance. The impact of using UTT on disease course or outcome could not be studied.

CONCLUSION

This study suggests that the 5-minute point-of-care urinary trypsinogen-2 test has high sensitivity and specificity, compares favourably with serum amylase and lipase measurements and appears to be suited for use as a screening test for AP. The test can also reduce diagnosis time and help in initiating therapy earlier.

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