Original Research Article

A comparative analytical study of the impact of urine trypsinogens-2 in the diagnosis of acute pancreatitis

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Abstract: Background: Acute pancreatitis is a very common disorder with a substantial burden on the healthcare system. Acute pancreatitis includes a wide spectrum of diseases varying from mild self-limiting symptoms to fulminant multi-organ failure and high mortality. Serum amylase and serum lipase, which are used for diagnosing acute pancreatitis, are relatively less sensitive and specific and give a lot of false positive or false negative values. Based on the immune-chromatographic method, the urinary trypsinogen-2 dipstick test is proposed to be a rapid method for diagnosing acute pancreatitis at the earliest.

Aim of the study: 1. To know the usefulness of urine trypsinogen-2 in accurately diagnosing acute pancreatitis 2. To compare the diagnostic role of urine trypsinogen-2 with that of serum amylase, serum lipase, and imaging studies in acute pancreatitis.

Materials and Methodology: 100 Patients presenting with acute upper abdominal symptoms like pain, vomiting, and abdominal distention, admitted to the emergency department of our hospital from January 2021 to January 2022, are enrolled in the study. Urine samples were obtained from all the patients and tested with Spot Urine trypsinogen-2 dipstick. Serum amylase and serum lipase tests were also simultaneously done in these patients. If required, patients are also evaluated with (USG) abdomen and (CECT) abdomen. The final diagnosis of acute pancreatitis is based on the clinical picture, serum amylase more than the threefold rise, and radiological findings. Urine trypsinogen-2 dipstick tests were compared with serum amylase, serum lipase, and imaging studies in patients with a final diagnosis of acute pancreatitis.

Results: Sensitivity of amylase and lipase was found to be 73.77% and 59.02%, respectively, whereas the sensitivity of trypsinogen was found to be 78.69%. The specificity of amylase and lipase was found to be 89.74% and 89.74%, respectively, whereas the specificity of trypsinogen was found to be 92.1%. Analyzing the data, it is found that the sensitivity and specificity of trypsinogen are higher than the routine investigations. Even though it has a low range of sensitivity, its high specificity ensures that the test can be used as a screening test to check the true negative cases.

Conclusion: 1. The urine Trypsinogen-2 dipstick test is a simple, rapid, easy, and noninvasive test that can diagnose or rule out most cases of acute pancreatitis. 2. Urine Trypsinogen-2 estimation does not require laboratory facilities. It is undertaken almost instantaneously (within 5 minutes) as opposed to serum amylase and lipase, results for which may require an hour to get back to the physician. 3. The urinary trypsinogen-2 test could be used as a screening test for acute pancreatitis. 4. Modification of the cutoff point of this assay increases the specificity to the point where it can be used for diagnosis. Qualitative rapid urine trypsinogen-2 test strip is easy to perform. Moreover, it is a reliable and useful screening test for acute pancreatitis in daily practice, particularly in healthcare units lacking laboratory facilities.

Keywords: Acute pancreatitis; Serum amylase; Serum lipase; Urine trypsinogen-2.

1. Introduction

A Cute pancreatitis is a very common disorder, with a substantial burden on the healthcare system [1]. Acute pancreatitis includes a wide spectrum of diseases varying from mild self-limiting symptoms to fulminant multi-organ failure and high mortality. The overall mortality rate is 3-10%, wherein 11-30% of cases are with severe disease manifested as pancreatic necrosis [2].

Since 1974, several scoring systems have been developed clinically and radiologically assessing the prognosis of the disease [3]. The rationale behind the severity assessment is mainly for practical purposes, where mild pancreatitis needs supportive care. However, severe pancreatitis needs intensive monitoring, and it has a guarded prognosis [4]. The key to reducing the mortality and morbidity of the disease is early detection and appropriate management [5]. An ideal diagnostic method can differentiate between patients with mild & severe disease, have easy usability, is widely available and accurate, and has low inter-observer variability. It should be able to detect early disease so that patients, before developing potential complications, could be monitored and treated, if possible empirically [6].

Serum amylase and serum lipase, used for diagnosing acute pancreatitis, are relatively less sensitive and specific and give a lot of false positive or false negative values [7]. Various scoring systems are used in acute pancreatitis to predict the severity and outcome of the disease. There is no single comprehensive test to aid in the early and accurate detection of acute pancreatitis [8].

The urinary trypsinogen-2 dipstick test is proposed to be a rapid method for diagnosing acute pancreatitis at the earliest, based on the immune-chromatographic method [9].

2. Aim

1. To know the usefulness of urine trypsinogen-2 in accurately diagnosing acute pancreatitis
2. To compare the diagnostic role of urine trypsinogen-2 with that of serum amylase, serum lipase and imaging studies in acute pancreatitis.

3. Material and Methods

- **Study design:** An institutional based Comparative Analytical study.
- **Study area:** Study was conducted at Alluri Sitarama Raju Academy of Medical Sciences (ASRAM) of Eluru District, Andhra Pradesh.
- **Study period:** Study was conducted for one year from January 2021 to January 2022.
- **Study Population:** Patients presenting with acute upper abdominal symptoms like pain, vomiting, abdominal distention, admitted in the general surgery department of our hospital from January 2021 to January 2022 are enrolled in the study.
- Sample size: 100 patients (who fits inclusion and exclusion criteria).

3.1. Methodology

- Urine sample were obtained from all the patients and tested with Spot Urine trypsinogen-2 dipstick.
- Serum amylase and serum lipase tests were also simultaneously done in these patients.
- Patients are also evaluated with (USG) abdomen and (CECT) abdomen,if required.
- Final diagnosis of acute pancreatitis is made on the basis of clinical picture, serum amylase more than threefold rise and radiological findings.
- Urine trypsinogen-2 dipstick test were compared with serum amylase, serum lipase and imaging studies in patients with final diagnosis of acute pancreatitis.
- Observations are tabulated according to the pre-designed proforma.
- The results are analyzed using Microsoft Excel for tabular transformation and graphical representation. For comparing the parameters, Chi Square test or Fischer's exact test are used. SPSS software will be used for statistical analysis.

3.2. Inclusion criteria

- Patients presenting with features suggestive of acute pancreatitis
- Male and female subjects of age between 20 - 60 years shall be selected.
- Adult subjects willing to give informed consent.

3.3. Exclusion criteria

- Subjects below the age of 20 years and above the age of 60 years
- Subject who are not willing to participate in the study.
- Individuals who are cognitively impaired and/or who are unable to give informed consent.
- Proven cases of chronic pancreatitis and pancreatic cancer
- Hereditary pancreatitis, cystic fibrosis

3.4. Statistical analysis

The data was entered in Microsoft Excel sheet. The results are analyzed using Microsoft Excel for tabular transformation and graphical representation. For comparing the parameters, Chi Square test or Fischer's exact test are used. SPSS software will be used for statistical analysis.

3.5. Ethical issues

Clearance from the institutional ethical committee was obtained prior to the start of the study. Written consent was taken beforehand for their participation.

4. Results

Table 1. Age Distribution

Age Range (years)	No. of patients	Percentage (%)
21yrs - 30yrs	24	24
31yrs - 40yrs	25	25
41yrs - 50yrs	36	36
51yrs - 60yrs	13	13
>60yrs	2	2
Total	100	100 %

Table 1: The age group of patients enrolled in this study ranges from 20 to 80 yrs. The peak incidence was noted in the 4th decade of life.
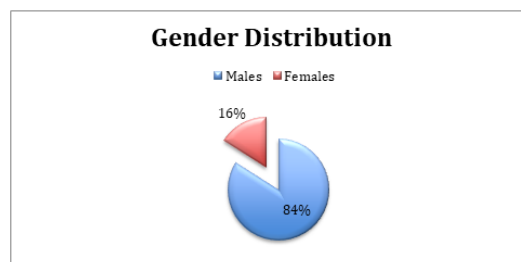

Figure 1. Gender Distributions

Figure 1: Out of 100 patients enrolled in this study, there were 84 male and 16 female patients.
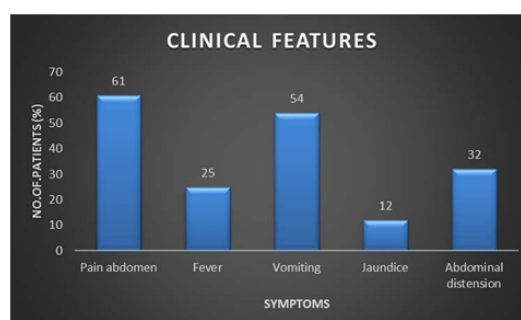

Figure 2. Gender Distributions

Figure 2: On clinical presentation,61% of patients presented with abdominal pain as chief complain. Rest of the patients had vomiting, abdomen distension and fever along with the presenting symptoms. Pain abdomen and vomiting are the predominant features followed by abdominal distention.
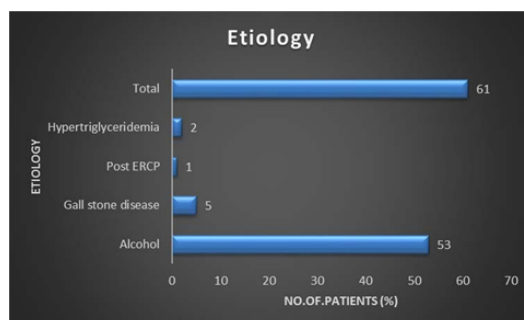

Figure 3. Gender Distributions

Figure 3: History of consumption of alcohol and the possibility of it being the etiological factor were found in 53 patients. Gall stone disease was attributed in 5 patients. Hyperlipidemia and Post ERCP, as a causative factor in 2 & 1 patients, respectively.

Table 2. Comparison of Different Parameters Used In The Diagnosis Of Acute Pancreatitis

Parameters	Acute Pancreatitis(61)	Others(39)	Total(100)
AMYLASE	45	4	49
LIPASE	36	4	40
USG	53	-	53
CECT	14	-	14
TRYPSINOGEN	48	3	51

Table 2: Urine trypsinogen found to be positive in 48 of 61 acute pancreatitis patients, it showed falsely positive in only 3 patients. Where amylase was positive in 45 of 61 cases and lipase in 36 of 61 cases. USG and CT are positive in 53 and 14 patients respectively.

Table 3. Estimation of Significance of The Test

Urine trypsinogen	Acute Pancreatitis	Non pancreatic causes	Total
Positive	48	3	51
Negative	13	36	49
TOTAL	61	39	100

Table 3: Sensitivity of the test is 78.6% and specificity is found to be 92.3%.

- Sensitivity= a/(a+c) ;48/61=78.6
- Specificity=d/(b+d);36/39=92.3
- Using Kappa statistics the value was found to be 0.67.
- (Based on criteria originally proposed by Landis and Koch: kappa values greater than about 0.75 are often taken as representing excellent agreement; those between 0.4 and 0.75 as fair to good agreement; and those less than 0.4 as moderate or poor agreement.
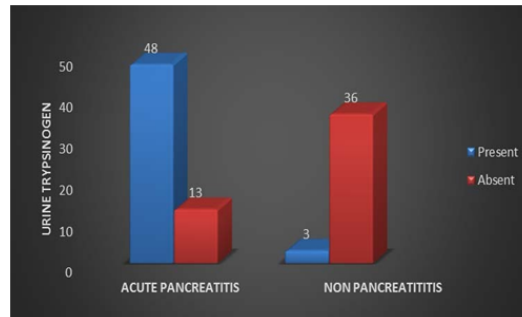
Figure 4. Gender Distributions

Figure 4: Out of 61 acute pancreatitis patients urine trypsinogen test was positive in 48 and negative in 13 patients. The test also showed false positives in 3 of 39 normal patients, and it is truly negative in 36 out of 39 normal patients.

5. Discussion

Trypsinogen, a precursor of trypsin, is required for protein digestion. Premature trypsin activation leads to pancreatic self-digestion. Trypsinogen is a 25-kd pancreatic proteinase. In human pancreatic juice, there are three trypsinogen (TPS) isoenzymes, namely, cationic (TPS-1) and anionic TPS (TPS-2), and a minor isoenzyme (TPS-3)[9].

The inactive form of trypsinogen, stored in the cytoplasmic zymogen granules of pancreatic acinar cells, is secreted into the adjacent duct lumen and is subsequently delivered to the small intestine. Within the intestine, they are activated by enterokinase. Premature activation of trypsinogen to trypsin within the pancreas is the prime pathophysiologic event in the development of acute pancreatitis [11]. Under normal conditions, trypsinogen is secreted into pancreatic fluid, and only a small amount enters the circulation. For unknown reasons, the tubular reabsorption of trypsinogen-2 is lower than trypsinogen-1 [12].

The urinary trypsinogen-2 dipstick test is proposed to be a rapid and simple method for the early diagnosis of acute pancreatitis [13].

Urine trypsinogen-2 concentrations were measured using a dipstick test (Actim Pancreatitis, Medix Biochemica Oy AB,Kauniainen, Finland) based on an immunochromatography assay. The detection limit of the test is approximately 50 ng/mL. The test strip has a control line, and two lines indicate a positive result. The test results can be read after 5 minutes [14].

The test strip dipped into the urine sample, contains trypsinogen-2 bound to monoclonal antibody labeled blue latex particles, which migrate across a nitrocellulose membrane with a zone containing another antibody specific for an epitope on trypsinogen-2 [15].

Though CT and USG showed 100% accurate results and are the best modalities, with CT being the gold standard to diagnose acute pancreatitis, the urine dipstick method is rapid and can be made easily available in primary setup also [16].

Two retrospective studies. [10,17] carried out by a Finnish group showed that Urine Trypsinogen-2 had better sensitivity and specificity in predicting Acute Pancreatitis than amylase and lipase. A later larger prospective cohort study [18] by the same group enrolling 53 patients with AP and 447 patients with AAD (non-pancreatic) showed that Urine Trypsinogen-2 has a sensitivity of 94% and a specificity of 95%, better than serum amylase (85% and 91%) and urinary amylase (83% and 88%) in predicting Acute Pancreatitis.

The sensitivity of amylase and lipase was found to be 73.77% and 59.02%, respectively, whereas as the sensitivity of trypsinogen was found to be 78.69%. The specificity of amylase and lipase was found to be 89.74% and 89.74%, respectively, whereas as the specificity of trypsinogen was found to be 92.1%. Analyzing the data, it is found that the sensitivity and specificity of trypsinogen are higher than the routine investigations. Even though it has a low range of sensitivity, its high specificity ensures that the test can be used as a screening test to check the true negative cases. False positive results are seen in 3 of the 39 non-pancreatitis cases, namely two cases of Gallstones and one case of renal failure. In case of renal failure, a defect in the excretion of trypsinogen makes the result positive. In the case of gallstone, studies with more sample size have to be conducted to analyze the etiology of the positivity of the test.

6. Conclusion

1. Urine Trypsinogen-2 dip stick test is a simple, rapid, easy, and noninvasive test which can diagnose or rule out, most of the cases of acute pancreatitis.
2. Urine Trypsinogen-2 estimation doesn't require laboratory facilities. It is undertaken almost instantaneously (within 5 minutes) as opposed to serum amylase and lipase, results for which may require an hour to get back to the physician.
3. The urinary trypsinogen-2 test could be used as a screening test for acute pancreatitis.
4. Modification of the cutoff point of this assay increases the specificity to the point where it can be used for diagnosis.
5. Qualitative rapid urine trypsinogen-2 test strip is easy to perform.

And hence it has been shown to be a reliable and useful screening test for acute pancreatitis in daily practice [12-16], particularly in healthcare units lacking laboratory facilities.

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Conflicts of Interest: "The authors declare that they do not have any competing interests."

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