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INTF ratio AIM MET depa RES	RODUCTION: Acute can be used to dist : To study serum am 'HOD: Prospective artment and subject ULTS: Amylase has p	pancreatitis patients are mostly caused by gall stone or chronic alcoholism. Serum Lipase/amylase inguish the etiology. ylase, lipase, lipase/amylase ratio in patients of acute pancreatitis. study of 549 patients suffering from acute pancreatitis. Blood sample collected in emergency et to analysis in SEIMENS auto analyser by spectrophotometry. positive correlation 0.252, 99% CI with alcoholic pancreatitis with mean lipase/amylase ratio=4.0.			

In gallstone pancreatitis mean lipase/amylase ratio=1.67. If L/A ratio =2.3, +LR=5.63, -LR=0.17, sensitivity85.7% & specificity84.8%, can be used as cutoff.

CONCLUSION: We find Lipase/Amylase ratio of >2.3 suggests alcoholic origin in whom no acute intervention is indicated while ratio< 2.3 of biliary origin in whom surgical intervention required.

Alcoholic Pancreatitis, Gallstone Pancreatitis, Lipase/Amylase(L/A) ratio

INTRODUCTION:

Acute pancreatitis includes a wide spectrum of disease, from mild self-limiting symptoms to a fulminant process with multiple organ failure and high mortality ^[1].

Approximately 80% of cases are associated with gall stone or chronic alcohol abuse. The course of disease is essentially the same but difference arises in management. Patients with gall stone pancreatitis undergo stone extraction through ERCP, followed by cholecystectomy to prevent recurrence. Patients with alcoholic origin for AP can be spared of such interventional procedure. These differences in therapeutic approach justify a quest for non-invasive tests that can help in differential diagnosis. Gumaste et al postulated lipase/amylase ratio as new index to distinguish alcoholic from non-alcoholic acute pancreatitis. ^[2]

The studies were conducted following conflicting results; while in some it was confirmed that the L / A ratio was useful in differentiating the alcohol because of other causes $^{[3,4,5]}$. The only study done in India by Anitha et al on South Indian population concluded that the serum L/A ratio greater than 3 can be used to differentiate alcoholic from non-alcoholic pancreatitis.^[22]

With this background we embarked on an study of studying serum amylase, lipase, amylase/lipase, in differentiating alcoholic from gallstone pancreatitis in Assam Medical College and hospital one of premier tertiary care hospital receiving patients from Assam and neighbouring sister states of north east region.

AIMS AND OBJECTIVES:

To study serum amylase, lipase, lipase/amylase ratio in patients of acute pancreatitis and to decide a possible cut off value for serum lipase/amylase to distinguish between gall stone (biliary) and alcohol as etiology.

MATERIALS AND METHODS:

We conducted a prospective study in Department of Biochemistry in Assam Medical College and hospital over 1 year duration and received patients admitted in Department of Surgery and Medicine from June 2015- May 2016. A total of 549 patients were enrolled for the study after application of inclusion and exclusion criteria and after taking approval of institutional ethical committee and patient's consent. Data was recorded using prestructured interview form. The diagnosis of acute pancreatitis based on patients with history of upper abdominal pain presenting within 72 hrs of onset, atleast 3 folds increase in serum amylase and lipase levels with diagnosis confirmed on Ultrasonography and/or CECT abdomen and/ or MRCP during the hospital stay. For diagnosis of gall stone pancreatitis in addition to the above mentioned criteria evidence of sludge or gall stones in gall bladder and/or common bile duct with no history of alcoholism was taken into account .For diagnosis of alcoholic pancreatitis all patients with alcohol consumption of 80 gm of ethanol per day for a period greater than 6 years with no evidence of gall stone in present scans or on history were taken.

Blood samples for determination of serum amylase lipase were collected on patient's presentation to emergency department. The serum Amylase and Lipase were analysed in Siemens Auto analyzer with its dedicated reagents.

LIPASE- LIPL method is an in vitro diagnostic test for the quantitative measurement of lipase in human serum.LIPL method is an adaptation of the colorimetric method described by Neumann et al.

PRINCIPLE OF PROCEDURE- The LIPL method uses as a substrate1, 2-O-dilauryl-rac-glycero-3glutaric acid-(6'methylresorufin) ester. Lipase catalyzes the hydrolysis of this substrate in presence of co lipase, bile salt and CaCl₂ at alkaline pH.The hydrolysis produces 1,2-O-dilauryl-rac-glycerol and glutaric acid-6'methylresorufin ester. Glutaric acid-6'methylresorufin ester is an unstable reaction intermediate and breaks down to yield chromogenic free methylresorufin in proportion to the activity of lipase in the sample. The rate of production of methylresorufin is measured by a bichromatic rate reaction at 577 and 700 nm spectrophotometrically.

AMYLASE-

The AMY method is an invitro diagnostic test intended for the quantitative determination of amylase activity in human serum.

PRINCIPLE OF PROCEDURE

- à-Amylase catalyzes the hydrolysis of a defined synthetic substrate CNPG3 (2-Chloro-4-Nitrophynyl-à-D-maltotrioside) to yield CNP (2-chloro-4-nitrophenol), CNPG2 (2-chloro-4-nitrophynyl-à-D-maltoside), maltotriose (G3) and glucose. After an incubation of 70 seconds at 37 degree Celsius, the absorbance due to formation of CNP is measured spectrophotometrically using a bichromatic (405,577nm) rate technique.

Exclusion criteria

1} Renal failure

2} DM

3} Congenital pancreaticobiliary anomaly

4} Chronic pancreatitis (pancreatic duct dilatation, calcification, history or evidence of malabsorption)

5} Dyslipidaemia

6} Trauma

7) Drug induced pancreatitis

8} Idiopathic pancreatitis

9} Hereditary pancreatitis

To calculate lipase to amylase ratio they were converted into multiples of upper reference limits that were used [lipase-393, amylase-115]

Statistical analysis performed using IBM SPSS(Version 16.0)

RESULT-

Age Distribution:

Considering the age distribution of all pancreatitis patients majority(n=210)belong to 30-40 years age group followed by 120 patients in age group 40-50 years followed by 90 patients in20-30 years. Hence majority of the patients belong to middle age group.51 patients (9.3%)belong to geriatric age group .2.2% of the patients belong to adolescent age group.



Figure 1: Pie representation of age interval wise distribution. While considering alcoholic pancreatitis 45.9%(n=252) group, majority of patients 16.9%(n=63) belong to age group 30-40 while 9.3%(n=51) are of 40-50 years age group.6%(n=33%) belong to geriatric age group while 1.6%(n=9)are adolescents. While comparing this statistics with gall stone pancreatitis 55.1%(n=297)group, majority 21.4%(n=117) belong to 30-40 years while 12.6%(n=69) belong to 40-50 years geriatric patients being 3.3%(n=18) while adolescents are 0.5%(n=3).Hence majority of patients (55.1%) suffered from gallstone pancreatitis with middle age group contributing the maximum burden of disease.

Gender distribution:

In alcoholic pancreatitis group out of 252 patients 63 %(n=159) are males and rest 37 %(n=93) being females. While in gall stone pancreatitis group out of 297 patients 64.6 %(n=192) are males while rest 35.4 %(n=105) being females.

3) Serum Amylase Levels:

The mean amylase in alcoholic pancreatitis group is 358.2(SD=390) which is significantly lower than mean of gall stone pancreatitis which is 690.8(SD=990.8) at 95% confidence interval.

Rise of serum amylase in gall stone pancreatitis is significantly higher than that in alcoholic pancreatitis.

4) Serum Lipase Levels:

The mean lipase level in alcoholic pancreatitis is 4329.5 which is higher but not statistically significant than that in gall stone pancreatitis 3435.7 at 95% confidence interval.

After running ANOVA Test we found that mean lipase levels in alcoholic pancreatitis is higher than in gall stone pancreatitis but not statistically significant at 95 % confidence interval.

5) Serum lipase/amylase ratio:

In alcoholic pancreatitis group mean lipase and amylase ratio is 4.0 while maximum being 12.5 and minimum 0.83 having SD of 2.19 and variance 4.80

In gallstone pancreatitis group mean lipase and amylase ratio is 1.67 while maximum being 5.66 and minimum 0.28 having SD of 0.91 and variance 0.82.

We find mean lipase to amylase ratio being significantly higher in alcoholic pancreatitis than in patients suffering from gall-stone pancreatitis .

Serum Lipasi						
DIAGNOSIS	Mean	Maxi- mum	Mini- mum	SD	Variance	Range
alcoholic pancreatitis	4.00	12.50	.83	2.19	4.80	11.67
gall stone pancreatitis	1.67	5.66	.28	.91	.82	5.38

Table 1: descriptive analysis of serum lipase/amylase ratio in gall stone and alcoholic pancreatitis.

6) Test of Normality of lipase/amylase ratio:

By applying Kolmogorov-Smirnov test normality to lipase /amylase ratio in acute pancreatitis values are found to have normal Gaussian distribution

7) Serum Amylase correlation:

By Kendall's tau b correlation analysis , amylase has got a positive correlation of 0.252 at 99% confidence interval with diagnosis of alcoholic pancreatitis.

8) Serum Lipase correlation:

According to Kendall tau b correlation statistics lipase has got a negative correlation of 0.148 at 99% confidence interval.

9)Serum Lipase/Amylase ratio:

Using Spearman's rho correlation method lipase/amylase ratio correlation is -0.700 at confidence interval of 99% means elevated lipase/amylase ratio is found in alcoholic pancreatitis

10) RECEIVER OPERATOR CHARACTERISTICS (ROC) Curve Analysis:

Analysis ROC curve characteristic for lipase/amylase ratio with respect for gallstone versus alcoholic acute pancreatitis at cut off level of lipase/amylase ratio 2.3 the sensitivity being 85.7% and specificity being 84.8.

The area under curve(AUC) of ROC plot is 0.906 at 99% significance.



Diagonal segments are produced by ties.

Figure 2: ROC curve if serum lipase/amylase ratio with gallstone and alcoholic pancreatitis.

Lipase/amylase ratio	Sensitivity(%)	Specificity(%)
2.0	95.2	73
2.3	85.7	84.8
2.5	73.5	90
3.0	62	94
3.5	46	96

Table 2: Diagnostics (at 95% confidence interval) fromROC curve analysis.

DISCUSSION

In western literature, alcohol is the most common cause of acute pancreatitis $^{\rm (15][16][17]}$

However biliary pancreatitis has been predominant in Japan, Taiwan and India. This is in accordance to our results being gall stone pancreatitis constituting 55.1% of patients. Majority of acute alcoholic pancreatitis patients 16.9 %(n=63) belong to age group 30-40 while 9.3%(n=51) are of 40-50 years age group.6%(n=33%) belong to geriatric age group while 1.6%(n=9)are adolescents. While comparing this statistics with gall stone pancreatitis 55.1% (n=297) group, majority21.4% (n=117) belong to 30-40 years while 12.6% (n=69) belong to 40-50 years. Hence majority of patients (55.1%) suffered from gallstone pancreatitis with middle age group contributing the maximum burden of disease.

Among both the groups there is significant difference (95% confidence interval) in gender affliction with males being more susceptible though there is no significant difference in likelihood of contracting gallstone vs alcoholic pancreatitis gender wise.

Earlier studies showed that rise in serum concentration of amylase in alcoholic pancreatitis was significantly lower than in gall stone pancreatitis. ^{[18][19][2]}.Serum lipase levels were not statistically different. Our study demonstrates similar results that is increase of serum amylase in gall stone pancreatitis is significantly higher than that in alcoholic pancreatitis while mean lipase levels in alcoholic pancreatitis is higher than in gall stone pancreatitis but not statistically significant at 95 % confidence interval. Hence we find that serum lipase to amylase ratio is significantly higher in alcoholic pancreatitis patients than in patients with gallstone pancreatitis

Gumaste et al ^[2] postulated that lipase /amylase ratio >2 in diagnosing acute alcoholic pancreatitis had sensitivity and specificity of 91.0% and 78.0% respectively. We found sensitivity 95.2% and specificity 73%.Tanner et al showed that L/A ratio>5 is exclusive for alcoholic pancreatitis with sensitivity 31%.We found that at L/A ratio greater than 5 diagnosis of alcoholic pancreatitis sensitivity 21.2% and high specificity99%.^[20]

Kazmierczak et al concluded L/A ratio>4.0 had a sensitivity of 92% but low specificity 60% while our result showed sensitivity of 33.3% and high specificity of 97%. ^[21]

While using L/A ratio 2.3 sensitivity is85.7% and specificity 84.8% and by using L/A 2.5, values are 73.5 and90% respectively. When we used L/A ratio3.0 as cut off, sensitivity was only62% but specificity at 94 %.Looking at these values we come to an agreement based on trade off between sensitivity and specificity that L/A ratio of 2.3 can be safely used as a cut off above which diagnosis of alcoholic pancreatitis can be predicted and below which diagnosis of biliary pancreatitis would be made.

By computing likelihood ratio to predict usefulness of various cut-off levels of serum lipase/amylase ratio we found that at cut-off level of 2 to diagnose acute alcoholic pancreatitis +LR=2.52,-LR=0.06. Hence this makes value L/A ratio of 2 only sometimes useful to accurately predict the diagnosis of acute alcoholic pancreatitis but when we use L/A ratio 2.3 we find +LR=5.63 AND –LR=0.17 which makes this cut-off value often useful to accurately diagnose the condition. While for cut-off values of L/A ratio=2.5 or 3.0 we found it to be only sometimes useful and at cut-off value of 3.5 it as only rarely useful

Lipase/Amylase	Positive Like- lihood ratio	Negative Likelihood ratio	Interpretation of test
2.0	2.51	0.06	Sometimes useful
2.3	5.63	0.17	Often useful
2.5	7.35	0.29	Sometimes useful
3.0	10.3	0.40	Sometimes useful
3.5	11.5	0.56	Rarely useful

Table 3: Likelihood ratio and interpretation of usefulness of various levels of L/A ratio

CONCLUSION

We would like to conclude that serum lipase to amylase ratio greater than 2.3 could be used to differentiate with values greater than 2.3 in favour of acute alcoholic pancreatitis and values less than 2.3 as acute gall stone pancreatitis. Hence serum lipase / amylase ratio may be useful as means of biochemical diagnostic tool to differentiate alcoholic from gallstone pancreatitis in acute pancreatitis setting in the background of relevant clinical and radiological assessment. This shall help in better triage of patient in emergency department.

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