VOLUME-6 | ISSUE-1 | JANUARY-2017 • ISSN No 2277 - 8179 | IF : 3.508 | IC Value : 78.46

Study of hyperbilirubinemia and serum aminotransferases level in patients of acute biliary pancreatitis.

Biochemistry

KEYWORDS: Acute biliary pancreatitis, Direct bilirubin, AST, ALT

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ABSTRACT

INTRODUCTION: Gallstones, being most common cause of acute pancreatitis, cause cholestasis leading to elevated levels of Aspartate Amino Transferase (AST), Alanine Amino Transferase (ALT) and bilirubin **AIM:** To study correlation of serum Total Bilirubin, Direct Bilirubin, ALT , AST in diagnosed cases of acute biliary pancreatitis. **METHOD:** Prospective study of 124 patients over 6 months. Blood samples collected and analysed. **RESULTS:** Mean total bilirubin is 2.31mg/dl. Mean Direct bilirubin is 1.5 mg/dl. A moderately strong positive correlations of serum direct bilirubin and ALT, R=0.597 & of direct bilirubin and AST levels,R=0.538 at CI= 99% exists. **CONCLUSION**: Serum direct bilirubin, Total bilirubin, AST, ALT can be used to predict Gallstones as etiology for Acute Pancreatitis. Serum Direct Bilirubin shows a fairly strong correlation with elevation of ALT & AST levels particularly more with serum ALT values in patients suffering from acute biliary pancreatitis.

INTRODUCTION: Gallstones are the most common cause of acute pancreatitis. There are no methods of differentiating acute biliary pancreatitis from alcoholic cause. Obstructions of biliary ducts, idiopathic pancreatitis may be related with biliary origin which needs identification for acute treatment. The obstructed common bile duct though at times transient causing pancreatic hypertension is implicated as central pathology for gall stone pancreatitis. The obstruction to bile ducts causes cholestasis with back pressure changes upstream causing hepatocyte injury. In a later stage of cholestasis Aspartate Amino Transferase (AST), Alanine Amino Transferase (ALT) and bilirubin may be elevated due to liver damage as a secondary effect of cholestasis.[4]

AIM : To study correlation of serum Total Bilirubin, Direct Bilirubin, ALT , AST in diagnosed cases of acute biliary pancreatitis.

METHODS: We conducted a prospective hospital based study in Department of Biochemistry in Assam Medical College and hospital over 6 months duration and received patients of diagnosed cases of biliary pancreatitis admitted in Department of Surgery from May 2016- October 2016. A total of 124 patients were included in study. The acute pancreatitis patients presented with history of upper abdominal pain within 72 hrs. of onset, with at least 2- 3 folds increase in serum amylase and lipase levels with diagnosis of gall stone pancreatitis confirmed on Ultrasonography or Contrast Enhanced Computed Tomography (CECT) abdomen or Magnetic Resonance Cholangiopancreatography (MRCP) during the hospital stay. For diagnosis of biliary pancreatitis in addition to the above mentioned criteria evidence of sludge or gall stones in gall bladder or common bile duct in the past.

Blood samples of biliary pancreatitis patients were analysed for estimation of serum total bilirubin, direct bilirubin, AST and ALT using Siemens Auto analyser in Advanced clinical biochemistry lab in Assam medical college and hospital

TOTAL BILIRUBIN (TBI): TBI method principle
Diazotized sulfanilic acid is formed by combining sodium nitrite and sulfanilic acid at low pH. Bilirubin (unconjugated) in the sample is solubilized by dilution in a mixture of caffeine/benzoate/acetate/EDTA. Upon addition of the diazotized sulfanilic acid, the solubilized bilirubin including conjugated bilirubins (mono and diglucoronides) and the delta form 4 (biliprotein-bilirubin covalently bound to albumin) is converted to diazo-bilirubin, a red chromophore representing the total bilirubin which absorbs at 540 nm and is measured using a bichromatic (540, 700 nm) endpoint technique.

Solubilized Bilirubin + Diazotized sulfanilic acid → Red chromophore (absorbs at 540nm)

DIRECT BILIRUBIN (DBI): DBI method principle
Diazotized sulfanilic acid is formed by combining sodium nitrite and sulfanilic acid at low pH. The sample is diluted in 0.5M HCl. A blank reading is taken to eliminate interference from non-bilirubin pigments. Upon addition of the diazotized sulfanilic acid, the conjugated bilirubin is converted to diazo-bilirubin, a red chromophore which absorbs at 540 nm and is measured using a bichromatic(540, 700 nm) endpoint technique.

Conjugated Bilirubin + Diazotized sulfanilic acid → Red chromophore (absorbs at 540 nm)

AST: Aspartate aminotransferase (AST) catalyzes the transamination from L-aspartate to α -ketoglutarate, forming L-glutamate and oxalacetate. The oxalacetate formed is reduced to malate by malate dehydrogenase (MDH) with simultaneous oxidation of reduced nicotinamide adenine dinucleotide (NADH). The change in absorbance with time due to the conversion of NADH to NAD is directly proportional to the AST activity and is measured using a bichromatic (340, 700 nm) rate technique

$$\text{L-aspartate} + \alpha - \text{ketoglutarate} \xrightarrow{\text{AST (pH 7.8)}} \text{L-glutamate} + \text{oxaloacetate}$$

$$\text{Oxaloacetate} + \text{NADH} \xrightarrow{\text{MDH}} \text{Malate} + \text{NAD}$$

ALT: Alanine aminotransferase (ALT) catalyzes the transamination of L-alanine to α-ketoglutarate (α-KG), forming L-glutamate and pyruvate. The pyruvate formed is reduced to lactate by lactate dehydrogenase (LDH) with simultaneous oxidation of reduced nicotinamide-adenine dinucleotide(NADH). The change in absorbance is directly proportional to the ALT activity and is measured using a bichromatic (340, 700 nm) rate technique.

$$\text{L-alanine} + \alpha - \text{ketoglutarate} \xrightarrow{\text{ALT(pH 7.4)}} \text{L-glutamate} + \text{pyruvate}$$

$$\text{Pyruvate} + \text{NADH}(H^+) \xrightarrow{\text{LDH}} \text{Lactate} + \text{NAD}^+$$

RESULT:

1) AGE DISTRIBUTION:

Out of 124 patients, majority belong to 30-40 years age group 33.8 %(n= 42), followed by 40-50 year age group 20.16 %(n=25), and 18.5 %(n=23) belonged to 20-30 years age group.

2) SEX DISTRIBUTION:

Out of 124 patients, 65 %(n=81) were females and rest 35 %(n=43) were males.

3) SERUM TOTAL BILIRUBIN :

Mean total bilirubin is 2.31(SD=2.65) at CI= 95% .Minimum value being 0.10 and maximum value 14.20 with a range of 14.10. (Table 1). 80.5% of patients suffering from acute biliary pancreatitis had hyperbilirubinemia i.e. total bilirubin> 1mg/dL. 19.4% had bilirubin between 1-2 mg/dL, 17.8% had bilirubin between 2-3mg/dL, 2.2% between 3-4mg/dL, 1.5% had between 4.0-5.0 mg/dL, and 13% patients had bilirubin levels > 5mg/dL.

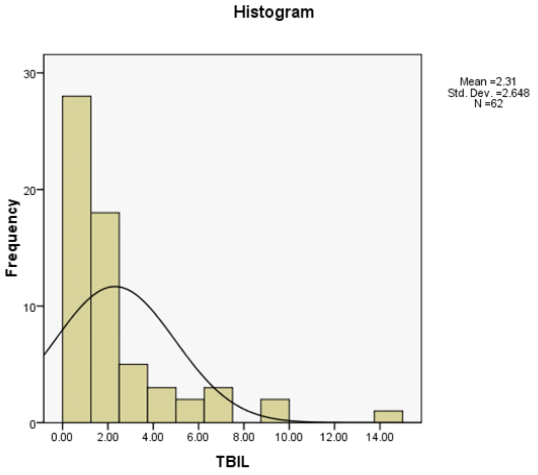


Table 1: Histogram showing frequency distribution of serum levels of Total Bilirubin in patients of Acute Biliary Pancreatitis. TBIL- Total Bilirubin in mg/dL.

4) SERUM DIRECT BILIRUBIN:

Mean Direct bilirubin is 1.5 mg/dL (SD=2.23) at CI= 95%. Minimum value is 0.09 mg/dL and maximum being 11.27 mg/dL.

56.5% of patients suffering from acute biliary pancreatitis had conjugated hyperbilirubinemia (Direct Bilirubin > 0.2mg/dL). Among them 45% had direct bilirubin between 0.2-1.0 mg/dL, 19.2% had direct bilirubin of 1.0-2.0 mg/dL, while only 0.9% had direct bilirubin of range 2.0-3.0 mg/dL , 5.8% patients had direct bilirubin of 3.0-4.0 mg/dL, 1% patients had between 4.0-5.0 mg/dL while 9% had direct bilirubin > 5.0 mg/dL.

5) SERUM ALANINE AMINOTRANSFERASE (ALT):

Mean ALT is 140.19 U/L (SD=227.9) at CI= 95%. Maximum value is 1305 U/L and minimum value is 11 U/L with range being 1294 U/L. (Table2)

41% of patients suffering from acute biliary pancreatitis demonstrated elevated ALT levels (> 78 U/L). Between 1-2 times ALT elevation was found in 22% patients, between 2-3 times ALT elevation in 7%, between 3-4 times in only 2% of patients. However between 4-5 times elevation of ALT levels were found in 3.5% while 6.5% demonstrated elevation greater than 5 times the normal upper range of ALT.

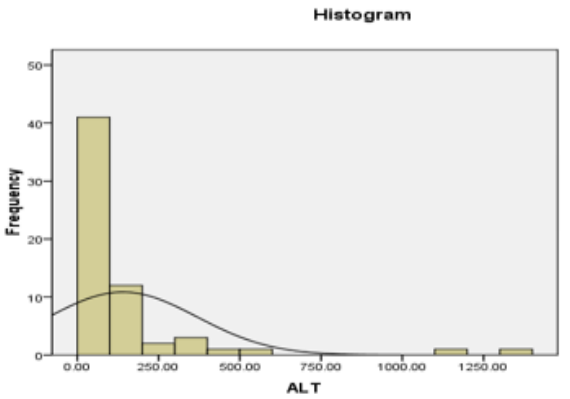


Table 2: Frequency Histogram of serum Alanine Transferase levels in patients of Acute Biliary Pancreatitis. ALT- Alanine Transferase in U/L.

6) SERUM ASPARTATE AMINOTRANSFERASE (AST)

Mean AST is 128.2 U/L (SD=155.5) at CI = 95%. Maximum value is 887 U/L and minimum value being 19 U/L with range of 858. (Table 3)

22.3% of patients suffering from acute biliary pancreatitis demonstrated elevated AST levels (> 37 U/L). Between 1-2 times AST elevation was found in 36.3% patients, between 2-3 times AST elevation in 16%, between 3-4 times in only 1% of patients. However between 4-5 times elevation of AST levels were found in 5% while 20% demonstrated elevation greater than 5 times the normal upper range of AST.

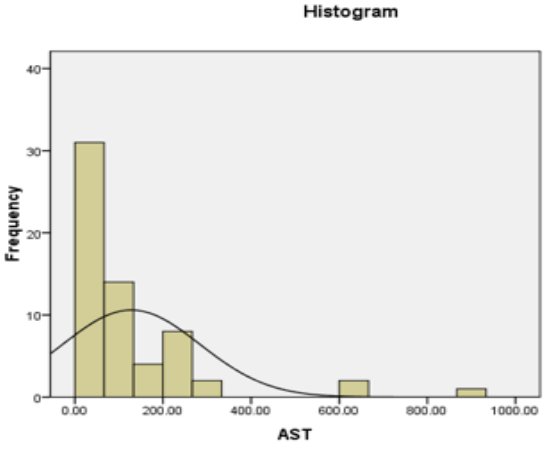
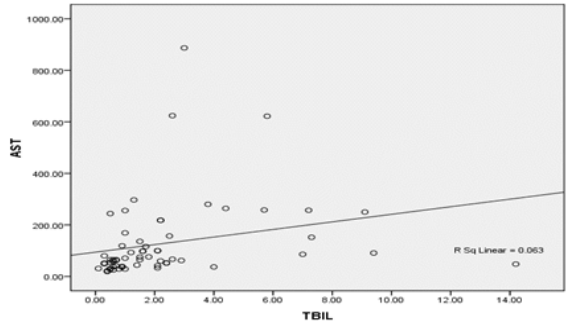


Table 3: Frequency Histogram of serum Aspartate Transferase levels in patients of acute Biliary Pancreatitis. AST- Aspartate Transaminase in U/L.

CORRELATIONS-

1) Total bilirubin and AST: There is a weak positive correlation of serum total Bilirubin with serum Aspartate amino transaminase levels. R=0.250 at CI= 95%.(Fig 1)



VOLUME-6 | ISSUE-1 | JANUARY-2017 • ISSN No 2277 - 8179 | IF : 3.508 | IC Value : 78.46

FIGURE 1: Scatter Plot of serum Total Bilirubin (TBIL) and Aspartate amino transaminase (AST)

2) Total bilirubin and ALT

There is a weak positive correlation of serum Total Bilirubin with serum Alanine amino transaminase levels. R=0.224 at CI= 95%.(Fig 2)

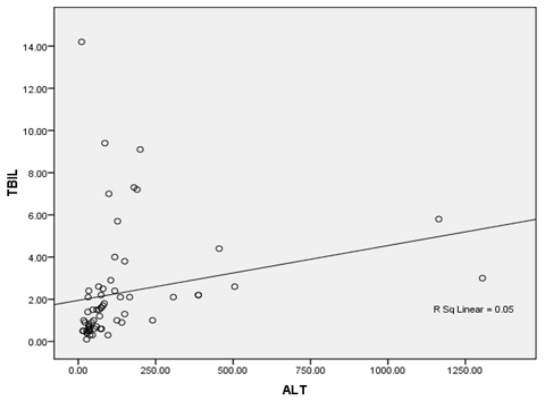


FIGURE 2: Scatter Plot of serum Total Bilirubin (TBIL) and Alanine amino transaminase (ALT)

3) Direct bilirubin and AST

Using Spearman's rho correlation , we found a moderately strong positive correlation of serum direct bilirubin and serum Aspartate amino transaminase levels. R=0.538 at CI= 99%.(Fig 3)

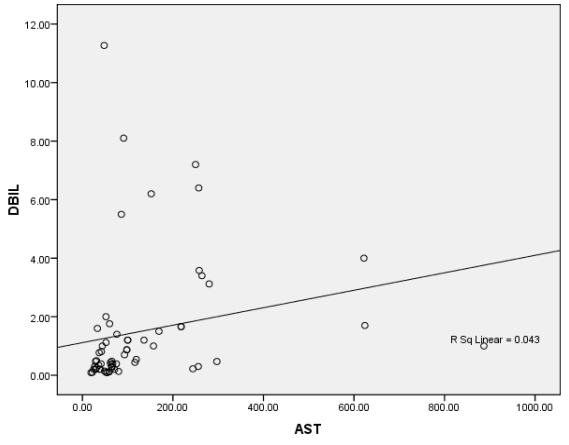


FIGURE 3: Scatter Plot of serum Direct Bilirubin (DBIL) and Aspartate amino transaminase (AST)

4) Direct bilirubin and ALT

Using Spearman's rho correlation , we found a moderately strong positive correlation of serum direct bilirubin and serum Alanine amino transaminase levels. R=0.597 at CI= 99%.(Fig 4)

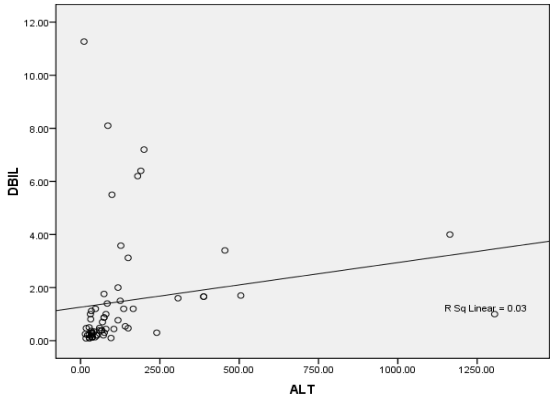


FIGURE 4: Scatter Plot of serum Direct Bilirubin (DBIL) and Alanine amino transaminase (ALT)

DISCUSSION

In our study, majority of patients are of age group 30-40 years, 33.8%(n=42) followed by age group 40-50 years,20.16%(n=25) and 18.5%(n=23) of age group 20-30 years. However Liu et al. reported majority of patients suffering from Acute Gall stone Pancreatitis were above 58 years [1]In a study of Zarnescu NO et al. significant association (p=0.001) with biliary pancreatitis of older age were found.[2].

We found majority of patients were female, 65%(n=81).This is in accordance with a study by Bohara TP et al who found females constituting majority of patient burden of Acute Biliary Pancreatitis. [3]Also significant differences were found in a study of B.R.Davidson et al between the biliary and non-biliary groups with respect to sex (139 versus 59 women, *P*<0·002) [4]

We found that mean serum levels of Total bilirubin were raised in patients of acute gall stone pancreatitis, mean (total bilirubin) = 2.31(SD=2.65 at CI= 95%), minimum value being 0.10 and maximum value 14.20. Mean Direct bilirubin is 1.5(SD=2.23) with minimum value 0.09 and maximum value 11.27. Similarly Davidson BR et al found serum Bilirubin levels significantly elevated in Biliary Pancreatitis. [4]Chang et al. showed total bilirubin as significant variables for biliary pancreatitis.[5]

In our study, mean serum AST level were elevated i.e128.2 (SD=155.5, CI-95%).Maximum value is 887 and minimum value being 19.Lin et al. identified AST level as significant predictors of biliary pancreatitis [6]

We found mean ALT elevated as 140.19(SD=227.9).Maximum value is 1305 and minimum value is 11.Similarly, Chang et al. showed ALT as significant variables for biliary pancreatitis[5].Similarly, ALT is elevated in biliary pancreatitis[4]Similarly, Liu et al. found that ALT value > 150 U/L was associated with biliary etiology in pancreatitis [7]. Matull WR et al found the positive predictive value of ALT value > 150 U/L is 95% for gall stone pancreatitis [8]Cohen et al. demonstrated rise in any serum biochemical marker within 24–48 hours of admission correlated with a persistent common bile duct stone in 31% of patients[9]. Van Gossum A et al found serum AST, ALT and bilirubin increased significantly more in patients with gallstone pancreatitis. Among them, the ALT was the most discriminant test between biliary and nonbiliary pancreatitis.[10]In Tenner S. et al study, it was found that ≥3 fold elevation of ALT in the presence of acute pancreatitis has a positive predictive value of 95% in diagnosing acute gallstone pancreatitis.[11]

We studied correlation between the four parameters which are elevated in acute biliary pancreatitis the total bilirubin, direct bilirubin, AST and ALT.

We found that Direct bilirubin has a moderately strong positive correlation with raised AST and ALT levels of 0.538, CI= 99.9% and 0.597, CI= 99.9% respectively. Raised total bilirubin has weak positive correlation with raised AST and ALT levels i.e. 0.250 and 0.224 respectively. This confirms the classical fact that in case of biliary pancreatitis serum direct bilirubin along with AST and ALT levels can be a good predictor rather than serum indirect bilirubin. Similarly, Bulent Gungor et al found Serum total bilirubin, direct bilirubin were significantly higher in biliary pancreatitis with a positive predictive value of 83.9%, 81.6%.[12]Similarly, Malfertheiner P et al found that determination of AST, bilirubin may allow to distinguish between biliary and non-biliary origin of the disease along with pancreatic enzymes[13]

However, Dammann HG et al showed that a valid prediction of Acute pancreatitis by AST, ALT, Bilirubin is not possible since (1) only about 40% of attacks of acute gallstone pancreatitis were associated with

elevated SGOT, bilirubin and alkaline phosphatase concentrations and (2) there was a considerable overlap with the other etiological groups especially with idiopathic pancreatitis.[14]

Grau F et al found that Serum increases of aminotransferases, especially alanine aminotransferase (ALT), were suggestive of microlithiasis in idiopathic acute pancreatitis, particularly when assessed early after the onset of abdominal pain. [15]

CONCLUSION: Various additional biochemical parameters like serum direct bilirubin, AST, ALT can be used to predict Gallstones as etiology for Acute Biliary Pancreatitis. Serum Direct Bilirubin shows a fairly strong correlation with elevation of ALT & AST levels particularly more with serum ALT values in patients suffering from acute biliary pancreatitis. assessed early after the onset of abdominal pain. [15]

CONCLUSION: Various additional biochemical parameters like serum direct bilirubin, AST, ALT can be used to predict Gallstones as etiology for Acute Biliary Pancreatitis. Serum Direct Bilirubin shows a fairly strong correlation with elevation of ALT & AST levels particularly more with serum ALT values in patients suffering from acute biliary pancreatitis.

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