

Evaluation of Deritis Ratio, Alkaline Phosphatase and Bilirubin in Liver Diseases

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Abstract

Liver diseases are common disorders encountered in clinical practice. Investigations in Liver diseases are used to detect hepatic abnormality, to measure its severity, to define its structural effect on the liver to look for specific causes of Liver diseases to assess prognosis and to evaluate therapy.

Aims and Objectives: To study and compare the altered serum levels of Alkaline phosphatase, Bilirubin and Deritis ratio in Liver disease and also to assess their usefulness in differential diagnosis of liver disease.

Method: The patients admitted to Chigateri General Hospital and Bapuji Hospital Davangere were selected for the present study. Total number of 124 subjects were chosen which include 50 controls and the cases of Liver diseases. The liver diseases were further grouped into Acute Hepatitis, chronic hepatitis and cirrhosis of liver.

Abbreviations: ALP-Alkaline phosphatase; Bil-Bilirubin; AST/ALT- Deritis ratio AST-Aspartate transaminase; ALT-Alanine Transaminase.

Results: The mean levels of Alkaline phosphatase was statistically significant in all liver diseases as compared to controls, the P value is highly significant $P < 0.001$. There is no statistical significance observed in non alcoholic and alcoholic cirrhosis and duration wise comparison in cirrhosis of liver. The mean levels of total Bilirubin was increased in all groups of liver diseases studied when compared to controls. Deritis ratio (AST/ALT ratio) It is statistically significant in chronic hepatitis, non alcoholic cirrhosis of liver, and alcoholic cirrhosis when compared to controls. It is statistically not significant in acute hepatitis when compared to controls.

Conclusion: The liver function test can be of value prognostically and screening for liver disease in response to treatment.

Key words: Deritis Ratio, ALP, Hepatitis, Cirrhosis of the Liver.

1. INTRODUCTION

The evaluation of modern medicine is attributed to the development of various investigative procedures in order to attain accurate diagnosis. The liver is the most important complex organ concerned with various metabolic activity of human body. It is known for its multiplicity of function with great reserve capacity and regenerating ability. It is important to recognize that no one liver function tests enable the clinician to assess accurately the total functional capacity of liver. In order to increase both the sensitivity and specificity of lab tests in the detection of liver diseases, it is helpful to use them as a battery. Selection of appropriate tests depends on particular clinical problem in understanding the use and limitation of application of each test.

Investigations of liver disease are used to detect hepatic abnormality, to measure its severity, to define its structural effect on the liver, to look for specific cause of liver disease, and to assess prognosis and evaluate therapy.

1.1 Alkaline Phosphatase:

AIP are membrane bound glycosylated

homodimeric enzyme present on different types of cells attached to plasma membrane where extensive transport takes place which indicates that they are involved in fundamental biological process¹. Two types of reactions are catalysed by AIP

a. It acts as an hydrolase with the cleavage of phosphdiester bond.

b. It acts as phosphotranferase in which phosphoryl group is transferred to acceptor molecule without the appearances of inorganic phosphate. zinc is a component of ALP acts as a cofactor in various catalytic reactions. ALP is present in bone placenta, small intestine, kidney, liver, mammary glands etc. Normal value is 5-15 king Armstrong units

In 1924 Mann et al used anhepatic dogs to the production of Bilirubin. In 1913 Ehrlich's diazo reaction distinguished 2 types of serum bilirubin. Bilirubin is an orange yellow pigment produced from protoporphyryn. A useful indicator of excretory ability of liver and derived from heme moiety of hemoglobin and from senescent RBC's by RES. Normal serum total bilirubin is 0.3-0.8 mg/dl. There are 2

forms of bilirubin in the body. Indirect or unconjugated normal level is 0.2 – 0.8mg, direct / conjugated bilirubin is upto 0.25mg/dl. Bilirubin elevations in Jaundice occurs due to Hepatic, obstructed or Haemolytic cause.

Deritis Ratio is the ratio between aspartate amino transferase and alanine aminotransferase³. This ratio has been useful in differential diagnosis and classification of hepatic disorders³. The ratio is particularly useful to differentiate intrahepatic >1.5 and extrahepatic < 1.4 lesions. For normal individual deritis ratio vary from 0.7-1.4 Elevated AST/ALT ratio in alcoholic and heavy drinkers indicate alcohol induced organ damage. AST/ALT > 1.5 is suggestive of alcoholic liver damage.

Also AST/ALT ratio is a good prognostic indicator of outcome in case of severe acute viral Hepatitis A low AST / ALT ratio 0.3-0.6 is found in survivors and higher ratio in 1.2 – 2.6 in non survivors.

2. MATERIALS AND METHODS

The present study was composed of 124 subjects out of which 50 were healthy controls, 21 were suffering from acute viral hepatitis, 23 patients were suffering from chronic hepatitis and 30 patients with cirrhosis of liver. The control and diseased subjects were in the age group of 15-65 yrs.

The control and diseased subjects were taken from the local population in and around Davangere. Informed consent was taken and approved by the ethical committee of J.J.M. Medical College. Patients and controls voluntarily participated for the study. A total number of 50 control subjects who were healthy non pregnant non alcoholics, non smoker, no past H/o liver diseases, not on any medications and no history of heart, renal, neurological and thyroid disorders, diabetes Mellitus, Pancreatic, bone diseases, secondaries, were excluded from the study.

The present study of estimation of Alkaline phosphatase Bilirubin and Deritis ratio in control subjects and patients with acute viral hepatitis, chronic hepatitis and cirrhosis of liver was conducted. On inpatients admitted for

treatment in Chigateri Hospital and Bapuji Hospital Davangere (which are teaching Hospital for J.J.M. Medical College, Davangere).

A careful history and through clinical examination was done in all the study groups according to the proforma prepared for the purpose of study. The cases were diagnosed by clinical signs and symptoms and confirmed by ultrasound and liver biopsy. Following admission 6ml of plain blood was drawn with aseptic precautions from large peripheral vein into sterile bulb before treatment; was started. Serum was separated by centrifugation and the test were carried out by standard biochemical procedures and chemicals and reagents used for the procedures were pure high grade analytical types. The lab parameter were recorded and subjected to statistical analysis. The observation were tabulated and conclusion obtained from the biochemical data.

Determination of total Bilirubin by the method of Jendrossick and Groff.

Its principle being – Bilirubin is coupled with diazotized sulfonilic acid in the presence caffeine to give an azo dye. Normal value of serum total Bilirubin upto 1mg/100ml = 17µmol/litre.

Determination of serum Alkaline phosphatase by king and Armstrong method.

Principle – phenol released by enzymatic hydrolysis from phenyl phosphate under defined conditioning of time temperature and patient is estimated calorimetrically .

Determinations of SGOT/AST and SGPT/ALT by Reitman and Frenkel method

Principle- The pyruvate produced by transamination by GPT reacts with 2-4 dinitrophenyl hydrazine (DNPH) to give a brown coloured hydrazine which is measured in the colorometry at 510nm. The oxalate formed in the reaction with GOT decarboxylates spontaneously to pyruvate which is again measured by hydrozone formation. Later converted to IU/L by reference table subsequently AST/ALT ratio called Deritis ratio is estimated.

3. RESULTS

It is evident from table 1 that there is increase in levels of AIP, Bil, AST/ALT ratio in patients with acute viral hepatitis when compared to healthy controls where P value is highly significant for ALP and Bilirubin and non significant for AST/ALT ratio.

According to table 1, there is increase in the levels of AIP, Bil and AST/ALT ratio in patients with chronic Hepatitis when compared to healthy controls where P value is very significant for AST/ALT ratio and highly significant for ALP and Bilirubin.

Table 1 shows that there is increase in the levels of AIP, Bil and AST/ALT ratio in patient with non alcoholic cirrhosis when compared to healthy controls. The P value is very significant for AST/ALT ratio and highly significant for ALP, Bilirubin.

Also according to Table 1 there is evidence showing increase in the levels of ALP, Bilirubin and AST/ALT ratio in patients with alcoholic cirrhosis when compared to healthy controls the P value is highly significant for ALP, Bilirubin and significant for AST/ALT ratio.

It is evident from table 1 that there is increase in the levels of AIP, Bil, AST/ALT in patients with alcoholic cirrhosis <10 yrs alcohol abuse when compared to healthy controls where the P value is highly significant for ALP, Bil and not significant for AST/ALT ratio.

Also according to table 1 there is increase in the levels of AIP, Bil and AST/ALT ratio in subjects with alcoholic cirrhosis >10yrs alcohol abuse compared to controls and the P value is highly significant for ALP, Bil and significant for AST/ALT ratio.

According to Table -2 it is evident that there is increase in the levels of Bil and decrease in levels of ALP in patients with alcoholic cirrhosis when compared to non alcoholic cirrhosis patients. AST/ALT ratio is same in non alcoholic cirrhosis patients and subjects with alcoholic cirrhosis.

The P value is non significant for ALP, Bil and AST/ALT ratio.

Table -1 showing levels of Mean \pm SD of ALP, BIL, AST/ALT Ratio in 50 control Subjects and Acute Hepatitis, Chronic Hepatitis and Cirrhosis

	No.	ALP IU/L	BIL IU/L	AST/ALT IU/L
Controls	50	9.71 \pm 3.3	0.5 \pm 0.2	1.6 \pm 1.0
Acute viral hepatitis	21	31.0 \pm 17.9	5.8 \pm 2.8	1.6 \pm 1.4
Chronic hepatitis	23	26.8 \pm 12.5	6.0 \pm 4.7	2.9 \pm 2.3
Cirrhosis of liver (non alcoholic)	10	28.3 \pm 14.3	2.1 \pm 1.9	2.6 \pm 1.9
Alcoholic cirrhosis (combined)	20	28.8 \pm 16.2	3.4 \pm 3.2	2.5 \pm 2.0
Alcoholic cirrhosis (> 10 Years)	10	33.6 \pm 12.9	4.6 \pm 4.1	2.9 \pm 2.7
Alcoholic cirrhosis (< 10 years)	10	24.0 \pm 11.2	2.2 \pm 1.3	2.2 \pm 1.2

Table - 2 showing Comparison of ALP, BIL, AST/ALT between Non-Alcoholic Cirrhosis and Alcoholic Cirrhosis

Group	ALPKA Units	BIL mg/100ml	AST/ALT
Non-alcoholic	28.3 \pm 14.3	2.1 \pm 1.9	2.6 \pm 1.9
Alcoholic	28.8 \pm 16.2	3.4 \pm 3.2	2.5 \pm 2.0
p-value	NS	NS	NS
Significance	NS	NS	NS

The value are expressed as their Mean \pm SD

P>0.05 NS Not significant
P<0.05 SS Statistically significant
P<0.001 HS High significant
P<0.01 VS Very significant

Table - 3 showing Comparison of ALP, BIL, AST/ALT between Alcoholic Cirrhosis of > 10 and < 10 years

Group	ALP IU/L	BIL mg/100ml	AST/ALT
> 10 years	33.6 \pm 12.9	4.6 \pm 4.1	2.9 \pm 2.7
< 10 years	24.0 \pm 11.2	2.2 \pm 1.3	2.2 \pm 1.2
p-value	> 0.05	> 0.05	> 0.05
Significance	NS	NS	NS

The values are expressed as their Mean \pm SD

P > 0.05 NS = Not Significant
P < 0.01 HS = Highly Significant

Table – 4 showing Comparison of ALP, BIL, AST/ALT between Acute Viral Hepatitis, Chronic Hepatitis and Cirrhosis

Group	ALP KA Units	BIL mg/100ml	AST/ALT
Acute Viral Hepatitis	31.0±17.9	5.8±2.8	1.6±1.4
Chronic Hepatitis	26.8±12.5	6.0±4.7	2.9±2.3
Cirrhosis	28.6±14.0	3.0±2.9	2.5±2.0
Acute Viral Hepatitis V/s Chronic Hepatitis	> 0.05, NS	> 0.05, NS	0.3 Sig
Acute Viral Hepatitis V/s Cirrhosis	> 0.05, NS	< 0.05, SS	>0.05, NS
Chronic Hepatitis V/s Cirrhosis	> 0.05, NS	< 0.05, SS	> 0.05, NS

The values are expressed as their Mean ± SD

P < 0.05 SS = Statistically Significant

P > 0.05 NS = Not Significant

P < 0.001 HS = Highly Significant

Table - 5 showing Comparison of ALP, BIL, AST/ALT between Chronic Hepatitis, Non Alcoholic Cirrhosis and Alcoholic Cirrhosis

Group	ALP KA Units	BIL mg/100ml	AST/ALT
Chronic Hepatitis	26.8±3.3	6.4±4.7	2.9±1.0
Non Alcoholic Cirrhosis	28.3±14.3	2.1±1.9	2.6±1.9
Alcoholic Cirrhosis	28.8±16.2	3.4±3.2	2.5±2.0
Chronic Hepatitis V/s Cirrhosis (NA)	HS < 0.001	> 0.05 NS	< 0.05, SS
Chronic Hepatitis V/s Cirrhosis (AL)	< 0.001, HS	< 0.001, HS	> 0.05, NS

The values are expressed as their Mean ± SD

P < 0.05, SS = Statistically Significant

P > 0.05, NS = Not Significant

P < 0.001, HS = Highly Significant

It is evident from Table -3 that there is increase in the levels of ALP, Bil and AST/ALT ratio in patients with alcoholic cirrhosis. (with alcoholic abuse of >10yrs) when compared to subjects suffering from alcohol cirrhosis with alcohol abuse of <10yrs. The P value is non significant for Bil, ALP, and AST/ALT ratio.

According to Table – 4 there is increased level of ALP and decreased Bilirubin and AST/ALT in acute viral hepatitis compared to chronic hepatitis. The P value is not significant for ALP and Bilirubin but significant AST/ALT.

According to Table-4 the estimated levels of ALP, and Bilirubin in acute viral hepatitis is increased compared to cirrhosis. Also AST/ALT ratio is decreased in acute viral Hepatitis compared to cirrhosis. P value is not significant for ALP and AST/ALT ratio but significant for bilirubin.

According to Table-4 the estimated level of ALP bilirubin and AST/ALT ratio in chronic hepatitis compared to cirrhosis where ALP is decreased, bilirubin is increased and AST/ALT is increased in chronic hepatitis compared to cirrhosis.

The P value is not significant for ALP and AST/ALT but significant for Bilirubin.

It is evident from table – 5 that there is decreased levels of ALP and increased Bilirubin and increased AST/ALT ratio in chronic hepatitis compared to non alcoholic cirrhosis. The P value is significant for AST/AST highly significant for ALP and insignificant for Bilirubin.

Also the table shows that the levels of Bilirubin and AST/ALT in chronic hepatitis increased when compared to alcoholic cirrhosis. The levels of ALP is decreased in chronic hepatitis when compared to alcoholic cirrhosis. The P value is highly significant for ALP and Bilirubin and not significant for AST/ALT.

4. DISCUSSION

Measurement of enzyme activities in serum are useful for diagnostic assessment of Hepatobiliary disease. Both the concentration and pattern of enzyme activities give valuable information regarding the type, extent and severity of liver disease. Several workers have conducted many studies to asses the usefulness

of serum enzymes estimation in the diagnosis and prognosis of various liver disease. In our study of liver disease there is no correlation with age and sex wise. Alkaline phosphatase are membrane bound glycosylated homodimeric enzymes involved in fundamental biological process. The mean levels of Alkaline phosphates was statistically higher significantly $P < 0.001$ in all liver disease in our study compared to healthy controls.

The mean levels of Alkaline phosphatase is increased (When compared to control subjects and) in acute hepatitis patients. P Value is highly significant < 0.001 2-3 fold above the upper limit of normal. Increase in serum ALP in Hepatic disease is due to on increase production of the enzyme by the liver, bileduct cells with impaired excretion.

The mean levels of ALP is increased when compared to healthy control subjects in chronic Hepatitis when P value is highly significant < 0.001 . In our study the level of ALP is marginally or mild to moderately increased.

Also the mean levels of Alkaline phosphatase in subjects with cirrhosis of liver (Non Alcoholic and alcoholic) are increased when compared to matched controls, P Value is significantly high $P < 0.01$. The mean ALP value was also increased in non-alcoholic cirrhosis compared to controls where P value was highly significant < 0.001 . Also the mean AIP was also increased in alcoholic cirrhosis compared to controls P value was higher significantly < 0.001 .

The mean alkaline phosphates showed no correlation with duration of Alcoholic cirrhosis. Elevation of serum AIP in subjects with Hepatic dysfunction is due to regurgitation of Alkaline phosphatase from damaged hepatocytes into serum. Its importance is only to detect cholestasis¹⁵. Increase in the activity of plasma AIP are not specific for liver diseases due to lack of tissue specificity.

4.1 AST/ALT Ratio:

This ratio is also called as deritis ratio. This ratio implies the degree of parenchymal damage caused to hepatocytes in the liver. The mean of AST/ALT ratio were increased and statistically high $P < 0.001$ in all liver disease in our study when compared to healthy control

subjects.

The mean levels of AST/ALT ratio is increased when compared to healthy control subjects in Acute viral hepatitis where P value is not significant $P > 0.05$.

This increase in AST/ALT ratio in our study indicates the progression to chronic hepatitis and or cirrhosis. Persistence of high AST/ALT ratio may indicate bad prognosis usually associated with non survivors¹¹.

In chronic hepatitis the mean levels of AST/ALT ratio are increased when compared to healthy subjects where P value is very significant < 0.01 In our study AST/ALT ratio > 1.0 .

In cirrhosis of Liver (Non alcoholic) the mean levels of AST/ALT ratio is increased when compared to healthy control subjects where P value is significant < 0.05 . In our study deritis ratio is > 2.0 .

In alcoholic cirrhosis the mean levels of AST/ALT ratio is increased when compared to healthy control subjects where P value is significant $P < 0.05$. $AST/ALT > 2.0$ implies the presence of alcoholic liver disease (cirrhosis).

In our study the deritis ratio is > 2.0 . The AST/ALT ratio does not correlate with the duration of alcoholic cirrhosis.

AST/ALT ratio is of most useful in the recognition of alcoholic liver disease if $ALT < 300$ IU / L and $AST/ALT > 2$ suggests alcoholic liver disease.

If $AST/ALT > 3$ suggests high incidence of alcoholic liver disease. The increase ratio is due to the low serum activity of ALT in patients with alcoholic liver disease secondary to deficiency of piridoxal-5 phosphate in patients with alcoholic liver disease.

AST/ALT ratio is usually of little value in distinguishing among various hepatobiliary disorders. Mitochondrial AST may be helpful in assessing the severity of liver disease. The ratio of m-AST/ t-AST helpful to distinguish alcoholic hepatitis of other liver disease.

The recent development of immunochemical methods for measurement of m-AST in serum has been of interest in the use of m-AST/t-AST ratio which is a sensitive marker than GGT. The ratio of m-AST/t-AST gave a sensitivity

ranging from 93-100% in alcoholics.

m-AST and its ratio to t-AST is being considered as most promising new biological marker for alcoholism detection¹¹.

4.2 Bilirubin:

The mean levels of bilirubin in liver disease under study are increased when compared to healthy control subjects. Where P value is highly significant being <0.001. The mean levels of bilirubin is increased in acute viral hepatitis when compared to healthy control subjects where P value is highly significant <0.001. Also the mean levels of bilirubin is increased, in chronic hepatitis when compared to healthy controls where P value is highly significant, 0.001.

The mean values of bilirubin is increased in cirrhosis of liver (non alcoholic) when compared to healthy control subjects and P value is highly significant <0.001.

In Alcoholic cirrhosis, the mean levels of bilirubin is increased when compared to healthy subjects and P value is highly significant <0.001. Therefore serum bilirubin is not useful as a diagnostic test. However if increase in bilirubin level continue after therapy, it indicate poor prognosis.

5. CONCLUSION

Low incidence of Alkaline phosphatase elevation in alcoholic liver damage indicate that this enzyme is of little value in detection of alcoholic liver disease. Also marginal to mild elevation of serum ALP in acute hepatitis, chronic hepatitis and cirrhosis implies that its estimation is not of much usefulness and important in the diagnosis of liver disease and in differential diagnosis of liver diseases¹⁶ in our study.

Persistence of high AST/ALT ratio may indicate bad prognosis and usually associated with non survivors. AST/ALT ratio is usually of little value in distinguish among various hepatobiliary disorder. Mitochondrial AST may be helpful in assessing the severity of liver disease and in detection of alcohol abuse as m-AST accounts for about 80% of total AST activity with in the liver cells¹⁴. Increase activity of enzyme in serum serves to identify

or confirm liver disorder. An abnormal result may be of limited value in differential diagnosis¹⁴. These tests can be of value prognostically in screening for liver disease and are vital in monitoring the liver disease in response to treatment.

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