

Assessing the utility of serum Cholinesterase, Lactate dehydrogenase and Albumin/Globulin ratio in differentiating liver diseases

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Abstract

Introduction: Variety of liver diseases including alcoholic liver disease (ALD) is responsible for liver related morbidity and mortality in India. Distinguishing ALD from non-alcoholic steatohepatitis (NASH) and viral hepatitis may not be easy. The history of alcohol intake may be unreliable. Clinico-pathological findings may not yield the correct diagnosis. So aim of our study was **a)** To compare the patients of ALD with those of NASH and acute viral hepatitis by biochemical parameters like serum Cholinesterase, Lactate dehydrogenase and Albumin/Globulin ratio and **b)** To assess the utility of these parameters in differentiating patients of ALD from NASH and acute viral hepatitis.

Materials and Method: Study was carried out on 50 patients of ALD, 35 patients of NASH and 35 patients of acute viral hepatitis. All the patients were in the age group of 20-70. Selection criteria were history of alcohol, clinical examination, raised serum bilirubin levels and viral studies. Blood sample analysis was done for bilirubin, cholinesterase, lactate dehydrogenase, total protein and albumin by Diazo, kinetic, biuret and BCG method respectively on fully automatic analyser. Statistical analysis was done by Student unpaired 't' test.

Results: The patients of NASH and acute viral hepatitis had normal serum ChE, albumin and LDH levels compared to patients of ALD who had lower serum ChE and albumin with higher LDH levels. These differences in the findings were statistically significant ($P < 0.05$). Lowered serum ChE and albumin levels with A/G ratio reversal might be because of long term alcohol induced hepatic injury resulting in lowered synthetic capacity. Increased LDH in ALD might suggest a hypoxic injury and a progression towards necrosis. Normal serum ChE, A/G ratio and LDH in patients of NASH as well as acute viral hepatitis suggested that hepatic synthetic capacity is intact with no hepatocyte hypoxia.

Conclusion: So our study concluded that NASH and viral hepatitis patients had less hepatocyte injury and fair prognosis as compared to ALD patients who had more hepatic loss with worse prognosis. Further our study has drawn a conclusion that we can biochemically differentiate NASH and acute viral hepatitis from ALD on the basis of parameters like ChE, LDH and A/G ratio. This finding can be used by clinicians to support their diagnosis whenever clinical dilemma occurs.

Keywords: Cholinesterase, Lactate dehydrogenase, Alcoholic liver disease, Non-alcoholic steatohepatitis

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Introduction

Liver plays vital role in the metabolism of our body. It is prone to many diseases like toxic, inflammatory, metabolic liver diseases. In India alcohol consumption is very common and so alcoholic liver disease is also prevalent. An early diagnosis of alcoholic liver disease (ALD) is very essential since it initiates screening programs to prevent life-threatening complications such as oesophageal varices or hepatocellular cancer and it helps to identify patients at genetic risk for ALD. Alcoholism is social and medical problem.

Alcoholic liver diseases affect millions of individuals worldwide and it remains one of the most common causes of chronic liver disease. A majority of adult population in India uses alcohol. Globally 5 million people die per year due to alcohol related problems.⁽¹⁾ Alcohol is readily absorbed by stomach and remainder from GI tract (Thompson, 1956).⁽²⁾ Only 2-10% of absorbed ethanol is eliminated through kidneys and lungs, the rest is oxidized in liver at rate of

50-180mg/kg body wt. per hour.⁽²⁾ Alcohol consumption increases liver disease mortality.

Alcoholic liver disease (ALD) is a disease with a known cause but a complicated pathogenesis. Alcohol dehydrogenase is the major enzyme responsible for alcohol metabolism. Alcohol dehydrogenase generates oxygen radicals causing hepatotoxicity.⁽³⁾ Metabolism of ethanol results in increased production of reducing equivalents with decrease in NAD/NADH ratio [Lieber,⁽⁴⁾ 1968]. The severity and prognosis of alcohol-induced liver disease depends on the amount and duration of alcohol consumption, as well as on the diet, nutritional status and genetic predisposition of an individual.

Physicians are in search of correct and cheaper means of identifying persons with excessive alcohol consumption. Chronic alcoholism is diagnosed on the basis of clinical examination, history of alcohol consumption and many other laboratory investigations. Differentiating non-alcoholic liver disease from alcoholic liver disease is although difficult but crucial in treatment point of view.

On the basis of some biochemical parameters we tried to differentiate alcoholic liver disease from other forms of diseases like acute viral hepatitis and non-alcoholic steatohepatitis. This study was undertaken primarily to compare the cholinesterase, lactate dehydrogenase and albumin/ globulin ratio in alcoholic liver disease, acute viral hepatitis and non-alcoholic steatohepatitis.

Due to rise in unhealthy dietary habits and sedentary lifestyles, non-alcoholic steatohepatitis (NASH) which is associated with insulin resistance, has emerged as a leading cause of chronic liver disease. The increasing prevalence of obesity, coupled with diabetes, dyslipidemia, hypertension and metabolic syndrome puts a very large population at risk of future liver failure.

Lactate dehydrogenase (LDH) activity is present in all cells of the body and invariably found only in cytoplasm of cell. Enzyme concentration in various tissues is 500 times greater than those normally found in serum. So leakage of enzyme from even a small mass of damaged tissue increases the serum activity of LDH to a significant extent. In liver and skeletal muscle, more cathodal LD-4 and LD- 5 isoforms predominate. LDH elevations occur in MI, hemolysis, liver disorders, kidney, lung and muscle disorders.⁽⁵⁾ Cholinesterase (ChE) is sensitive indicator of synthetic capacity of liver. Any decrease in ChE level reflects impaired synthesis of enzyme by liver. Serial measurements of ChE are used as prognostic marker for long-term survival in patients with liver failure.⁽⁶⁾ Serum albumin is synthesized exclusively by hepatocytes.⁽⁷⁾ It has a long half life (15-20 days) with 4% degraded daily. Because of slow turnover it is not a good indicator of acute or mild hepatic dysfunction. Only minimal changes in sr. albumin are seen in acute conditions such as viral hepatitis, drug related hepato-toxicity and obstructive jaundice.⁽⁸⁾ Albumin levels < 3 gm/dl increase the chances of chronic liver disease. Hypoalbuminemia is common in chronic liver disorders such as cirrhosis and reflects severe liver damage and decreased albumin synthesis.⁽⁹⁾

Although a lot of information is available about these biochemical parameters in ALD, NASH and acute viral hepatitis separately, but a comparative study, taking these three forms of hepatitis into consideration is lacking. This study aims to assess the usefulness of above parameters in differentiating NASH and acute viral hepatitis from alcoholic liver disease.

Aims and Objectives

- a. To compare the patients of alcoholic liver disease with those of non-alcoholic steatohepatitis and

acute viral hepatitis on the basis of ChE, LDH and A/G ratio

- b. To assess the use of these parameters in biochemically differentiating the patients of alcoholic liver disease from non-alcoholic steatohepatitis and acute viral hepatitis.

Materials and Method

The present study was carried out in the department of Biochemistry in tertiary care hospital.

To compare the groups, male patients with diagnosis of alcoholic liver disease (n = 50), NASH (n=35) and acute viral hepatitis (n=35) admitted in the ward were included in our study. All the patients in our study were male as it is rare to find a female patient with alcoholism reporting to outpatient department in our region.

Patients of ALD in the age group of 20–70 years were diagnosed on the basis of history of alcohol consumption (daily amount and duration), clinical examination and raised serum bilirubin. Each member of this group had history of alcohol intake for at least 10 years with average daily drinking of 60-80 gm of alcohol. The patients with liver disease other than alcoholic origin were excluded from the study.

Patients of NASH and acute viral hepatitis in the age group of 20–70 years were diagnosed on the basis of clinical examination, viral antigen/antibody study, no alcohol consumption and raised serum bilirubin. Patients with history of alcoholism and other diseases were excluded from the study.

Serum samples from the patients were analyzed for total bilirubin (normal 0.1–1.2 mg%) and direct bilirubin (0–0.3mg%) by Diazo method, LDH(normal 240-480 IU/L) by DGKC kinetic method, ChE (normal 3700-11,500U/L) by kinetic method, total protein (normal 6-8 gm%) by Biuret method and albumin (normal 3.5-5gm%) by BCG method in clinical biochemistry laboratory of our hospital using autoanalyzer (Erba Excel-300) and semi-autoanalyzer (Erba chem 5-plus).

Parametric data of this study was analyzed statistically by Student unpaired 't' test. A 'P' value of <0.05 was considered to be statistically significant. P<0.01 was considered as highly significant.

Results

Comparative data of various biochemical parameters in ALD and NASH is presented below in Table 1 and that in ALD and acute viral hepatitis is presented in Table 2.

Table 1: Comparison of patients of Alcoholic liver disease with NASH

Parameter	ALD (n=50) [mean ± SD]	NASH (n=35) [mean ± SD]	P- Value
Age (years)	48.18 ± 7.56	47.92 ± 9.57	P> 0.05, NS
Bil. (T) (mg%)	3.94 ± 2.88	2.56 ± 0.715	NS
Bil. (D) (mg%)	2.01 ± 1.86	1.47 ± 0.578	NS
CHE (U/lit)	2986.12 ± 790	5200 ± 5273.48	P<0.01, S
LDH (IU/lit)	732.3 ± 155.35	495.16 ± 118.60	P<0.01, S
Total Protein (gm%)	6.22 ± 1.091	6.99 ± 0.37	NS
Albumin (gm%)	2.53 ± 0.45	4.092 ± 0.21	P<0.01, S
A/G ratio	0.702 ± 0.15	1.41 ± 0.106	P<0.01, S

Table 2: Comparison of patients of ALD with Acute viral hepatitis

Parameter	ALD (n=50) [mean ± SD]	Acute viral hep. (n=35) [mean ± SD]	P- Value
Age (years)	48.18 ± 7.56	46.76 ± 4.70	P> 0.05, NS
Bil. (T) (mg%)	3.94 ± 2.88	5.79 ± 6.1	NS
Bil. (D) (mg%)	2.01 ± 1.86	3.38 ± 4.68	NS
CHE (U/lit)	2986.12 ± 790	6982.64 ± 1000	P<0.01, S
LDH (IU/lit)	732.3 ± 155.35	439.4 ± 150.2	P<0.01, S
Total Protein (gm%)	6.22 ± 1.09	6.86 ± 0.737	NS
Albumin (gm%)	2.53 ± 0.454	4.1 ± 0.65	P<0.01, S
A/G ratio	0.702 ± 0.15	1.51 ± 0.50	P<0.01, S

Discussion

Alcohol consumption is social and medical problem worldwide. Early diagnosis and management ALD is of utmost importance to prevent further complications. But sometimes it becomes difficult to differentiate it from diseases like non-alcoholic steatohepatitis and acute viral hepatitis, as clinical appearance may be same. Also the history of alcohol consumption often misleads the clinicians. So with the help of biochemical parameters we tried to compare patients of ALD and NASH in table no.1. We observed the fall in serum ChE levels in patients of ALD. Serum ChE levels in patients of NASH were within normal limits. This difference was statistically significant (P<0.01). In ALD patients serum ChE levels are more lowered than in non-alcoholic liver disease patients.^(10,11,12) Serum ChE is a marker of synthetic function of liver as well as a prognostic factor in advanced liver disease.^(13,14,15,16,17) Low serum ChE in ALD patients of our study might be because of decreased synthetic function of hepatocytes owing to alcohol induced injury to hepatocytes and also it might be a sign of progression from alcoholic fatty liver to fibrosis. Serum ChE levels may be normal or high in NASH or fatty liver patients.^(12,16) Normal ChE levels in NASH patients of our study revealed that hepatic synthetic function in these patients is maintained. LDH levels were found to be increased in ALD group as compared to NASH group. This difference was statistically significant (P<0.01). LDH levels can be used to detect hepatocellular necrosis.⁽⁸⁾ LDH concentration is high under low oxygen level in

hepatocytes.⁽¹⁸⁾ The rise in LDH level in ALD patients of our study suggested hypoxic and necrotic condition of hepatocytes. This may be due to alcohol induced hypoxic injury to hepatocytes. High normal levels of LDH in NASH patients rule out any hypoxic injury to hepatocytes. Serum total protein levels in patients of ALD and NASH were within normal limits. But albumin levels in ALD patients were found to be reduced as compared to that in NASH patients. This difference was statistically significant (P<0.01). So A/G ratio was reversed in ALD group while it was normal in NASH group. This finding is supported by Majhi S. et al⁽¹⁹⁾ who found that there was A/G ratio reversal in ALD patients. In advanced liver disease there is decreased synthetic capacity of liver leading to low levels of albumin.^(15,16) Low levels of serum albumin in ALD group of our study indicates that there is alcohol induced hepatocellular injury of chronic duration and it is of advanced stage as compared to that of NASH group. Fall of serum albumin level in NASH patients indicate the progression towards fibrosis.⁽¹⁶⁾ In our study NASH patients might be in the initial stage of steatosis, so no alteration in albumin level was observed.

This comparison of patients of ALD with NASH gives us an idea that these two forms of liver diseases can be differentiated with the help of biochemical parameters. So parameters like ChE, LDH and albumin/globulin ratio are useful in differentiating these two conditions. These parameters can be used in support where history, clinical examination and other invasive investigations provide no clear diagnosis.

Sometimes clinicians face problems in diagnosing ALD from acute viral hepatitis, as clinical presentation may be similar. Also the patient may tell lies regarding history of alcohol consumption. Serological tests may not confirm the diagnosis of viral hepatitis. So we tried to compare the patients of ALD and acute viral hepatitis on the basis of parameters like ChE, LDH, A/G ratio. Our aim was to find a definitive biochemical test to make the diagnosis more clear whenever there is difficulty clinically and pathologically. In Table 2 we observed that there was rise in serum bilirubin in both ALD and viral hepatitis groups but it was not statistically significant. There was fall in serum ChE level in ALD group with normal level in viral hepatitis group and this was statistically significant ($P < 0.01$). ChE level is a diagnostic and prognostic marker in advanced liver disease.^(20,17) Serum ChE is a marker of synthetic function of liver.^(13,14,15) So decreased ChE levels in ALD group of our study signifies a diminished synthetic function of liver. It may be due to chronic alcohol induced hepatocyte injury. ChE is a sensitive indicator of diminished hepatic synthetic capacity and its decrease favours the shift of liver disease towards fibrosis or chronicity.⁽¹⁶⁾ So ALD patients of our study may be at risk for fibrosis and advanced liver disease. Serum LDH levels were found to be raised in ALD group compared to normal levels in viral hepatitis group and it was statistically significant ($P < 0.01$). LDH production increases under low oxygen concentration in ischemic and drug induced liver injury than viral hepatitis.^(18,21) LDH levels in viral hepatitis group were in the normal range in our study. Alcohol induced hypoxic and necrotic condition of hepatocytes raises the serum LDH level in ALD group. This finding is supported by the study⁽⁸⁾ which states that LDH level is used to detect hepatic necrosis. But normal LDH levels in acute viral hepatitis group of our study rules out any hypoxic and necrotic injury to hepatocytes. Serum total proteins were within normal limits in both the groups. But serum albumin is decreased in ALD group and it was normal in viral hepatitis group. So A/G ratio was reversed in ALD group while it was normal in other group and this difference was statistically significant ($P < 0.01$). These findings in our study is supported by findings in other studies.⁽¹⁹⁾ Serum albumin levels are normal in acute liver diseases while it is decreased in chronic diseases.^(15,16,8) This is in conjunction with our findings of normal albumin levels in acute viral hepatitis group and low albumin levels in ALD group. This fact may be due to alcohol induced fall in synthetic capacity of liver after long term alcohol use. At the same time acute injury of short duration does not hamper serum albumin levels in viral hepatitis group.

This comparative study of patients of ALD and acute viral hepatitis on the basis of parameters like ChE, LDH and A/G ratio gives us an idea that these two conditions can be very well differentiated. Although clinico-pathologically sometimes there exist dilemma

about diagnosis, biochemically the picture becomes much clear.

Conclusion

This study concluded that we can differentiate the patients of ALD from non-alcoholic steatohepatitis and acute viral hepatitis on the basis of biochemical parameters like ChE, LDH and albumin/globulin ratio (A/G ratio). Our study further indicated that these parameters should be used whenever there is any doubt in diagnosis of liver diseases clinically. These parameters are cheaper and non-invasive, hence should be used in evaluating and differentiating the patients of various liver diseases. Prompt diagnosis and management can reduce the mortality in alcoholic liver disease. The patients of NASH and acute viral hepatitis have much less mortality. The limitation of our study is that we evaluated very few numbers of patients with only three to four parameters in differentiating these groups. Further studies using more parameters with large sample size in differentiating these liver diseases are advised.

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