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Assessing The Utility Of Serum Cholinesterase, LDH, AST /ALT Ratio, Albumin /Globulin Ratio In Differentiating Alcoholic Liver Disease From Non- Alcoholic Steatohepatitis.

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ABSTRACT

Alcoholic liver disease is responsible for liver related mortality & morbidity in India. An early diagnosis of Alcoholic Liver Disease is crucial ininitiating screening programs to prevent life threatening complications. To compare the patientsof ALD with those of NASH by biochemical parameters like serum Cholinesterase, Lactate dehydrogenase and Albumin / Globulin ratio AST/ALT ratio. This study was carried out in Govt Stanley Medical College in 24 hrs. Clinical Biochemistry lab & Department of Medical Gastroenterology. 40 patients of ALD (Group 1) and 30 patients of NASH (Group 2) were included in this study. Selection criteria include male patients of age group 20-70 years, history of alcohol for ALD (Group 1), no history of alcohol consumption for NASH (Group 2). Blood sample was analysed for bilirubin, cholinesterase, lactate dehydrogenase, total protein andalbumin by Diazo, kinetic, biuret and BCG method respectively on fully automatic analyzer. Statistical analysis was done by Student unpaired 't' test, Chi-square test. The patients of ALD is found to have low S.CHE & high LDH levels, high Bilirubin levels and low albumin levels whereas patients of NASHis found to have normal S.CHE, LDH levels and also found to have minimally low albumin levels, normal A/G ratio, high cholesterol & FBS levels. We also noted a reversal of A/G ratio in ALD patients. The difference in the findings were found to be statistically significant. Our study concluded that ALD patients had more hepatic injury when compared to that of NASH. We also came in to conclusion that NASH and ALD can be differentiated by means of these biochemical parameters. These findings may help the clinicians in arriving at prompt diagnosis and instigate earlier management.in ALD and NASH patients.

Keywords: Alcoholic liver disease, Aspartate aminotransferase, Alanine aminotransferase, Nonalcoholic steatohepatitis.

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INTRODUCTION

The liver is one of the largest and most complex organs in the body. It performs multiple functions, including the production of protein and enzymes, detoxification, metabolic functions, and the regulation of cholesterol and blood clotting. Because the liver is primarily responsible for alcohol metabolism, it is especially vulnerable to alcohol-related injury [1]. Liver diseases are quite prevalent throughout the globe. Their early and correct diagnosis is always a concern among physicians, especially the residual liver function [2]. Liver disease develops silently; there may be no signs or symptoms until the complications of liver failure or portal hypertension develop [3]. Liver function test including enzymes is a good tool but it is usually difficult in screening patients exhibiting or concealing signs of liver disease. This article presents the relationship between liver diseases with increase serum levels of various enzymes and their importance in clinical diagnosis while highlighting a system for assessing abnormal liver test results [4].

MATERIALS AND METHODS

This study was carried out in Govt Stanley Medical College in 24 hrs. Clinical Biochemistry lab & Department of Medical Gastroenterology. 40 patients of ALD (Group 1) and 30 patients of NASH (Group 2) were included in this study. Selection criteria include male patients of age group 20-70 years, history of alcohol for ALD (Group 1), no history of alcohol consumption for NASH (Group 2). Inclusion Criteria For This Study: Male patients in the age group of 20-70 years. H/O Alcohol consumption (daily amount &duration) (ALD). Average daily intake of 60-80gm.Patients with positive radiological features of fatty liver. Patients with positive Viral antigen /Antibody markers. No history of alcohol consumption &raised serum bilirubin. Exclusion Criteria For This Study: ALD: Patients with liver disease other than alcoholic origin. NASH: Patients with history of alcoholism. Patient with any other malignant diseases. After obtaining prior permission from ethical committee, the study was conducted in Govt Stanley Medical College in Dept of Biochemistry and Dept of Medical Gastroenterology. All the participants will be informedabout the study and informed consent will be obtained from the participants.There is no risk for the participants in conducting this study. After obtaining informed consent from the patient, under aseptic precaution, 5ml blood sample will be collected in red Plain tube randomly. Blood in the plain red tube was allowed to clot and centrifuged at 2000-2500 rpm for 15 minutes. Serum samples will be separated immediately from the cells and analyzed for total bilirubin, Direct Bilirubin, LDH, ChE, Total protein, AST, ALT, AST/ALT ratio, Albumin and globulin were analyzed in our 24 hours central Biochemistry lab using auto analyzer - EM 360

Statistical Analysis

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

RESULTS

Table 1: FBS

	ALD (GROUP 1) N=40	NASH (GROUP 2) N=30	TOTAL(N=70)	P value
	N (%)	N (%)	N (%)	
Normal	17 (42.5)	3 (10)	20 (28.6)	0.001*
Abnormal (high)	23 (57.5)	27 (90)	50 (71.4)	

Table :1shows comparison of Fasting blood sugar levels between two groups group1 -ALD & group 2 NASH. 90% of NASH and57% of ALD patients is found to have abnormal (high) fasting blood sugar levels. the difference between the two groups is found to be statistically significant(P value :0.001℃) (Chi -square test)

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Table 2: TGL

	ALD (GROUP 1)N=40	NASH (GROUP 2)N=30	TOTAL(N=70)	P value
	N (%)	N (%)	N (%)	
Normal	38 (95)	30 (100)	68 (57.1)	0.214
Low	2 (5)	0	2	

Table:2 shows comparison of Triglycerides levels between two groups ALD & NASH 100% NASH. pts and 95% of ALD pts have normal triglycerides level. only 5% of ALD has low triglycerides level. the. difference between the two groups is not statistically significant (P value :0.214) (Chi -square test) comparison of triglycerides level between ALD &NASH Groups.

Table 3: Cholesterol

	ALD (GROUP 1) N=40	NASH (GROUP 2) N=30	TOTAL(N=70)	P value
	N (%)	N (%)	N (%)	
Low	32 (80)	6 (20)	38 (54.3)	0.001*
Normal	8 (20)	17 (56.7)	25 (35.7)	
High	0	7 (23.3)	7 (10)	

Table:3 shows the cholesterol levels between two groups ALD (group1) & NASH. 23% of NASH Patients reported to have high cholesterol levels. whereas 80% of ALD Patients reported to have low cholesterol. 56.7% of NASH patients found to have normal cholesterol levels. the difference between the two groups is found to be statistically significant (Chi -square test).

Table 4: HDL

	ALD (GROUP 1)N=40	NASH (GROUP 2)N=30	TOTAL(N=70)	P value
	N (%)	N (%)	N (%)	
Low	30 (75)	25 (83.3)	55 (78.6)	0.300
Normal	7 (17.5)	5 (16.7)	12 (17.1)	
High	3 (7.5)	0	3 (4.3)	

This table shows comparison of HDL levels between two groups - ALD & NASH .83.3% of NASH & 75% of ALD shows low HDL Values.7.5% of ALD patients show high HDL values .16.7% of NASH and 17.5% of ALD patients show normal HDL Values. the difference between the two groups is not statistically significant (Chi -square test).

Table 5: Total Protein

	ALD (GROUP 1)N=40	NASH (GROUP 2)N=30	TOTAL(N=70)	P value
	N (%)	N (%)	N (%)	
Low	25 (62.5)	19 (63.3)	44 (62.9)	0.177
Normal	11 (27.5)	11 (36.7)	22 (31.4)	
High	4 (10)	0	4 (5.7)	

This table shows comparison of total protein levels between two groups ALD & NASH .36.7% of NASH &27.5% of ALD patients show normal values of protein.63.3% of NASH & 62.5% of ALD patients show low protein values. the difference between the two groups is not statistically significant (Chi -square test).

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Table 6: Albumin

	ALD (GROUP 1)N=40	NASH (GROUP 2) N=30	TOTAL(N=70)	P value
	N (%)	N (%)	N (%)	
Normal	2 (5)	0	2 (2.9)	0.214
Low	38 (95)	30 (100)	68 (97.1)	

Table:6 shows comparison of ALBUMIN between two groups ALD & NASH. 95% of ALD & 100% of NASH patients is noticed to have low albumin levels. only 5% of ALD patients found to have normal albuminlevels. the difference between the two groups is not statistically significant (Chi –square test).

Table 7: AST

	ALD (GROUP 1) N=40	NASH (GROUP 2)N=30	TOTAL(N=70)	P value
	N (%)	N (%)	N (%)	
Normal	6 (15)	1 (3.3)	7 (10)	0.107
High	34 (85)	29 (96.7)	63 (90)	

Table:7 shows comparison of AST between two groups ALD & NASH .96.7% of NASH & 85% of ALD Patients found to have high AST VALUES. the difference between the two groups is not statistically significant (Chi -square test).

Table 8: ALT

	ALD (GROUP 1) N=40	NASH (GROUP 2) N=30	TOTAL(N=70)	P value
	N (%)	N (%)	N (%)	
Normal	11 (27.5)	3 (10)	14 (20)	0.05*
High	29 (72.5)	27 (90)	56 (80)	

Table:8 shows comparison of ALT between two groups ALD &NASH. 90% of NASH and 72.5 % of ALD Patients found to have high ALT levels . 10% of NASH & 27.5 % of ALD patients found to have normal ALT levels. the difference between the two groups is found to be statistically significant (Pvalue 0.05*) Chi -square test).

Table 9: Total Bilirubin

	ALD (GROUP 1)N=40	NASH (GROUP 2) N=30	TOTAL(N=70)
	N (%)	N (%)	N (%)
Normal	40	30	70

Table 10: Direct Bilirubin

	ALD (GROUP 1) N=40	NASH (GROUP 2) N=30	TOTAL(N=70)	P value
	N (%)	N (%)	N (%)	
Low	1 (2.5)	3 (10)	4 (5.7)	0.01 **
Normal	39 (97.5)	27 (90)	66 (94.3)	

Table :10 shows comparison of direct bilirubin between two groupsALD & NASH. 90% of NASH & 97.5 of ALD showed normal direct bilirubin values. only 10% of NASH & 2.5 % ALD showed Low direct bilirubin values. The difference between the two groups is found to be significant (P Value :0.01*) Chi -square test).

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Table 11: Serum Lactate Dehydrogenase

	ALD (GROUP 1) N=40	NASH (GROUP2) N=30	TOTAL(N=70)	P value
	N (%)	N (%)	N (%)	
Low			0	0.001 **
Normal	0	1(3.3)	1 (1.4)	
High	40 (100)	29 (96.7)	69 (98.6)	

Table:10 shows comparison of serum lactate dehydrogenase levels between two groups ALD & NASH. 96.7 % of NASH patients & 100% of ALD is noted to have high serum levels of lactate dehydrogenase. Only 3.3%of NASH Patients is noticed to have normal levels of serum cholinesterase. The difference between the two groups is found to bestatistically significant (Chi -square test).

Table 11: Cholinesterase

	ALD (GROUP 1) N=40	NASH (GROUP 2) N=30	TOTAL(N=70)	P value
	N (%)	N (%)	N (%)	
Low	39 (97.5)	25 (83.3)	64 (91.4)	0.03*
Normal	1 (2.5)	5 (16.7)	6 (8.6)	

Table:11 shows comparison of serum cholinesterase levels between two groups ALD & NASH.97.5 %of ALD patients & 83.3% of NASH Patients is noticed to have low serum cholinesterase levels. only 2.5% of ALD & 16.7 % of NASH revealed to have normal serum cholinesterase levels. The Difference Between the two groups is found to be statistically significant (P value 0.03*) (Chi -square test).

Table 12: A/G Ratio

	ALD (GROUP 1) N=40	NASH (GROUP 2) N=30	TOTAL(N=70)	P value
	N (%)	N (%)	N (%)	
<1	15 (37.5)	5 (16.7)	20 (28.6)	0.05*
>1	25(83.3)	25 (62.5)	50 (71.4)	

TABLE: 12 This table shows comparison of A/G ratio in two groupsALD & NASH. 83.3% of ALD patients & 62.5% of NASH patients is notedto have A/G ratio more than 1 which is found to be statistically significant (Chi square test).

Table 13: AST / ALT Ratio

	ALD (GROUP 1) N=40	NASH (GROUP 2) N=30	TOTAL(N=70)	P value
	N (%)	N (%)	N (%)	
<2	14 (35)	11 (36.7)	25 (35.7)	0.885
>2	26 (65)	19 (63.3)	45 (64.3)	

TABLE :13 This table shows comparison of AST/ALT ratio in two groups ALD & NASH .63.3% of NASH & 65% of ALD patients found to have AST/ALT ratio more than 2. The difference between the two groups isnot statistically significant (Chi -square test).

Table 14: ALD & NASH

	(ALD) (Mean + SD)	(NASH) (Mean + SD)	P VALUE
TGL	111 ± 41	176.4 ± 47.3	0.001**

This Table shows mean comparison of triglycerides level in both group ALD & NASH . The average Mean of triglycerides level is found to be high in NASH groups when compared to that of ALD . The difference between the two group is found to be statistically significant.

Table 14: Mean Comparison Cholesterol

	CASES (ALD) (Mean + SD)	CONTROLS (NASH) (Mean + SD)	P VALUE
CHOLESTEROL	111.2 ± 42	212 ± 71.6	0.001**

This table shows mean comparison of cholesterol levels between two groups ALD & NASH. The average Mean of cholesterol levels is found to be high in NASH groups when compared to that of ALD. The difference between the two group is found to be statistically significant (P Value 0.001**).

Table 15: Mean Comparison HDL

	CASES (ALD) (Mean + SD)	CONTROLS(NASH) (Mean + SD)	P VALUE
HDL	35.8 ± 19.6	20.6 ± 9.8	0.05**

This table shows mean comparison of HDL levels between two groups ALD & NASH The average Mean of HDL levels is found to be normal in ALD groups when compared to that of NASH. The difference between the two group is found to be statistically significant(P Value 0.05**).

Table 16: Mean Comparison Of Total Protein Between ALD & NASH

	CASES(ALD) (Mean + SD)	CONTROLS(NASH) (Mean + SD)	P VALUE
TP	6.3 ± 1.52	5.9 ± 0.64	0.265

This table shows mean comparison of TOTAL PROTEIN levels between two groups ALD & NASH. The average Mean of Total Protein levels is found to be normal in ALD when compared to that of NASH. The difference between the two group is not statistically significant

Table 17: Mean Comparison Of Globulin Between ALD & NASH

	CASES(ALD) (Mean + SD)	CONTROLS(NASH) (Mean + SD)	P VALUE
GLOBULIN	2.7 ± 0.79	3.2 ± 0.66	0.256

This table shows mean comparison of Globulin levels between two groups ALD & NASH. The average Mean of GLOBULIN levels is found to be normal in both the groups ALD & NASH. The difference between the two group is not statistically significant (P Value 0.256)

Table 18: Mean Comparison Of AST Between ALD & NASH

	CASES(ALD) (Mean + SD)	CONTROLS(NASH) (Mean + SD)	P VALUE
AST	82.3 ± 55.3	91.8 ± 47.4	0.458

This table shows mean comparison of AST levels between two groups ALD & NASH The average Mean of AST levels is found to be high in NASH when compared with that of ALD. The difference between thetwo group is not statistically significant (P Value 0.458).

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Table 19: Mean comparison of Albumin between ALD & NASH

	CASES(ALD) (Mean + SD)	CONTROLS(NASH) (Mean + SD)	P VALUE
ALB	1.7 ± 0.57	2.8 ± 0.53	0.045*

This table shows mean comparison of ALBUMIN levels between two groups ALD & NASH The average Mean of ALBUMIN levels is found to be Low in both the groups ALD& NASH. The difference between the two group is found to be statistically significant

Table 20: Mean comparison of ALT between ALD & NASH

	CASES(ALD) (Mean + SD)	CONTROLS(NASH) (Mean + SD)	P VALUE
ALT	51.7 ± 37.8	65.2 ± 28.8	0.109

This table shows mean comparison of ALT LEVELS between two groups ALD &NASH. The average mean values of ALT levels are found to be similar in both groups. the difference between the two groups is not statistically significant (P Value 0.109).

DISCUSSION

Alcohol is a well-known toxin that primarily affects the liver. It is also found to affect millions of people worldwide. ALD is still found to be a leading cause of liver-related morbidity and mortality and the burden it is exerting on medical systems with hospitalization and management costs rising constantly worldwide [5]. In our country, alcohol consumption is found to be very common and hence Alcoholic liver disease is found to be widely prevalent. It is essential to diagnose alcoholic liver disease earlier so that we can prevent the complication that is arising due to the disease and also in identifying the riskfactors that is attributable to the disease and also in initiating screening programs to prevent further complication and improving status of life [6]. Alcoholic liver disease includes a spectrum of simple steatosis or fatty liver, alcoholic hepatitis, hepatitis, fibrosis, and cirrhosis. Diagnosis of alcoholic liver disease from other liver disease like Non-Alcoholic Steatohepatitis is found to be difficult because clinical picture will be resembling somewhat similar and history will be difficult to be obtained as some patients may deny the use of alcohol [7]. Alcohol is found to affect the liver depending upon the dose and duration of alcohol use or abuse. Progression of ALD will lead to scarring and development of cirrhosis. NASH is found to be a widely emerging cause of chronic liver disease, which is due to increase in unhealthy dietary habits, sedentary life style and it is also associated with obesity, diabetes, dyslipidemia, hypertension and metabolic syndrome.Liver biopsy is found to be the gold standard in diagnosing both NASH and ALD, since it is a highly invasive procedure and cost effective, time consuming and a painful procedure to the patient, so clinicians are in need of a simple means of differentiating the alcoholic liver disease from Non - Alcoholic Steatohepatitis by means of certain biochemical markers [8]. In this study with the help of following biochemical parameters AST/ALT Ratio, albumin, globulin, albumin/globulin ratio serum cholinesterase, serum lactate dehydrogenase total and direct bilirubin we compared the patients of alcoholic liver disease from Non- Alcoholic Steatohepatitis. We included all male patients aged from 20 - 60 years with h/o alcohol intake and with positive radiological findings in the ALD group. We included all male patients with no history of alcoholism in the NASH group [9]. In our study we observed a decline in serum cholinesterase levels in Alcoholic liver disease patients when compared to that of NASH population. where it is found to be normal. This difference was found to be statistically significant (P value 0.05*S). Serum cholinesterase is an early sensitive marker of synthetic function of liver and it is also considered to be good prognostic marker of advanced liver disease. This low serum cholinesterase levels in Alcoholic Liver disease is probably due to decreased synthetic capacity of liver which may be due to prolonged alcohol induced hypoxic injury to the hepatocytes, which may also indicate sign of progression of alcoholic fatty liver to fibrosis [10]. Serum cholinesterase levels is found to be normal or high normal in NASH patients, which reflects that hepatic synthetic function of liver is maintained and unaltered. In our study we also observed that Serum Lactate Dehydrogenase levels tend to be high in Alcoholic Liver disease compared to that of Non-Alcoholic steatohepatitis. This difference is also found to be statistically highly significant (P value 0.001**) [11]. The major cause for marked rise in Serum LDH Levels in Alcoholic liver disease is mainly due to hypoxic and necrotic condition of hepatocyte that has been caused

by prolonged alcohol related hypoxic injury to the hepatocytes. LDH levels are mainly measured to identify the level of necrosis in the hepatocytes [12]. LDH concentration is found to be high in hypoxic condition of liver. In NASH, the LDH levels were found to be normal which rules out hypoxic injury to the hepatocytes. In our study serum protein levels were found to be in normal limits between both the groups ALD & NASH. We observed that serum albumin levels are found to be in decreasing trend in Alcoholic liver disease in comparison with non-alcoholic steatohepatitis [13]. This difference is found to be statistically significant (P Value 0.05*). These low levels of serum albuminin alcoholic liver disease in our study is mainly due to alcohol related hepatocellular injury probably of chronic duration and is leading to advanced stage of fibrosis. In Non-Alcoholic Steatohepatitis, we noticed a minimal fall in albumin levels which may indicate initial progression to fibrotic stage [14]. We also noted there was reversal of A/G RATIO in the alcoholic liverdisease patients compared to that of NASH patients which is also found to be statistically significant (P Value 0.05*). This finding has been supportedby a study conducted by Majhi S et al who has reported in his that there is reversal of A/G Ratio in Alcoholic Liver Disease [15]. In this study, we observed that serum AST and ALT levels are found to be high in case of both ALD and NASH which is found to be statistically non-significant. In our study we also observed that Bilirubin levels (both direct and total) were found to be increased in both the groups ALD & NASH and the difference is found to be statistically significant [16]. This is probably due to chronic alcoholic consumption leading to advanced stage of liver disease that may contribute to progression of fibrosis in case of alcoholic liver disease. Whereas in NASH, it may be probably due to negative relation with metabolic syndrome and obesity, which is part of NASH definition. By comparing the patients of ALD & NASH we came in to a decisionthat these two forms of liver disease can be differentiated with the help of biochemical parameters like serum cholinesterase, serum lactate dehydrogenase, A/G ratio, AST/ALT ratio [17, 18]. These parameters can be used asa supportive means where history, clinical examination and other invasive investigations provide no clear-cut diagnosis [19, 20].

CONCLUSION

In Our study we came in to a conclusion that alcoholic liver disease can be differentiated from non-alcoholic steatohepatitis with the help of biochemical parameters like serum cholinesterase, serum lactate dehydrogenase, A/G ratio, AST, ALT, AST/ALT ratio. Our study also shows that the above biochemical parameters can be used instead of invasive procedures like liver biopsy to differentiate various liver diseases. So, our study also concluded that NASH patients had less hepatocyte injury and fair prognosis as compared to ALD patients who had more hepatic loss with worse prognosis. Prompt diagnosis and management will help in reducing the morbidity and mortality in both alcoholic liver disease and non - alcoholic steatohepatitis.

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