

Evaluation of Biochemical, Haematological Parameters and Noninvasive Prognostic Scores in Alcoholic Liver Disease with and without Complications

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ABSTRACT

Background: Chronic and excessive alcohol ingestion is one of the major causes of liver disease in the industrialized nations. The spectrum of pathology of alcoholic liver injury ranges from fatty liver to alcoholic hepatitis and cirrhosis. Fatty liver is present in over 90% of binge and chronic drinkers and 10 to 20 % of alcoholics develop alcoholic hepatitis. The mortality of patients with alcoholic hepatitis concurrent with cirrhosis is nearly 60% at 4 years. Various risk factors implicated in alcoholic liver disease include quantity and duration of alcohol intake, type of alcohol consumption and drinking pattern, gender, co-existent HCV infection, gene polymorphism and nutritional factors such as malnutrition/obesity.

Aims and Objective: To identify the role of biochemical, haematological parameters and noninvasive prognostic scores in alcoholic liver disease with or without complications.

Materials and Methods: In this study, sixty alcoholic patients with (n=32) or without (n=28) radiologic and/or clinical evidence of cirrhosis were, retrospectively evaluated to identify the role of various biochemical, haematological parameters and noninvasive prognostic scores.

Results: Patients having higher values of total bilirubin (≥ 1.9), AST ALT ratio (≥ 2.3) and Lower value of albumin (< 2.9) shown to have the higher risk of complications (ORs are 7.5, 5.0 and 7.5 respectively) developed in alcoholic liver disease. Similarly lower value of Hb, and higher values of TLC, MCV, PT, INR, BCDS and Maddrey's DF shown to have higher risk of

complications among the alcoholic liver disease. Multiple regression analysis revealed that the higher values of MCV (≥ 96.2), TLC (≥ 10.4) and MDR (≥ 24.2) are independently positively associated with the complications (ORs are 5.5, 7.6 and 29.3 respectively) developed in ALD.

Conclusion: Higher values of MCV, TLC and Maddrey's DF are playing their independent role of positive association with the complications developed in the ALD. Early evaluation and management of these easily available parameters and non-invasive scoring system may be more appropriate in prevention of development of complications among ALD cases.

Key words: Alcoholic Liver Disease, Biochemical, Haematological, Scoring, Prognosis.

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Article History:

Received: 30-08-2018, Revised: 20-09-2018, Accepted: 13-11-2018

Access this article online

Website: www.ijmrp.com	Quick Response code 
DOI: 10.21276/ijmrp.2018.4.6.025	

INTRODUCTION

Alcoholism constitutes one of the major health and socioeconomic issues worldwide. Excessive alcohol ingestion belongs to top five risk factors for preventable mortality causing 2.5 million deaths and 69.4 million annual disability adjusted life years globally.¹

Liver disease, heart disease, pancreatitis, central nervous system disorders and certain forms of cancers are the major health hazards of alcoholism. Chronic alcoholism capable of producing diseases depends on various factors like amount and pattern of

alcohol consumption, gender, ethnicity, age, obesity, malnutrition, co-existing chronic viral hepatitis, smoking and host genetic factors.² Alcohol abuse is associated with a spectrum of liver injury known as alcoholic liver disease (ALD) & includes fatty liver, alcoholic hepatitis (AH) and cirrhosis and may eventually lead to carcinoma of liver. Diagnosis of ALD can be made based on history, clinical and laboratory findings and distinguishing alcoholic from non-alcoholic liver disease is important as their treatment & management are different. However, it can sometimes be challenging as there is no single specific diagnostic test for ALD, both the physical findings and laboratory evidence for ALD may be non-specific. Besides liver disease is multifactorial and patients are usually not truthful about their degree of alcohol consumption.^{3,4}

Therefore, the clinician has to rely on indirect evidence of alcohol abuse, such as questionnaires, information from family members and group of laboratory tests to strengthen or confirm a clinical suspicion ALD. The CAGE questionnaire remains the most widely used screening instruments for diagnosis of ALD.⁵ Chronic liver diseases frequently are associated with range of biochemical and hematological abnormalities. Raised serum gamma glutamyl transferase (GGT), hyperbilirubinemia and hypoalbuminemia are markers of acute & chronic liver injury. Serum aspartate aminotransferase (AST) is typically elevated to a level of 2 – 6 times the upper limits of the normal in severe AH. Levels of AST >500 IU / l or ALT (Alanine Aminotransferase) >200 IU / l are rarely seen with AH and suggest another etiology. AST / ALT ratio >3 are highly suggestive of ALD & AST / ALT >2, seen in 70 % of alcoholics without cirrhosis.⁶ Patients with severe hepatocellular disease develop thrombocytopenia, deficiencies of coagulation factors leading to prolongation of the prothrombin time (PT), splenomegaly causing secondary hemolysis; macrocytosis and megaloblastic anemia due to malnutrition, aplastic anemia due to bone marrow failure.⁷

In the present study we have investigated changes in biochemical and haematological parameters in patients with alcoholic liver injury (both moderate and heavy drinkers). Four noninvasive scoring systems for assessing severity of illness have been used here namely AST/ALT (AAR), AST to platelet ratio index (APRI), Bonacini cirrhosis discriminant score (BCDS) and Maddrey's discriminant function (DF) for the stratification of patients and to appraise the need of treatment. The APRI index has been proposed as a simple and noninvasive predictor to evaluate hepatic fibrosis in several liver diseases. It can be a good alternative diagnostic method to FibroScan or liver biopsy.⁸ The Maddrey's DF ($DF = 4.6(PT_{sec} - control PT_{sec}) + \text{serum total bilirubin in mg/dL}$) is a predictor of significant mortality risk in patients with AH. This test is in use for long time, but it uses PT as a variable which depends on the sensitivity of the thromboplastin with its laboratorial variations.⁹

BCDS uses four laboratory tests (AST/ALT, PT, INR and platelet count) to calculate a discriminant score capable of identifying patients with cirrhosis. This is helpful for those who cannot undergo liver biopsy.¹⁰

Several prospective studies, in the recent past, have studied the predictive utility of various biochemical and haematological parameters & scoring systems in patients with non-alcoholic liver disease (NALD). However, data from patients with alcoholic liver diseases are limited.

AIMS AND OBJECTIVES

To evaluate the significance of different biochemical and haematological parameters and scoring systems in alcoholics and to study their predictive ability in development of complications associated with it.

- To evaluate the biochemical parameters (Total bilirubin, direct bilirubin, AST, ALT, GGT, Total protein, Albumin), haematological parameters (Hb, TLC, TPC, MCV, PT, INR) & scores (APRI, AAR, BCDS, Maddrey's DF) in alcoholics with and without complications.
- To determine the predictive value of these parameters in alcoholics with complications.
- To evaluate which non-invasive marker has the highest prognostic significance in determining liver cirrhosis.

MATERIALS & METHODS

This retrospective study was conducted on 60 patients diagnosed as alcohol induced liver cirrhosis of 1 year duration from January 2015 to December 2015.

Data pertaining to the patient details like duration of alcohol intake, clinical findings, presence or absence of co morbidities and various investigations; with special emphasis to biochemical markers including AST, ALT, GGT, Total Bilirubin, Direct bilirubin, Total protein and Albumin were noted along with haematological parameters like Hemoglobin (Hb), Total leukocyte count (TLC), Total platelet count (TPC), Mean Corpuscular Volume (MCV), PT & International normalized ratio (INR) were collected from the medical records department.

Four scoring systems were calculated based on these parameters namely AST/ALT (AAR), AST/Platelet count (APRI), Bonacini cirrhosis discriminant score [$\text{platelet} \times 10^3 / \mu\text{L} + \text{ALT/AST} + \text{INR}$], Maddrey's discriminant function ($4.6 \times (PT_{\text{patient}} - PT_{\text{control}}) + \text{total Bilirubin}$). The cut off value for severe fibrosis were APRI >1 & AAR ≥ 0.8 .⁶

For Bonacini cirrhosis discriminant score minimum discriminant score was 0, maximum discriminant score was 11. A score > 3 (≥ 4) was taken to have best sensitivity and specificity for cirrhosis. The score was not used in patients treated with warfarin or with thrombocytopenia due to a non-hepatic cause.¹⁰

Maddrey's DF score of 32 or greater was predictive of a high short-term mortality with improved clinical outcomes after receiving corticosteroids.¹¹

The cases were selected based on the inclusion and exclusion criteria mentioned below. Inclusion criteria were all males, age group ranging from 20-70 years, history of alcohol intake for ≥ 6 months with clinical and/or radiological evidence of presence of liver disease.

Exclusion criteria were all female patients, age less than 20 years, occasional drinkers and presence of associated co-morbidities like hepatitis B virus infection (HBV), hepatitis C virus infection (HCV), drug induced liver disease and obesity.

Statistical Analysis

The results are presented as mean \pm SD and those of qualitative variables as numbers and percentages to arrive at the final results about the pattern of variations of these biochemical parameters in cirrhosis. Independent sample t test and binary logistic regression test was used. The level of significance was considered when p value <0.05.

Table 1: Comparison of clinical & biochemical parameters among alcoholics with and without complications:

Parameters	Alcoholics without complication (N=28)	Alcoholics with complications (N=32)	P-value
Age (years); (Mean ± SD)	41.93 ± 10.88	46.78 ± 9.150	0.066
Duration of alcohol intake (yrs); (Mean ± SD)	14.30 ± 9.298	13.57 ± 7.006	0.858
Smoker; n (%)			0.969
No	18 (45.0)	22 (55.0)	
Yes	8 (44.4)	10 (55.6)	
Type DM			0.442
No	18 (41.9)	25 (58.1)	
Yes	8 (53.3)	7 (46.7)	
Total Bilirubin (mg/dL); (Mean ± SD)	3.76 ± 6.33	8.22 ± 7.4	<0.001
Direct Bilirubin (mg/dL); (Mean ± SD)	2.26 ± 4.45	5.62 ± 5.84	<0.001
AST (IU/L); (Mean ± SD)	69.25 ± 40.90	99.88 ± 88.24	0.296
ALT (IU/L); (Mean ± SD)	38.93 ± 16.31	34.97 ± 24.54	0.044
GGT (IU/L); (Mean ± SD)	219.46 ± 226.07	119.84 ± 110.21	0.101
Total protein (g/dL); (Mean ± SD)	6.85 ± 0.90	6.30 ± 0.85	0.028
Albumin (g/dL); (Mean ± SD)	3.50 ± 0.94	2.50 ± 0.68	<0.001

*; P≤0.05- statistically significant, **; P≤0.005- statistically very significant

Table 2: Comparison of hematological parameters among alcoholics with and without complications:

Parameters	Alcoholics without complication (N=28)	Alcoholics with complications (N=32)	P-value
Haemoglobin (g/dL)	13.0 ± 3.99	9.23 ± 3.04	<0.001
Total leukocyte count (x 10 ⁹ /L)	7.74 ± 2.95	11.20 ± 4.40	0.001
Total platelet count (x 10 ⁹ /L)	164.29 ± 60.70	153.03 ± 66.43	0.230
Mean corpuscular volume (fL)	90.96 ± 9.12	97.40 ± 11.05	0.018
Prothrombin time (seconds)	17.04 ± 4.13	24.35 ± 9.21	<0.001
INR	1.24 ± 0.35	1.92 ± 0.89	<0.001

*; P≤0.05- statistically significant, **; P≤0.005- statistically very significant

Table 3: Comparison of scoring systems among alcoholics with and without complications:

Scores	Alcoholics without complication (N=28)	Alcoholics with complications (N=32)	P-value
APRI	1.26 ± 0.95	2.08 ± 2.32	0.252
AAR	1.93 ± 1.09	3.25 ± 2.23	0.003
Bonacini cirrhosis discriminant score (BCDS)	6.61 ± 1.64	8.12 ± 1.80	<0.001
Maddrey's discriminant function (DF)	22.19 ± 21.53	60.46 ± 43.34	<0.001

*; P≤0.05- statistically significant, **; P≤0.005- statistically very significant

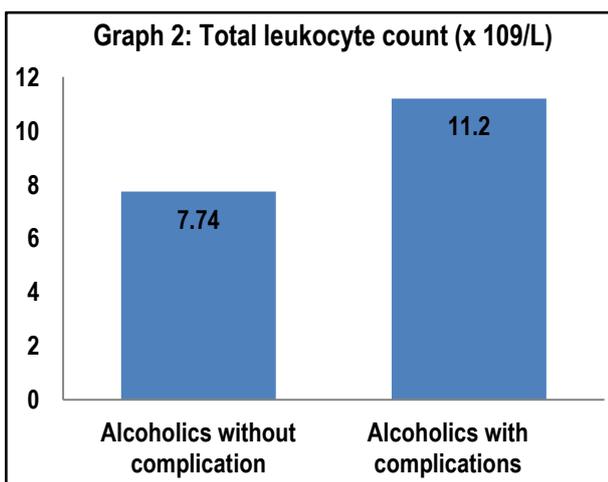
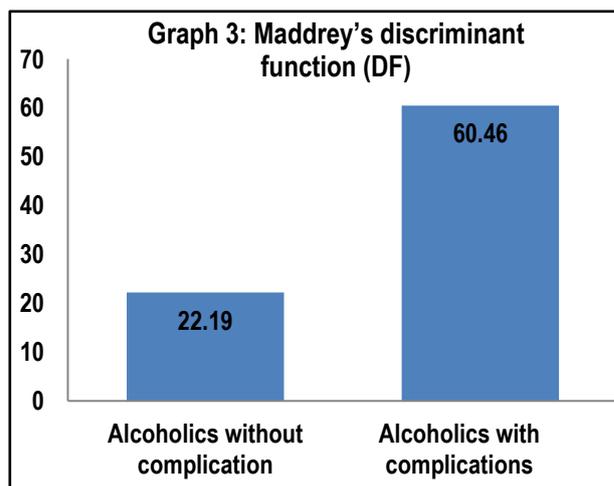
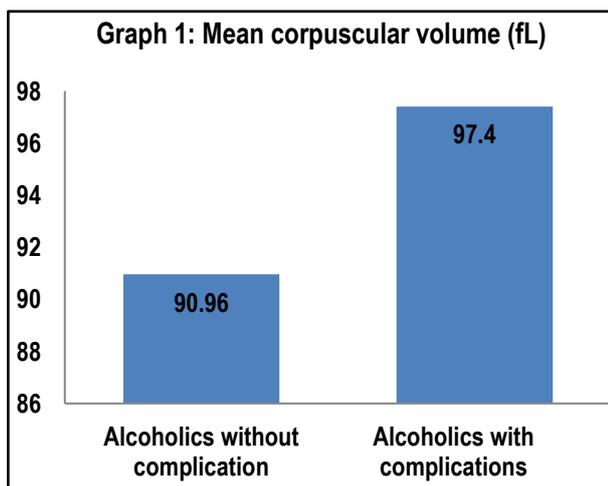
Table 4: Diagnostic performance of clinical characteristics and scores to predict the complications

Parameters	Cut-offs	AUROC (95% CI)	Sensitivity (%)	Specificity (%)
Total bilirubin(mg/dl)	≥1.9	76.7 (63.8 – 89.6)	81.25	67.86
AAR	≥2.3	72.7 (59.6 – 85.8)	71.88	64.29
Total protein(g/dl)	<6.8	66.6 (52.6 – 80.5)	60.71	62.50
Albumin(g/dl)	<2.9	79.5 (67.7 – 91.2)	71.43	75.00
Hb(g/dl)	<10.9	78.5 (66.4 – 90.6)	75.00	71.88
TLC(x10 ⁹ /l)	≥10.4	72.1 (59.3 – 85.0)	56.25	82.14
MCV(fl)	≥96.2	72.7 (59.3 – 86.0)	65.63	82.14
PT(sec)	≥17.8	84.3 (73.6 – 95.0)	90.63	75.00
INR	≥1.5	86.7 (76.6 – 96.9)	81.25	85.19
BCDS	≥8	77.8 (65.1 – 90.5)	78.13	75.00
Maddrey's DF	≥24.2	85.5 (75.7 – 95.2)	93.75	67.86

Table 5: Binary logistic regression used to identify the factors associated with ALD with complications

Parameters		COR (95% CI)	P Value
Total bilirubin(mg/dl)	<1.9	1.0	0.001
	≥1.9	7.5 (2.4 – 23.9)	
AAR	<2.3	1.0	0.005
	≥2.3	5.0 (1.6 – 15.2)	
Total protein(g/dl)	≥6.8	1.0	0.075
	<6.8	2.6 (0.9 – 7.3)	
Albumin(g/dl)	≥2.9	1.0	0.001
	<2.9	7.5 (2.4 – 23.6)	
Hb(g/dl)	≥10.9	1.0	0.001
	<10.9	7.7 (2.4 – 24.2)	
TLC(x10 ⁹ /l)	<10.4	1.0	0.007
	≥10.4	5.2 (1.6 – 17.1)	
MCV(fl)	<96.2	1.0	0.003
	≥96.2	5.9 (1.8 – 19.5)	
PT(sec)	<17.8	1.0	<0.001
	≥17.8	21 (5.4 – 81.2)	
INR	<1.5	1.0	<0.001
	≥1.5	19.2 (4.7 – 77.7)	
BCDS	<8	1.0	<0.001
	≥8	10.7 (3.2 – 35.5)	
Maddrey's DF	<24.2	1.0	<0.001
	≥24.2	10.7 (3.2 – 35.5)	

COR – Crude odds ratio



Graphs 1, 2, 3: Stepwise logistic regression analysis to identify the independent predictors of ALD with complications

RESULTS

Mean age group of alcoholics with complications (46.78 ± 9.150) were higher than alcoholics without complications (41.93 ± 10.88). AST, Total bilirubin and direct bilirubin were higher in group associated with complications. Albumin and total protein significantly lower in complication group. Parameters such as total protein, ALT were found to be statistically significant with $P < 0.05$. Total & direct bilirubin, Albumin ($P < 0.001$) were statistically very significant. Age, duration of alcohol intake, AST, GGT were found to be insignificant.

PT, INR, MCV & TLC were higher in group associated with complications. TPC and haemoglobin significantly lower in complication group. Parameters such as MCV was found to be statistically significant with $P < 0.05$. PT ($P < 0.001$), INR ($P < 0.001$), Hb ($P < 0.001$), TLC ($P < 0.001$) were statistically very significant between two groups. All haematological parameters except total platelet count ($P = 0.23$) were significant between two groups.

Table 3 shows that APRI score is not significantly associated with the ALD patients who developed complications whereas AAR, BCDS and Maddrey's DF were observed significantly higher in group associated with complications (3.25, 8.12 and 60.46 respectively).

Thresholds for clinical characteristics were identified using area under the ROC curve analysis at optimal values of sensitivity and specificity (Table 4).

Table 5 shows the crude/unadjusted odds ratio of different biochemical and clinical characteristics to quantify the risk of developing the complications among the alcoholic liver disease. Patients having total bilirubin of more than 1.9 mg/dl had 7.5 times higher risk of developing the complications as compared to the patients who had less than 1.9 mg/dl. AST/ALT ratio of more than 2.3 showing 5 times higher risk of developing complications. Lower value of albumin (< 2.9) showing 7.5 times higher risk of developing complications. Similarly lower value of Hb, and higher values of TLC, MCV, PT, INR, BCDS and Maddrey's DF shown to have higher risk of complications among the alcoholics with liver disease as compared to their lower reference values.

Results of stepwise logistic regression analysis revealed that MCV, TLC and Maddrey DF are independently associated with the complications developed in the alcoholic liver disease (graphs 1, 2, 3). Higher value of Maddrey's DF (≥ 24.2) shown to have 29.3 times higher risk of complications among ALD. Increased MCV and TLC count also shown to have independent association with higher risk (OR 5.5 and 7.6 respectively) of complications among ALD.

DISCUSSION

Alcoholism is a major public health problem all over the world implicated in 25% of all hospital admissions, liver and upper gastrointestinal cancers, crimes and >40% of all fatal traffic accidents. Severity of organ damage is often directly proportional to the amount of alcohol consumption.¹²

In our study group alcoholics with or without associated complications mostly belonged to age group 30-50 yrs (63.30%), similar to Bajaj et al in which the mean age group of those having fatty liver was 40.11 ± 1.1 whereas the age range was higher in the study by Nepal et al at 47.04 ± 11.32 .¹³ Duration of alcohol intake was not associated significantly with development of complications in liver disease in our study echoing toruella et al & hatton et al postulating no absolute threshold of alcohol consumption is necessary for inducing liver injury, and no direct correlation between level of alcohol consumption and severity of ALD has been established.^{14,15} Alcoholic liver disease (ALD) not only depends on the total amount of alcohol consumed, also on the drinking patterns and type of alcoholic beverage ingested; presence and extent of other associated factors like protein calorie malnutrition, obesity and genetic factors.^{5,15}

Total bilirubin, direct bilirubin, AST, GGT were higher in group associated with complications with similar observation by Toruella

et al & Nepal et al that patients with alcoholic hepatitis will typically have moderately elevated aminotransferases (< 500 IU/mL) and elevated serum bilirubin (> 5 mg/dL). Patients with elevated GGT levels has the highest likelihood of having fatty liver.^{13,16} Chronic alcoholism induces rise in serum GGT and is a widely used index for excessive alcohol intake. However, elevated GGT alone has both low sensitivity and specificity for alcohol abuse similar to our study.⁵ Albumin and total protein were lower in complication group in our study and lower value of albumin (< 2.9) showed 7.5 times higher risk of developing complications. As per Das et al a common feature of chronic alcoholic liver disease is progressive hypoalbuminemia as exposure to alcohol depresses albumin. The decrease in serum albumin level is also attributed to nutritional status of subjects.¹⁷

MCV, TLC, PT, INR were higher in group with complications as noted by Das et al. Low Hb, higher MCV, TLC, PT and INR value predicted higher risk of ensuing complications in our study. Raised MCV or macrocytosis is multi factorial and results from toxicity of alcohol on bone marrow, folate or vitamin B12 deficiency, weak anti folate action of ethanol. Higher MCVs reflect the severity of underlying liver disease.^{7,18} Raised TLC can be explained by the fact that alcohol interferes with the normal production and function of WBC's and alcoholics commonly develop bacterial infections. However Deepak Jain et al have opined that alcohol impairs neutrophil development in the bone marrow. So bone marrow of alcoholic patients shows lack of maturation of neutrophil precursors. The liver secretes several clotting factors like factors I (fibrinogen), II (prothrombin), V, VII and IX. Hepatic injury caused by alcohol causes diminished synthesis of the clotting factors causing prolongation of prothrombin time. TPC and haemoglobin were significantly lower in complication group. Decreased platelet production & enhanced splenic sequestration is cause of lowered TPC in alcoholics. Decreasing trend of hemoglobin in our study is due to the fact that patients with ALD, the iron is not incorporated properly into the hemoglobin molecules. It is converted into ferritin causing iron overload.¹⁹

The non-invasive scores AAR, APRI, BCDS & Maddrey's DF were higher in complications group. Higher values of BCDS and Maddrey's DF score were associated with higher risk of complications. AAR was 3.25 ± 2.23 in alcoholics with complications & 1.93 ± 1.09 without complications; in accordance with Rossman et al noting that an AST/ALT > 2 is highly suggestive of ALD & most patients with non-ALD have AST to ALT ratios below one. Deficiency of pyridoxal-5'-phosphate decreases hepatic ALT to a greater extent than AST, with corresponding changes in serum concentration. Elevation of the AST/ALT ratio in cirrhotic patients may be explained by the reduction in AST clearance, which leads to an increase in serum levels. In addition, advanced liver disease may be associated with mitochondrial injury, resulting in increased release of AST. AST/ALT ratio had a diagnostic accuracy of 65% in alcoholic liver disease by Afdhal et al similar to our finding of 71.88% sensitivity & 64.29% sensitivity in predicting worse prognosis in alcoholics.^{20,21} APRI score was 2.08 ± 2.32 in alcoholics with complications. In fatty liver patients APRI values suggests degree of fibrosis & APRI values tend to increase with the degree of fibrosis. The lesser the APRI score (< 0.5), the greater ability to rule out cirrhosis and the higher the value (> 1.5) the greater the positive predictive value to rule in cirrhosis. APRI cutoff of 1.0 was

76% sensitive and 71% specific in identification of cirrhosis by Shaheen et al.²²⁻²⁴

BCDS score of 8.12 ± 1.80 observed in our study with complications group. The Bonacini score has 100% specificity only for alcoholic cirrhosis & is an indicator that can be useful for prediction of cirrhosis independent of etiology. At the cutoff value of ≥ 8 , the BCDS has a sensitivity of 46% and specificity of 98% for the diagnosis of histological fibrosis scores of 3 to 4. Limitations of the score is it cannot be used in a patients being treated with warfarin or with thrombocytopenia due to a non-hepatic cause.²⁵ Maddrey's DF score was much higher (60.46 ± 43.34) in complication group. In stepwise logistic regression analysis Maddrey's DF was strongest independent predictor of development of complications in alcoholics. It is the extensively used score to assess the severity of AH & identify patients who might benefit from steroid therapy. A score greater than 32 indicates a 30-day mortality risk more than 50%.^{11, 26}

LIMITATIONS

- 1) It's retrospective design. We could not use many other useful parameters due to that like validated questionnaire & liver biopsy.
- 2) Only 60 patients have been included in the study. Further studies with more number of patients are required to support the results of this study. It also limited our ability to identify independent predictors of complications and to show significant differences in the scores.
- 3) Only patients with alcoholic liver disease have been considered in the present study. Studies are needed in patients having end stage liver disease due to other causes in relation the parameters & scores used here.
- 4) Our selection criteria were biased by referral to the liver center and our results are specifically intended to describe features of the referral population.

CONCLUSION

ALD is an appalling public health problem in the world. Early recognition is essential for diagnosis and taking corrective measures to change the disease course and delaying its complications. Our study revealed deranged haematological & biochemical parameters in cases of ALD group. The prognostic scores were very useful as non-invasive alternate methods to assess the degree of liver injury. Hence, it's of paramount importance to conduct further prospective as well as retrospective studies in relation to this with larger sample size to measure the extent & magnitude of effect of alcohol abuse. In conclusion, the MCV, TLC, PT, INR, AAR, BCDS & Maddrey's DF showed good accuracy, moderate sensitivity and high specificity for the diagnosis of complicated ALD.

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Source of Support: Nil.

Conflict of Interest: None Declared.

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Cite this article as: Rajlaxmi Sarangi, Sarojini Raman, Somanath Padhi, Bhaskar Thakur. Evaluation of Biochemical, Haematological Parameters and Noninvasive Prognostic Scores in Alcoholic Liver Disease with and without Complications. *Int J Med Res Prof*. 2018 Nov; 4(6):121-27.
DOI:10.21276/ijmrp.2018.4.6.025