Original paper

Non-invasive assessment of liver fibrosis in alcoholic liver disease

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Abstract

Aim of the study: To assess the severity of fibrosis in patients with alcoholic liver disease (ALD) by a non-invasive method (transient elastography – TE).

Material and methods: A cross sectional study was conducted on 130 cases of ALD over a period of 2 years. Upper gastrointestinal (GI) endoscopy and transient elastography were done. Liver fibrosis was staged with the METAVIR system and severity of fibrosis was correlated with complications, duration of alcohol abuse, aspartate transaminase (AST) to platelet ratio index (APRI) and Child-Turcotte-Pugh (CTP) score. To establish the relationship between various parameters, Spearman's correlation coefficient (r) and their associated probability (p) were used.

Results: Distribution in 130 patients according to the METAVIR stage (median liver stiffness measurement [LSM]) was: F0: $n = 16$ (5.08 kPa); F1: $n = 19$ (6.6 kPa); F2: $n = 9$ (9.3 kPa); F3: $n = 26$ (16.3 kPa) and F4: $n = 60$ (50.5 kPa) ($p < 0.0001$). Liver stiffness measurement (LSM) score was significantly correlated with CTP score ($r = 0.492$, $p < 0.0001$), APRI ($r = 0.435$, $p < 0.0001$), duration of alcohol consumption ($r = 0.816$, $p < 0.0001$), presence of ascites ($r = 0.756$, $p < 0.0001$), presence of esophageal varices ($r = 0.567$, $p < 0.0001$), and presence of variceal bleeding ($r = 0.383$, $p < 0.0001$).

Conclusions: TE is an inexpensive and non-invasive modality to assess the severity of liver fibrosis in ALD. It can be used as a good screening tool to identify patients with cirrhosis without the use of invasive liver biopsy, enabling better prognostication for the development of complications.

Key words: fibrosis, cirrhosis, transient elastography, alcoholic liver disease.

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Introduction

Alcoholic liver disease (ALD) comprises a spectrum of conditions which range from reversible changes such as fatty liver to irreversible changes associated with increased morbidity such as alcoholic hepatitis (AH), cirrhosis, and hepatocellular carcinoma (HCC) [1]. Alcohol and its metabolites induce cell injury and release of endotoxin, thereby activating both innate and adaptive immunity pathways and resulting in release of pro-inflammatory cytokines (e.g., tumor necrosis factor α – TNF-α) and chemokines, and proliferation of T and B cells along with activation of stellate cell and production of collagen [2]. Thus, a characteristic feature of alcohol-related liver disease is chronic liver inflammation resulting in slowly progressing hepatic fibrosis, thereby resulting in cirrhosis in a considerable number of patients [3].

Amongst various complications of cirrhosis, major ones are ascites, coagulopathy, hepatorenal syndrome, varices, hepato-pulmonary hypertension, splenomegaly, hepatic encephalopathy, spontaneous bacterial peritonitis, and hepatocellular carcinoma. These complications can be a result of either abnormal synthetic function, secondary to portal hypertension, or a combination of both [4].

Liver biopsy is regarded as the gold standard for the evaluation and quantification of fibrosis but has the risk of well-known complications in a few patients [5]. Compared to a single liver biopsy, transient elastography (TE) better represents the hepatic parenchyma, as it assesses a larger liver area. Thus, to avoid biopsy in a large number of patients, TE (FibroScan) can be used to assess cirrhotic patients. Moreover, as a result of changed mechanical properties of the fibrotic liver, FibroScan (TE) helps in identifying the disease severity [6].

Non-invasive modalities such as TE have been demonstrated to be effective in ruling in advanced fibrosis or ruling out fibrosis. Very few studies have been done worldwide to assess the severity of liver fibrosis in a non-invasive manner in patients with ALD. To the best of our knowledge, liver fibrosis has not been assessed exclusively by TE in ALD patients in India. Therefore, in this study we aimed to assess patients with ALD in a tertiary care rural hospital. The objectives were to correlate severity of fibrosis with complications of ALD such as esophageal varices, upper gastrointestinal (GI) bleeding, splenomegaly and ascites; with duration of alcohol abuse and aspartate transaminase (AST) to platelet ratio index (APRI); and with Child-Turcotte-Pugh (CTP) score in patients with alcoholic cirrhosis.

Material and methods

This study was carried out in the Department of Medicine, Acharya Vinoba Bhave Rural Hospital (AVBRH) of JNMC, Sawangi (Meghe) Wardha, over a period of 2 years from October 2017 to September 2019. This was a single-center, cross-sectional study conducted after obtaining approval of the Institutional Ethics Committee (IEC) and signed informed consent of study subjects.

A total of 150 consecutive patients suspected of having alcohol liver disease attending the out-patient department (OPD) or admitted in the wards of the Department of Medicine, AVBRH, Wardha were screened for eligibility and 130 patients meeting the inclusion criteria were included in the study. ALD was defined as chronic daily consumption of minimum 30 grams of alcohol in men or 20 grams in women along with evidence of liver injury.

The study included patients aged 18-70 years, and diagnosed cases of alcoholic liver disease. Patients with HBsAg/anti-HCV (hepatitis C virus) antibody positive status; patients with body mass index (BMI) > 30 kg/m^2 (obesity class II according to the WHO Asian classification), and/or patients who have large amounts of

chest wall fat; patients with HCC; and patients with congestive cardiac failure, hepatic encephalopathy or on invasive mechanical ventilation were excluded from the study.

Clinical characteristics of the patients recorded in the study were duration of alcohol intake, ascites, and grade of varices, and whether bleeding or not. Laboratory investigations performed were complete blood count, kidney function test (KFT) and liver function test (LFT), prothrombin time-international normalized ratio (PT-INR), and viral markers for hepatitis B virus (HBV) and HCV. Patients underwent ultrasound of the abdomen to assess liver size and echotexture, absence or presence of ascites (mild, moderate, or gross) and splenomegaly. Patients also underwent upper GI endoscopy to assess the presence or absence of varices, their grade (i.e., small or large varices), and the bleeding status (i.e., whether bleeding or not). Finally, patients underwent transient elastography by a FibroScan 402 machine (EchoSens, Paris, France) to measure the liver stiffness, and liver fibrosis was staged with the METAVIR system [7] i.e., F0 to F4 as:

- F0 (0-5.9 kPa): no fibrosis,
- F1 (6-7.9 kPa): mild fibrosis,
- F2 (8-10.9 kPa): moderate or significant fibrosis,
- F3 (11-19.5 kPa): severe fibrosis,
- F4 (> 19.5 kPa): cirrhosis.

Sample size was calculated on the basis of the prevalence of ALD and the following formula was used [8]:

$$\frac{Z_{1-\frac{\alpha}{2}}^2\, p\, (1-p)}{d^2} = \frac{(1.96)\, (1.96)^2 \times 0.094\, (1-0.094)}{(0.05)^2} =$$

$$= \frac{3.84 \times 0.085}{0.0025} = 130.56$$

where

p = prevalence of ALD = 9.4% = 0.094 [9],

d = absolute precision required on either side of the proportion = 5% points = 0.05 (2-sided),

$Z_{0.05}$ = 1.96 for 95% confidence interval.

Thus, sample size was calculated to be 130 patients.

Statistical analysis

Data were collected and graphics were designed by Microsoft Office Excel 2013. The data were analyzed with SPSS (IBM, Armonk, NY, USA) version 23.0 for Windows, with the help of a statistician. Variables with a normal distribution are represented as mean ± SD. Skewed variables are presented as the median (interquartile range). To establish the relationship between various parameters, Spearman's correlation coefficient and the associated probability (p) were used. According to Evans' classification, correlation strength was

interpreted by using r, < 0.20 was considered as very weak, between 0.20 and 0.39 as weak, between 0.40 and 0.59 as moderate, between 0.60 and 0.79 as strong, and ≥ 0.80 as a very strong correlation [10]. A two-tailed probability value of < 0.05 was considered as statistically significant.

Results

The baseline characteristics of the patients are shown in Table 1. The median age of the patients was 40.00 (35.00-49.00) years and the majority belonged to the age group of 40-60 years (52.31%). The majority were male (i.e., 96.92%). The median values of PT-INR, AST, alanine transaminase (ALT), total bilirubin, platelet count, serum albumin, and serum creatinine were 1.28, 85 IU/l, 36 IU/l, 2.23 mg/dl, 1.29 per mm³, 3.20 g/dl, and 0.95 mg/dl, respectively. The mean hemoglobin was 11.05 ±2.66 g%, the median value of APRI was 1.60 and the median LSM value was 17.65 kPa. In cirrhotic patients, the median CTP score was 9.

The clinico-morphological characteristics are shown in Table 2. The median duration of alcohol intake was 10 years. On upper GI endoscopy, the majority (i.e., 70%) of the patients had no esophageal varices, while 16.92% and 13.08% of patients had large and small varices, respectively; 26 (20%) patients had bleeding varices. On USG, the majority (55.38%) of the patients had no ascites, while 26.16% and 18.46% of the patients had moderate and mild ascites; 58 (44.62%) patients had splenomegaly. The majority of the patients had liver cirrhosis (46.15%), followed by fatty liver (45.38%) and hepatitis (8.46%).

The LSM scores, duration of alcohol abuse and APRI in different stages of fibrosis are shown in Tables 3 and 4. The majority of the patients had stage F4 fibrosis [$n = 60$ (46.15%)] followed by stage F3 [$n = 26$ (20%)], stage F1 [$n = 19$ (14.62%)], stage F0 [$n = 16$ (12.31)], and stage F2 [$n = 9$ (6.92)]. The mean LSM scores of patients with stages F0, F1, F2, F3, and F4 were 5.08 ±0.61, 6.64 ±0.60, 9.32 ±0.88, 16.37 ±217, and 50.56 ±21.76 respectively. The duration of alcohol abuse was highest in patients with stage F4 followed by stages F2 and F3. The mean APRI score was maximum in stage F3 followed by stage F1.

The complications of ALD in various stages of fibrosis and their mean or median LSM scores are shown in Table 5. The majority of the patients with complications had stage F4 fibrosis; i.e. 82.35% of patients with small varices, 95.45% of patients with large varices, 80.77% of patients with variceal bleeding, 77.59% of patients with splenomegaly, and 87.93% of patients with ascites were found to have F4 fibrosis.

Table 1. Baseline characteristics

Baseline characteristics	Mean/median ($N = 130$)
Age (years)	40.00 (35.00-49.00)
Sex	
Males	126 (96.92%)
Females	4 (3.08%)
PT-INR	1.28 (1.14-1.45)
Aspartate aminotransferase (AST) (IU/l)	85.00 (50.00-102.00)
Alanine aminotransferase (ALT) (IU/l)	36.00 (25.00-51.25)
Total bilirubin (mg/dl)	2.23 (1.19-4.32)
Platelet count (per mm³)	1.29 (0.82-2.17)
Serum albumin (g/dl)	3.20 (2.77-3.83)
Serum creatinine (mg/dl)	0.95 (0.80-1.13)
Hemoglobin (g%)	11.05 ±2.66
APRI	1.60 (0.70-2.90)
CTP score ($n = 60$)	9 (8-11)
LSM (kPa)	17.65 (7.55-48.20)

Variables with a normal distribution are represented as mean ± SD; non-parametric variables presented as frequency (percentages); skewed variables presented as median (interquartile range).
PT-INR – prothrombin time-international normalized ratio, APRI – AST to platelet ratio index, CTP – Child-Turcotte-Pugh, LSM – liver stiffness measurement.

Table 2. Clinico-morphological characteristics

Clinico-morphological characteristics	Included patients ($N = 130$)
Duration of alcohol intake (years)	10.00 (5.75-15.00)
Variceal bleeding [n (%)]	26 (20%)
Splenomegaly [n (%)]	58 (44.62%)
Ascites	
No [n (%)]	72 (55.38%)
Mild [n (%)]	24 (18.46%)
Moderate [n (%)]	34 (26.16%)
Esophageal varices	
No [n (%)]	91 (70%)
Small [n (%)]	17 (13.08%)
Large [n (%)]	22 (16.92%)
Stages of ALD	
Fatty liver [n (%)]	59 (45.38%)
Hepatitis [n (%)]	11 (8.46%)
Cirrhosis [n (%)]	60 (46.15%)

Skewed variables presented as median (interquartile range); non-parametric variables presented as frequency (percentages); ALD – alcoholic liver disease.

The correlation of LSM score with various parameters is shown in Table 6. LSM score was significantly correlated with CTP score ($r = 0.492$, $p < 0.0001$), APRI ($r = 0.435$, $p < 0.0001$), duration of alcohol con-

Table 3. Liver stiffness measurement (LSM) score, duration of alcohol consumption, and AST to platelet ratio index (APRI) in different stages of fibrosis

Characteristics (total *N* = 130)	F0 (*n* = 16)	F1 (*n* = 19)	F2 (*n* = 9)	F3 (*n* = 26)	F4 (*n* = 60)
LSM (kPa)	5.08 ±0.61	6.64 ±0.60	9.32 ±0.88	16.37 ±2.17	50.56 ± 21.76
Duration of alcohol consumption (years)	5 (3.5-5)	5 (3.5-7.5)	8 (6-10)	7.44 ±2.24	18.33 ±6.61
APRI	0.5 (0.3-1.9)	2.98 ±3.21	1.11 ±0.48	3.46 ±2.81	1.7 ±0.94

Variables with a normal distribution are represented as mean ± SD; skewed variables presented as median (interquartile range).

Table 4. Liver stiffness measurement (LSM) score and duration of alcohol consumption

Duration of alcohol consumption	*n*	Mean LSM (kPa)	SD	*P*-value
< 5 years	13	6.74	2.594	0.0001, S
5-15 years	95	26.35	23.04	
> 15 years	22	58.64	20.19	

sumption ($r = 0.816$, $p < 0.0001$), presence of ascites ($r = 0.756$, $p < 0.0001$), presence of esophageal varices ($r = 0.567$, $p < 0.0001$), and presence of variceal bleeding ($r = 0.383$, $p < 0.0001$).

Discussion

Alcohol is the most commonly abused substance worldwide with a predilection to cause liver injury, especially with increasing duration of abuse. In India alcohol consumption is on the rise in both urban and rural populations as well as irrespective of gender. The consumption of country liquor is on the rise in the Indian rural population, it being inexpensive and readily available. Country liquor is licensed distilled spirits made locally from molasses. Common varieties include *desi sharab*, *arrack and tari* [11].

Non-invasive assessment of liver fibrosis has been studied widely in chronic viral hepatitis as well as non-alcoholic fatty liver disease (NAFLD). There is a paucity of studies on non-invasive assessment of liver fibrosis in ALD patients and there is no study from rural India to date.

The study population included a much higher number of males and the male : female ratio was around 30 : 1, as compared to a study by Sen *et al.* where the male : female ratio in India was 4.5 : 1 [12]. A higher median value of age was observed in another study by Pathak *et al.* as compared to our study [13]. The median duration of alcohol abuse in our study population was 10 years. The average duration of alcohol intake observed by Sen *et al.* was 18.09 years [12]. Our study revealed an impaired liver function test and decreased mean hemoglobin and platelet counts, suggesting an altered hematology profile. Khatroth studied the clinical and biochemical profile of acute ALD and reported that bilirubin > 2 mg/dl, AST > 40 IU/l, and albumin < 3 g/dl were seen in 83.3%, 66.6%, and 41.6% of cases, respectively [14].

Physical examination showed that 44.62% of patients had ascites, 44.62% had splenomegaly, 30% had esophageal varices, and 20% of patients had variceal bleeding. In another study, Nand *et al.* reported that ascites (72%) followed by splenomegaly (57%), HRS syndrome (35%), HE (59%) and upper GI bleeding (59%) were the most commonly observed complications [15].

The median value of APRI in our study (1.6) was significant for presence of severe fibrosis. APRI more than 1.5 has area under receiver operating curve (AUROC) of 80% and 89% for advanced fibrosis F3-F4 and cirrhosis, respectively [16]. Vanbiervliet *et al.* reported that APRI score was independently related to significant fibrosis ($p < 0.05$) and ALD patients with significant fibrosis had statistically significantly higher APRI scores [17]. CTP score is a marker of prognosis in patients with cirrhosis. In our study, in cirrhotic patients, the median value of CTP score was 9 and suggestive of poor prognosis in the study population,

Table 5. Complications of alcoholic liver disease (ALD) in various stages of fibrosis and their mean or median Liver stiffness measurement (LSM) scores

Complications	F0	F1	F2	F3	F4	LSM (kPa)
Small varices (*n* = 17)	0	0	0	3 (17.65%)	14 (82.35%)	36.9 ±16.39
Large varices (*n* = 22)	0	0	0	1 (4.55%)	21 (95.45%)	69.1 (52.55-75.00)
Variceal bleeding (*n* = 26)	0	0	0	5 (19.23%)	21 (80.77%)	48 (23.55-75.00)
Splenomegaly (*n* = 58)	0	3 (5.17%)	1 (1.72%)	9 (15.51%)	45 (77.59%)	48 (23.55-69.10)
Ascites (*n* = 58)	0	2 (3.44%)	0	5 (8.62%)	51 (87.93%)	48.4 (33.35-70.58)

Variables with a normal distribution are represented as mean ± SD; non-parametric variables presented as frequency (percentages); skewed variables presented as median (interquartile range).

the median being in the CTP class B. In a study by Radisavljevic *et al.* in cirrhotic patients, the CTP score was associated with higher mortality [18]. The mean LSM was 17.65 kPa and suggestive of significant fibrosis (stage F3). In a meta-analysis of 17 studies by Singh *et al.* on chronic liver disease, increased liver stiffness was associated with higher risk of hepatic decompensation, hepatocellular carcinoma development, and death [19].

The FibroScan test showed that 46.14% of the patients had stage F4 fibrosis, which is a very significant number. Fernandez *et al.* in their study observed that the majority of the patients belonged to stage F4 (30.4%), followed by stage F2 (24.4%), stage F1 (20%), stage F3 (17.8%), and stage F0 (7.4%) [20]. Correlating the LSM score with various parameters showed its positive correlation with complications such as ascites, splenomegaly, esophageal varices and variceal bleeding as well as with duration of alcohol abuse, APRI and CTP score. Fernandez *et al.*, in their study, observed a significant correlation between FibroScan score and APRI score ($r = 0.355$, $p = 0.001$) [20]. Foucher *et al.* reported a statistically significant correlation between FibroScan score and CTP score ($r = 0.590$, $p < 0.0001$), ascites ($r = 0.447$, $p < 0.0001$), esophageal varices ($r = 0.492$, $p < 0.0001$), and bleeding varices ($r = 0.387$, $p < 0.0001$) [21]. We included all of these parameters in our study and found statistical significance with LSM score. This study attempted to correlate the FibroScan score with complications of cirrhosis as well as with APRI and CTP score.

Thus, transient elastography is a good predictor of hepatic complications in patients with ALD. It can be concluded that these combined methods can be useful and could allow for the development of a scoring system in the future for the prognosis of patients of ALD. This will provide valuable information to physicians and assist them in counseling the patients regarding their prognosis and may aid them in their follow-up and their categorization for future need of liver transplant.

Limitations

This is a single-center study but the findings are relevant to a national setting.

Conclusions

Transient elastography (TE) is an inexpensive and non-invasive modality to assess severity of liver fibrosis in ALD. It can be used as a good screening tool to identify patients with cirrhosis without the use of invasive liver biopsy, enabling better prognostication as well as

Table 6. Correlation of FibroScan score with various parameters

Parameters	*r* value[#]	*P*-value
FibroScan score vs. CTP score	0.492*	< 0.0001
FibroScan score vs. APRI	0.435*	< 0.0001
FibroScan score vs. duration of alcohol consumption	0.816*	< 0.0001
FibroScan score vs. ascites	0.756*	< 0.0001
FibroScan score vs. esophageal varices	0.567*	< 0.0001
FibroScan score vs. variceal bleeding	0.383*	< 0.0001

*correlation was significant at 0.01 levels (2-tailed), #Spearman's correlation coefficient, CTP – Child-Turcotte-Pugh, APRI – aspartate aminotransferase to platelet ratio index.

predicting complications such as esophageal varices, upper GI bleeding and ascites. Prevention goals such as alcohol abstinence and follow-up TE can be part of the treatment strategy in patients with ALD. Patients in stage F3 or F4 can be categorized for liver transplant considering the validity of this screening tool.

Disclosure

The authors report no conflict of interest.

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