Evaluation of Shear Wave Elastography along with Biomarker Parameters as an Algorithm for Early Detection of Liver Fibrosis in Type 2 Diabetes Mellitus and Alcohol Abuse Patients

ABSTRACT

Introduction: Hazardous alcohol use and diabetes are the major risk factors for development of liver cirrhosis. The presence and progression of hepatic fibrosis into cirrhosis is a prognostic variable having impact on survival in people with hazardous alcohol use and Type 2 Diabetes Mellitus. Liver biopsy is an invasive method for diagnosis and staging of hepatic fibrosis. Shear Wave Elastography (SWE) is a non-invasive method for assessing and staging hepatic fibrosis.

Aim: To evaluate SWE as a non-invasive technique along with biomarker parameters as an algorithm for early detection of liver fibrosis in Type 2 Diabetes and hazardous alcohol use patients and to compare the results of shear wave ultrasound elastography in liver fibrosis with AST/ALT ratios, AST Platelet Ratio Index (APRI scores), and Fibrosis-4 (FIB-4) scores.

Materials and Methods: A prospective cross-sectional study was conducted in a tertiary care hospital attached to Mysore Medical College and Research Institute, Mysore, Karnataka, India, from July 2018 to June 2019. Based on inclusion and exclusion criteria 460 patients were recruited for the study. For each patient, AST, ALT, platelet count and SWE values were measured. Patients with normal serum biomarkers and elevated SWE results were subjected to liver biopsy to confirm the diagnosis. Student’s t-test, Analysis of Variance (ANOVA), Chi-square/fisher-exact test was used to find the significance of study parameters. ANOVA was used to compare the SWE findings with the biochemical parameters.

Results: SWE identified 75 patients with normal values, <1.3 m/sec (Group A); 239 patients with mildly elevated liver stiffness -1.3-2.2 m/sec (Group B) and 146 patients with markedly elevated liver stiffness >2.2 m/sec (Group C). SWE showed positive correlation with APRI (p<0.001, r=0.464); AST/ALT ratio (p<0.001, r=0.462); AST (p<0.001, r=0.479); ALT (p<0.001, r=0.321) and FIB-4 (p<0.001, r=0.409). Forty seven patients with normal serum biomarkers levels and elevated SWE results, were advised liver biopsy. Twenty three patients who underwent successful liver biopsy at a cut-off value of 1.9 m/sec for significant fibrosis, the Area Under the Receiver Operating Characteristic (AUROC) was 0.793 and elastography had 71% sensitivity and 90% specificity.

Conclusion: Incorporating SWE and liver enzyme assay as a non-invasive algorithm in routine helps in detection of a large number of previously undisclosed, significant chronic liver disease cases. This novel diagnostic approach resulted in increased detection of asymptomatic but clinically significant liver disease cases as compared to traditional liver enzyme-based algorithms. SWE along with liver enzymes assay as an algorithm can replace 80% of liver biopsies within a community setting.

INTRODUCTION

Chronic, excessive alcohol consumption is a major public health problem. Alcoholic Liver Disease (ALD) is characterised by a spectrum of damage, ranging from hepatic steatosis to cirrhosis [1]. Alcohol now accounts for 50% of all deaths from liver cirrhosis [2,3]. South-East Asia accounts for one-fifth (19%) of the total number of people with diabetes in the world. Out of 5 adults with diabetes one lives in this region. Almost $4 million people have diabetes which is estimated to rise to 156 million (86%) by 2045 [4].

Patients with Type 2 Diabetes Mellitus (T2DM) are at increased risk for developing Non-Alcoholic Fatty Liver Disease (NAFLD) and have a higher rate of mortality and progression to cirrhosis than non-diabetics with NAFLD. Insulin resistance, in part explains the aggressive nature of NAFLD in diabetes [5-7]. The prevalence rate of NAFLD in T2DM is now accounts for 50% of all deaths from liver cirrhosis [2,3]. South-East Asia accounts for one-fifth (19%) of the total number of people with diabetes in the world. Out of 5 adults with diabetes one lives in this region. Almost $4 million people have diabetes which is estimated to rise to 156 million (86%) by 2045 [4].

It is probable that severe fibrosis and alcoholic cirrhosis are under-diagnosed in routine clinical practice [9], notably because the invasiveness and potential morbidity and mortality of liver biopsy make it poorly accepted by patients [10,11]. However, patients with severe fibrosis or cirrhosis are completely clinically asymptomatic for a long period of time and can be difficult to diagnose, that is why ALD patients have disproportionately more advanced disease than other liver disease patients [12]. For example, only 25% of patients with alcoholic cirrhosis are diagnosed with compensated disease [13]. In chronic liver disease, the prognosis and management decisions are dependent on the stage of fibrosis. Early intervention can prevent serious long-term outcomes [14].

Cirrhosis patients have greater risk of complications (portal hypertension, hepatocellular carcinoma, ascites, etc.) and need closer follow-up. Informing patients of their disease stage and prognosis could encourage them to stop alcohol consumption [15]. Liver biopsy is the standard reference for the diagnosis and staging of fibrosis in ALD and NAFLD [14]. However, this procedure is invasive, expensive and associated with high risk of complications. The liver biopsy yields only a small sample of the liver and a sampling bias can occur especially when fibrosis is heterogeneously distributed, as happens in the mild stage. Moreover, the results can be impaired...
by sampling errors, and intra and inter observer variability in a specimen readings [16].
Utility of many simple markers like BARD score (weighted sum of BMI>28, AST/ALT ratio and diabetes) is limited in a population with type 2 diabetes and led to over-estimation of the prevalence of fibrosis and high levels of indeterminate results [17]. For all these reasons, the development of reliable non-invasive imaging modalities that assess liver fibrosis by measuring liver stiffness has generated great clinical interest. Ultrasound elastography is one such method [18]. Though abnormal transaminases indicate liver disease, patients diagnosed with liver disease within 5 years of an abnormal test is only 3.9% which is very low [19].

Due to the absence of symptoms in early stage of liver disease combined with poor sensitivity of blood liver enzymes and liver biopsy to detect early hepatic fibrosis, there is a need to develop a strategy that results in early detection of hepatic fibrosis while patients are still asymptomatic. This is acceptable to the patients and economically efficient. Hence the present study was undertaken with an aim to design an algorithm combining serum biomarkers and SWE to diagnose liver fibrosis in a community setting, targeting high risk group patients (Type II DM and Alcohol Abuse Patients).

MATERIALS AND METHODS
A prospective cross-sectional study was conducted in Krishnarajendra tertiary care hospital attached to Mysore Medical College and Research Institute, Mysore, Karnataka, India, patients who were referred to the radiology department from July 2018 to June 2019 were involved in this study. The IEC approval was obtained with ref number ECR/134/Inst/KA/2013/RR-16. Patients aged above 18 years, patients with prior or current chronic alcohol overuse defined as >24 g/day for women and >36 g/day for men for >1 year (corresponding to two and three units of alcohol, respectively) and patients with Type 2 diabetes were included. According to BARD score criteria score of ≥2 were considered as high risk patients and were included in the study [20,21]. Patients with already known cirrhosis or obvious cirrhosis due to clinical and biological signs (ascites, increased prothrombin time or oesophageal varices), patients with other comorbid causes of hepatic disease (viral, autoimmune or cholestatic disease) and cancer or other debilitating disease with an expected survival of <12 months were excluded from the study. Patients with percutaneous liver biopsy, hepatic congestion or bile duct dilatation evidenced by ultrasound, positive for human immunodeficiency virus with altered enzyme pattern, on-going substance abuse other than alcohol and inability to comply with the study protocol were also excluded from the study.

Methods of Collection of Data
I. After taking informed consent of all the patients, abdominal ultrasonography using 1-5.0 MHz high frequency curvilinear transducer on PHILIPS Affiniti 70 ultrasound machine was done. Ultrasound elastography of liver was performed in all the patients included in the study. Examination was done using SWE (Elast PQR technique) in Philips Affiniti 70 machine (PHILIPS medical systems, Bothell, WA) using 1-5.0 MHz high frequency curvilinear transducer (CS-1). Elastography measurements were obtained by positioning a sample box in B mode image. To avoid reverberation artifacts the Region Of Interest (ROI) is placed 1.5 to 2.0 cm beneath Glisson’s capsule. Multiple measurements in the same location were made. Median of 10 valid measurements was taken. The values of liver stiffness were expressed in meters per second (m/s). Elastography results were divided into, no clinically significant fibrosis<1.3 m/sec (Group A), moderate to severe fibrosis1.3-2.2 m/sec (Group B) and advanced fibrosis and/or cirrhosis:>2.2 m/sec (Group C) based on Elastography Assessment of Liver Fibrosis Society of Radiologists in Ultrasound Consensus Conference Statement [22].

The following biomarker parameters were derived and are as follows:
a) Aspartate Aminotransferase to Alanine Aminotransferase Ratio (AST/ALT) calculated as AST (U/L)/ALT (U/L) [23]; b) APRI calculated as [AST (U/L)/upper limit normal]/platelets (X10^9/L)] X 100; c) FIB-4 Score calculated as age (yr) X AST (U/L)/[Platelets (10^9/L)] X [ALT (U/L)]^2 [24].

II. Patients with normal blood serum markers with elevated SWE results underwent liver biopsy to confirm the diagnosis. Ultrasound-assisted percutaneous liver biopsy was performed through an intercostal approach with a 17G Menghini needle. All biopsy specimens were fixed in formalin and embedded in paraffin. Liver fibrosis and necroinflammatory activity were evaluated semi quantitatively according to the METAVIR system [22].

Diagnostic Algorithm
III. Patients who met the inclusion criteria were included in the diagnostic algorithm.
IV. All the patients with high risk for chronic liver disease underwent simple blood serum marker tests (i.e., AST, ALT and Platelet count) followed by SWE.
V. Patients with normal blood serum markers with elevated wave elastography results underwent liver biopsy to establish the diagnosis.
VI. Patients with elevated liver enzymes and shear wave SWE results were considered to be having chronic liver disease.
VII. Patients with AST: ALT cut-off of ≥0.8 were considered as high risk patients. Local cut-offs for elevated ALT (>35 U/L for women and >45 U/L for men) was considered.
VIII. APRI>1.5 and FIB-4 score >3.25 were compared with SWE values for identifying elevated liver stiffness and cirrhosis in the study population.
IX. Further investigations including ultrasonography and liver biopsy wherever needed were performed.

Number of patients included in the study is depicted in [Table/Fig-1].
Statistical Analysis

For the analysis of the data, Statistical software namely SPSS 18.0, and R environment ver. 3.2.2 was used. To find the significance of study parameters on continuous scale between two groups (Inter group analysis) on metric parameters Student’s t-test (two tailed, independent) has been used. Analysis of variance (ANOVA) was used to find the significance of study parameters between three or more groups of patients. To find the significance of study parameters on continuous scale between two groups (Inter group analysis) on metric parameters, Student’s t-test (two tailed, independent) was used. Levene’s test for homogeneity of variance has been performed to assess the homogeneity of variance.

Results

Of the 600 patients with defined risk factors considered for the study, 460 patients fulfilled the inclusion, criteria including 359 (78%) males and 101 (22%) females. Majority of the patients belonged to above 40 years of age with mean age 57.68±15.32. The mean age for male patients was 57.9 years and female patients was 56.6 years. A considerably larger percentage of patients with type 2 diabetes (74.1%; p<0.001) had eminrent liver stiffness as compared with hazardous alcohol users (30.1%). Compared with patients with Type 2 diabetes alone or with hazardous alcohol use alone, patients with more than one defined risk factor (n= 103) had no significantly greater percentage of elevated liver stiffness. Patients with elevated liver stiffness (n=385) were older, had a higher BMI and higher prevalence of the metabolic syndrome than those with normal liver stiffness (n=75). On laboratory testing, patients with elevated liver stiffness had lower platelet count, greater prevalence of thrombocytopenia, than patients with normal liver stiffness [Table/Fig-2].

The mean APRI score was higher in Group B (2.98±2.32) and Group C (4.41±2.63) patients compared to Group A patients (0.96±1.37). There was a significant correlation between shear wave and APRI (r=0.464, p<0.001). Similarly, FIB-4 score was higher in Group B patients than patients with normal liver stiffness [Table/Fig-2].

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Predictive Value of Liver Stiffness Measurement for Liver Fibrosis and Non Alcoholic Steatohepatitis (NASH)

Analysis of the subgroup of patients who underwent liver biopsy indicated that the Positive Predictive Value (PPV) for the presence of SWE values <1.7 m/sec for early fibrosis (F1) is 80% (4/5); for values between 1.7-2.2 m/sec for significant hepatic fibrosis (F2) was 87% (7/8); for values >2.2 m/sec for advanced fibrosis / cirrhosis (F3, F4) is 90% (9/10).

SWE was able to discriminate between patients with grade 2 and those with grade 3 with an AUROC of 0.793 (95% Confidence Interval (CI), Standard Error (SE) 0.105, p<0.005). With an SWE velocity 1.9 m/s as the cut-off between patients with grade 3 and those with grade 2, elastography had 71% sensitivity and 90% specificity [Table/Fig-5].

Discussion

The algorithm combining serum biomarkers and SWE to diagnose liver fibrosis in a community setting targeting high risk group patients (Type II DM and Alcohol Abuse Patients) detected a large number of previously undiscovered subclinical but significant chronic liver disease cases. A total of 47 patients with normal ALT levels had elevated SWE results, these cases would had been missed had we followed traditional methods based on blood serum markers alone.

In present study, we have used an algorithm wherein patients with high risks for chronic liver disease are stratified by non-invasive

<table>
<thead>
<tr>
<th>Variable</th>
<th>SWE (less than 1.3 m/sec)</th>
<th>SWE-Elevated liver stiffness (1.3-2.2 m/sec)</th>
<th>SWE-Cirrhosis (&gt;2.2 m/sec)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (Years)</td>
<td>47.65±15.34</td>
<td>60.16±14.78</td>
<td>58.77±14.19</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>Male- n (%)</td>
<td>65 (86.7%)</td>
<td>188 (78.7%)</td>
<td>106 (72.6%)</td>
<td>0.054+</td>
</tr>
<tr>
<td>Body Mass Index (kg/ m²)</td>
<td>Underweight</td>
<td>18.5-24.9</td>
<td>25.0-29.9</td>
<td>30.0-34.9</td>
</tr>
<tr>
<td></td>
<td>&lt;18.5</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>0</td>
<td>3</td>
<td>5</td>
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<tr>
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<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Type 2 Diabetes - n (%)</td>
<td>54 (72%)</td>
<td>177 (74.1%)</td>
<td>103 (70.5%)</td>
<td>0.749</td>
</tr>
<tr>
<td>Hazardous Alcohol - n (%)</td>
<td>28 (37.3%)</td>
<td>72 (30.1%)</td>
<td>46 (31.5%)</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>28 (37.3%)</td>
<td>72 (30.1%)</td>
<td>46 (31.5%)</td>
<td>0.503</td>
</tr>
<tr>
<td>Multiple Risk Factors n (%) (i.e., both HTN, DM, and obesity-grade 2,3,4)</td>
<td>21 (28%)</td>
<td>52 (21.8%)</td>
<td>30 (20.5%)</td>
<td>0.428</td>
</tr>
<tr>
<td>Raised ALT - n (%)</td>
<td>31 (41.3%)</td>
<td>177 (74.1%)</td>
<td>131 (89.7%)</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>Platelet Count (10^11/L)</td>
<td>358.7±239.70</td>
<td>169.6±104.45</td>
<td>135.8±92.17</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>Thrombocytopenia (Platelets&lt;150x10^11/L)</td>
<td>18 (24%)</td>
<td>154 (64.4%)</td>
<td>120 (82.2%)</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>56.8±40.66</td>
<td>104.9±63.03</td>
<td>122.7±58.71</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>AST: ALT Ratio</td>
<td>63.8±53.28</td>
<td>133.8±78.17</td>
<td>182.2±89.60</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>APRI</td>
<td>0.98±1.37</td>
<td>2.98±2.32</td>
<td>4.41±2.63</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>FIB4</td>
<td>2.02±2.52</td>
<td>6.82±5.33</td>
<td>9.41±6.22</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>FIB4&lt;3.25</td>
<td>16 (21.3%)</td>
<td>152 (63.6%)</td>
<td>124 (84.9%)</td>
<td>&lt;0.001**</td>
</tr>
</tbody>
</table>

[Table/Fig-2]: Clinical variables comparison in relation to SWE of patients studied.

Significant figures; * Suggestive significance (p-value: 0.05≤P<0.10); ** Moderately significant (p-value: 0.01≤P<0.05); *** Strongly significant (p-value: P<0.01)
biomarkers. By this novel diagnostic algorithm we are able to detect patients with chronic liver disease who are asymptomatic but with significant liver fibrosis thus helping in early diagnosis and preventing further progression of disease [Table/Fig-6-8].

A prospective cross-sectional study done by Harman DJ et al., concluded that identification of substantial number of patients with previously undetected cirrhosis can be done by targeting risk factors as using a non-invasive biomarker approach [25]. Of the 378 patients in their study with defined risk factors who agreed to undergo elastography 71 had elevated liver stiffness with normal liver enzymes which would have been missed by traditional investigation algorithms. Among them, 25 patients in whom liver biopsy was performed, 20 patients had cirrhosis and 5 patients were confirmed to have steatohepatitis.

In particular, liver SWE has high Negative Predictive Value (NPV). Thus, liver SWE may be a useful tool in DM/alcohol abuse patients for initial diagnosis of patients with the disease. SWE hence can be used as a screening tool. Similar results were seen in a prospective study done by Kiani A et al., which presented the possibility of using ultrasound elastography as a screening test rather than a diagnostic test with excellent negative value of ARFI (98.2% for F=4) [26].

In present study SWE showed positive correlation with APRI (p<0.001,
A 43-year-old male, with DM and history of alcohol abuse. AST = 37 U/L, ALT = 41 U/L, median elastography values were elevated (1.9 m/sec) corresponding to moderate fibrosis which was confirmed by liver biopsy. ([Table/Fig-6] shows averaged elastography values).

A 62-year-old female, DM. AST = 40 U/L, ALT = 35 U/L, median elastography values were elevated (3.4 m/sec) corresponding to advanced fibrosis/cirrhosis which was confirmed by liver biopsy. ([Table/Fig-6a-d] shows averaged elastography values).

In present study correlation between platelet count and stiffness showed a strong negative correlation (p<0.001, r= -0.404), as thrombocytopenia is seen in patients with liver disease with advanced fibrosis [27].

In our study, patients with higher BMI (overweight) were more likely to have higher elastography results compared to patients with lower BMI (230 patients had higher elastography results compared with 121 patients with normal BMI). Wong VW et al., showed that presence of increased BMI, abdominal waist circumference did not affect SWE results, thus the presence increased BMI with high Liver Stiffness Measurement (LSM) can be attributed to increased fibrosis in these patients [28].

The diagnosis of advanced fibrosis may motivate patients to abstain from alcohol. Advanced fibrosis and early cirrhosis may be completely asymptomatic. Evidence shows that alerting patients of possible ALD using non-invasive tests reduces their drinking levels after a one-year follow-up [29]. Liver biopsy is affected by intraobserver and interobserver variability in assessing fibrosis hence it is considered an imperfect gold standard and it represents only 1/50000 of entire liver parenchyma [30]. Recent study by Poynard T et al., showed that liver biopsy exhibited a relative lower level of performance compared with biomarkers and elastography when evaluated similarly for the diagnosis of advanced fibrosis [31]. In our study there was low acceptance for liver biopsy when compared to a non-invasive procedure of liver elastography which further reinstates the notion that liver biopsy is an imperfect gold standard.

**LIMITATION**

While subjects with no known liver disease were enrolled into the study, the authors cannot exclude selection or referral bias relating to recruitment for the study. Second, liver biopsy was used as the gold standard although it is well recognised that sampling variability can occur. The size of the liver biopsies obtained in the study was small (range 12-19 mm). While this has potential implications for the accuracy of staging liver disease, it also highlights the limitations of percutaneous liver biopsies in the routine assessment of patients at risk of liver disease. As no subjects in Group A were offered liver biopsies, the authors cannot exclude the possibility that some of these subjects may also have had evidence of hepatic fibrosis. Therefore, the true prevalence of liver fibrosis/cirrhosis in this population is unknown. Although other ultrasound techniques can be used to measure liver stiffness, such as transient elastography (Fibro Scan, Echosens), and LSMs determined using transient elastography might also be affected by inflammation, we evaluated only SWE. The comparison with Transient Elastography (TE) was not done and would also be useful. We did not assess the reproducibility of SWE because previous studies had reported excellent reproducibility and inter-observer agreement. The information sort in the data collection form to analyse the secondary objectives was sensitive in nature.
REFERENCES


