Fibroscan Compared to Aspartate Aminotransferase to Platelet Ratio Index (APRI) and Aspartate Aminotransferase to Alanine Aminotransferase Ratio (AST to ALT) in the Assessment of Liver Fibrosis in Patients with Non Alcoholic Fatty Liver Disease in a Tertiary Care Hospital

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ABSTRACT

Objectives: To compare the extent of liver fibrosis using fibroscan and subsequently compare it with AST/ALT ratio and AST to platelet ratio index (APRI).

Study Design: Comparative cross-sectional study.

Place and Duration of Study: Chemical Pathology Department, Pak Emirates Military Hospital Rawalpindi in collaboration with Armed Forces Institute of Radiology & Imaging and Gastroenterology Department, Pak Emirates Military Hospital Rawalpindi Pakistan, from Jan to Apr 2021.

Methodology: A total of 50 patients in group-1 and 24 healthy subject participants in group-2 as controls were studied. Serum AST, ALT, platelet count, APRI and AST to ALT ratio were assessed along with fibroscan results to determine stages of liver fibrosis and results were compared for the association.

Results: There were 49 (66%) males and 25 (34%) females included in the study with the mean age of 49.77 ± 8.3 years. 11 (14.9%) patients had advanced fibrosis. There was a significant negative relationship (p-value <0.001 and r=-0.637) between platelet count and increasing fibroscan score (F1, F2) and a significant positive correlation between fibroscan score and AST (p-value <0.001 and r=0.623), ALT (p-value <0.001 and r=0.596), fibroscan result and APRI (p-value <0.001 and r=0.771) and fibroscan scores and AST/ALT ratio (p-value= 0.037 and r=0.243). One third of the study subjects had fibrosis in early and relatively advanced stages (F1, F2 respectively) correlating with serum and blood markers.

Conclusion: Early fibro scan testing and serum markers can help in early diagnosis and timely initiation of treatment.

Keywords: APRI, AST/ALT ratio (DeRitis ratio), Non-alcoholic fatty liver disease (NAFLD), Transient elastography.

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INTRODUCTION

Chronic liver disease is linked to non-alcoholic fatty liver disease (NAFLD) and has been associated with decompensated liver cirrhosis and hepatocellular carcinoma. NAFLD has also been a major culprit of morbidity and mortality associated with liver disease and cardiovascular disease.[1] There is considerable variability in NAFLD causes among different populations and the difference in methods used for diagnostic purposes.[2,3] NAFLD has also been linked with diabetes and is an important feature of metabolic syndrome and insulin resistance.[4,5] NAFLD severity may range from mild disease to varying degrees of inflammation, non-alcoholic steatohepatitis or severely decompensated liver cirrhosis which occurs in only a few patients.[6]

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The tests used for checking liver fibrosis severity are key to predicting prognosis and outcome in various forms of fibrosis of the liver caused by varying etiologies.[6] Presently gold standard for liver fibrosis assessment is a liver biopsy, but life-threatening complications have limited its use. Other methods used include fibroscan, AST to ALT ratio, and AST platelet ratio index (APRI). Out of these, transient elastography (fibroscan) is used often and is a well-validated non-invasive method for detecting and assessing liver fibrosis.[7]

Fibroscan/transient elastography is a non-invasive and specialized test of liver scarring and fibrosis, and its results are reported in terms of stiffness scores and range from F0 to F4, with F0 being normal liver with no fibrosis and F1 to F4 indicating increasing degrees of liver fibrosis/scarring. Fibroscan has its limitations, including limited diagnostic yield in obese, which includes about one-third of patients who are

advised fibroscan.[7,8] Recent development in probe technology which includes the design of an XL-probe, especially for patients with BMI greater than 30 kg per meter square, has improved the accuracy of diagnosis compared to the previously used M probe among obese.[9]

The results of fibroscan are usually interpreted, especially in case of impending liver fibrosis, with results of other noninvasive biological tests. These are markers of liver damage which are routinely performed as part of liver function tests and include ALT and AST. AST/ALT ratio is commonly used and is called the de-rites ratio. The significance of the rise of these markers, especially in various liver pathologies, is well established.[10]

In this study, liver fibrosis was assessed in patients with grade three NAFLD using fibroscan and compared to the APRI scores, and AST to ALT ratios in cases diagnosed three months prior to the study and followed up at the radiology and gastroenterology dept.of Military Hospital Rawalpindi.

METHODOLOGY

This comparative cross-sectional study was conducted from January to April 2021 at the Department of Chemical Pathology, Military Hospital Rawalpindi, collaborating with the Armed Forces Institute of Radiology & Imaging and Gastroenterology Department. Study was initiated after obtaining approval from the Ethical Review Committee (ERC/ID/121) of Army Medical College. Informed consent was obtained from all study participants. Non-probability consecutive sampling technique was used for sample collection. The sample size was calculated using an online calculator (www.calculator.net). Prevalence of NAFLD grade 3 was taken as 5% from the previous literature. Globally a prevalence of NAFLD grade 3 is 3-7% .[11] A confidence level of 95% and a margin of error of 5% were used for sample size calculation. Calculated sample size came out to be 73, and we took a sample size of 74 for the study.

Inclusion Criteria: The study population consisted of two groups. Group-1 included patients with NAFLD grade 3, and group-2, included healthy patients for comparison.

Exclusion Criteria: Patients with even a single missing test were excluded from the study. Patients with evidence of other chronic liver diseases like hepatitis B or hepatitis C or autoimmune hepatitis suspected clinically or alcoholic liver disease, patients on hepatotoxic medications were also excluded. Patients with cardiac failure, patients who could not undergo fibroscan because of extreme obesity or other reasons and those with clinical and USG documentation of decompensated cirrhosis were also excluded from the study.

Group-1 patients were diagnosed with grade 3 non Alcoholic fatty liver disease on USG and their serum markers AST, ALT and platelet counts had been carried out. Group-2 included healthy individuals with no NAFLD and their AST, ALT and platelet counts were carried out for comparison with the cases.

Fatty liver grades on USG include grade-0 (absence of steatosis with normal liver echogenicity); grade-1 (mild steatosis, the liver had higher echogenicity than the right renal cortex, but the echogenic wall of the main portal vein was preserved); grade-2 (moderate steatosis, impaired echogenicity of the main portal vein wall); grade-3 (severe steatosis, impaired visualization of the posterior hepatic parenchyma or the diaphragm).[12] In our study, cases included those with grade 3 NAFLD. Two classified radiologists did all the ultrasono grams, and only those patients were included in the study who had severe fatty liver, i.e., grade 3 non-alcoholic fatty liver disease. Two radiologists carried out ultrasonograms.

The lab tests included in the study were those carried out in the hospital laboratory and were obtained within one week of fibroscan examination. The normal reference range for ALT was taken as <42 IU/L, whereas that of AST and platelets was from <40 IU/L and 150 to 400 X 109 platelets per microliter, respectively. ALT and AST were measured using spectrophotometry based on Beer-Lambert law on Microlab 300, a semi-automated chemistry analyzer, whereas platelets were measured on a semi-automated Hematology analyzer Sysmex XN 350, USA. Both the external and internal quality controls were checked before running each test. Samples were collected in the Radiology Department in a serum separator tube and immediately centrifuged and carried by the principal investigator by hand to the Chemical Pathology and Hematology departments for carrying out ALT, AST and platelet counts. The sample for platelet count was taken in an EDTA tube.

AST to ALT ratio was measured for each patient, and the APRI score was determined using the following equation. APRI equals divided by Platelet count multiplied by 100.

APRI score was studied by keeping two numbers in mind, i.e., 0.5 and 1.5. A score of less than or equal

to 0.5 indicated that the liver is either completely free of fibrosis or has a tiny bit of scarring. An APRI score of 1.5 or greater indicated that the liver has scarring and likely some cirrhosis. Midrange values are less helpful.[7]

A fibroscan machine was also used in the study. Manufacturers' guidelines and recent studies in this regard were followed. To eliminate bias, a fibroscan was carried out by two investigators. The following ranges of fibrosis, from nil to advanced fibrosis, were observed as provided by the manufacturer and established by studies. F0 on fibroscan was taken as no liver scarring, whereas F1 was taken as mild liver scarring, F2 as moderate liver scarring, F3 was taken as severe liver scarring, and F4 was taken as advanced liver scarring.[7,8]

Statistical Package for Social Sciences (SPSS) version 21.0 was used for the data analysis. Descriptive statistics were generated, and independent sample t-test was used to compare lab values in the study groups. ANOVA was applied to compare the quantitative data of more than two groups. Pearson correlation was applied between fibroscan results and AST, ALT, platelet count, APRI and AST/ALT ratios. The *p*-value of <0.05 was taken to be statistically significant.

RESULTS

According to inclusion criteria, a total of 74 patients were included in the study. Out of them, fifty (50) patients of NAFLD grade 3 were included in group-1 and 24 healthy subjects were included in the study in group-2. There were 49 males (66.2%)and 25 females (33.8%). The mean age of group-1 and group-2 was 49.77 ± 8.3 years and 48.08 ± 7.8 years, respectively.

NAFLD stage among group-2 was zero, while in group-1, all the patients had stage 3 NAFLD. None of the subjects in group-2 had fibrosis as was shown in Table-I.

Mean AST and ALT levels were higher in group-1 than in group-2 (mean AST 46.75 + 25.91 U/l among group-2 versus 56.98 + 33.63 U/l among group-1 and mean ALT 78.33 ± 18.05 U/l among group- 2 and 82.78 + 49.48 U/l among group-1).

However, the raised level of AST and ALT was not statistically significant (*p*-value 0.193 and 0.672 for AST and ALT, respectively). Platelet count showed a significant inverse relationship with increasing degrees of fibrosis (mean platelet count of 165.44X109 ± 60.42/L among group-1 and 196.67X109 ± 51.23/L among group-2 with the *p*-value of 0.032). APRI (AST to platelet ratio index) showed significantly increased levels in group-1 (cases with NAFLD) as compared to group-2 (healthy subjects), i.e.,1.21 ± 0.90 and 0.70 ± 0.54, respectively, with the *p*-value of 0.012. AST/ALT ratio was significantly (*p*-value 0.002) increased in group-1 as compared to group-2 i.e., 0.69 ± 0.10 among group-1 and 0.57 ± 0.21 among group-2 as shown in the Table-II.

Table-I: Fibroscan result of the study groups.

	Group 2	Group 1
	n (%)	n (%)
F0	24 (32.4%)	18 (24.3%)
F1	-	21 (28.4%)
F2	-	11 (14.9%)

Table-II: Lab investigations and APRI score of study groups.

Lab Investigations	Group-2	Group-1	*p*-value
	Mean ± SD	Mean ± SD	
AST evel (U/l)	46.75 ± 25.91	56.98 ± 33.63	0.193
ALT evel (U/l)	78.33 ± 18.05	82.78 ± 49.48	0.672
Platelet Level	196.67 x 10^9 ± 51.23	165.44 x 10^9 ± 60.42	0.032
APRI	0.70 ± 0.54	1.21 ± 0.90	0.012
AST/ALT Ratio	0.57 ± 0.21	0.69 ± 0.10	0.002

Table-III showed that group-1 with increasing degrees of fibrosis on fibroscan, and there was a statistically significant positive association between platelet count, APRI and AST/ALT ratio among Group-1, i.e., cases with increasing degrees of fibrosis F0 through F2 (*p*-value of 0.032, 0.012, and 0.002 respectively). The rise in AST and ALT with increasing degrees of fibrosis on fibroscan results (F0, F1 and F2) was insignificant (*p*-value 0.193 and 0.672, respectively).

Table-III: Lab distribution according to fibro-scan result.

Lab Distribution	Group1 (F0)	Group 1 (F1)	Group 1 (F2)	*p*-value
	Mean ± SD	Mean ± SD	Mean ± SD	
AST (U/l)	46.75 ± 25.91	68.90 ± 29.84	87.36 ± 22.77	0.193
ALT (U/l)	78.33 ± 18.05	102.38 ± 45.51	122.55 ± 35.63	0.672
Platelet Count	196.67 ± 51.23	146.14 ± 47.35	111.18 ± 41.77	0.032
APRI	0.70 ± 0.54	1.50 ± 0.58	2.19 ± 0.70	0.012
AST/ALT Ratio	0.57 ± 0.21	0.67 ± 0.10	0.72 ± 0.07	0.002

Table-IV showed the direct relationship, whereas platelet count showed a negative relationship with increasing fibroscan grades, i.e., from F0 through F1 and F2.

Table-IV: Correlation between various parameters of study and fibroscan results.

Fibro scan result		Values
AST level(U/l)	Pearson Correlation	0.623
	p-value	<0.001
ALT level(U/l)	Pearson Correlation	0.596
	p-value	<0.001
Platelet level	Pearson Correlation	-0.637
	p-value	<0.001
APRI	Pearson Correlation	0.771
	p-value	<0.001
AST/ALT ratio	Pearson Correlation	0.243
	p-value	0.037

Correlation study of parameters of study i.e. AST, ALT, APRI and AST to ALT ratio, showed a significant positive relationship, whereas platelet count showed a significant negative relationship with fibroscan results.

DISCUSSION

Our study established that fibroscan can be used comparatively to AST platelet ratio index and AST to ALT ratio for early fibrosis diagnosis in non-alcoholic fatty liver disease (NAFLD), especially in advanced cases, i.e., prolonged grade 3 NAFLD, especially among those with advanced degrees of fibrosis on fibroscan. Furthermore, there was a strong positive association between fibroscan results and AST/ALT ratios and APRI scores. Moreover, platelet count and advanced fibroscan results F1 and F2 had an inverse relationship, thus indicating that thrombocytopenia in liver disease is associated with advancing fibrosis.[13,14]

The relationship between advanced NAFLD and the extent of fibrosis determined in our study was higher than those reported by other authors using fibroscan examinations to assess liver fibrosis.[15]

All the patients in the study had abdominal USG done along with liver function tests that showed steatohepatitis. However, a USG scan alone cannot be used to determine the advancement of hepatic disease.[16] Razavizade *et al,* showed that varying degrees of severity of NAFLD could be established by adding serum markers to USG examination.[17] This finding supports the use of simple biomarkers in addition to USG abdomen in assessing NAFLD when more advanced methods such as transient elastog-raphy are not available or if the case may be that liver biopsy cannot be done.[18]

It was also shown that male patients were more likely to suffer from advanced fibrosis at a younger age than females. This is not surprising, as many studies have consistently shown that male patients are more likely to suffer from varying aetiologies of liver disease than females. This relationship between gender, fibrosis and advanced liver disease may be explained by the protective effect of female sex hormones on the progression of hepatic fibrosis.[19] In a Saudi study carried out at KAUH, it has already been shown that in the case of NAFLD and associated fibrosis, males are more commonly affected than females.[18,19] Furthermore, our findings were consistent with previous NAFLD studies that age was associated with more advanced stages of the disease, i.e., NAFLD and hepatic fibrosis.[19]

The AST/ALT ratio or de ritis ratio was also found to indicate the difference between mild, moderate and advanced fibrosis indicated by increasing levels of this ratio, respectively. Our study establishes the use of APRI, AST/ALT ratio and fibroscan examinations for follow up on early-stage NAFLD patients when a liver biopsy cannot be used. These tests can also be used for follow up on patients who have undergone bariatric surgery or other treatment for NAFLD.[20]

The mechanism of NAFLD has been shown by various theories and is commonly looked at as one is the "two-step/two-hit theory". The first hit is fatty change/hepatic steatosis of the liver and the second hit is inflammation and liver injury. Both hits are promoted by adipocytokines.[21] Recent study has indicated that adipocytokines such as IL6, IL8, visfatin, TNF alpha, and TNF beta are associated with a high probability of developing NAFLD and NASH. These adipokines can be used as future biomarkers for asse-ssing NAFLD patients.[22] This indicates an area where these new non-invasive biomarkers can be studied.

CONCLUSION

Early fibroscan testing and serum markers can help in early diagnosis and timely initiation of treatment.

Conflict of Interest: None.

Authors' Contribution

HI: Principle investigator, SRJ: Responsible for all aspects, AH: Critical analysis, MK: Data compilation, NM: Statistical analysis, AN: Data analysis.

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