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Observational Study
Metabolic Puzzle: Exploring Liver Fibrosis Differences in Asian MAFLD Subtypes

Liver Fibrosis in MAFLD Subtypes

Abstract
BACKGROUND
Metabolic-associated fatty liver disease (MAFLD) is a medical condition characterized by the presence of fatty liver along with overweight/obesity and/or diabetes and/or metabolic dysfunction. However, whether the subtypes of MAFLD based on the metabolic disorder differentially impact on liver fibrosis is not well explicated, especially in the Asian population.

AIM
This study aimed to compare the severity of liver fibrosis among different MAFLD subtypes.

METHODS
A total of 322 adult patients of either gender, with fatty liver on ultrasound were enrolled between January to December 2021. MAFLD was defined as per the APASL guidelines. Fib-4 and NFS scores were employed to evaluate liver fibrosis.

RESULTS
The mean age was 44.84±11 years. 72% were females. 273 patients were classified as MAFLD. Out of which, 110 (40.3%) carried a single, 129 (47.3%) had two, and 34 (12.5%)
had all three metabolic conditions. The cumulative number of metabolic conditions was related to elevated BMI, triglyceride (TG) levels, & Hb1Ac, lower HDL levels, higher liver inflammation (by AST & GGT) and higher likelihood of fibrosis (by NFS & Fib-4 scores) (all p<0.05). Among MAFLD patients, those with Diabetes alone were the eldest and had the highest mean value of NFS score and FIB-4 score (p<0.05). While MAFLD patients diagnosed with lean metabolic dysfunction exhibited the greatest levels of TG and ALT, with the lowest HDL levels (p<0.05).

CONCLUSION
The increased number of metabolic conditions increases the likelihood of fibrosis in patients with MAFLD. The severity of liver fibrosis varies among different subtypes of MAFLD. Patients with diabetes and MAFLD have the highest risk of developing fibrosis, while those with lean body type and MAFLD tend to have a worse metabolic profile.

Key Words: Metabolic syndrome; Diabetes; Fatty liver disease; Dyslipidemia; Obesity.


Core Tip: This is the first study on the South-Asian population on assessment of fibrosis among MAFLD patients. The study highlights that as the number of risk factors increases in a patient with MAFLD, the more is the progression of liver fibrosis among such patients.

INTRODUCTION
Non-alcoholic fatty liver disease (NAFLD) is a continuum of diseases ranging from benign accumulation of excessive fat in the liver (steatosis) to the inflammation of liver
cells (steatohepatitis, i.e., NASH). It can lead to advanced fibrosis, cirrhosis, and even hepatocellular carcinoma (HCC). NAFLD is one of the main reasons for liver transplantation. It is a diagnosis of exclusion that requires the absence of other causes of liver fat accumulation, for instance, alcohol, drugs, viral hepatitis, and autoimmune liver disease.[1]

The progression from benign fatty liver to inflammation and ultimately liver fibrosis is linked with the presence of diabetes mellitus (DM), obesity and metabolic syndrome (MS).[2] This steered the proposal of a terminology swap from NAFLD to MAFLD (Metabolic-dysfunction associated fatty liver disease).[3] The APASL (Asian Pacific Association for the Study of the Liver) also endorsed this change in nomenclature and the development of “positive criteria” for MAFLD, unlike NAFLD, which was a diagnosis of exclusion.[4]

To determine fatty liver and fibrosis, liver biopsy has historically remained the gold standard. However, given its invasive nature, various noninvasive diagnostic modalities (based on imaging or biomarkers) are increasingly being used. Among them, the NAFLD Fibrosis Score (NFS) and Fibrosis-4 index (Fib-4) are endorsed by various guidelines as preferred screening panels for assessing advanced fibrosis.[4, 5]

It has been shown that MAFLD outperformed NAFLD in identifying significant liver fibrosis.[6, 7] However, whether the subtypes of MAFLD differentially influence liver fibrosis, is not well explained, especially in the Asian population. Therefore, given the recent notion of MAFLD, our objective was to compare the severity of liver fibrosis among different MAFLD subtypes.

**MATERIALS AND METHODS**

For this study, a cross-sectional investigation was carried out at the National Institute of Liver and GI Diseases (NILGID), located at Dow University Hospital, in Karachi, Pakistan. All patients aged between 18 and 65 years, either gender, who were diagnosed with fatty liver between January and December 2021 were included. Patients with decompensated liver disease, HCC, acute hepatitis, acute-on-chronic liver disease
and other concomitant liver disease (chronic active viral, alcohol, autoimmune or metabolic liver diseases) were excluded. Pregnant or lactating female patients and patients with concomitant systemic diseases such as tuberculosis, autoimmune disorders, and extra-hepatic malignancies were also excluded from the study.

Demographic, clinical and laboratory information was gathered and analyzed. Indications to perform an ultrasound examination were dyspepsia, right upper quadrant abdominal pain and deranged liver enzymes. The Fatty liver was identified on ultrasound. Evaluation of hepatic steatosis using ultrasound is based on the echo change in hepatic parenchyma. Hepatic steatosis appears as a diffuse increased hepatic parenchymal echogenicity, or “bright liver” on ultrasound. According to the Asia Pacific Association for the Study of the Liver (APASL) guidelines, MAFLD is defined as the presence of fatty liver in conjunction with at least one of the following three conditions: overweight/obesity, type 2 DM, or evidence of metabolic dysfunction (MD) such as increased waist circumference or an abnormal lipid or glycemic profile. Fib-4 and NFS were used to assess liver fibrosis. Asian cutoffs were used for body mass index (BMI) to classify as overweight/obese vs lean/normal weight MAFLD groups. Figure 1 describes the study flow chart.

The study was undertaken following approval from the Institutional Review Board of Dow University of Health Sciences (IRB#1842). Informed consent was obtained in writing from all eligible participants. The methods employed complied with the ethical standards of the responsible committee on human experimentation and with the Helsinki Declaration of 1975, as revised in 2000.

The statistical analyses were executed using Statistical Package for Social Sciences (SPSS) software version 26.0. The mean ± SD was used to represent quantitative variables, while frequency and percentages were used for categorical variables. The Chi-square test was used to assess categorical variables. The Mann-Whitney U-test was applied to compare the two groups, while the Kruskal-Wallis’s test was performed to evaluate the difference among all three groups. A p-value of 0.05 or less was considered as significant.
RESULTS

A total of 322 patients with fatty liver were included. The mean age was 44.84±11 years. The majority were females (72%). The mean BMI was 29.83±5.53 kg/m². 29.8% had DM, and 9.6% had hypertension.

Out of 322 patients with fatty liver, 273 were classified as MAFLD. The MAFLD patients were segregated into three categories corresponding to their number of metabolic conditions (i.e., one, two and three). Out of 273 participants with MAFLD, 110 (40.3%) carried a single metabolic condition, 129 (47.3%) had two metabolic conditions, and 34 (12.5%) had all of the three metabolic conditions. (Figure 1)

With an increase in the cumulative number of metabolic conditions, the patients exhibited an elevation in their metabolic parameters such as BMI (28.99±5.19 vs. 31.63±5.19 and 33.59±4.75; p<0.001), triglyceride (TG) levels (182.45±109.5 vs. 198.13±98.8 vs. 221.85±102.38, p=0.002), and Hb1Ac (5.97±1.13 vs. 6.82±1.86 vs. 8.22±1.58, p<0.001) for 1, 2 and 3 conditions respectively. In contrast, high-density lipoprotein (HDL) levels showed a negative trend with the increasing number of metabolic conditions among MAFLD patients (41.65±15.08 vs. 36.05±18.93 vs. 32.38±6.62, p<0.001). Furthermore, increasing liver inflammation (reflected by aspartate aminotransferase - AST 28.62±20.74 vs 32.29±23.36 vs 40.06±26.74, P = 0.021, and gamma-glutamyl transferase - GGT 34.93±21.08 vs 51.50±36.44 vs 65.41±38.02, p<0.001) and fibrosis (reflected by the NFS score -2.59±1.59 vs -2.00±1.69 vs -1.39±1.60, P = 0.002 and Fib-4 score 0.79±0.45 vs 0.94±0.86 vs 1.11±0.66, P = 0.041) was seen as the cumulative number of metabolic conditions increased. (Table 1) Notably, the proportion of significant fibrosis also increased with the cumulative number of metabolic conditions. For the NFS score, advanced fibrosis was 4.1%, 25.5%, 35.6%, and 44.1% for No MAFLD, MAFLD with 1, 2, & 3 conditions, respectively while for FIB-4 score, advanced fibrosis was 6.1%, 10.9%, 17%, and 26.5% for No MAFLD, MAFLD with 1, 2, & 3 conditions, respectively. (Figure 2)
Furthermore, MAFLD patients with a single metabolic condition (n = 110, 40.3%) were sub-classified into three categories; Obesity alone (n = 61, 55.5%), lean MD (n = 34, 30.9%) and DM alone (n = 15, 13.6%). Among MAFLD patients with solitary metabolic condition, those established with DM alone were the oldest (mean age 50.73±9.04 vs 45.53±10.60 for lean MD and 41.72±10.03 for obesity alone, P = 0.005), with normal but lowest platelet counts (245.40±50.70 vs 275.44±81.92 vs 314.85±97.95, P = 0.004) and had the highest mean value of the NFS score (-1.61±0.81 vs -2.50±1.40 vs -2.86±1.74 P = 0.017) and the FIB-4 score (0.95±0.48 vs 0.92±0.56 vs 0.68±0.35, P = 0.027). MAFLD diagnosed by lean MD had the highest levels of TG (269.02±120.03 vs 176.13±132.33 vs 135.75±57.72, p<0.001), and alanine transaminase (ALT) (43.94±28.41 vs 34.13±19.04 vs 33.89±30.47, P = 0.043) while lowest HDL levels (39.96±21.71 vs 42.30±8.73 vs 42.50±11.14, P = 0.026) as compared to the patients with DM alone and obesity alone respectively. As expected, diabetic MAFLD had the highest HbA1c levels (8.03±1.71 vs 5.77±0.48 vs 5.56±0.46, p<0.001) than others. (Table 2)

When compared among the three subtypes of MAFLD, the proportion of advanced liver fibrosis was significantly higher among diabetic MAFLD patients according to the NFS score (46.6% vs 26.5% for MD alone and 19.7% for Obesity alone), whereas patients with lean MD had the highest proportion of advanced fibrosis according to the FIB-4 score (14.7% vs 9.8% for Obesity alone vs 6.7% for DM alone). (Figure 3)

No significant differences were observed in gender, education level, and history of hypertension, blood pressure, and blood levels of cholesterol, low-density lipoprotein (LDL), bilirubin, albumin, AST, and alkaline phosphate (ALP) between these three groups.

**DISCUSSION**

The substitution of nomenclature from NAFLD to MAFLD led to an increase in the prevalence of fatty liver disease, and the identification of more at-risk patients for advanced liver disease.[9] The current study highlighted the importance of subgrouping the MAFLD patients based on the number and type of metabolic conditions. Different
subgroups present distinct clinical spectrums and risks of advanced liver fibrosis, which can influence their treatment strategies.

MS is related to higher deaths in NAFLD patients. Moreover, the high fibrotic burden in fatty liver disease is associated with a higher risk of development of HCC, liver-related mortality and cardiovascular disease. Hence, it is worth classifying the MAFLD patients depending on the number of metabolic conditions at the beginning. This helps to stratify patients with MAFLD according to the long-term risk of significant liver fibrosis. We showed that the fibrosis scores and proportion of advanced fibrosis patients increased with an increasing number of metabolic conditions. In a recent study of the NHANES III database, more than 70% of all patients with MAFLD had more than one metabolic condition. In addition, having more than one metabolic condition was associated with increased levels of liver and kidney dysfunctions. The same study found that the number of metabolic conditions was associated with increased age. In our study, there was only a non-significant increase in age with the number of comorbidities. This could be due to the difference in population (north American vs Southeast Asian). This is further supported by a recent meta-analysis on clinical profiles of Asians with NAFLD. Overall, the pooled mean age of NAFLD patients was 52.07 (95% CI: 51.28–52.85) years, with those from Southeast Asia (42.66, 95% CI: 32.23–53.11) being significantly younger than other Asians.

The burden of liver fibrosis is known to differ across MAFLD subtypes, and there is an exponential increase in the risk of liver-related death as the fibrosis stage progresses. Hence, we further classified the MAFLD into subtypes according to the type of metabolic conditions. Diabetic patients were the eldest among the three groups, with normal but comparatively lowest platelet count and highest risk of fibrosis, as depicted by the Fib-4 and NFS scores. Diabetes is thought to promote fibrotic response by activating hepatic stellate cells due to excess insulin resistance induced excess free fatty acid leading to inflammation, mitochondrial dysfunction and increased oxidative stress. In a recent study, a higher proportion of advanced liver fibrosis was found in diabetic MAFLD (6.6%), as compared to overweight- (2.0%), lean- (1.3%), and no
MAFLD (0.2%) based on NFS score. Similarly, the proportion of patients with significant liver fibrosis was greatest in diabetic MAFLD (8.6%), when compared to lean- (3.9%), Overweight- (3.0%), and no MAFLD (2.0%) based on Fib-4 score. Further, the aOR (95%CI) for NFS-defined significant liver fibrosis was 4.46 (2.09 to 9.51), 2.81 (1.12 to 6.39), and 9.52 (4.46 to 20.36) in Overweight-, lean-, and diabetic- MAFLD. At the same time, for Fib-4 defined significant liver fibrosis the aOR (95%CI) were 1.03 (0.78 to 1.36), 1.14 (0.82 to 1.57), and 1.97 (1.48 to 2.62) in Overweight-, lean-, and diabetic- MAFLD.[17] In another recent NHANES III database study, diabetic MAFLD patients were elderly and had more liver fibrosis risk.[15] In another larger South Korean study on 6775 subjects, DM with MAFLD carried a high (9.5%) rate of advanced fibrosis.[20] This relationship between DM, MAFLD and advanced fibrosis could be multifactorial, including the impact of insulin resistance on the liver, and advanced age as fibrosis is a chronic process.[21, 22] Further, age is one of the variables in Fib-4 and NFS scores; thus the greater the age, the higher the Fib-4 and NFS scores.

Findings of elevated TG, ALT and AST levels in lean MD signify that lean MD is not benign and is linked with liver inflammation/injury. In previous studies, the metabolic outline of lean MAFLD was more unfavourable than lean non-MAFLD.[23] Further, lean MAFLD has been autonomously related with a greater chance of all-cause mortality (HR: 1.296; 95%CI: 1.064 - 1.578) as compared to overweight/obese MAFLD (HR: 0.992; 95%CI: 0.893 - 1.102) and diabetic MAFLD (HR: 1.275; 95%CI: 1.075 - 1.512).[24] It has also been associated with liver-specific mortality (HR: 2.84; 95%CI: 2.72 - 2.97) as compared to overweight/obese MAFLD (HR: 1.76; 95%CI: 1.70 - 1.82).[25] Further, this impact appears to continue even post-liver transplant, as lean NASH patients showed worse post-liver transplant overall survival in a recent study (HR: 0.17; 95%CI: 0.03-0.86, P = 0.0142).[26]

The performance of Fib-4 and NFS in diagnosing advanced fibrosis can be influenced by various factors, including age and diabetes mellitus. Additionally, the inclusion of overweight or obesity as a criterion for MAFLD may impact the BMI component in NFS but not in the Fib-4 score. The difference observed in identifying advanced fibrosis in
the current study using these two scores could be attributed to this discrepancy. This hypothesis is supported by a recent study in which although the performance of FIB-4 and NFS in diagnosing liver fibrosis was found to be similar between lean and non-lean individuals, the sensitivity and specificity of NFS varied according to BMI quartiles, showing an increasing trend (P for trend < 0.001), while no such trend was observed with FIB-4 (P for trend = 0.05 for sensitivity; P = 0.20 for specificity). Although there were no significant differences in the areas under the curve (AUROC) between FIB-4 and NFS in the lean group (0.807 vs 0.790; P = .09), it was found that the current cutoff values of NFS had lower sensitivity compared to those of FIB-4 among lean individuals (54.4% vs 81.8%; P = .03). Another study found that overall diagnostic performance did not differ between FIB-4 and NFS for subjects with MAFLD. Nevertheless, the performance of NFS was lower specifically among those with diabetes (AUROC 0.809 vs 0.717; P = 0.002). No significant differences were found between FIB-4 and NFS AUROCs for Obese MAFLD (0.801 vs 0.778; P = 0.351) or Lean MAFLD (0.777 vs 0.802; P = 0.659). In a recent study, FIB-4 demonstrated higher performance than the NFS score (AUROC 81.5% vs 73.7%, p < 0.001) in accurately classifying non-obese NAFLD patients with F2-4 fibrosis. Meanwhile, another study found that while FIB-4 and NFS can effectively rule out advanced fibrosis in overweight, obese, and severely obese individuals, their clinical utility in lean and morbidly obese patients is uncertain. It’s important to note that this analysis was conducted on a Caucasian population, so caution should be exercised when generalizing the results as lower BMI thresholds for obesity have been established for Asians.

The study is limited by factors such as sample size, single-center design, and the unavailability of liver biopsy or transient elastography. Despite the lack of these methods, Fib-4 and NFS have been widely used and endorsed by various guidelines for screening MAFLD patients for advanced fibrosis and are superior to other scores like APRI and BARD. Nonetheless, their effectiveness may be reduced by age and BMI limitations.
However, this is the first study on the South Asian population to highlight the importance of subtyping MAFLD, validating previous reports from Western datasets. As the severity of liver fibrosis varies across the MAFLD subtypes and is correlated with mortality in fatty liver disease,[33, 34] identifying the patients at greater risk for significant liver fibrosis and complications would help physicians strategize the patient-centred management plan from the outset.

CONCLUSION

The increased number of metabolic conditions increases the likelihood of fibrosis in patients with MAFLD. The severity of liver fibrosis varies among different subtypes of MAFLD. Patients with diabetes and MAFLD have the highest risk of developing fibrosis, while those with lean body type and MAFLD tend to have a worse metabolic profile.

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ARTICLE HIGHLIGHTS

Research background

Metabolic-associated fatty liver disease (MAFLD) is a medical condition characterized by the presence of fatty liver along with overweight/obesity and/or diabetes and/or metabolic dysfunction. However, whether the subtypes of MAFLD based on the metabolic disorder differentially impact on liver fibrosis is not well explicated, especially in the Asian population.

Research motivation

Different subgroups of MAFLD present distinct clinical spectrums and risks of advanced liver fibrosis, which can influence their treatment strategies. Metabolic Syndrome is related to higher deaths in NAFLD patients. Moreover, the high fibrotic burden in fatty liver disease is associated with a higher risk of development of HCC, liver-related mortality and cardiovascular disease. Hence, it is worth classifying the MAFLD patients depending on the number of metabolic conditions at the beginning.
This helps to stratify patients with MAFLD according to the long-term risk of significant liver fibrosis.

**Research objectives**
To compare the severity of liver fibrosis among different MAFLD subtypes.

**Research methods**
This was a cross-sectional investigation carried out at the National Institute of Liver and GI Diseases (NILGID), located at Dow University Hospital, in Karachi, Pakistan. All patients aged between 18 and 65 years, either gender, who were diagnosed with fatty liver between January and December 2021 were included. Patients with decompensated liver disease, hepatocellular carcinoma, acute hepatitis, acute-on-chronic liver disease and other concomitant liver disease (chronic active viral, alcohol, autoimmune or metabolic liver diseases) were excluded. Pregnant or lactating female patients and patients with concomitant systemic diseases such as tuberculosis, autoimmune disorders, and extra-hepatic malignancies were also excluded from the study. MAFLD was defined according to the Asia Pacific Association for the Study of the Liver (APASL) guidelines, Fib-4 and NFS were used to assess liver fibrosis. Asian cutoffs were used for body mass index (BMI) to classify as overweight/obese vs lean/normal weight MAFLD groups. The proportion of significant fibrosis increased with the cumulative number of metabolic conditions. For the NFS score, advanced fibrosis was 4.1%, 25.5%, 35.6%, and 44.1% for No MAFLD, MAFLD with 1, 2, & 3 conditions, respectively while for FIB-4 score, advanced fibrosis was 6.1%, 10.9%, 17%, and 26.5% for No MAFLD, MAFLD with 1, 2, & 3 conditions, respectively.

**Research results**
Out of 322 patients with fatty liver, 273 were classified as MAFLD. The MAFLD patients were segregated into three categories corresponding to their number of metabolic conditions (i.e., one, two and three). Out of 273 participants with MAFLD, 110 (40.3%)...
carried a single metabolic condition, 129 (47.3%) had two metabolic conditions, and 34 (12.5%) had all the three metabolic conditions. Furthermore, MAFLD patients with a single metabolic condition (n = 110, 40.3%) were sub-classified into three categories; Obesity alone (n = 61, 55.5%), lean MD (n = 34, 30.9%) and DM alone (n = 15, 13.6%). When compared among the three subtypes of MAFLD, the proportion of advanced liver fibrosis was significantly higher among diabetic MAFLD patients according to the NFS score (46.6% vs 26.5% for MD alone and 19.7% for Obesity alone), whereas patients with lean MD had the highest proportion of advanced fibrosis according to the FIB-4 score (14.7% vs 9.8% for Obesity alone vs 6.7% for DM alone).

**Research conclusions**
The increased number of metabolic conditions increases the likelihood of fibrosis in patients with MAFLD.
The severity of liver fibrosis varies among different subtypes of MAFLD.
Patients with diabetes and MAFLD have the highest risk of developing fibrosis,

**Research perspectives**
The direction of future research in this area involves several key questions that need to be addressed.
1. Investigating the specific diagnostic markers for different subgroups within MAFLD, such as obesity, lean individuals, and those with type 2 diabetes.
2. Further exploration is needed regarding the pathogenesis of MAFLD/MASH. By conducting thorough investigations into these areas, researchers can gain a better understanding of the complexities surrounding non-alcoholic fatty liver disease and its associated metabolic dysfunction.
3. Future research should focus on identifying effective pharmacotherapeutic interventions for MAFLD/MASH, as there is currently no approved treatment for this condition.
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