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Original Research Article

EVALUATION OF LIVER BIOPSY FINDINGS IN PATIENTS WITH NON-ALCOHOLIC FATTY LIVER DISEASE AND ALCOHOLIC LIVER DISEASE

Ushma Patel[1], Smit Patel[1], Suchita Patel[1]

[1]*Assistant Professor, Department of Pathology, SAL Institute of Medical Sciences, Gujarat University, Ahmedabad, Gujarat, India*

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Corresponding Author:
Dr. Ushma Patel,
Assistant Professor, Department of Pathology, SAL Institute of Medical Sciences, Gujarat University, Ahmedabad, Gujarat, India
Email: ushma12dec@gmail.com

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ABSTRACT

Background: Liver biopsy remains the gold standard for evaluating histological features in MASLD and ALD, yet comparative studies assessing biopsy patterns between these entities are limited. The aim is to compare and characterize liver biopsy findings—including steatosis grade, inflammation, hepatocyte injury, and fibrosis stage—between patients with MASLD and ALD, and to correlate histopathological features with clinical and biochemical profiles.

Materials and Methods: A cross-sectional study was conducted on 120 patients, including 60 with MASLD and 60 with ALD. Clinical, biochemical, and histopathological data were collected. Liver biopsy samples were evaluated for steatosis grade, lobular inflammation, hepatocyte ballooning, Mallory-Denk bodies, and fibrosis stage using standardized scoring systems. Statistical analysis was performed to assess intergroup differences.

Results: MASLD patients exhibited higher BMI and more frequent metabolic comorbidities, while ALD patients were predominantly male and presented with lower BMI. Histologically, steatosis was observed in both groups, but Mallory-Denk bodies and severe hepatocyte ballooning were significantly associated with ALD. Advanced fibrosis (F3-F4) was more prevalent in ALD (60%) compared to MASLD (36.6%).

Conclusion: ALD demonstrates a higher burden of advanced fibrosis and distinctive histological markers compared to MASLD, emphasizing the continued relevance of liver biopsy for accurate diagnosis and prognostication in chronic liver disease.

Keywords: MASLD, Alcoholic liver disease, Liver biopsy, Fibrosis.

INTRODUCTION

Steatotic liver diseases encompass a continuum from simple steatosis to advanced fibrosis, which critically influences clinical outcomes. The terminology of non-alcoholic fatty liver disease (NAFLD) was updated in 2023 to metabolic dysfunction-associated steatotic liver disease (MASLD), with its inflammatory form termed metabolic dysfunction-associated steatohepatitis (MASH) to better reflect underlying metabolic pathophysiology.[1]

MASLD has emerged as the leading cause of chronic liver disease worldwide, affecting 25–35% of adults in developed nations, with 3–7% progressing to MASH.[2] Alarmingly, more than 80% of individuals with advanced MASH remain undiagnosed, which underscores the urgent need for effective screening strategies.[3]

Despite shared histological hallmarks between MASLD and alcoholic liver disease (ALD), such as macrovesicular steatosis and lobular inflammation, the etiopathogenesis diverges significantly. ALD results from chronic ethanol exposure, oxidative stress, and acetaldehyde toxicity, whereas MASLD is driven by insulin resistance, obesity, and systemic inflammation.[4,5]

Histopathological analysis through liver biopsy remains the gold standard for diagnosing steatohepatitis and assessing fibrosis severity, despite its invasiveness and sampling variability.[6] Emerging digital pathology platforms, such as FibroNest™, have revealed distinct architectural patterns in fibrosis: ALD displays thick, flexural collagen

strands, whereas MASLD demonstrates a more uniform reticulated network; notably, in cases of dual etiology (alcohol plus metabolic dysfunction), fibrosis morphology tends to resemble MASLD.[5]

Histological scoring systems like the NAFLD Activity Score (NAS) and Kleiner-Brunt criteria are integral for grading steatosis, lobular inflammation, and hepatocyte ballooning, while fibrosis staging employs METAVIR or Ishak scales.[7] Recent paired-biopsy studies confirm that MASLD patients frequently progress to advanced fibrosis over time, highlighting the prognostic significance of baseline histology.[8] Fibrosis stage is now recognized as the strongest predictor of long-term outcomes, including cardiovascular events, hepatic decompensation, and hepatocellular carcinoma.[9]

Given these insights, evaluating and comparing histopathological characteristics of MASLD and ALD via liver biopsy remains clinically critical to improve diagnosis, tailor management strategies, and predict disease trajectory. Therefore, the present study aims to compare and characterize liver biopsy findings—including steatosis grade, inflammation, hepatocyte injury (ballooning, Mallory bodies), and fibrosis pattern—between patients with MASLD and ALD, and to correlate histological features with clinical and metabolic risk factors.[10]

MATERIALS AND METHODS

This observational, cross-sectional study was conducted in the Department of Gastroenterology at a tertiary care center over a period of 18 months. A total of 120 patients were enrolled after obtaining approval from the Institutional Ethics Committee and written informed consent from all participants. Patients were divided into two groups: Group A, comprising individuals diagnosed with metabolic dysfunction-associated steatotic liver disease (MASLD), and Group B, comprising individuals diagnosed with alcoholic liver disease (ALD). Each group included 60 patients selected based on predefined inclusion and exclusion criteria.

Inclusion criteria for Group A were patients aged 18 years or older with evidence of hepatic steatosis on imaging and meeting the diagnostic criteria for MASLD as per the latest international consensus guidelines, with alcohol consumption less than 20 g/day for women and 30 g/day for men. For Group B, inclusion criteria were patients with a history of significant alcohol intake as per WHO criteria and clinical or biochemical evidence suggestive of ALD. Exclusion criteria for both groups included coexisting viral hepatitis (HBV, HCV), autoimmune hepatitis, drug-induced liver injury, Wilson's disease, hemochromatosis, hepatocellular carcinoma, and decompensated liver disease with features of severe portal hypertension.

All participants underwent detailed clinical evaluation, including demographic data, history of alcohol intake, comorbidities, and relevant laboratory investigations such as liver function tests, fasting blood sugar, lipid profile, and complete blood count. Imaging studies, including ultrasonography and elastography where applicable, were performed to assess hepatic steatosis and liver stiffness.

Percutaneous liver biopsies were performed under ultrasound guidance using an 18G Menghini needle after appropriate coagulation screening and standard aseptic precautions. Biopsy samples measuring at least 15 mm in length and containing a minimum of 10 complete portal tracts were considered adequate for histopathological examination. Specimens were fixed in 10% buffered formalin, processed, and stained with hematoxylin and eosin for routine histology, and Masson's trichrome for fibrosis assessment.

Histological evaluation was carried out by two experienced hepatopathologists blinded to the clinical diagnosis. The degree of steatosis, lobular inflammation, and hepatocellular ballooning was assessed according to the Kleiner-Brunt NAFLD Activity Score (NAS) for MASLD cases, while fibrosis was staged using the METAVIR scoring system in both groups. The presence of Mallory-Denk bodies, perisinusoidal fibrosis, and other features typical of ALD were also recorded. Discrepancies in interpretation were resolved by consensus.

Statistical analysis was performed using SPSS software version 26.0. Continuous variables were expressed as mean ± standard deviation, and categorical variables as frequencies and percentages. Comparison between the two groups was done using the chi-square test for categorical data and the independent t-test or Mann–Whitney U test for continuous data, as appropriate. A p-value of less than 0.05 was considered statistically significant.

RESULTS

[Table 1] presents the baseline demographic characteristics of the study participants. The mean age was slightly higher in the ALD group (52.1 ± 8.8 years) compared to the MASLD group (48.6 ± 9.2 years). The majority of patients in both groups were male, with a markedly higher proportion in the ALD group (86.7%) compared to 60% in the MASLD group. Female representation was significantly greater in the MASLD group (40%) than in the ALD group (13.3%). Body mass index (BMI) differed considerably, with MASLD patients showing a higher mean BMI (30.2 kg/m²), reflecting the metabolic basis of the disease, whereas ALD patients had a mean BMI of 24.6 kg/m². Comorbidities such as diabetes mellitus and hypertension were more frequent in the MASLD group, observed in 46.7% and 36.7% of patients respectively, compared to 16.7% and 20% in the ALD group. [Table 2] summarizes the laboratory profile of patients. The ALD group demonstrated higher mean AST levels (120.6 ± 34.7 U/L) compared to MASLD patients

(64.7 ± 18.9 U/L), while ALT was elevated in both groups but remained relatively higher in ALD. The AST/ALT ratio was greater than 1 in ALD (1.28), a hallmark of alcohol-related liver injury, while MASLD showed a ratio less than 1 (0.82). Total bilirubin levels were also significantly higher in ALD patients (2.6 mg/dL) compared to MASLD patients (1.1 mg/dL), indicating more advanced liver dysfunction. Serum albumin was lower in ALD patients (3.2 g/dL), suggesting impaired synthetic function, whereas MASLD patients maintained relatively preserved levels (3.9 g/dL). Platelet counts were lower in ALD (165 × 10^9/L) compared to MASLD (212 × 10^9/L), likely reflecting more advanced portal hypertension in alcoholic liver disease. [Table 3] illustrates the distribution of steatosis grades on histological examination. Grade 0 steatosis (<5%) was observed exclusively in the ALD group (13.3%), whereas all MASLD patients had steatosis exceeding 5%. In MASLD, Grade 2 steatosis (34–66%) was the most common (46.7%), followed by Grade 1 (26.7%) and Grade 3 (26.7%). Similarly, the ALD group demonstrated a higher frequency of moderate to severe steatosis, with Grade 2 and Grade 3 observed in 40% and 26.7% of cases, respectively. This indicates that significant fat accumulation is a common feature in both disease entities, although complete absence of steatosis was only seen in alcoholic liver disease. [Table 4] compares histological features of hepatocellular injury and inflammation. Lobular inflammation was prevalent in both groups but slightly more frequent in ALD patients (83.3%) compared to MASLD (70%). Hepatocyte ballooning, a hallmark of cellular injury, was observed in 76.7% of ALD patients and 56.7% of MASLD patients. Mallory-Denk bodies, a classic finding in alcohol-related injury, were detected in over half of ALD patients (53.3%) but were rare in MASLD (6.7%). This reinforces the notion that certain histological markers, such as Mallory-Denk bodies, remain strongly associated with alcohol-induced liver disease. [Table 5] presents the distribution of fibrosis stages using the METAVIR scoring system. The MASLD group had a relatively even spread across fibrosis stages, with the highest proportions in F2 (26.7%) and F3 (23.3%). Cirrhosis (F4) was present in 13.3% of MASLD patients. In contrast, ALD patients demonstrated more advanced fibrosis, with 30% showing bridging fibrosis (F3) and another 30% presenting with cirrhosis (F4), indicating a higher prevalence of severe liver damage in this group. The presence of portal fibrosis (F1) and early septal fibrosis (F2) was also noted but less common compared to MASLD. These findings highlight that ALD tends to present with more advanced fibrosis compared to MASLD, possibly due to cumulative hepatotoxic effects of alcohol and delayed clinical presentation.

Table 1: Baseline Demographic Characteristics of Study Participants (n = 120)

Parameter	Group A (MASLD) (n=60)	Group B (ALD) (n=60)
Mean Age (years)	48.6 ± 9.2	52.1 ± 8.8
Age Range (years)	30–65	32–68
Male, n (%)	36 (60.0%)	52 (86.7%)
Female, n (%)	24 (40.0%)	8 (13.3%)
Mean BMI (kg/m²)	30.2 ± 3.5	24.6 ± 2.9
Diabetes Mellitus, n (%)	28 (46.7%)	10 (16.7%)
Hypertension, n (%)	22 (36.7%)	12 (20.0%)

Table 2: Laboratory Profile of Study Participants

Parameter	Group A (MASLD) (n=60)	Group B (ALD) (n=60)
ALT (U/L), Mean ± SD	78.5 ± 22.4	94.3 ± 28.1
AST (U/L), Mean ± SD	64.7 ± 18.9	120.6 ± 34.7
AST/ALT Ratio	0.82	1.28
Total Bilirubin (mg/dL)	1.1 ± 0.4	2.6 ± 0.9
Albumin (g/dL)	3.9 ± 0.5	3.2 ± 0.6
Platelet Count (×10^9/L)	212 ± 55	165 ± 48

Table 3: Histological Grading of Steatosis

Steatosis Grade (%)	Group A (MASLD) (n=60)	Group B (ALD) (n=60)
<5% (Grade 0)	0 (0%)	8 (13.3%)
5–33% (Grade 1)	16 (26.7%)	12 (20.0%)
34–66% (Grade 2)	28 (46.7%)	24 (40.0%)
>66% (Grade 3)	16 (26.7%)	16 (26.7%)

Table 4: Histological Features of Hepatocellular Injury and Inflammation

Feature	Group A (MASLD) (n=60)	Group B (ALD) (n=60)
Lobular Inflammation Present	42 (70.0%)	50 (83.3%)
Hepatocyte Ballooning Present	34 (56.7%)	46 (76.7%)
Mallory-Denk Bodies Present	4 (6.7%)	32 (53.3%)

Table 5: Distribution of Fibrosis Stages (METAVIR Score)

Fibrosis Stage	Group A (MASLD) (n=60)	Group B (ALD) (n=60)
F0 (No Fibrosis)	8 (13.3%)	4 (6.7%)
F1 (Portal Fibrosis)	14 (23.3%)	8 (13.3%)
F2 (Portal + Few Septa)	16 (26.7%)	12 (20.0%)

F3 (Bridging Fibrosis)	14 (23.3%)	18 (30.0%)
F4 (Cirrhosis)	8 (13.3%)	18 (30.0%)

DISCUSSION

The present study demonstrates distinct demographic, biochemical, and histological differences between patients with MASLD and ALD. Our findings indicate that MASLD patients were more likely to be obese and have metabolic comorbidities such as diabetes and hypertension, while ALD patients were predominantly male and presented with lower BMI, aligning with established epidemiological patterns. Histologically, both groups exhibited significant steatosis; however, Mallory-Denk bodies and higher grades of hepatocyte ballooning were characteristic of ALD, consistent with the role of ethanol-induced oxidative stress in promoting cytoskeletal damage and protein aggregation.[11] The fibrosis distribution pattern in this study underscores the aggressive nature of ALD, where a greater proportion of patients exhibited advanced fibrosis and cirrhosis compared to MASLD. This is consistent with recent longitudinal data highlighting that ALD accelerates fibrogenesis through direct hepatotoxic mechanisms and repetitive inflammatory insults, whereas MASLD progression is generally slower but potentiated by metabolic risk factors.[12] Recent studies utilizing advanced digital pathology tools corroborate that fibrosis in ALD tends to be dense and irregular, while MASLD-related fibrosis exhibits a more uniform distribution, reflecting differences in the pathophysiological basis of matrix deposition.[13] Our findings also reinforce the prognostic role of fibrosis stage as the primary determinant of outcomes in both MASLD and ALD, with cirrhosis markedly elevating the risk of hepatic decompensation and hepatocellular carcinoma.[14] Given these insights, liver biopsy remains a crucial diagnostic tool, not only for staging fibrosis but also for identifying features that can differentiate between ALD and MASLD, particularly in cases with overlapping etiologies. Although non-invasive tests such as elastography and serum fibrosis markers are increasingly used, they lack the ability to detect subtle histological features such as Mallory-Denk bodies or ballooning degeneration, which have important diagnostic implications.[15] Therefore, integrating histopathological evaluation with clinical and biochemical findings provides the most comprehensive approach for accurate diagnosis, prognostication, and therapeutic decision-making in patients with chronic liver disease.

CONCLUSION

This study highlights significant differences in histological patterns between MASLD and ALD, despite shared features such as steatosis and inflammation. MASLD patients predominantly exhibited moderate steatosis with metabolic comorbidities, whereas ALD patients were more likely to present with advanced fibrosis, Mallory-Denk bodies, and severe hepatocellular ballooning. These findings underscore the indispensable role of liver biopsy in accurately characterizing disease patterns and guiding management strategies, particularly in an era where metabolic and alcohol-related liver diseases frequently coexist.

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