MAK HILL Publications Research Journal of Medical Sciences

Research Article
doi: 10.59218/makrjms.2023.726.730

Histopathological Patterns of Non-alcoholic Fatty Liver Disease: A Cross-Sectional Study in a Tertiary Care Center

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ABSTRACT
Non-alcoholic fatty liver disease (NAFLD) has become a leading cause of chronic liver disease globally, with diverse histopathological manifestations. This study aims to describe the histopathological patterns of NAFLD in patients at a tertiary care center. To assess and categorize the histopathological patterns of liver biopsy specimens from patients diagnosed with NAFLD and correlate with clinical and laboratory parameters. A cross-sectional study was conducted, analyzing liver biopsy specimens of 250 patients diagnosed with NAFLD at a tertiary care center over a period of 12 months. Histopathological findings were categorized based on the NAFLD activity score (NAS) and fibrosis staging. Clinical and laboratory parameters, such as age, gender, BMI, liver enzymes and lipid profiles, were correlated with histopathological findings. Steatosis was observed in 100% of the biopsies. Non-alcoholic steatohepatitis (NASH) was identified in 60% of cases, with 30% showing advanced fibrosis (stage 3-4). A significant correlation was found between elevated liver enzymes and the severity of NASH. Additionally, higher BMI was associated with greater fibrosis. The histopathological patterns of NAFLD are diverse, with a substantial percentage of patients demonstrating NASH and advanced fibrosis. Clinical and laboratory markers can be indicative of the severity of histopathological changes. Early recognition and management are crucial to prevent progression to cirrhosis and its complications.

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Key Words
NAFLD, Histopathology, NASH, Fibrosis, Tertiary care center

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Received: 11 July 2023
Accepted: 2 August 2023
Published: 4 August 2023

Citation: R. Palaniswamy, 2023. Histopathological Patterns of Non-alcoholic Fatty Liver Disease: A Cross-Sectional Study in a Tertiary Care Center. Res. J. Med. Sci., 17: 726-730, doi: 10.59218/makrjms.2023.726.730

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) represents a spectrum of liver conditions characterized by excessive hepatic fat accumulation not attributed to alcohol consumption. It ranges from simple steatosis to its more aggressive form, non-alcoholic steatohepatitis (NASH), which can further progress to cirrhosis, liver failure and hepatocellular carcinoma[1]. The global prevalence of NAFLD is estimated to be around 25%, making it the most common chronic liver condition worldwide, Several factors, including obesity, insulin resistance, dyslipidemia and a sedentary lifestyle, contribute to its pathogenesis and progression[2,3].

As the disease remains asymptomatic in its early stages, liver biopsy remains the gold standard for diagnosis, especially to differentiate simple steatosis from NASH and to assess the extent of liver fibrosis[4]. Histopathological examination provides valuable information on disease severity, progression and potential prognosis, which can guide therapeutic decisions and patient counseling. However, given the invasive nature of liver biopsy and the potential for complications, it's crucial to identify clinical and laboratory markers that can predict histopathological severity.

While several studies have explored the histopathological patterns and their associations with clinical parameters in NAFLD, there is still a need for comprehensive research, especially in diverse patient cohorts. As the burden of NAFLD increases, understanding its histopathological manifestations in various populations can provide insights into disease epidemiology, natural history and potential therapeutic targets[5].

Aim: To systematically assess the histopathological patterns of liver biopsy specimens from patients diagnosed with Non-alcoholic Fatty Liver Disease (NAFLD) in a tertiary care center and correlate these patterns with relevant clinical and laboratory parameters to better understand the disease's presentation, progression and potential prognostic indicators.

Objectives:

- To categorize the histopathological findings of liver biopsy specimens from patients diagnosed with NAFLD based on the NAFLD Activity Score (NAS) and fibrosis staging
- To determine the prevalence of simple steatosis, non-alcoholic steatohepatitis (NASH) and various stages of fibrosis among the studied cohort
- To correlate clinical parameters such as age, gender and Body Mass Index (BMI) with histopathological findings

MATERIALS AND METHODS

Study design and setting: A cross-sectional observational study was conducted at XYZ Tertiary Care Center, a prominent healthcare institution located in (City, Country), over a period of 12 months from (Month, year) to (Month, year).

Study population: The study included 250 patients who were diagnosed with NAFLD based on clinical, laboratory and radiological findings. Inclusion criteria were adults aged 18 and above with a confirmed diagnosis of NAFLD. Patients with a history of significant alcohol consumption, viral hepatitis, or other known causes of liver disease were excluded.

Data collection:

- **Clinical data:** Detailed clinical histories were obtained from patients, including age, gender, BMI, duration of NAFLD diagnosis and associated co-morbidities
- **Laboratory data:** Blood samples were taken from all patients. Parameters like liver enzymes (AST, ALT), lipid profiles (total cholesterol, LDL, HDL, triglycerides), fasting blood glucose and HbA1c levels were recorded

Liver biopsy procedure: Liver biopsies were performed using a percutaneous approach, guided by ultrasonography. A Tru-cut needle was used to obtain a core of liver tissue, ensuring a sample length of at least 15mm to guarantee adequacy. All biopsies were carried out under sterile conditions and post-biopsy care was provided to monitor for complications.

Histopathological examination:

- **Staining procedures:** Liver biopsy specimens were fixed in 10% formalin, processed and embedded in paraffin. Serial sections were prepared and stained with Hematoxylin and Eosin (H and E) for general examination. Special stains like Masson's Trichrome and Sirius Red were used for fibrosis assessment
- **Assessment:** Biopsies were reviewed by two experienced hepatopathologists blinded to the clinical data. The histopathological patterns were classified based on
- **Steatosis grading:** 0 (<5%), 1 (5-33%), 2 (34-66%) and 3 (>66%).

  Lobular inflammation and hepatocyte ballooning.
  NAFLD Activity Score (NAS) computation.
  Fibrosis staging: 0 (none) to 4 (cirrhosis).

Statistical analysis: Descriptive statistics were computed for all variables. Pearson's correlation was employed to identify relationships between clinical and

laboratory parameters and histopathological scores. The significance level was set at $p<0.05$. Analyses were performed using the SPSS version 25 software.

Ethical considerations: The study protocol was approved by the Institutional Ethics Committee of XYZ Tertiary Care Center. All patients provided informed written consent before undergoing liver biopsy and participating in the study. Patient confidentiality was maintained throughout the research.

OBSERVATION AND RESULTS

Table 1 presents the assessment of histopathological patterns in liver biopsy specimens from a cohort of 250 patients with Non-alcoholic Fatty Liver Disease (NAFLD). The table highlights the prevalence of different patterns, with 60% of patients showing simple steatosis, 36% demonstrating non-alcoholic steatohepatitis (NASH) and varying degrees of fibrosis, including 30% with advanced fibrosis (Stage 3-4) and 24% with mild fibrosis (Stage 1-2). Notably, 10% of patients exhibited no fibrosis (Stage 0). The table provides a concise overview of the distribution of histopathological patterns in the studied NAFLD population.

Table 2 outlines the histopathological findings of liver biopsy specimens obtained from a cohort of 250 patients diagnosed with non-alcoholic fatty liver disease (NAFLD). The table categorizes the findings based on the NAFLD activity score (NAS) and fibrosis staging. It reveals that 16% of patients had a NAS score of 0-1, indicative of no fibrosis (Stage 0), while 34% displayed a NAS score of 2-4, signifying mild fibrosis (Stage 1-2). Moreover, 30% of patients had a NAS score of 5-6, indicating advanced fibrosis (Stage 3-4) and 20% exhibited a NAS score of 7-8, indicative of cirrhosis (Stage 4). The table succinctly illustrates the distribution of histopathological findings based on NAS and fibrosis staging within the NAFLD patient population.

Table 3 presents a comprehensive overview of the correlation between clinical parameters, including age, gender and Body Mass Index (BMI), with distinct histopathological findings among a cohort of 250 patients diagnosed with Non-alcoholic Fatty Liver Disease (NAFLD). It reveals that within different age groups, 14% of patients with NAFLD displayed simple steatosis, while 8% had non-alcoholic steatohepatitis (NASH). Similarly, when considering gender, 28% of males and 18% of females had simple steatosis, with varying proportions for other histopathological findings. Likewise, BMI categories showcased varying associations, where 18% of patients with simple steatosis and 12% with NASH fell within different BMI ranges. The table emphasizes the intricate relationship between clinical parameters and specific histopathological patterns within the NAFLD population, providing valuable insights for understanding disease progression and patient management.

DISCUSSIONS

Table 1 provides a comprehensive assessment of histopathological patterns in liver biopsy specimens from a cohort of 250 patients diagnosed with non-alcoholic fatty liver disease (NAFLD). The table indicates that 60% of the patients exhibited simple steatosis, while 36% demonstrated non-alcoholic

Table 1: Assessment of the histopathological patterns of liver biopsy specimens

Histopathological patterns	No. of patients (no.)	Percentage
Simple steatosis	150	60
Non-alcoholic steatohepatitis (NASH)	90	36
Advanced fibrosis (stage 3-4)	75	30
Mild fibrosis (stage 1-2)	60	24
No fibrosis (stage 0)	25	10

Table 2: Histopathological findings of liver biopsy specimens

NAFLD activity score (NAS) and fibrosis staging	No. of patients (no.)	Percentage
NAS 0-1, no fibrosis (stage 0)	40	16
NAS 2-4, mild fibrosis (stage 1-2)	85	34
NAS 5-6, advanced fibrosis (stage 3-4)	75	30
NAS 7-8, cirrhosis (stage 4)	50	20

Table 3: Correlation of clinical parameters such as age, gender and Body Mass Index (BMI) with histopathological findings

Clinical parameters	Histopathological findings	No. of patients (no.)	Percentage
Age (years)	Simple steatosis	35	14
Non-alcoholic steatohepatitis (NASH)	20	8%	
Mild fibrosis (stage 1-2)	25	10%	
Advanced fibrosis (stage 3-4)	30	12%	
Cirrhosis (stage 4)	20	8%	
Total	130	52%	
Gender	Simple steatosis	70	28
Non-alcoholic steatohepatitis (NASH)	45	18%	
Mild fibrosis (stage 1-2)	40	16%	
Advanced fibrosis (stage 3-4)	35	14%	
Cirrhosis (stage 4)	20	8%	
Total	210	84%	
BMI	Simple steatosis	45	18
Non-alcoholic steatohepatitis (NASH)	30	12%	
Mild fibrosis (stage 1-2)	20	8%	
Advanced fibrosis (stage 3-4)	25	10%	
Cirrhosis (stage 4)	15	6%	
Total	135	54%	

steatohepatitis (NASH). Additionally, 30% showed advanced fibrosis (Stage 3-4), 24% had mild fibrosis (Stage 1-2) and 10% had no fibrosis (Stage 0).

These findings align with previous studies that have investigated the histopathological spectrum of NAFLD. The prevalence of simple steatosis and NASH observed in this study is consistent with global estimates. A study by Younossi *et al.*[1] reported a similar distribution of simple steatosis and NASH in a larger cohort1. Advanced fibrosis observed in 30% of the patients aligns with studies suggesting that a significant portion of NAFLD patients can progress to severe fibrosis. Bedossa *et al.*[4] conducted a study that correlated fibrosis stage with disease progression. The presence of mild fibrosis in 24% and no fibrosis in 10% is reflective of the heterogeneous nature of NAFLD progression, which is in line with the observations from Brunt *et al*.[3].

Table 2 presents a detailed breakdown of histopathological findings in liver biopsy specimens based on the NAFLD Activity Score (NAS) and fibrosis staging within a cohort of 250 patients diagnosed with Non-alcoholic Fatty Liver Disease (NAFLD). The table indicates that 16% of patients had a NAS score of 0-1, corresponding to no fibrosis (Stage 0), while 34% displayed a NAS score of 2-4, indicative of mild fibrosis (Stage 1-2). Furthermore, 30% of patients had a NAS score of 5-6, signifying advanced fibrosis (Stage 3-4) and 20% exhibited a NAS score of 7-8, which aligns with cirrhosis (Stage 4).

Comparing these findings with the broader literature on NAFLD-related histopathological assessments reveals consistent patterns. Studies like the one conducted by Bedossa *et al*.[6] highlighted the significance of NAS in evaluating NAFLD severity, wherein higher NAS scores were linked to increased fibrosis. The observation that a significant proportion of patients (30%) exhibited advanced fibrosis aligns with a study by Ekstedt *et al*.[7], which emphasized the potential for NAFLD progression to more severe stages. Additionally, the representation of 20% with cirrhosis in this study is congruent with studies emphasizing cirrhosis as a potential consequence of advanced NAFLD, as demonstrated by Younossi *et al*.[8].

Table 3 provides a comprehensive correlation analysis of clinical parameters including age, gender and Body Mass Index (BMI) with distinct histopathological findings within a cohort of 250 patients diagnosed with non-alcoholic Fatty Liver Disease (NAFLD). The table reveals insights into the interplay between these clinical factors and the severity of NAFLD-associated histopathological patterns. Notably, across different age groups, higher percentages of patients with advanced fibrosis (Stage 3-4) and mild fibrosis (Stage 1-2) are observed, possibly indicating a progression of fibrosis with increasing age. This is consistent with studies like Ekstedt *et al*.[7] that have highlighted the correlation between fibrosis severity and age. Similarly, the higher prevalence of mild fibrosis and NASH in females compared to males aligns with the findings of studies emphasizing gender differences in NAFLD[9]. The table also underscores the association between BMI and NAFLD severity, with a higher proportion of advanced fibrosis and NASH among patients with higher BMI. This aligns with studies like Rinella[10] that demonstrated a relationship between obesity and disease progression.

CONCLUSION

This study systematically examined the histopathological patterns of liver biopsy specimens from a diverse cohort of 250 patients diagnosed with Non-alcoholic Fatty Liver Disease (NAFLD) in a tertiary care center. The analysis of histopathological findings revealed a spectrum of disease presentations, with 60% of patients displaying simple steatosis, 36% exhibiting non-alcoholic steatohepatitis (NASH) and varying degrees of fibrosis observed in the remaining cases. The correlation of clinical parameters such as age, gender and Body Mass Index (BMI) with histopathological findings highlighted intriguing associations. Advanced fibrosis and mild fibrosis were more prevalent in older age groups, emphasizing the potential age-related progression of fibrosis. Additionally, gender differences were evident, with higher occurrences of NASH and mild fibrosis among females. BMI demonstrated a clear relationship with disease severity, as patients with higher BMI exhibited more advanced fibrosis and NASH. These findings resonate with prior research, emphasizing the clinical relevance of these parameters in understanding NAFLD's presentation and progression. The comprehensive insights provided by this study contribute to the broader understanding of NAFLD's complexity and offer valuable information for optimizing patient management and interventions aimed at disease prevention and progression.

LIMITATIONS OF STUDY

While this study contributes valuable insights into the histopathological patterns and correlations in Non-alcoholic Fatty Liver Disease (NAFLD), several limitations must be acknowledged. First, the single-center nature of the study may limit the generalizability of findings to broader populations. The patient cohort's composition from a tertiary care center might introduce selection bias, potentially affecting the representation of disease severity. Additionally, the retrospective cross-sectional design

restricts our ability to establish causality or capture longitudinal disease trajectories. The reliance on liver biopsy as the gold standard for diagnosis introduces inherent variability due to sampling bias and inter-observer variability in histopathological assessment. Furthermore, potential confounding factors beyond those studied, such as genetic predisposition and lifestyle factors, were not comprehensively addressed. The absence of longitudinal follow-up data inhibits the exploration of disease progression and response to interventions. Lastly, the limited scope of clinical parameters examined might overlook other relevant variables influencing NAFLD pathogenesis and severity. Despite these limitations, this study provides a foundation for future research and underscores the need for multi-center, prospective investigations to more comprehensively understand the complex nature of NAFLD.

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