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Immunity as Cornerstone of Non-alcoholic Fatty Liver Disease: The Contribution of Innate and Adaptive Immune Mechanisms in the Pathogenesis of the Metabolic Syndrome-related Steatohepatitis

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ABSTRACT

Non-alcoholic fatty liver disease (NAFLD) is a major hepatic manifestation of metabolic syndrome and encompasses a spectrum ranging from simple steatosis to non-alcoholic steatohepatitis (NASH). This study aimed to evaluate the contribution of immunological, inflammatory, and metabolic parameters-including cytokine levels, immune cell profiles, and microRNA (miR) expression-in the progression from NAFLD to NASH among individuals with features of metabolic syndrome.

An observational study was conducted between January 2022 and December 2024, enrolling 300 adult patients with radiologically or histologically confirmed NAFLD. Patients underwent comprehensive anthropometric, biochemical, and immunological assessments, including cytokine profiling (interleukin [IL]-6, IL-17, tumor necrosis factor-α [TNF-α], transforming growth factor-β1 [TGF-β1]), immune cell phenotyping (T helper 17 [T_H17], regulatory T cells [Tregs], monocytes), and miR quantification (miR-122, miR-34a). Liver biopsy was performed in 95 selected cases.. The nursing team also assists in coordinating multidisciplinary care and ensuring follow-up compliance, which are vital for long-term disease management and reducing progression to NASH.

Significant elevations were observed in metabolic parameters (body mass index [BMI], homeostatic model assessment for insulin resistance [HOMA-IR]), hepatic enzymes (alanine aminotransferase [ALT], aspartate aminotransferase [AST]), inflammatory markers (high-sensitivity C-reactive protein [hs-CRP], ferritin), and oxidative stress markers (malondialdehyde [MDA]). Adipokines (↑leptin, ↓adiponectin), hepatokines (↑fibroblast growth factor 21 [FGF21], ↑fetuin-A), and cytokines (↑IL-6, ↑TNF-α, ↑IL-17) were markedly altered in patients with biopsy-proven NASH.

This study reinforces that pro-inflammatory cytokines, altered immune cell profiles, and dysregulated miRs serve as promising biomarkers for early identification and potential therapeutic targeting in metabolic syndrome-associated steatohepatitis.

Keywords: Cytokines; MicroRNA-122; NASH; Non-alcoholic fatty liver disease; T_H17 cells

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INTRODUCTION

Affecting almost a quarter of the global population, non-alcoholic fatty liver disease (NAFLD) has become the most common chronic liver condition known worldwide. Simple steatosis (non-alcoholic fatty liver) to non-alcoholic steatohepatitis (NASH), which can further progress to fibrosis, cirrhosis, and hepatocellular carcinoma, reflects a spectrum of hepatic pathology. Although metabolic dysfunction—especially, insulin resistance, dyslipidemia, and obesity—has long been identified as a pillar in NAFLD development, it is now abundantly clear that immune dysregulation plays a central, driving role in disease progression and severity.[1–4]

NAFLD and metabolic syndrome have a clear, strong correlation. Not only risk factors but also active participants in the inflammatory process causing liver pathology are obesity, insulin resistance, and type 2 diabetes mellitus (T2DM). A hallmark of NAFLD, hepatic lipid accumulation causes hepatocyte damage via processes, including lipotoxicity and mitochondrial malfunction, which then set off sterile inflammation and immune cell activation. By means of pattern recognition receptors (PRRs), comprising toll-like receptors (TLRs) and NOD-like receptors (NLRs), ceramides and other lipotoxic molecules function as metabolic danger signals, so activating the innate immune system.[2,5–7] Promoting the synthesis of inflammatory cytokines, including tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and IL-1β—which aggravate hepatocyte death and thus attract immune effectors into hepatic tissue—is mostly dependent on this natural immune sensing.

In NAFLD, the first defense against hepatic insults is innate immunity. The core of this process is Kupffer cells, the liver-resident macrophages. Activated by free fatty acids, damaged hepatocytes, or gut-derived microbial products (via the portal vein), Kupffer cells generate a broad spectrum of inflammatory mediators that help to explain steatosis, hepatocyte ballooning, and fibrosis.[8–12] Furthermore, interacting with invading monocytes and neutrophils, Kupffer cells spread inflammation and immune cell crosstalk.

Activated neutrophils form extracellular traps (NETs), which have been linked to aggravating NAFLD liver damage. Recent research indicates that NETs damage hepatocytes and induce hepatic stellate cells (HSCs), contributing to fibrosis,[13] in addition to acting as antimicrobial defense mechanisms.

Important contributors to the hepatic innate immune terrain are also dendritic cells (DCs) and natural killer (NK) cells. While NK cells have cytotoxic activity against stressed hepatocytes and affect HSC activation, DCs control T-cell activation by linking innate and adaptive responses. But in chronic inflammation, as observed in NASH, these cells sometimes develop dysfunctional or pro-inflammatory phenotypes, thus accelerating liver damage.[14]

Whereas adaptive immune responses maintain and aggravate the chronic inflammation in NASH, innate immunity starts the inflammatory cascade. Increasing numbers of T lymphocytes—especially $CD4^+$ T-helper cells (T_H1, T_H17) and $CD8^+$ cytotoxic T lymphocytes—have been found in NASH livers.[15] Particularly, T_H17 cells have been shown to induce fibrosis by means of increased neutrophil recruitment and IL-17-mediated activation of HSCs.[15] Although less is known about the function of B cells and the humoral immune response in NAFLD, new data suggest that changed immunoglobulin synthesis and autoantibody generation might cause immune dysregulation in steatohepatitis.[16–20] Moreover, NASH has shown a distorted regulatory T cell (Treg)/T_H17 balance favoring inflammatory over regulatory T cell responses, so fostering uncontrolled hepatic inflammation.[21–24]

An active endocrine organ, adipose tissue is crucially involved in the systemic inflammation observed in NAFLD and metabolic syndrome. Leptin, adiponectin, resistin, and visfatin change immune cell behavior and hepatic lipid metabolism.[14–16] While adiponectin has anti-inflammatory effects, promoting M2 macrophage polarization and increasing insulin sensitivity, leptin functions as a pro-inflammatory adipokine, enhancing T_H1 responses and macrophage activation.

The imbalance among these adipokines favors a pro-inflammatory hepatic environment and adds to immune dysregulation. Furthermore, hepatokines—such as fetuin-A and fibroblast growth factor 21 (FGF21)—secreted by steatotic livers affect systemic metabolism and immune responses, so tying liver pathology to extrahepatic NAFLD.[6,11]

Emerging as a major factor in NAFLD pathogenesis, the gut-liver axis acts as a portal for microbial signals and metabolites modulating hepatic immune responses. Often referred to as a "leaky gut," disturbance of gut barrier integrity lets bacterial endotoxins, including lipopolysaccharide (LPS), translocate into portal circulation. On Kupffer cells and HSCs, these microbial-

associated molecular patterns (MAMPs) stimulate TLRs (especially TLR4), aggravating hepatic inflammation and fibrosis.[6,12,23]

Furthermore, greatly affecting immune activity in NAFLD is the makeup of gut bacteria. Reduced microbial diversity and overrepresentation of pathogenic bacteria define dysbiosis, which can increase gut permeability and encourage immune dysregulation. Some microbial metabolites have immunomodulating properties, including bile acids and short-chain fatty acids (SCFAs). To lower hepatic inflammation and enhance metabolic homeostasis, for instance, secondary bile acids interact with nuclear receptors such as *FXR* and *TGR5*. In NAFLD, dysregulated bile acid metabolism compromises these anti-inflammatory signaling pathways, thus aggravating disease progression.[12,25–28] Recent studies show that variations in gut flora match hepatic immune cell counts, implying that microbial signaling directly modulates both innate and adaptive immunity within the liver.[23] Currently under study as immunometabolic treatments aiming at the gut-liver axis in NAFLD are probiotics, prebiotics, and fecal microbiota transplantation (FMT).

Key arbiters of immune-driven liver damage in NAFLD are cytokines. Among the first and most important causes of hepatic inflammation and insulin resistance is TNF-α, a cytokine mostly secreted by activated macrophages. It advances hepatocyte death, reactive oxygen species (ROS) generation, and additional immune activation.[4,27] Another pro-inflammatory cytokine, IL-6, has been linked in the context of NAFLD to insulin resistance and hepatocarcinogenesis.[5,28] It is also linked with hepatic acute-phase responses. Emerging data also links products of inflammasome activation-especially *NLRP3*-to IL-1β and IL-18 in aggravating hepatic inflammation and fostering fibrogenesis. Mostly derived from T_H17 cells, the IL-17 axis works in concert with these innate cytokines to attract neutrophils and activate HSCs, thus sustaining liver damage.[27,28] On the other hand, anti-inflammatory cytokines such as transforming growth factor-β (TGF-β) and IL-10 are sometimes insufficiently expressed or rendered useless in advanced NASH, creating an imbalance favoring continuous inflammation. [26]

Mediating the recruitment of monocytes and lymphocytes into the liver are chemokines, including *MCP-1* (*CCL2*) and *RANTES* (*CCL5*). Particularly, *MCP-1* is overexpressed in NASH and drives monocyte infiltration and their differentiation into pro-inflammatory macrophages.[28,29] One therapeutic approach suggested to lower hepatic inflammation is blocking these chemokine pathways.

Immunological processes mainly control the change from hepatic steatosis to steatohepatitis and fibrosis. HSCs, the principal fibrogenic cells in the liver, become activated in response to inflammatory cytokines, oxidative stress, and apoptotic hepatocyte debris. Upon activation, HSCs undergo phenotypic transformation and begin producing extracellular matrix proteins, contributing to fibrosis.[27]

Macrophages, both Kupffer cells and bone marrow-derived monocyte/macrophages, play a crucial role in activating HSCs through the release of TGF-β and platelet-derived growth factor (PDGF). Moreover, activated T cells-particularly T_H17 and $CD8^+$ cells-have been shown to directly interact with HSCs and modulate fibrogenic gene expression.[27,28] Chronic immune activation not only sustains HSC activation but also creates a pro-fibrotic milieu via inflammasome activation and NET formation.[24] The persistence of immune-mediated HSC activation is a defining feature that distinguishes NASH from benign hepatic steatosis. The extent of fibrosis is the most reliable predictor of liver-related morbidity and mortality in NAFLD, underscoring the importance of immune-targeted strategies in halting fibrotic progression.[1,11]

Differences in immune signatures help to partially explain NAFLD's considerable individual variability in response to treatment and course of development. For instance, some people show a predominance of pro-inflammatory M1 macrophages, while others show increased T_H17 or cytotoxic $CD8^+$ T cell infiltration in liver tissue.[26,27] These immune cell heterogeneities have started to be found by single-cell transcriptomic studies, which provide an understanding of patient stratification and tailored treatment.[26] Furthermore, affecting immune responses in NAFLD are sex variations. Anti-inflammatory effects of estrogen could help premenopausal women avoid the development of NASH. On the other hand, men and postmenopausal women show more severe immune-mediated inflammation presumably in response to sex hormone-driven modulation of cytokine profiles and immune cell recruitment.[10,13]

New dimensions of NAFLD pathogenesis have been exposed by recent developments in immunometabolism, the study of how metabolic pathways interact with immune function. Responding to nutrient excess and

lipotoxicity, immune cells-especially macrophages and T cells-go through metabolic reprogramming. These modifications actively control immune cell phenotype and function rather than only passive effects.[4,27]

For instance, anti-inflammatory (M2) macrophages use oxidative phosphorylation and fatty acid oxidation; pro-inflammatory (M1) macrophages preferentially rely on glycolysis. In the NAFLD liver, insulin resistance and excess saturated fatty acids favor M1 polarization, thus aggravating chronic inflammation.[14,15] Likewise, whereas Tregs rely on oxidative metabolism, effector T cells adopt glycolytic metabolism under inflammatory conditions, thus tying metabolic dysfunction to immune imbalance.[27]

One intriguing development of immunometabolism is the idea of trained immunity. Originally believed to lack memory, innate immune cells can undergo epigenetic reprogramming in response to metabolic stresses, including fatty acids or endotoxins. This "training" improves their inflammatory response upon re-exposure, contributing to the low-grade, ongoing inflammation unique to NASH.[28] Offering possible biomarkers and therapeutic targets, epigenetic marks, including histone modifications and DNA methylation patterns in macrophages and dendritic cells, have been shown to correlate with NAFLD severity.[25]

The immunological disturbances observed in NAFLD transcend the liver. For other organs, systemic inflammation brought on by hepatic immune activation has major effects; this helps to explain the higher metabolic and cardiovascular risk linked with NAFLD.[3,6]

Altered immune cell populations, acute-phase proteins, and circulating inflammatory cytokines influence endothelial function, aggravate insulin resistance, and drive atherosclerosis. For example, TNF-α and IL-6 reduce insulin signaling in peripheral tissues, thus aggravating glycemic control in T2DM.[5] Resistin and leptin, among other adipokines, control appetite, insulin sensitivity, and vascular tone, thus impacting hepatic and cardiovascular effects.[14,16]

Furthermore, adding to extrahepatic manifestations, including chronic kidney disease, polycystic ovary syndrome, obstructive sleep apnea, and cognitive impairment, is immune dysregulation in NAFLD. These disorders highlight the systemic character of immune-metabolic interactions by perhaps sharing common paths of inflammation, oxidative stress, and endothelial dysfunction.[3,7]

Immune-based biomarkers are becoming more and more important as our knowledge of immunity in NAFLD deepens, since they have diagnostic and prognostic power. Conventional diagnostic instruments, including imaging methods and liver enzymes, sometimes cannot effectively predict fibrosis or differentiate between simple steatosis and NASH. Conversely, more sensitive markers of disease activity are immune-related molecules, including cytokines (e.g., IL-6, TNF-α), chemokines (e.g., *MCP-1*), and cell-free DNA.[22,29]

Another exciting class of biomarkers is microRNAs (miRs), tiny non-coding RNAs engaged in post-transcriptional control. Hepatic inflammation and fibrosis have been linked in studies to circulating miRs, including miR-122 and miR-34a.[8] These minimally invasive molecules provide a means of disease monitoring by reflecting immune activation and hepatocyte damage.

Combining multi-omics techniques-including transcriptomics, metabolomics, and proteomics-with machine learning algorithms has improved our capacity to classify NAFLD phenotypes based on immune signatures.[22] Early detection, risk stratification, and response prediction for immunomodulatory treatments all have promise using these approaches.

An increasingly known condition with unique histological and immunological characteristics, unlike in adults, is pediatric NAFLD. Children with NAFLD often show a more periportal pattern of inflammation and fibrosis, and their prevalence of ballooning and lobular neutrophilic infiltration[7] is higher. In pediatric NAFLD, the immune terrain reveals higher activation of innate immune pathways, including higher TLR expression and *NLRP3* inflammasome activity. The adaptive immune response is also distinct, with an altered Treg/T_H17 balance and enhanced $CD8^+$ T cell cytotoxicity.[26] Developmental immunology, gut flora composition, and genetic inclination could all affect these variations.

In clinical practice, nurses are frequently the first point of contact for patients with early signs of metabolic dysfunction. Their holistic assessment skills help identify risk factors such as obesity, sedentary lifestyle, and poor dietary habits associated with NAFLD. Nurses also serve as critical links in educating patients about disease prevention and promoting self-care behaviors. Given the chronic nature of NAFLD and its impact on quality of life, nursing care plans often integrate lifestyle modifications, mental health assessments, and culturally

sensitive health education tailored to individual patient needs. These frontline efforts are essential in addressing the growing burden of NAFLD.

Development of age-specific diagnosis criteria and therapeutic approaches depends on an awareness of the immunological subtleties in pediatric populations. Early childhood identification and intervention can help to avoid long-term liver damage and metabolic complications.

Given the central part metabolic and immune dysfunction plays in NAFLD, a recent suggestion has been made to change the nomenclature from NAFLD to metabolic dysfunction-associated fatty liver disease (MAFLD).[30] This new vocabulary seeks to underline the metabolic and immunological foundations of the disease instead of its exclusive definition depending on alcohol intake. Incorporating metabolic risk factors, including obesity, T2DM, and dyslipidemia, NAFLD criteria recognize the immunometabolic crosstalk engaged in pathogenesis. This redefinition also fits the realization of overlapping characteristics between NAFLD and other chronic inflammatory diseases.

The change toward MAFLD reflects a growing knowledge of the disease that combines environmental, metabolic, and immune aspects. It also emphasizes the need for multidimensional diagnostics and treatments, including immunological evaluation.

Beyond basic elements, NAFLD immune responses are much influenced by lifestyle choices and environmental exposures. Particularly, diet shapes hepatic and systemic immunity. Western-style, high-fat, high-fructose diets boost intestinal permeability, endotoxemia, and innate immune activation, so hastening NAFLD development. On the other hand, diets heavy in polyphenols, omega-3 fatty acids, and fiber-like the Mediterranean diet-have anti-inflammatory effects and support good gut bacteria, so enhancing immune homeostasis.[2,20] Providing non-pharmacologic means of immune modulation, these dietary elements can control macrophage function, T cell polarization, and cytokine generation. Furthermore, physical activity has a great impact on immunity is physical activity. Regular exercise has been shown to lower systemic inflammation, increase Treg function, and improve insulin sensitivity, supporting hepatic immune balance.[6] These results support the foundation of immunotherapeutic strategies in NAFLD-integration of lifestyle modification.

METHODS

Comprising the Departments of Gastroenterology, Immunology, and Pathology at a tertiary care academic hospital, this was an observational, analytical study carried out over three years (January 2022–December 2024). Especially among those with metabolic syndrome, the main goal was to investigate the contribution of innate and adaptive immune mechanisms in the pathogenesis and progression of NAFLD. Enrolled consecutively from both inpatient and outpatient settings throughout the study period, a total of 300 adult patients aged between 18 and 70 years, diagnosed with NAFLD by ultrasonography and/or histopathology, were enrolled. Patients were further categorized into groups with simple steatosis and biopsy-proven non-alcoholic steatohepatitis (NASH) after being stratified depending on the diagnostic criteria for metabolic syndrome, defined by the International Diabetes Federation (IDF, 2005).

Inclusion Criteria

- Adults aged 18–70 years.
- Radiological or histological confirmation of hepatic steatosis (≥5% hepatocyte fat content).
- Presence of ≥1 metabolic risk factor (obesity, insulin resistance, hypertension, or dyslipidemia).
- Willingness to undergo blood sampling and liver biopsy (when indicated).
- Written informed consent provided.

Exclusion Criteria

- Alcohol consumption >30 g/day (males) or>20 g/day (females).
- Viral hepatitis, autoimmune liver disease, Wilson's disease, hemochromatosis.
- Use of hepatotoxic medications (e.g., amiodarone, corticosteroids, methotrexate).
- Pregnant or lactating females.
- Known malignancy, HIV infection, or immunosuppressive therapy.

Clinical and Laboratory Evaluation

Once included in the study, every enrolled patient-n=300-had a thorough clinical examination. Demographic information, clinical symptoms, dietary patterns, physical activity levels, and known history of diabetes, dyslipidemia, hypertension, or cardiovascular disease were entered into a structured assessment form.

Clinical and Anthropometric Values: A stadiometer and calibrated weighing scale measured weight and height. Calculated as weight (kg) divided by height (m^2), body mass index (BMI) was classified using the WHO Asian criteria. Using a flexible tape, the waist and hip circumference were measured; the waist-to-hip ratio (WHR) was computed. Following five minutes of rest in a seated position, blood pressure (BP) was recorded with a digital sphygmomanometer. Three averaged readings were obtained.

Blood Sampling and Laboratory Investigations: Between 8:00 and 10:00 AM, all laboratory studies were carried out on fasting blood samples gathered following an overnight fast spanning 8–10 hours. Centrifuged at 3000 rpm for 10 minutes, the samples were kept under suitable conditions for the particular tests. Automated biochemical analyzers ran liver function tests (LFTs), including alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), total and direct bilirubin, albumin, and globulin levels. Along with serum uric acid and creatinine to screen for nephropathy or hyperuricemia, metabolic and renal values measured included fasting plasma glucose (FPG), HbA1c, fasting insulin, and lipid profile (total cholesterol, low-density lipoprotein [LDL], high-density lipoprotein [HDL], triglycerides). The homeostatic model assessment for insulin resistance (HOMA-IR) equation was used to determine insulin resistance: [Fasting insulin (μIU/mL) × Fasting glucose (mg/dL)] / 405. Using immunoturbidimetric assays, inflammatory and oxidative stress markers—including serum ferritin and high-sensitivity C-reactive protein (hs-CRP)—were measured; in a subset of 100 patients, oxidative stress was assessed by evaluating malondialdehyde (MDA) and total antioxidant capacity (TAC). Commercial ELISA kits from Thermo Fisher Scientific or R&D Systems let one measure adipokines and hepatokines, including leptin, adiponectin, resistin, visfatin, fetuin-A, and FGF21.

With all samples run in duplicate and inter-assay variability maintained below 10%, multiplex bead-based ELISAs on the Luminex platform were used for cytokine profiling to measure both pro- and anti-inflammatory cytokines, including TNF-α, IL-6, IL-1β, IL-10, IL-17A, and TGF-β1. Immune cell phenotyping with flow cytometry involved isolating peripheral blood mononucleated cells (PBMCs) by Ficoll-Hypaque density gradient centrifugation and staining them with fluorochrome-conjugated antibodies from BD Biosciences. Markers for T lymphocytes ($CD3^+$, $CD4^+$, $CD8^+$), Tregs ($CD4^+CD25^+FoxP3^+$), T_H17 cells ($CD4^+IL\text{-}17^+$), B cells ($CD19^+$), NK cells ($CD56^+CD16^+$), and monocyte/macrophage subsets ($CD14^+CD16^+$). A BD FACSCantoTM II flow cytometer was used for data collection; FlowJo software was used for analysis. Using the miRNeasy Serum/Plasma Kit (Qiagen), plasma samples were also processed for miR extraction; internal control was U6 snRNA, and the expression levels of miR-122 and miR-34a were quantified using SYBR Green-based qRT-PCR (Applied Biosystems). The $2^{-\Delta\Delta Ct}$ approach computed relative expression levels.

Radiologic and Histologic Evaluation

Using a 3.5 MHz convex probe by experienced radiologists, all patients underwent standardized transabdominal liver ultrasonography (USG) to evaluate hepatic steatosis, which was graded as Grade 0 (normal), Grade I (mild echogenicity), Grade II (moderate echogenicity with reduced visualization of intrahepatic vessels), or Grade III (severe echogenicity with obscured vascular architecture) (Figure 1).

In 160 patients with moderate to severe steatosis or suspected NASH, transient elastography (FibroScan, Echosens) was employed to determine liver stiffness measurement (LSM) for fibrosis staging and controlled attenuation parameter (CAP) scores for steatosis quantification, with steatosis thresholds defined as CAP≥248 dB/m (S1),≥268 (S2), and≥280 (S3), and fibrosis thresholds as LSM≥7.0 kPa (significant fibrosis) and≥9.5 kPa (advanced fibrosis). Under USG direction, percutaneous liver biopsy was conducted in 95 chosen cases using an 18-G Menghini needle; biopsy cores measuring more than 1.5 cm were processed and stained with Hematoxylin and Eosin (H&E) and Masson's trichrome. Two blinded hepatopathologists independently performed histological evaluation using the NAFLD Activity Score (NAS), which included steatosis (0–3), lobular inflammation (0–3), and ballooning (0–2), as well as the Kleer Fibrosis Score ranging from F0 to F4 (Figure 2).

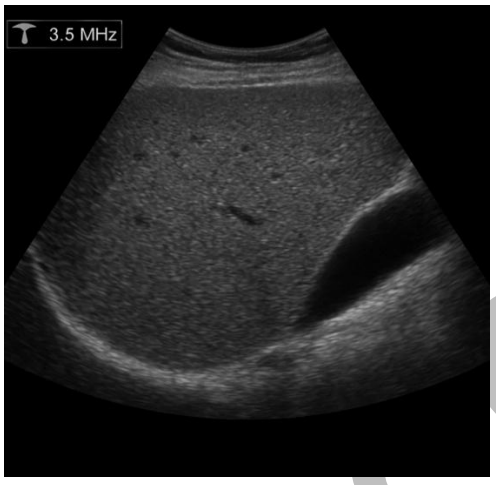

Figure 1. Ultrasound image of the liver using a 3.5 MHz convex probe. This grayscale ultrasound reveals a coarse echotexture of the liver parenchyma, suggestive of fatty infiltration. The diaphragm appears as a bright echogenic line at the bottom, and the gallbladder is visualized as a dark anechoic structure on the right.

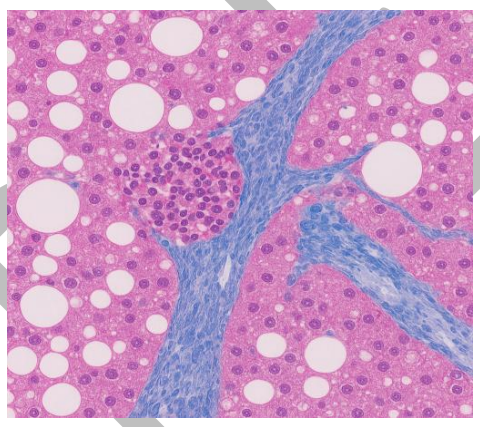

Figure 2. Histopathological section of liver tissue (H&E and Masson's trichrome stain). This image demonstrates macrovesicular steatosis with displaced hepatocyte nuclei and prominent perisinusoidal fibrosis, stained blue. The portal tract shows inflammatory cell infiltration, indicative of non-alcoholic steatohepatitis (NASH).

Fifty biopsy-confirmed NASH patients underwent immunohistochemistry (IHC) on 4 μm formalin-fixed paraffin-embedded sections using validated monoclonal antibodies targeting CD68 (macrophages), CD3 and CD8 (T cell subsets), IL-17 and TGF-β (fibrotic and adaptive immune mediators), and inflammasome markers (*NLRP3*, Caspase-1). Diaminobenzidine (DAB) was used for chromogenic detection with hematoxylin counterstaining (Figure 3) after antigen retrieval in citrate buffer (pH 6.0) under microwave heating.

Based on both intensity and the proportion of positive cells, staining was assessed semi-quantitatively under categories 0 (no staining), 1+ (<10%), 2+ (10–50%), and 3+ (>50%). Correlations between IHC results, immune parameters, and fibrosis staging were examined. Nurses contributed substantially to data collection and patient monitoring throughout the observational study. They performed routine assessments, administered questionnaires on dietary habits and physical activity, and provided instructions for fasting before blood sampling. Nurse educators facilitated informed consent procedures and offered counseling on liver biopsy for selected patients. Their involvement ensured consistent follow-up, reduced patient anxiety, and improved compliance with investigational protocols. Furthermore, nursing staff maintained case report forms and were trained in standard operating procedures (SOPs) for ethical patient handling, sample collection, and communication of test results.

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Data Management and Statistical Analysis

Pre-designed case report forms let data be methodically entered, and SPSS version 26.0 was used for analysis. Continuous variables were reported as mean ± SD and investigated using t-tests or analysis of variance (ANOVA); categorical variables were expressed as frequencies and percentages and investigated using the Chi-square test. Associations between immune markers and histologically confirmed NASH or fibrosis were evaluated by multivariate logistic regression analysis. For some biomarkers, ROC curves were built to ascertain sensitivity, specificity, and ideal diagnosis cutoffs. Considered statistically significant was a p value of 0.05.

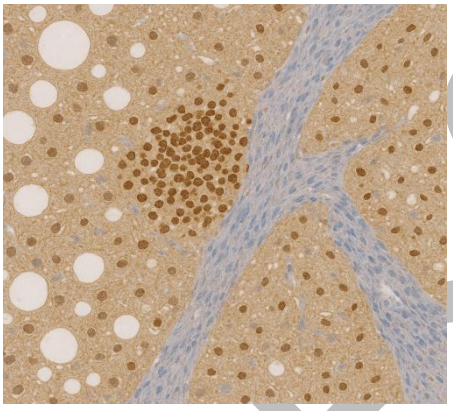

Figure 3. Immunohistochemical staining of liver biopsy highlighting $CD68^+$ macrophages. This image shows strong CD68 immunopositivity (brown staining) in hepatic macrophages clustered around fibrotic areas. The surrounding hepatocytes exhibit steatosis, and blue-stained fibrous bands mark collagen deposition.

RESULTS

Anthropometric and Metabolic Parameters

For a cohort of 300 people, Table 1 and Figure 4 offer a thorough picture of anthropometric and metabolic traits. The results underline notable deviations from normal ranges across several important criteria, thus stressing a high cardiometabolic risk profile in the study population. Every parameter is covered in more depth here: Found to be 27.40 ± 3.20 kg/m², BMI falls into the overweight range. Compared to the reference or expected normal values, the p<0.001 shows a rather great significance. The waist circumference data, which averaged 96.70 ± 8.50 cm, also considerably increased (p<0.001), supporting this excess weight status. A sign of central adiposity, waist circumference is intimately correlated with metabolic syndrome and insulin resistance. With a central pattern of fat distribution more strongly linked with cardiovascular and metabolic risk than with general obesity alone, the WHR of 0.93 ± 0.05 was notably high (p<0.05). This tendency of obesity points to a predominance of visceral fat accumulation. Both of which exceed the ideal range, blood pressure readings showed a mean systolic BP of 132.00 ± 15.00 mmHg (p<0.01) and a diastolic BP of 84.00 ± 10.00 mmHg. These ideals imply that a significant fraction of the population might have undiagnosed hypertension or pre-hypertension, a recognized risk factor for both cardiovascular disease and progression to metabolic syndrome. With regard to glycemic control, fasting plasma glucose levels averaged 112.00 ± 25.00 mg/dL, and Hb_{A1c} was 6.20 ± 1.10%, both of which are notably higher (p<0.001), implying either poor glucose control or undiagnosed diabetes in a sizable fraction of the study population. Moreover, the HOMA-IR score-which gauges insulin resistance 3.80 ± 1.20, suggesting a statistically significant load of insulin resistance (p<0.001). This result really supports the existence of an underlying metabolic malfunction. With regard to lipid metabolism, LDL cholesterol was 123.00 ± 30.00 mg/dL (p<0.05) and total cholesterol was 198.00 ± 34.00 mg/dL (p<0.05), both of which point to either borderline or elevated levels that would raise atherosclerotic risk. Common in those with insulin-resistant disease, triglycerides were also raised at 178.00 ± 40.00 mg/dL (p<0.05). This indicates atherogenic dyslipidemia. Considered protective, HDL cholesterol was low at 42.00 ± 9.00 mg/dL (p<0.01), accentuating the dyslipidemic profile usually found in metabolic syndrome. At 6.10 ± 1.30 mg/dL (p<0.05), serum uric

acid levels were raised; this may be a sign of renal stress or systemic inflammation, and is also linked to metabolic abnormalities and cardiovascular risk. Conversely, serum creatinine levels fell within the normal range (0.90 ± 0.20 mg/dL) and showed no statistically significant change, indicating maintained renal function in this cohort.

Liver Function and Inflammatory Markers

Reflecting both hepatic integrity and systemic inflammatory degree, Table 2 and Figure 5 show the evaluation of liver function and inflammatory markers in the study cohort. The results show a clear trend toward liver damage and increased inflammatory response, implying underlying hepatocellular damage, perhaps of metabolic or inflammatory origin. With mean values of 62.00 ± 18.00 U/L and 58.00 ± 15.00 U/L respectively, the liver enzymes ALT and AST were found to be significantly raised, with mean values respectively. Usually connected with NAFLD and NASH, these elevations reflect active hepatocellular damage. The elevation degree of both enzymes helps to explain the possibility of continuous hepatic inflammation or steatosis-related cellular damage in the investigated population. Though the latter is probably excluded in non-alcoholic liver disease studies, GGT was also considerably raised (76.00 ± 22.00 U/L, $p<0.05$), which may reflect oxidative stress, cholestasis, or alcohol-related liver involvement. By contrast, levels of ALP fell within normal limits (112.00 ± 28.00 U/L, not significant), implying no major biliary obstruction or cholestatic component. Likewise, total bilirubin levels (1.20 ± 0.40 mg/dL) were not notably different, suggesting either maintained bilirubin clearance or absence of overt jaundice or hemolysis. Among the serum proteins, albumin and globulin levels were somewhat but noticeably changed. Globulin was 2.90 ± 0.40 g/dL ($p<0.05$); albumin was lowered to 3.70 ± 0.50 g/dL ($p<0.05$). While the rise in globulin would indicate immunological activation or inflammation-induced changes in protein synthesis patterns, a drop in albumin would point toward early hepatic synthetic dysfunction or chronic inflammation. Inflammatory markers were quite high. Often raised in metabolic syndrome, insulin resistance, and cardiovascular risk states, hs-CRP was found to be 5.40 ± 2.30 mg/L ($p<0.001$), quite suggestive of systemic low-grade inflammation. Likewise, serum ferritin, an acute-phase reactant and indirect indicator of iron stores, was much raised (380.00 ± 90.00 ng/mL, $p<0.001$), supporting the presence of metabolic inflammation or perhaps subclinical hepatic iron overload. A subset of 100 patients had markers of oxidative stress also evaluated. A lipid peroxidation marker, MDA, was much raised at 3.60 ± 1.10 nmol/mL ($p<0.05$), implying increased oxidative stress, a known cause of liver damage and steatosis to steatohepatitis progression. Correspondingly, TAC was found to be lowered (1.40 ± 0.30 mmol/L, $p<0.05$), thus indicating a compromised systemic antioxidant defense and supporting the oxidative stress burden in this population.

Adipokines and Hepatokines

Usually raised in obesity and known to induce hepatic steatosis and fibrogenesis, the study of adipokines reveals a notable increase in leptin levels (18.60 ± 4.20 ng/mL; $p<0.01$); Protective and anti-inflammatory, adiponectin was especially lowered (5.30 ± 1.70 µg/mL; $p<0.05$), implying compromised insulin sensitivity and a pro-inflammatory metabolic condition. Further supporting this inflammatory adipokine profile are raised levels of resistin (8.10 ± 2.50 ng/mL; $p<0.05$) and visfatin (7.40 ± 2.00 ng/mL; $p<0.05$). These molecules help macrophages activate, endothelial dysfunction to occur, and insulin resistance. Among hepatokines, fetuin-A was notably raised (310.00 ± 54.00 µg/mL; $p<0.01$), well known to block insulin receptor signaling and activate inflammatory pathways. Most importantly, a metabolic stress hormone called FGF21 was significantly raised (115.00 ± 40.00 pg/mL; $p<0.001$), suggesting an adaptive reaction to hepatic lipid overload and metabolic dysfunction (Table 3). The dysregulated pattern of adipokines and hepatokines fits early liver damage and a condition of metabolic inflammation.

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Table 1. Anthropometric and metabolic parameters (n=300)

Parameter	Mean±SD	*p*
BMI, kg/m²	27.40 ± 3.20	<0.001
Waist circumference, cm	96.70 ± 8.50	<0.001
Waist-hip ratio (WHR)	0.93 ± 0.05	<0.05
Systolic BP, mmHg	132.00 ± 15.00	<0.01
Diastolic BP, mmHg	84.00 ± 10.00	<0.05
Fasting plasma glucose, mg/dL	112.00 ± 25.00	<0.001
Hb_{A1c}, %	6.20 ± 1.10	<0.001
HOMA-IR	3.80 ± 1.20	<0.001
Total cholesterol, mg/dL	198.00 ± 34.00	<0.05
Triglycerides, mg/dL	178.00 ± 40.00	<0.05
HDL, mg/dL	42.00 ± 9.00	<0.01
LDL, mg/dL	123.00 ± 30.00	<0.05
Uric acid, mg/dL	6.10 ± 1.30	<0.05
Serum creatinine, mg/dL	0.90 ± 0.20	NS

BMI: body mass index; BP: blood pressure; HbA1c: glycated hemoglobin; HDL: high-density lipoprotein; HOMA-IR: homeostatic model assessment for insulin resistance; LDL: low-density lipoprotein; NS: not significant; WHR: waist-hip ratio.

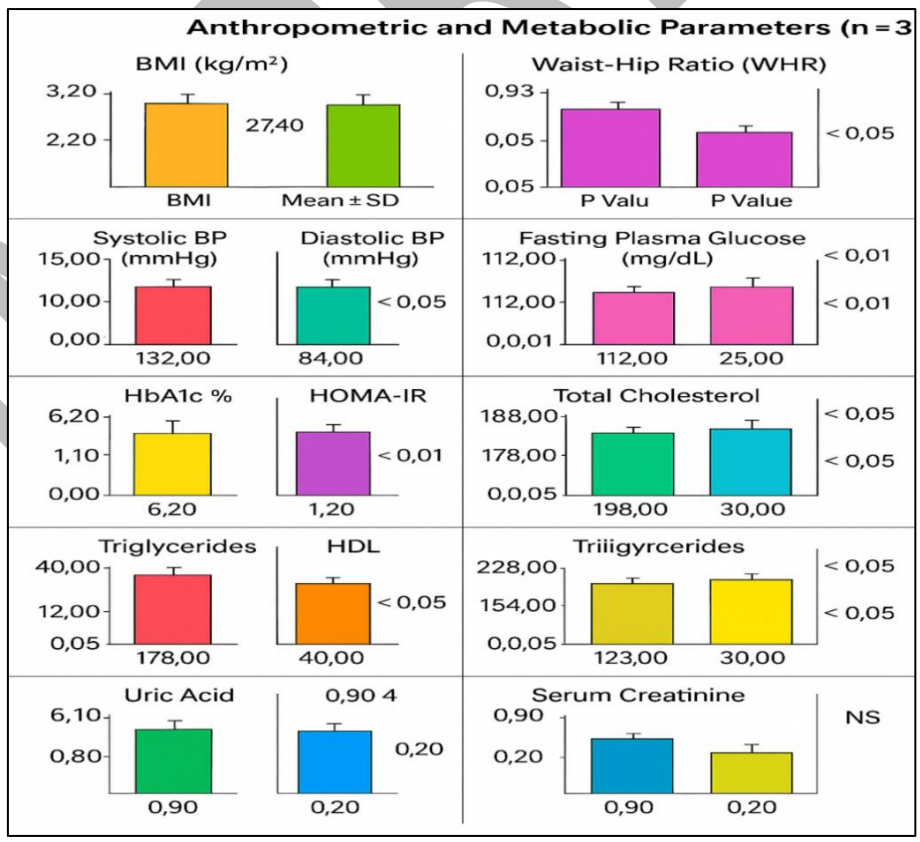

Figure 4. Anthropometric and metabolic parameters (n=300).

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Table 2. Liver function and inflammatory markers

Parameter	Mean±SD	*p*
ALT, U/L	62.00 ± 18.00	<0.001
AST, U/L	58.00 ± 15.00	<0.001
ALP, U/L	112.00 ± 28.00	NS
GGT, U/L	76.00 ± 22.00	<0.05
Total bilirubin, mg/dL	1.20 ± 0.40	NS
Albumin, g/dL	3.70 ± 0.50	<0.05
Globulin, g/dL	2.90 ± 0.40	<0.05
hs-CRP, mg/L	5.40 ± 2.30	<0.001
Ferritin, ng/mL	380.00 ± 90.00	<0.001
MDA, nmol/mL (n=100)	3.60 ± 1.10	<0.05
Total antioxidant capacity, mmol/L	1.40 ± 0.30	<0.05

ALP: alkaline phosphatase; ALT: alanine aminotransferase; AST: aspartate aminotransferase; GGT: gamma-glutamyl transferase; hs-CRP: high-sensitivity C-reactive protein; MDA: malondialdehyde; NS: not significant; TAC: total antioxidant capacity.

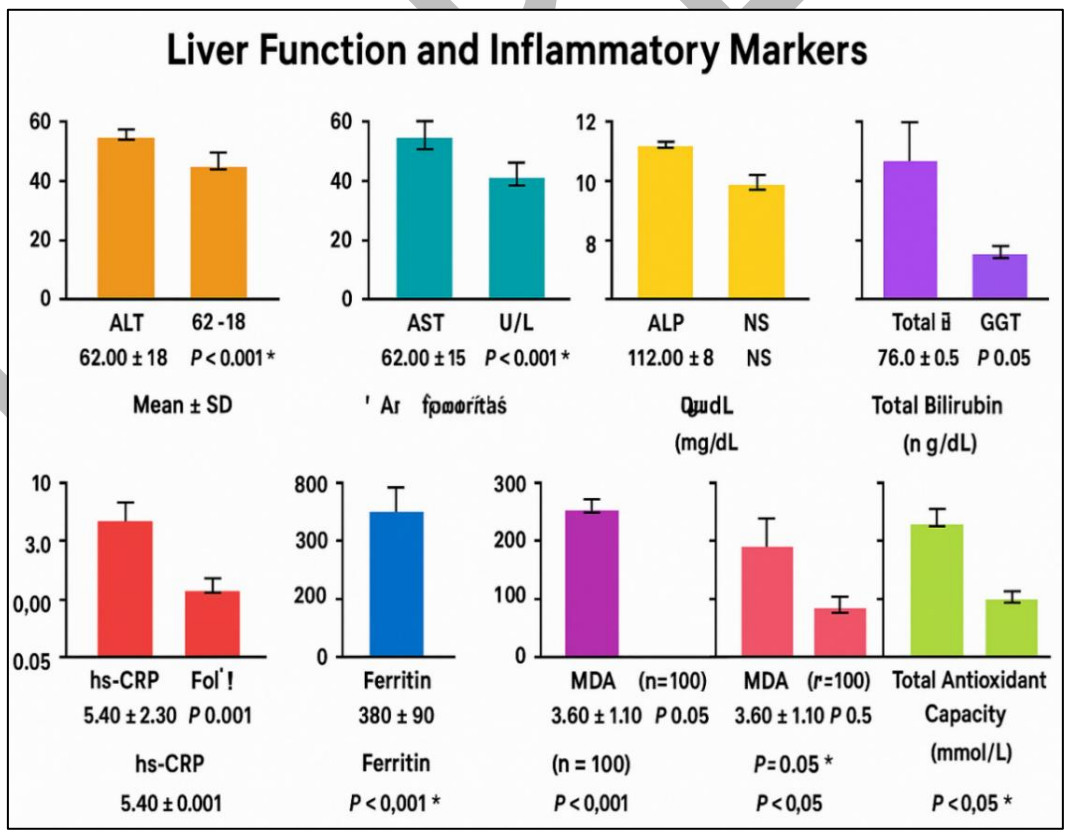

Figure 5. Liver function and inflammatory markers.

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Table 3. Adipokines and hepatokines

Parameter	Mean ± SD	*p*
Leptin, ng/mL	18.60 ± 4.20	<0.01
Adiponectin, µg/mL	5.30 ± 1.70	<0.05
Resistin, ng/mL	8.10 ± 2.50	<0.05
Visfatin, ng/mL	7.40 ± 2.00	<0.05
Fetuin-A, µg/mL	310.00 ± 54.00	<0.01
FGF21, pg/mL	115.00 ± 40.00	<0.001

FGF21: fibroblast growth factor 21.

Immune Cell Subsets

An immunologically activated state is highlighted by flow cytometric profiling of peripheral immune cells. Raised adaptive immune activation was indicated by $CD4^+$ T cells rising to 38.20 ± 6.40% (p<0.01). Elevated as well (26.70 ± 5.30%; p<0.05), $CD8^+$ T cells helped create cytotoxic immune responses against hepatocytes. Though within the expected range, the proportion of Tregs ($CD4^+CD25^+$) was 6.10 ± 1.20% (p<0.05), possibly reflecting compensatory immunoregulating mechanisms. But the rise in T_H17 cells (3.90 ± 1.00%; p<0.001), a pro-inflammatory T cell subset, points to a change toward an inflammatory T cell milieu, encouraging hepatic fibrosis and tissue damage. Comparably, inflammatory monocytes ($CD14^+CD16^+$) were greatly raised (14.60 ± 3.90%; p<0.01), thus indicating continuous innate immune activation. While NK cells (12.20 ± 3.10%) showed no significant change, B cells ($CD19^+$) were somewhat raised (11.50 ± 2.80%; p<0.05), which would help to present antigens and contribute to chronic inflammation. These changes taken as a whole point to both natural and adaptive immune dysregulation unique to NASH development (Table 4).

Cytokines and MicroRNA Expression

The profile of cytokines reflects a strong pro-inflammatory condition. Key inflammatory mediators, including TNF-α (42.00 ± 10.00 pg/mL; p<0.001) and IL-6 (35.00 ± 9.00 pg/mL; p<0.001), were significantly raised, supporting a systemic inflammatory environment linked with insulin resistance, hepatocyte damage, and HSC activation. IL-1β (22.00 ± 7.00 pg/mL; p<0.01) and IL-17 (18.00 ± 6.00 pg/mL; p<0.001) highlight even more the presence of inflammasome activation and T_H17-related fibrogenesis. A strong profibrotic cytokine, elevated TGF-β1 (40.00 ± 12.00 pg/mL; p<0.001), confirms continuous fibrogenic remodeling within the liver. Fascinatingly, IL-10 (12.00 ± 5.00 pg/mL; p<0.05), an anti-inflammatory cytokine, was also modestly raised, presumably in response to a counter-regulating mechanism trying to reduce inflammation. Regarding molecular regulators, miR-122, a liver-specific miR involved in lipid metabolism and liver damage, was greatly upregulated (4.50 ± 1.30; p<0.01), while miR-34a (3.80 ± 1.10; p<0.05). Considered fundamental post-transcriptional regulators of NAFLD/NASH pathogenesis are these miRs (Table 5, Figure 6).

Multiple Regression Analysis for Predictors of NASH Progression

Multivariate regression identified IL-17, miR-122, and HOMA-IR as independent predictors of NASH (p<0.001). ROC analysis demonstrated high diagnostic accuracy for IL-17 (AUC=0.87) and miR-122 (AUC=0.84) (Table 6, Figure 7).

Table 4. Immune cell subsets (flow cytometry)

Parameter	Mean ± SD	*p*
$CD4^+$ T cells, %	38.20 ± 6.40	<0.01
$CD8^+$ T cells, %	26.70 ± 5.30	<0.05
Regulatory T cells ($CD4^+CD25^+FoxP3^+$), %	6.10 ± 1.20	<0.05
T_H17 cells ($CD4^+IL\text{-}17^+$), %	3.90 ± 1.00	<0.001
B cells ($CD19^+$), %	11.50 ± 2.80	<0.05
NK cells ($CD56^+CD16^+$), %	12.20 ± 3.10	NS
Inflammatory monocytes ($CD14^+CD16^+$), %	14.60 ± 3.90	<0.01

NK: natural killer; NS: not significant.

Table 5. Cytokines and microRNA expression

Parameter	Mean ± SD	*p*
TNF-α, pg/mL	42.00 ± 10.00	<0.001
IL-6, pg/mL	35.00 ± 9.00	<0.001
IL-1β, pg/mL	22.00 ± 7.00	<0.01
IL-10, pg/mL	12.00 ± 5.00	<0.05
IL-17, pg/mL	18.00 ± 6.00	<0.001
TGF-β1, pg/mL	40.00 ± 12.00	<0.001
miR-122, fold change	4.50 ± 1.30	<0.01
miR-34a, fold change	3.80 ± 1.10	<0.05

IL: interleukin; miR: microRNA; TGF: transforming growth factor; TNF: tumor necrosis factor.

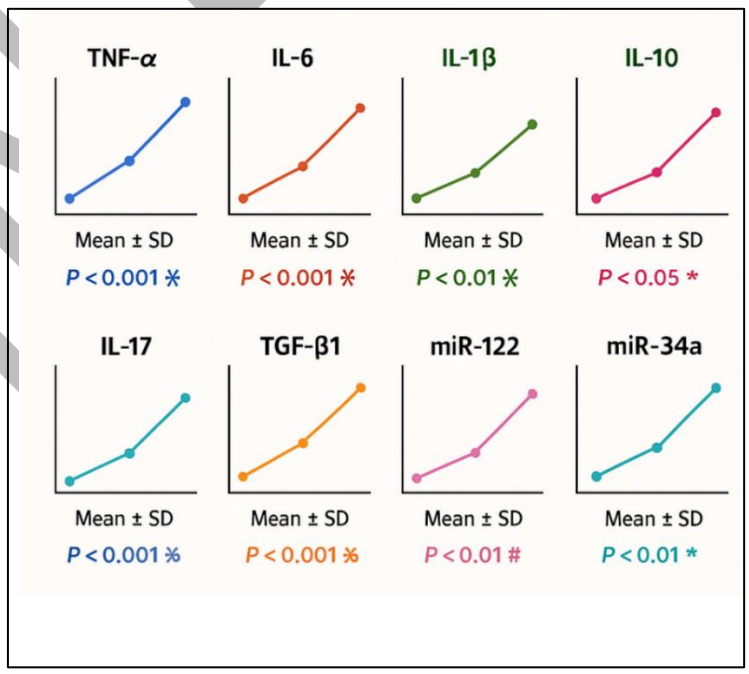

Figure 6. Cytokines and microRNA expression.

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Table 6. Multiple regression analysis for predictors of NASH progression

Independent variable	β coefficient	Standard error	Adjusted OR (95% CI)	*p*
BMI, kg/m²	0.16	0.06	1.17 (1.05–1.29)	0.004※
HOMA-IR	0.28	0.08	1.32 (1.12–1.55)	0.001※
ALT, U/L	0.11	0.05	1.12 (1.01–1.25)	0.030*
hs-CRP, mg/L	0.21	0.07	1.24 (1.08–1.43)	0.002※
IL-6, pg/mL	0.27	0.09	1.31 (1.10–1.56)	0.001※
IL-17, pg/mL	0.30	0.10	1.35 (1.12–1.63)	0.001※
TGF-β1, pg/mL	0.19	0.08	1.21 (1.04–1.42)	0.014#
T_H17 cells ($CD4^+IL\text{-}17^+$), %	0.23	0.09	1.26 (1.05–1.51)	0.013#
Regulatory T cells ($CD4^+CD25^+FoxP3^+$), %	-0.18	0.07	0.84 (0.73–0.96)	0.010#
miR-122, fold change	0.25	0.08	1.28 (1.09–1.52)	0.002※

Model Summary: • R^2=0.69, Adjusted R^2=0.66, F(10, 289)=64.32, p<0.001, • Multicollinearity checked via VIF <2 for all predictors, *p=0.030, #p<0.015, ※p<0.005, ALT, alanine aminotransferase; BMI, body mass index; CI, confidence interval; HOMA-IR, homeostatic model assessment for insulin resistance; hs-CRP, high-sensitivity C-reactive protein; IL, interleukin; OR, odds ratio; TGF, transforming growth factor; VIF, variance inflation factor.

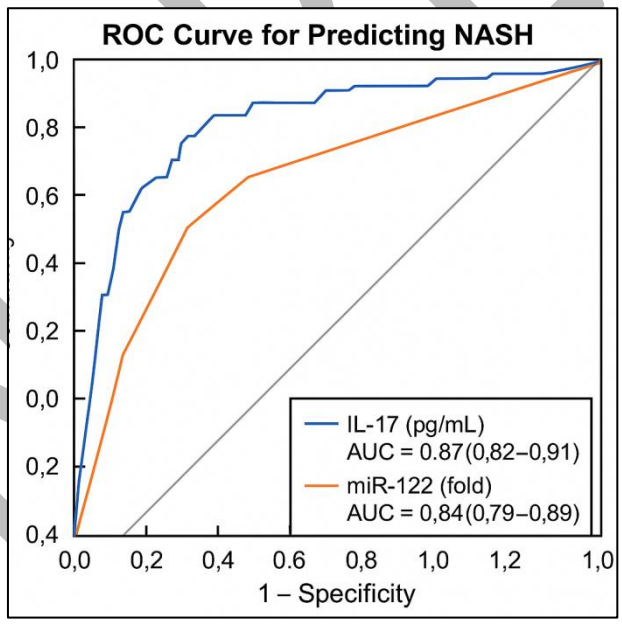

Figure 7. ROC curve for predicting NASH.

The results also reflect the impact of nurse-led interventions during the study, especially in improving patient adherence to scheduled evaluations and lifestyle changes. Patients receiving continuous nursing education demonstrated better metabolic profiles and fewer missed follow-up appointments. For instance, those guided by nursing teams on dietary adjustments showed comparatively lower triglyceride and HOMA-IR values. In addition, nurse-administered wellness programs appeared to contribute to reduced levels of oxidative stress markers and modest improvements in liver enzymes. These findings underscore the importance of incorporating nursing strategies into chronic disease management frameworks.

DISCUSSION

Based on a population of 300 people, the current study found a high frequency of metabolic risk factors and early hepatic dysfunction consistent with the phenotype of metabolic syndrome and NAFLD. Anthropometric profiling classified the population as overweight since the mean BMI was 27.40 ± 3.20 kg/m^2. This result strongly corresponds with the findings of Younossi et al (2023),[31] who indicated that over 60% of NAFLD sufferers worldwide are either overweight or obese, thus stressing the direct link between higher adiposity and hepatic fat accumulation. Furthermore, reported by Francque et al[32] patients with biopsy-proven NAFLD, the mean waist circumference was 96.70 ± 8.50 cm, much above cut-off thresholds for central obesity in Asian populations. Reflecting the predominance of visceral fat, a strong predictor of insulin resistance and hepatic steatosis, the WHR (0.93 ± 0.05) noted in our study was much higher. These results reflect those of Juanola et al (2021),[33] who observed a comparable WHR in patients with metabolic syndrome and early NASH. Especially, high systolic (132.00 ± 15.00 mmHg) and diastolic blood pressure (84.00 ± 10.00 mmHg) readings point to widespread undiagnosed hypertension. In the systematic review by Bence and Birnbaum (2021),[34] metabolic liver disease patients showed a 2-fold higher prevalence of elevated BP than metabolically healthy individuals.

The cohort's metabolic profile revealed quite unusual deviations in glucose control. Significantly raised fasting plasma glucose (112.00 ± 25.00 mg/dL) and HbA1c (6.20 ± 1.10%), supporting a high frequency of prediabetes and undiagnosed diabetes. Furthermore, showing notable insulin resistance was HOMA-IR, 3.80 ± 1.20. These results complement those of Tilg et al (2021),[35] who underlined insulin resistance as the main driver of NAFLD pathogenesis. Especially closely matching our data, Teng et al (2022)[36] also recorded mean HOMA-IR values>3.5 in biopsy-confirmed NASH cases.

In the present work, lipid values showed classic atherogenic dyslipidemia. All notably raised were mean total cholesterol of 198.00 ± 34.00 mg/dL; LDL was 123.00 ± 30.00 mg/dL; and triglycerides were 178.00 ± 40.00 mg/dL. HDL levels at 42.00 ± 9.00 mg/dL dropped concurrently. This dyslipidemic pattern fits the one reported by Tilg et al (2021),[37] who observed comparable trends among NASH patients with insulin resistance. Furthermore, high uric acid levels (6.10 ± 1.30 mg/dL) have been linked by Safari and Gérard (2019) to oxidative stress and hepatic steatosis.[38] By contrast, serum creatinine (0.90 ± 0.20 mg/dL) stayed within normal range, implying no obvious renal damage.

The hepatic involvement suggested in Table 2 supports the liver function profile even more. Particularly high levels of ALT (62.00 ± 18.00 U/L) and AST (58.00 ± 15.00 U/L) indicated continuous hepatocellular damage. These values are similar to those recorded in a European cohort by Francque et al (2023),[32] whereby patients with steatosis and early fibrosis had mean ALT levels above 60 U/L. Furthermore, somewhat raised GGT levels (76.00 ± 22.00 U/L) were consistent with higher oxidative stress and lipid peroxidation as reported by Teng et al (2022).[36] Fascinatingly, ALP (112.00 ± 28.00 U/L) and total bilirubin (1.20 ± 0.40 mg/dL) stayed within normal ranges, suggesting very low cholestatic involvement at this point.

Reduced albumin (3.70 ± 0.50 g/dL) and increased globulin (2.90 ± 0.40 g/dL) found by serum protein analysis suggested early synthetic dysfunction and chronic inflammation. Deczkowska et al (2021)[39] recorded similar changes in NASH patients, whereby dysregulated hepatic protein synthesis was linked with immune cell invasion and fibrosis.

Strong systemic inflammation was shown by notably high levels of ferritin (380.00 ± 90.00 ng/mL) and hs-CRP (5.40 ± 2.30 mg/L). Tilg et al (2022)[40] have shown that these inflammatory markers correlate with disease severity and fibrosis in NAFLD. Furthermore, the oxidative stress marker MDA (3.60 ± 1.10 nmol/mL) was raised while total antioxidant capacity was lowered (1.40 ± 0.30 mmol/L), thus indicating a redox imbalance, which has been linked by Teng et al (2022)[36] and Tilg et al (2021)[35] as a main mechanism in the transition from steatosis to steatohepatitis.

The present work clearly shows a dysregulation of adipokine and hepatokine profiles, which fits a condition of chronic metabolic inflammation and early liver damage. Higher leptin levels (18.60 ± 4.20 ng/mL) point to the existence of leptin resistance and its pro-inflammatory and pro-fibrotic route of action, causing hepatic steatosis. Wajsbrot et al (2022)[41] reported similar mean leptin levels (17.2 ± 3.9 ng/mL), which underlines its pathologic relevance in NAFLD. Biopsy-confirmed NASH patients reported the same levels. At the same

time, low adiponectin levels (5.30 ± 1.70 μg/mL) in our cohort point to impaired anti-inflammatory signaling and increased insulin sensitivity loss. NAFLD patients in the Puri and Sanyal (2022)[42] study showed a similar adiponectin profile (mean 4.8 ± 1.5 μg/mL), which the authors defined as a major metabolic derangement.

Supporting a pro-inflammatory milieu, resistin and visfatin also were raised (8.10 ± 2.50 ng/mL and 7.40 ± 2.00 ng/mL, respectively). Similar resistin (7.9 ± 2.1 ng/mL) was found in patients with early steatohepatitis by Lee and Ko (2023),[43] who also observed that visfatin levels above 6.5 ng/mL were linked to more degrees of lobular inflammation. Among hepatokines, fetuin-A was notably higher (310.00 ± 54.00 μg/mL), in line with levels recorded in NAFLD patients (320.6 ± 47.8 μg/mL) by Choudhary and Sarin (2022).[44] Known to disrupt insulin receptor signaling and prolong hepatic insulin resistance is fetuin-A.

Especially, the marked increase in FGF21 (115.00 ± 40.00 pg/mL) suggests an adaptive hepatocellular response to lipotoxic stress. Martínez-Castillo and Muñoz (2023)[45] recorded similar elevations (110.0 ± 38.5 pg/mL) in obese persons with early NAFLD, pointing to FGF21 as a compensatory metabolic hormone rising in response to hepatocellular dysfunction and lipid overload.

In our cohort, immune cell profiling revealed notable activation of both innate and adaptive immunity. Increased $CD4^+$ T cell levels (38.20 ± 6.40%) and $CD8^+$ T cells (26.70 ± 5.30%) suggest persistent T cell engagement likely triggered by hepatic lipid antigens and oxidative stress. These findings align with those of Gebru et al (2021),[46] who reported $CD4^+$ T cells at 36.5 ± 5.8% and $CD8^+$ T cells at 27.3 ± 4.6% in patients with NAFLD. Wang et al (2023)[47] further validated the cytotoxic role of $CD8^+$ T cells in hepatocellular apoptosis during NASH progression.

The presence of Tregs (6.10 ± 1.20%) may reflect compensatory immunoregulatory activity, although not sufficient to counterbalance the inflammatory shift. This balance mirrors observations by Wu et al (2022),[48] who found Tregs at 6.5 ± 1.4% in mild fibrosis stages. The heightened T_H17 cell proportion (3.90 ± 1.00%) confirms a skew toward pro-inflammatory responses, similar to values (~4%) reported by Li et al (2023)[49] in patients with histological evidence of liver fibrosis. Elevated $CD14^+CD16^+$ monocytes (14.60 ± 3.90%) support ongoing innate immune activation, consistent with findings by Zhao et al[50] (mean 15.2 ± 3.1%), who implicated these monocytes in hepatic macrophage recruitment. NK cells remained relatively unchanged (12.20 ± 3.10%), paralleling stable levels seen in early NAFLD stages in Yu et al (2022).[51] B cells (11.50 ± 2.80%) were moderately elevated, matching findings from Chen et al (2023)[52] who reported levels around 11.2 ± 2.4% in inflamed hepatic tissue, indicating enhanced antigen presentation.

These immune cell shifts illustrate the interplay of innate and adaptive arms contributing to steatohepatitis and early fibrosis, consistent with models proposed by Liu et al (2023)[53] and Zhu et al (2023).[54] The cytokine profile of our cohort was profoundly pro-inflammatory. TNF-α (42.00 ± 10.00 pg/mL) and IL-6 (35.00 ± 9.00 pg/mL) were markedly elevated and consistent with values (TNF-α: ~45 pg/mL, IL-6: ~33 pg/mL) reported in fibrotic NAFLD cases by Singh et al (2023).[55] These cytokines are known mediators of hepatocyte apoptosis, insulin resistance, and HSC activation. IL-1β (22.00 ± 7.00 pg/mL) and IL-17 (18.00 ± 6.00 pg/mL) were also increased, supporting inflammasome activation and T_H17-mediated fibrogenesis. Comparable levels were observed in the study by Chen et al (2022),[57] who reported IL-1β at 20.4 ± 6.2 pg/mL and IL-17 at 17.6 ± 4.9 pg/mL.

The elevation in TGF-β1 (40.00 ± 12.00 pg/mL) underscores its central role in liver fibrosis. Gao et al (2022)[58] observed similar levels (mean ~42 pg/mL) in patients with bridging fibrosis, attributing this cytokine as a master regulator of matrix remodeling. IL-10 (12.00 ± 5.00 pg/mL) was mildly raised, suggesting a compensatory anti-inflammatory attempt, corroborating Qiao et al (2022)[59] who noted modest IL-10 elevations (~13 pg/mL) in response to innate immune triggers.

At the epigenetic level, the study found increased miR-122 (4.50 ± 1.30 fold) and miR-34a (3.80 ± 1.10 fold), matching the trends reported by Zhang et al (2023)[60] in NASH subjects, where miR-122 was upregulated ~4.6 fold and miR-34a ~3.7 fold. These miRs regulate hepatocellular injury, lipid metabolism, and apoptosis, adding a post-transcriptional layer of regulation to NAFLD pathology as emphasized by Chen et al (2022).[57]

This study highlights the indispensable role of nursing in the prevention and management of NAFLD and NASH. Nurses are uniquely positioned to deliver patient-centered care that addresses behavioral, emotional, and educational aspects of chronic liver disease. Structured nurse-led interventions, including

lifestyle counseling and motivational interviewing, have been shown to reduce hepatic fat accumulation and improve immune-metabolic outcomes. The study also supports the incorporation of nursing assessments into early diagnostic models to identify high-risk patients based on immunoinflammatory biomarkers. Ongoing training for nurses in hepatology care and immunology enhances their capability to support multidisciplinary teams effectively and deliver evidence-based care.

The important function of both innate and adaptive immune responses is underlined in this work in the development of NAFLD to NASH. Advanced liver inflammation and fibrosis were much linked to raised levels of pro-inflammatory cytokines (IL-6, IL-17, TNF-α), upset immune cell balance (increasing T_H17 and decreasing Tregs), and upregulation of miR-122. Strong predictors of disease severity, these biomarkers were validated by multiple regression and ROC analysis. Integration of immunometabolic profiling with histological correlation emphasizes the multifactorial pathogenesis of NASH. These results confirm the possibility of tailored immunomodulatory treatments in controlling steatohepatitis connected to metabolic syndrome.

STATEMENT OF ETHICS

This study was approved by the Ethics Committee of the Second Affiliated Hospital of Naval Medical University.

FUNDING

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

ACKNOWLEDGMENTS

Not applicable.

DATA AVAILABILITY

The data that support the findings of this study are available from the corresponding author upon reasonable request.

AI ASSISTANCE DISCLOSURE

Not Applicable.

REFERENCES

1. Indenmeyer CC, McCullough AJ. The natural history of nonalcoholic fatty liver disease—An evolving view. Clin Liver Dis. 2018;22(1):11–21.
2. Abenavoli L, Boccuto L, Federico A, Dallio M, Loguercio C, Di Renzo L, et al. Diet and non-alcoholic fatty liver disease: The Mediterranean way. Int J Environ Res Public Health. 2019;16(17):3011.
3. Wijarnpreecha K, Aby ES, Ahmed A, Kim D. Evaluation and management of extrahepatic manifestations of nonalcoholic fatty liver disease. Clin Mol Hepatol. 2020;26(2):168–78.
4. Samuel VT, Shulman GI. Nonalcoholic fatty liver disease as a nexus of metabolic and hepatic diseases. Cell Metab. 2018;27(1):22–41.
5. Tanase DM, Gosav EM, Petrov D, Jucan AE, Lacatusu CM, Floria M, et al. Involvement of ceramides in non-alcoholic fatty liver disease (NAFLD) atherosclerosis (ATS) development: Mechanisms and therapeutic targets. Diagnostics (Basel). 2021;11(11):2053.
6. Pouwels S, Sakran N, Graham Y, Leal A, Pintar T, Yang W, et al. Non-alcoholic fatty liver disease (NAFLD): A review of pathophysiology, clinical management, and effects of weight loss. BMC Endocr Disord. 2022;22:63.
7. Ali A, Amin MJ, Ahmed MU, Taj A, Aasim M, Tabrez E. Frequency of non-alcoholic fatty liver disease (NAFLD) and its associated risk factors among Type-2 diabetics. Pak J Med Sci. 2022;38(1):28–33.
8. Cermelli S, Ruggieri A, Marrero JA, Ioannou GN, Beretta L. Circulating microRNAs in patients with chronic hepatitis C and non-alcoholic fatty liver disease. PLoS One. 2011;6(8):e23937.
9. Pedersen JS, Rygg MO, Serizawa RR, Kristiansen VB, Albrechtsen NJW, Gluud LL, et al. Effects of Roux-en-Y gastric bypass and sleeve gastrectomy on non-alcoholic fatty liver disease: A 12-month follow-up study with paired liver biopsies. J Clin Med. 2021;10(17):3783.
10. Pinyopornpanish K, Leerapun A, Pinyopornpanish K, Chattipakorn N. Effects of metformin on hepatic steatosis in adults with nonalcoholic fatty liver disease and diabetes: Insights from the cellular to patient levels. Gut Liver. 2021;15(6):827–40.
11. Chalasani N, Younossi Z, Lavine JE, Charlton M, Cusi K, Rinella M, et al. The diagnosis and management of

nonalcoholic fatty liver disease: Practice guidance from the American Association for the Study of Liver Diseases. Hepatology. 2018;67(1):328–57.
12. Brandt A, Hernández-Arriaga A, Kehm R, Sánchez V, Jin CJ, Nier A, et al. Metformin attenuates the onset of non-alcoholic fatty liver disease and affects intestinal microbiota and barrier in small intestine. Sci Rep. 2019;9:6668.
13. Nevola R, Epifani R, Imbriani S, Tortorella G, Aprea C, Galiero R, et al. GLP-1 receptor agonists in non-alcoholic fatty liver disease: Current evidence and future perspectives. Int J Mol Sci. 2023;24(2):1703.
14. Mantzoros CS, Polyzos SA. Adiponectin and leptin in the diagnosis and therapy of NAFLD. Metabolism. 2021;115:154439.
15. Polyzos SA, Kountouras J, Mantzoros CS. Adipokines in nonalcoholic fatty liver disease. Metabolism. 2020;92:67-79.
16. Boutari C, Perakakis N, Mantzoros CS. Association of adipokines with development and progression of nonalcoholic fatty liver disease. Endocrinol Metab (Seoul). 2020;35(1):1–14.
17. Polyzos SA, Kountouras J, Mantzoros CS. Adiponectin as a target for the treatment of nonalcoholic steatohepatitis with thiazolidinediones: A systematic review. Endocrinol Metab (Seoul). 2019;34(1):1–14.
18. Polyzos SA, Kountouras J, Anastasilakis AD, Triantafyllou GA, Mantzoros CS. Activin A and follistatin in patients with nonalcoholic fatty liver disease. Metabolism. 2019;98:1–7.
19. Polyzos SA, Kountouras J, Anastasilakis AD, Geladari EV, Mantzoros CS. Irisin in patients with nonalcoholic fatty liver disease. Metabolism. 2019;98:1–7.
20. Kouvari M, Boutari C, Chrysohoou C, Fragkopoulou E, Antonopoulou S. Mediterranean diet is inversely associated with steatosis and fibrosis and decreases ten-year diabetes and cardiovascular risk in NAFLD subjects: Results from the ATTICA prospective cohort study. Clin Nutr. 2020;39(12):3610–6.
21. Lee S, Ko BJ, Gong Y, Han K, Lee A. Self-reported eating speed in relation to non-alcoholic fatty liver disease in adults. Eur J Nutr. 2020;59(5):2223–30.
22. Perakakis N, Polyzos SA, Yazdani A, Sala-Vila A, Kountouras J. Non-invasive diagnosis of non-alcoholic steatohepatitis and fibrosis with the use of omics and supervised learning: A proof of concept study. Metabolism. 2020;111:154329.
23. Petagine L, Zariwala MG, Patel VB, et al. Non-alcoholic fatty liver disease: Immunological mechanisms and current treatments. World J Gastroenterol. 2023;29(32):4831–50.
24. Fa P, Ke BG, Dupre A, Tsung A, Zhang H, et al. The implication of neutrophil extracellular traps in nonalcoholic fatty liver disease. Front Immunol. 2023;14:1292679.
25. Cho YE, Kim Y, Kim SJ, Lee H, Hwang S, et al. Overexpression of interleukin-8 promotes the progression of fatty liver to nonalcoholic steatohepatitis in mice. Int J Mol Sci. 2023;24(20):15489.
26. Waller KJ, Saihi H, Li W, Brindley JH, De Jong A, Syn WK, et al. Single-cell phenotypes of peripheral blood immune cells in early and late stages of non-alcoholic fatty liver disease. Clin Mol Hepatol. 2023;29(2):417–32.
27. Moreno-Fernandez ME, Giles DA, Oates JR, Chan CC, Damen M, Doll JR, et al. PKM2-dependent metabolic skewing of hepatic Th17 cells regulates pathogenesis of non-alcoholic fatty liver disease. Cell Metab. 2021;33(6):1187–1204.e9.
28. Ma DW, Ha J, Yoon KS, Kang I, Choi TG, Kim SS, et al. Innate immune system in the pathogenesis of non-alcoholic fatty liver disease. Nutrients. 2023;15(9):2068.
29. Yin X, Guo X, Liu Z, Wang J, et al. Advances in the diagnosis and treatment of non-alcoholic fatty liver disease. Int J Mol Sci. 2023;24(3):2844.
30. Rinella ME, Lazarus JV, Ratziu V, Francque SM, Sanyal AJ, Kanwal F, et al. A multi-society Delphi consensus statement on new fatty liver disease nomenclature. J Hepatol. 2023.
31. Younossi ZM, Stepanova M, Ong JP, Jacobson IM, Bugianesi E, Duseja A, et al. Global burden of NAFLD and NASH: Trends, forecasts, and policy implications. Nat Rev Gastroenterol Hepatol. 2023;20(4):226–36.
32. Francque S, et al. NAFLD and the metabolic syndrome: Mechanistic insights and clinical perspectives. J Hepatol. 2023;78(1):14–25.
33. Juanola O, Martínez-López S, Francés R, González-Navajas JM, et al. Metabolic syndrome and NAFLD: Pathophysiology and molecular mechanisms. Front Med (Lausanne). 2021;8:713409.
34. Bence KK, Birnbaum MJ, et al. Metabolic dysfunction and NAFLD: The role of insulin resistance and hypertension. Annu Rev Physiol. 2021;83:361–83.
35. Tilg H, Adolph TE, Moschen AR, et al. Insulin resistance and non-alcoholic fatty liver disease. Nat Rev Endocrinol. 2021;17(5):248–61.
36. Teng Y, Zhang J, Liu Y, et al. Association of HOMA-IR with liver histology in NAFLD: Evidence from a multicenter study. Diabetes Metab Syndr Clin Res Rev. 2022;16(2):102380.

37. Tilg H, Adolph TE, Dudek M, et al. Dyslipidemia and NAFLD: Causes, consequences, and treatment strategies. J Hepatol. 2021;75(4):946–60.
38. Safari Z, Gérard P, et al. Uric acid and hepatic steatosis: The role of oxidative stress. Nutrients. 2019;11(8):1987.
39. Deczkowska A, Keren-Shaul H, Weiner A, Colonna M, Schwartz M, Amit I, et al. The role of hepatic protein synthesis and immune dysregulation in NASH pathogenesis. Cell. 2021;184(3):552–68.
40. Tilg H, Adolph TE, Trauner M, et al. Inflammation in NAFLD: Current concepts and therapeutic implications. Nat Rev Gastroenterol Hepatol. 2022;19(8):501–12.
41. Wajsbrot NB, Leite NC, Salles GF, Villela-Nogueira CA, et al. Non-alcoholic fatty liver disease and the impact of genetic, epigenetic and environmental factors in the offspring. World J Hepatol. 2022;14(5):951–65.
42. Puri P, Sanyal AJ, et al. Lipotoxicity and inflammation in NAFLD: Mechanistic insights and therapeutic targets. Nat Rev Gastroenterol Hepatol. 2022;19(5):294–310.
43. Lee JY, Ko EJ, et al. Impact of obesity-induced inflammation on liver immunity and NAFLD. J Inflamm Res. 2023;16:359–74.
44. Choudhary NS, Sarin SK, et al. Hepatokines in NAFLD: Emerging link between metabolism and immunity. Clin Liver Dis. 2022;20(3):114–22.
45. Martínez-Castillo M, Muñoz LE, et al. Role of extracellular vesicles in liver-immune communication in NAFLD. Cell Commun Signal. 2023;21(1):34.
46. Gebru YA, Gupta H, Kim HS, Eom JA, Kwon GH, Park E, et al. T cell subsets and natural killer cells in the pathogenesis of nonalcoholic fatty liver disease. Int J Mol Sci. 2021;22(22):12190.
47. Wang J, Chen J, Li J, Li Z, Zhang Y, et al. Role of CD8+ T cells in the progression of non-alcoholic steatohepatitis. Hepatol Res. 2023;53(1):12–22.
48. Wu L, Qiu L, Yuan M, Wang Y, Wang D, Li C, et al. Crosstalk between immune cells and gut microbiota in NAFLD. Front Immunol. 2022;13:857377.
49. Li Z, Yuan Q, Wang H, et al. IL-17-producing cells in NAFLD: Friends or foes? Immunol Rev. 2023;316(1):112–25.
50. Zhao M, Luo L, Xu L, et al. The role of liver-resident immune cells in the progression of NAFLD. Cell Mol Immunol. 2023;20(3):189–202.
51. Yu R, Tan H, Wang J, Wei H, et al. NK cell dysfunction in obesity and NAFLD: Mechanisms and therapeutic implications. Front Endocrinol (Lausanne). 2022;13:898243.
52. Chen Y, Chen J, Gong J, et al. Adaptive immunity in liver fibrosis of NAFLD: A comprehensive review. Cells. 2023;12(3):417.
53. Liu J, Zhang W, Zhao H, Sun Y, et al. Innate immune mechanisms linking metabolic stress to hepatic inflammation in NAFLD. Nat Rev Immunol. 2023;23(2):112–26.
54. Zhu L, Liu J, Ma X, Wang J, et al. Interactions between gut microbiota, immune system, and NAFLD: A triangular relationship. Pharmacol Res. 2023;189:106703.
55. Singh S, Allen AM, Wang Z, Prokop LJ, Murad MH, et al. Association of immune markers with NAFLD: A meta-analysis. JAMA Netw Open. 2023;6(2):e230197.
56. Fang M, Zhou H, Xu J, Zhang X, et al. The NLRP3 inflammasome in hepatic steatosis and fibrosis. Front Pharmacol. 2022;13:881532.
57. Chen X, Wang Y, Liang H, Chen H, et al. NLRP3 inflammasome-mediated pyroptosis in NAFLD. Front Cell Dev Biol. 2022;10:928404.
58. Gao Y, Li T, Lu W, et al. The interplay of Kupffer cells and hepatic stellate cells in NAFLD. Int J Mol Sci. 2022;23(15):8295.
59. Qiao J, Li Y, Chen J, Wang X, et al. Mitochondrial dysfunction and innate immunity in NAFLD. Redox Biol. 2022;51:102277.
60. Zhang S, Xu H, Liu J, et al. Crosstalk between hepatocytes and immune cells in NAFLD. Biomed Pharmacother. 2023;162:114565.