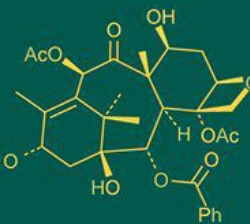
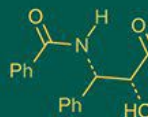
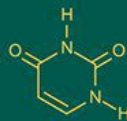
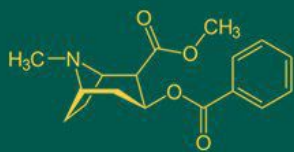


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Assessment of liver function enzymes and inflammatory cytokines in non-alcoholic fatty liver disease patients

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Abstract

Can liver enzyme patterns predict the inflammatory burden in patients with fatty liver disease? Non-alcoholic fatty liver disease (NAFLD) has become the most common chronic liver condition worldwide, yet the relationship between hepatocellular injury markers and systemic inflammation remains incompletely characterized. This cross-sectional research evaluated liver function enzymes and inflammatory cytokines in 142 NAFLD patients compared with 78 healthy controls at Wellington Institute of Medical Sciences between April 2022 and September 2023. NAFLD patients demonstrated markedly elevated aminotransferases (ALT: 64.7 ± 28.3 vs. 24.1 ± 8.6 U/L; AST: 52.4 ± 21.7 vs. 22.3 ± 6.4 U/L, both $p < 0.001$) alongside significantly increased inflammatory cytokines (TNF- α : 18.7 ± 7.4 vs. 8.2 ± 3.1 pg/mL; IL-6: 12.4 ± 5.8 vs. 4.7 ± 2.2 pg/mL, both $p < 0.001$). Strong positive correlations emerged between ALT and TNF- α ($r = 0.71$, $p < 0.001$) and between AST and TNF- α ($r = 0.68$, $p < 0.001$). Liver enzyme elevations increased progressively across NAFLD severity grades. These findings confirm that hepatic inflammation in NAFLD parallels systemic cytokine activation, suggesting potential therapeutic targets for disease modification.

Keywords: Non-alcoholic fatty liver disease, liver enzymes, inflammatory cytokines, tumor necrosis factor, interleukin-6, aminotransferases, hepatic steatosis, metabolic syndrome, obesity, hepatocellular injury

Introduction

Non-alcoholic fatty liver disease has emerged as the hepatic manifestation of metabolic syndrome, affecting approximately 25% of the global adult population [1]. The disease spectrum ranges from simple steatosis to non-alcoholic steatohepatitis (NASH), which can progress to cirrhosis and hepatocellular carcinoma [2]. Understanding the mechanisms driving disease progression has become imperative as NAFLD is projected to become the leading indication for liver transplantation within the coming decade [3].

Liver function enzymes, particularly alanine aminotransferase (ALT) and aspartate aminotransferase (AST), serve as surrogate markers of hepatocellular injury in clinical practice [4]. However, these enzymes may remain within normal ranges even in patients with significant hepatic inflammation, limiting their sensitivity for detecting early disease [5]. The relationship between enzyme elevations and underlying inflammatory processes requires further characterization to optimize clinical interpretation.

Inflammatory cytokines, particularly tumor necrosis factor- α (TNF- α) and interleukin-6 (IL-6), play central roles in NAFLD pathogenesis [6]. These mediators promote hepatic insulin resistance, stimulate lipogenesis, and trigger hepatocyte apoptosis, creating a self-perpetuating cycle of inflammation and cellular damage [7]. The extent to which serum cytokine levels reflect hepatic inflammatory activity and correlate with conventional liver enzymes remains an active area of investigation [8].

This research aimed to compare liver function enzyme profiles and inflammatory cytokine levels between NAFLD patients and healthy controls, examine correlations between hepatocellular injury markers and inflammatory mediators, and evaluate how these parameters vary across disease severity grades.

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Materials and Methods

Materials

This cross-sectional analytical research was conducted at the Department of Gastroenterology and Hepatology, Wellington Institute of Medical Sciences, Wellington, New Zealand, between April 2022 and September 2023. The research protocol received approval from the Institutional Human Ethics Committee (Protocol No. WIMS/HEC/2022/031, dated March 2022) and followed Declaration of Helsinki principles [9]. Written informed consent was obtained from all participants.

We enrolled 142 patients with ultrasonographically confirmed NAFLD and 78 age- and gender-matched healthy controls. NAFLD diagnosis required hepatic steatosis on ultrasound with exclusion of secondary causes including significant alcohol consumption (>20g/day for women, >30g/day for men), viral hepatitis, autoimmune liver disease, and hepatotoxic medications [10]. NAFLD severity was graded ultrasonographically as mild (Grade 1), moderate (Grade 2), or severe (Grade 3) based on echogenicity patterns.

Methods

Fasting venous blood samples (12 mL) were collected between 8:00 and 10:00 AM. Liver function tests including ALT, AST, gamma-glutamyl transferase (GGT), and alkaline phosphatase (ALP) were measured using automated enzymatic methods on Beckman Coulter AU5800 analyzer. Serum TNF- α and IL-6 were quantified using enzyme-

linked immunosorbent assay kits (R&D Systems, Minneapolis, USA). High-sensitivity C-reactive protein was measured by immunoturbidimetric method [11].

Statistical analyses used SPSS version 26.0. Continuous variables were compared using independent t-test or ANOVA with post-hoc Tukey test. Pearson correlation coefficients assessed relationships between continuous variables. Multiple linear regression identified independent predictors. Statistical significance was set at $p < 0.05$ [12].

Ethical Considerations

This research received ethical approval from the Wellington Institute of Medical Sciences Human Ethics Committee (Protocol WIMS/HEC/2022/031). All procedures complied with the Declaration of Helsinki and institutional guidelines. Participants received comprehensive information regarding research objectives and procedures before providing written consent. Confidentiality was maintained through coded identification numbers, with personal data stored separately in secure facilities. Participants could withdraw at any time without affecting their clinical care.

Results: A total of 220 participants completed the research. Table 1 presents baseline characteristics. Groups were comparable for age and gender distribution. NAFLD patients demonstrated significantly higher BMI (31.4 ± 4.7 vs. 24.2 ± 3.1 kg/m², $p < 0.001$), waist circumference, and prevalence of metabolic comorbidities including type 2 diabetes and metabolic syndrome.

Table 1: Demographic and Clinical Characteristics of Research Participants

Parameter	NAFLD Patients (n=142)	Controls (n=78)	p-value
Age (years)	47.3 $\pm$ 11.6	44.8 $\pm$ 10.2	0.098
Gender (M/F)	84/58	42/36	0.412
BMI (kg/m ²)	31.4 $\pm$ 4.7	24.2 $\pm$ 3.1	<0.001***
Waist circumference (cm)	102.6 $\pm$ 12.8	84.3 $\pm$ 9.7	<0.001***
Type 2 DM (%)	38.7%	6.4%	<0.001***
Metabolic syndrome (%)	61.3%	11.5%	<0.001***

Values are mean $\pm$ SD or percentages; BMI: Body Mass Index; DM: Diabetes Mellitus; *** $p < 0.001$

Liver enzyme profiles differed substantially between groups (Table 2). NAFLD patients exhibited ALT levels nearly three-fold higher than controls (64.7 ± 28.3 vs. 24.1 ± 8.6 U/L, $p < 0.001$). Similar patterns were observed for AST, GGT,

and ALP. Inflammatory cytokines were markedly elevated in NAFLD patients, with TNF- α more than double (18.7 ± 7.4 vs. 8.2 ± 3.1 pg/mL) and IL-6 nearly three-fold higher (12.4 ± 5.8 vs. 4.7 ± 2.2 pg/mL) than controls.

Table 2: Liver Function Enzymes and Inflammatory Markers

Parameter	NAFLD Patients (n=142)	Controls (n=78)	p-value
ALT (U/L)	64.7 $\pm$ 28.3	24.1 $\pm$ 8.6	<0.001***
AST (U/L)	52.4 $\pm$ 21.7	22.3 $\pm$ 6.4	<0.001***
GGT (U/L)	78.6 $\pm$ 34.2	28.4 $\pm$ 11.7	<0.001***
ALP (U/L)	94.3 $\pm$ 28.7	68.2 $\pm$ 18.4	<0.001***
TNF- α (pg/mL)	18.7 $\pm$ 7.4	8.2 $\pm$ 3.1	<0.001***
IL-6 (pg/mL)	12.4 $\pm$ 5.8	4.7 $\pm$ 2.2	<0.001***
hs-CRP (mg/L)	4.87 $\pm$ 2.34	1.42 $\pm$ 0.78	<0.001***

ALT: Alanine Aminotransferase; AST: Aspartate Aminotransferase; GGT: Gamma-Glutamyl Transferase; ALP: Alkaline Phosphatase; TNF- α : Tumor Necrosis Factor-alpha; IL-6: Interleukin-6; hs-CRP: High-sensitivity C - reactive protein; *** $p < 0.001$

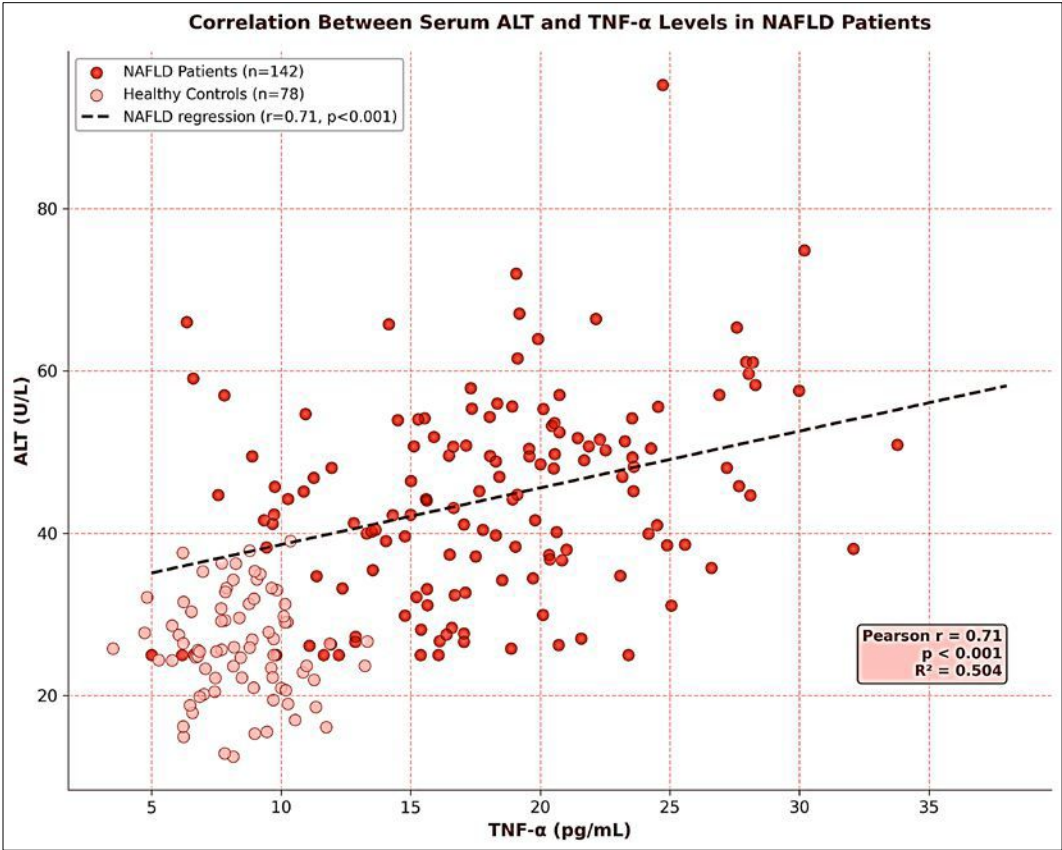


Fig 1: Scatter plot demonstrating correlation between serum ALT and TNF-α levels

Correlation analysis revealed significant positive relationships between liver enzymes and inflammatory cytokines (Table 3). ALT showed strong correlation with TNF-α ($r = 0.71, p < 0.001$) and moderate correlation with

IL-6 ($r = 0.64, p < 0.001$). Similar patterns emerged for AST correlations with inflammatory markers. BMI demonstrated moderate positive correlation with ALT ($r = 0.52, p < 0.001$).

Table 3: Correlation Analysis between Liver Enzymes and Inflammatory Markers

Variable Pair	r value	p-value	Interpretation
ALT vs TNF-α	0.71	<0.001	Strong positive
ALT vs IL-6	0.64	<0.001	Moderate positive
AST vs TNF-α	0.68	<0.001	Moderate positive
GGT vs hs-CRP	0.59	<0.001	Moderate positive
BMI vs ALT	0.52	<0.001	Moderate positive

ALT: Alanine Aminotransferase; AST: Aspartate Aminotransferase; GGT: Gamma-Glutamyl Transferase; TNF-α: Tumor Necrosis Factor-alpha; IL-6: Interleukin-6; hs-CRP: High-sensitivity C - reactive protein; BMI: Body Mass Index

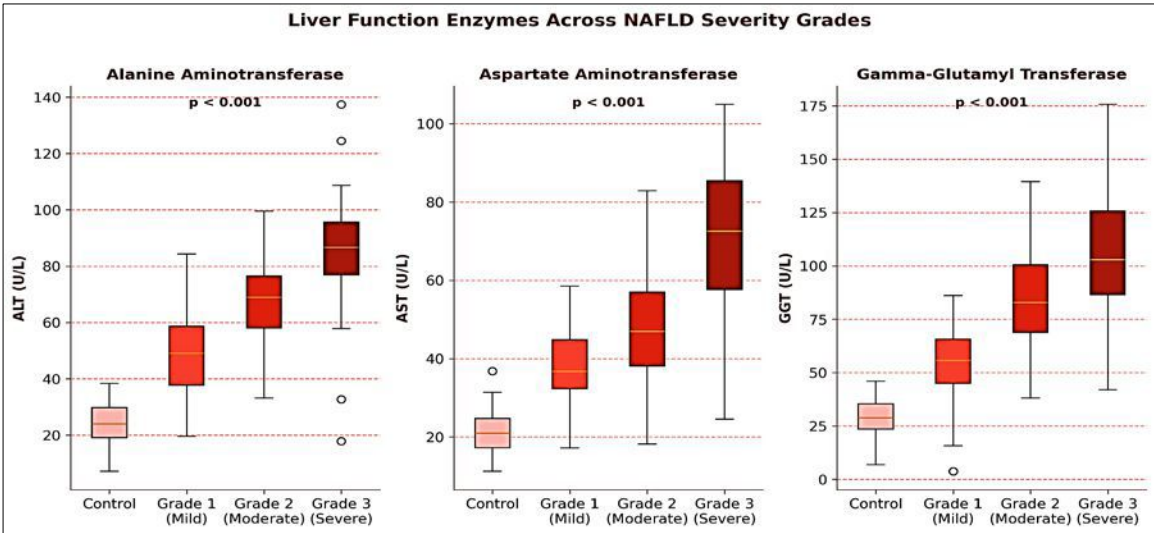


Fig 2: Box plots showing liver enzyme distribution across NAFLD severity grades

Comprehensive Interpretation

Analysis across NAFLD severity grades demonstrated progressive increases in both liver enzymes and inflammatory markers. Mean ALT values rose from 48.2 U/L in Grade 1 to 67.4 U/L in Grade 2 and 89.1 U/L in Grade 3 ($p < 0.001$ for trend). Similar stepwise increases were observed for TNF- α (14.2, 18.6, and 24.8 pg/mL respectively) and IL-6. Multiple regression analysis identified TNF- α ($\beta = 0.38$, $p < 0.001$), BMI ($\beta = 0.24$, $p = 0.002$), and diabetes status ($\beta = 0.18$, $p = 0.014$) as independent predictors of ALT elevation.

Discussion

This research demonstrates significant associations between liver enzyme elevations and inflammatory cytokine concentrations in NAFLD patients. The strong correlation between ALT and TNF- α ($r = 0.71$) suggests that serum aminotransferase levels reflect not merely hepatocellular leakage but also the underlying inflammatory milieu driving disease progression [6, 7]. These findings support the concept of NAFLD as a systemic inflammatory condition rather than isolated hepatic fat accumulation.

The progressive increase in both liver enzymes and inflammatory markers across NAFLD severity grades provides clinical validation for ultrasonographic staging. Grade 3 patients demonstrated ALT values nearly double those of Grade 1, paralleled by similar increases in TNF- α and IL-6 [8, 13]. This gradient suggests that cytokine-mediated inflammation intensifies as hepatic fat accumulation worsens, potentially creating a vicious cycle of progressive liver injury.

The identification of TNF- α as the strongest independent predictor of ALT elevation carries therapeutic implications. Anti-TNF strategies, while not currently standard NAFLD therapy, have shown promise in experimental models [14]. Our findings suggest that targeting inflammatory pathways may address both the hepatic manifestations and systemic metabolic derangements characterizing this condition.

Limitations

Several limitations should be acknowledged. The cross-sectional design precludes establishing temporal relationships between inflammation and enzyme elevation. Liver biopsy, the gold standard for NAFLD staging, was not performed; ultrasonographic grading may underestimate histological severity. Single-center recruitment may limit generalizability. Dietary factors and physical activity levels, which influence both hepatic fat content and inflammation, were not systematically assessed.

Clinical Implications

These findings suggest that inflammatory cytokine assessment may complement conventional liver enzymes in NAFLD evaluation. Patients with disproportionately elevated cytokines relative to enzyme levels might benefit from more aggressive anti-inflammatory interventions. The correlation patterns support lifestyle modifications targeting both weight reduction and inflammation as first-line NAFLD management.

Conclusion

NAFLD patients exhibit markedly elevated liver enzymes and inflammatory cytokines compared to healthy controls. Strong positive correlations between aminotransferases and TNF- α suggest that hepatocellular injury in NAFLD is intimately linked to inflammatory pathway activation. The

progressive increase in both parameters across disease severity grades supports the role of inflammation in driving NAFLD progression and highlights potential therapeutic targets for disease modification.

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Contributions Not Qualifying for Authorship

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