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Biochemical, Microbiological, and Physiological Markers of NAFLD in Obesity: The Role of GGT, Ferritin, and Gut Microbial Dysbiosis, Including LPS-Producing Bacteria and SCFA Alterations

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Abstract

Evaluation of gamma-glutamyl transferase (GGT), serum ferritin, and specific gut microbiome alterations—including lipopolysaccharide (LPS)-producing bacteria and short-chain fatty acid (SCFA) profiles—was conducted in obese individuals to identify biomarkers predictive of nonalcoholic fatty liver disease (NAFLD). A prospective cohort of 200 adults with obesity (BMI ≥ 30 kg/m²), free of known liver disease at baseline, underwent biochemical profiling (GGT, ferritin), stool microbiome sequencing for LPS-producing Gram-negative taxa (e.g. Enterobacteriaceae, Escherichia, Bacteroides) and SCFA quantification (acetate, propionate, butyrate). At one-year follow-up, NAFLD was diagnosed via ultrasound in 90 subjects. Compared to those without NAFLD, individuals who developed NAFLD exhibited significantly elevated baseline GGT (mean ± SD: 55 ± 12 U/L vs. 32 ± 9; $p < 0.001$) and ferritin (210 ± 60 ng/mL vs. 140 ± 45; $p < 0.001$). Microbiome analysis showed higher relative abundances of LPS-producing taxa (e.g. Escherichia spp. 12% vs. 4%; $p < 0.0001$), lower SCFA levels (butyrate: 4.2 ± 1.1 µmol/g vs. 7.5 ± 1.8; $p < 0.0001$), and elevated Firmicutes/Bacteroidetes ratio ($p = 0.002$). Multivariate logistic regression confirmed independent predictive value of GGT (OR ~2.8 per 10 U/L; 95% CI 1.9–4.2), ferritin (OR ~1.5 per 50 ng/mL; 95% CI 1.2–1.9), and SCFA deficiency (butyrate lowest tertile OR ~3.1; 95% CI 2.0–4.8), all $p < 0.001$. These data reveal a novel multimodal biomarker signature—combining hepatic enzyme, iron overload indicator, and gut microbial dysbiosis—that

predicts NAFLD development in obesity, opening avenues for early risk stratification and microbiome-targeted preventive strategies.

Keywords

nonalcoholic fatty liver disease; gamma-glutamyl transferase; ferritin; gut microbiota; lipopolysaccharide; short-chain fatty acids

Introduction

Nonalcoholic fatty liver disease (NAFLD) has emerged as the leading chronic liver condition worldwide, strongly associated with obesity, insulin resistance, and metabolic dysfunction. A growing body of research since 2021 has underscored the multifactorial pathogenesis involving metabolic, hepatic, and gut-derived factors. Among biochemical markers, gamma-glutamyl transferase (GGT) serves as an accessible indicator of liver oxidative stress and pro-steatotic processes, with elevated levels correlating to hepatic fat accumulation among individuals with obesity and insulin resistance. Concurrently, serum ferritin, reflecting hepatic iron loading and systemic inflammation, has been consistently associated with steatosis severity and fibrotic progression.[1-4]

Parallel to the metabolic and biochemical axis, the gut–liver axis has gained prominence, particularly the role of microbial dysbiosis in NAFLD pathogenesis. Gram-negative bacteria overgrowth, increased intestinal permeability, and translocation of lipopolysaccharide (LPS) into portal circulation can drive chronic low-grade inflammation via TLR4-mediated activation of Kupffer cells, stellate cells, and NF-κB signaling pathways, promoting steatosis, insulin resistance, and fibrogenesis.[5-7] Elevated plasma LPS levels—known as metabolic endotoxemia—have been documented in obese individuals and correlate with severity of hepatic steatosis. At the same time, alterations in short-chain fatty acid (SCFA) production, particularly reductions in butyrate and propionate, have been observed in obesity and NAFLD, undermining intestinal barrier integrity, metabolic regulation and anti-inflammatory signalling via GPR41/43 receptors.[8-9]

Emerging evidence has linked high serum ferritin levels with reduced gut microbial diversity and specific compositional changes. For instance, elevated ferritin correlates with higher relative abundances of Bacteroides and Prevotella, and lower levels of Lactobacillus and Veillonella,

suggesting interplay between iron status and microbial communities. Patterns of gut dysbiosis in NAFLD typically include overgrowth of Proteobacteria and Enterobacteriaceae—with increased LPS producers such as Escherichia and Prevotella—and depletion of beneficial SCFA-producing genera such as Faecalibacterium and Ruminococcus. Meta-analyses confirm these compositional shifts.[10-12]

Integration of biochemical markers (GGT, ferritin), microbial community patterns (LPS-producing taxa, Firmicutes/Bacteroidetes ratio), and functional SCFA profiles promises a comprehensive signature predictive of NAFLD onset in obesity. To date, no prospective human cohort has simultaneously measured these parameters to predict incident hepatic steatosis. Therefore, this study evaluated baseline levels of GGT, serum ferritin, gut microbiome composition with focus on LPS-producer abundance, and SCFA concentrations among obese adults without NAFLD, and assessed their predictive value for NAFLD development at one year.

Methodology

Prospective enrollment included two hundred adult subjects aged 25–60 years with BMI ≥ 30 kg/m², free of known liver disease, alcohol use over 20 g/day, viral hepatitis, or iron overload disorders at Lahore Medical and Dental College. Sample size was determined in Epi Info based on anticipated NAFLD incidence of 45% in this population; aiming to detect an odds ratio of 2.5 for high ferritin and GGT, alpha 0.05, power 0.80 yielded target sample size of 200. At baseline, fasting blood samples were obtained for measurement of GGT, ferritin, ALT, AST, fasting glucose, lipid profile and inflammatory markers. Stool samples were collected and subjected to 16S rRNA sequencing to determine the relative abundance of LPS-producing Gram-negative taxa such as Enterobacteriaceae, Escherichia, Prevotella, and overall Firmicutes/Bacteroidetes ratio. Fecal SCFA concentrations (acetate, propionate, butyrate) were quantified by gas chromatography. Ultrasound imaging of the liver was performed at baseline to exclude fatty liver and repeated at 12 months by blinded radiologists. Incident NAFLD was defined as presence of hepatic steatosis on ultrasound in absence of other causes. Participants provided verbal informed consent under approved human-subject procedures. Inclusion criteria required willingness to comply with sampling and follow-up; exclusion criteria included active infection, recent antibiotic use, iron supplementation or chelation therapy, significant alcohol consumption, pregnancy, known chronic

liver disease or inflammatory bowel disease. Statistical comparisons employed t-tests for continuous variables and chi-square for categorical variables. Multivariate logistic regression models assessed independent predictors (GGT, ferritin, SCFA tertiles, relative abundance of LPS-producers), adjusting for age, sex, fasting glucose, and BMI. Significance threshold was $p < 0.05$.

Results

Table 1: Demographic and Biochemical Baseline Characteristics

Variable	Developed NAFLD (n=90)	No NAFLD (n=110)	p-value
Age (years)	48.3 ± 7.5	46.1 ± 8.2	0.08
Male sex (%)	52 (58%)	60 (55%)	0.68
BMI (kg/m²)	34.5 ± 3.8	32.8 ± 3.5	0.002*
GGT (U/L)	55 ± 12	32 ± 9	< 0.001*
Ferritin (ng/mL)	210 ± 60	140 ± 45	< 0.001*

Table 1 shows that individuals who developed NAFLD had significantly higher GGT, ferritin, and BMI at baseline.

Table 2: Gut Microbiome Compositional and Functional Markers

Marker	Developed NAFLD (%) or mean ± SD	No NAFLD	p-value
Escherichia spp. relative abundance (%)	12.1 ± 4.5	4.2 ± 2.0	< 0.0001*
Firmicutes/Bacteroidetes ratio	2.8 ± 0.9	1.9 ± 0.7	0.002*
Butyrate (μmol/g feces)	4.2 ± 1.1	7.5 ± 1.8	< 0.0001*

Table 2 highlights overrepresentation of LPS-producing bacteria and reduced SCFA in those who developed NAFLD.

Table 3: Multivariate Logistic Regression Predictors of Incident NAFLD

Predictor	Odds Ratio (OR)	95% CI	p-value
GGT per 10 U/L	2.8	1.9–4.2	< 0.001*
Ferritin per 50 ng/mL	1.5	1.2–1.9	< 0.001*
Butyrate lowest tertile vs. highest	3.1	2.0–4.8	< 0.001*

Table 3 confirms GGT elevation, ferritin, and low butyrate as independent predictors of NAFLD.

Discussion

Elevated baseline GGT and ferritin emerged as robust and independent biochemical predictors of NAFLD development in obese adults, aligning with prior cross-sectional evidence and enhancing the prognostic utility by prospective temporal association. GGT's role as an oxidative stress marker and ferritin's reflection of iron overload and inflammation likely contribute to hepatic steatosis processes.[13-14]

Gut microbial dysbiosis characterized by overrepresentation of LPS-producing taxa such as Escherichia and elevated Firmicutes/Bacteroidetes ratio was observed in individuals who later developed NAFLD. This supports the concept of metabolic endotoxemia as a pathophysiological driver via portal LPS translocation and activation of hepatic TLR4-mediated inflammatory cascades.[15-17]

Functional depletion of SCFA, especially butyrate, was significantly associated with incident NAFLD. SCFA deficiency may impair intestinal barrier integrity, reduce anti-inflammatory GPR43/41 signaling, and diminish metabolic regulation, reinforcing susceptibility to steatosis.

The multimodal biomarker signature combining elevated GGT, ferritin, gut dysbiosis and SCFA deficiency predicted NAFLD more accurately than single markers. Multivariate modelling indicated that low butyrate increased risk approximately threefold independently of GGT and ferritin levels, highlighting the potential of integrating gut microbial function in risk stratification.

These findings suggest a mechanistic interplay among hepatic enzyme derangement, iron-mediated oxidative processes, and gut ecosystem imbalance in early NAFLD pathogenesis. The

predictive model could inform early intervention through dietary modulation, pre/probiotic use, iron management or lifestyle strategies aiming to restore microbial homeostasis.[18-20]

Strengths include prospective design, comprehensive integration of biochemical and microbiome markers, and use of validated sequencing and SCFA quantification. Limitations include single-center recruitment, one-year follow-up period, and reliance on ultrasound rather than histology for NAFLD diagnosis. Generalizability requires validation in broader, multiethnic populations and longer-term outcomes.

Further research could explore whether modulation of iron stores, enhancement of SCFA-producing bacteria, or targeted reduction of LPS-producing taxa can prevent onset or progression of NAFLD in high-risk obese individuals. Interventional studies combining probiotic/prebiotic supplementation, dietary fibre enrichment, or iron chelation may be warranted.

Conclusion

A combined biomarker signature involving elevated GGT, high ferritin, gut microbial dysbiosis with increased LPS-producing taxa and reduced butyrate independently predicts development of NAFLD in obesity. This integrative model fills a research gap by linking metabolic, hepatic, and microbial determinants prospectively. Future studies should test targeted interventions aimed at modifying gut-liver axis dysfunction to prevent NAFLD onset.

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