E-ISSN 2250-0944
ISSN 2250-1150
doi: 10.48047/ijprt/16.01.534

Research Article

Serum Ghrelin and Leptin Levels as Predictors of Disease Severity in Patients with Non-Alcoholic Fatty Liver Disease

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Abstract: Non-alcoholic fatty liver disease (NAFLD) has emerged as one of the most prevalent metabolic liver disorders worldwide, closely associated with obesity, insulin resistance, and systemic inflammation. The present experimental study aimed to evaluate serum ghrelin and leptin levels as potential predictors of disease severity in NAFLD patients and to determine their association with metabolic and anthropometric parameters. A total of 180 participants were categorized into control, mild, moderate, and severe NAFLD groups based on ultrasonographic grading and fibrosis assessment. Serum ghrelin levels demonstrated a progressive decline with increasing disease severity, whereas leptin levels showed a significant upward trend across the disease spectrum. Statistical analysis revealed a strong inverse correlation between ghrelin and NAFLD severity and a positive correlation between leptin and disease progression ($p<0.001$ for both). The leptin-to-ghrelin ratio was found to be the most robust marker for distinguishing disease stages, indicating superior predictive capability compared to individual biomarkers. These findings suggest that dysregulation of appetite-regulating hormones plays a central role in NAFLD progression. The study highlights that combined assessment of ghrelin and leptin may serve as a valuable non-invasive tool for early detection, risk stratification, and monitoring of disease severity in NAFLD

patients, offering potential clinical utility in metabolic hepatology.

Keywords: Non-alcoholic fatty liver disease, ghrelin, leptin

Introduction

Non-alcoholic fatty liver disease has become a major global health concern due to its rapidly increasing prevalence and strong association with metabolic syndrome components such as obesity, dyslipidemia, hypertension, and type 2 diabetes mellitus. It encompasses a wide spectrum of liver abnormalities ranging from simple steatosis to non-alcoholic steatohepatitis, fibrosis, cirrhosis, and hepatocellular carcinoma. In recent years, lifestyle transitions characterized by high-calorie diets and reduced physical activity have significantly contributed to the rising burden of NAFLD across both developed and developing countries. The disease is often asymptomatic in its early stages, which complicates timely diagnosis and increases the risk of late-stage complications [1-3].

The pathogenesis of NAFLD is complex and multifactorial, involving lipid accumulation in hepatocytes, oxidative stress, mitochondrial dysfunction, and chronic low-grade inflammation. The classical "multiple-hit hypothesis" suggests that hepatic fat accumulation is not solely responsible for disease progression but interacts with genetic, environmental, and hormonal factors to drive inflammation and fibrosis. Insulin resistance plays a central role in promoting hepatic lipogenesis and inhibiting fatty acid oxidation, thereby contributing to progressive hepatic injury [4-6].

Adipose tissue functions as an active endocrine organ, secreting a variety of biologically active peptides known as adipokines. Among these, leptin is a key regulator of energy homeostasis and appetite suppression. In obese individuals and NAFLD patients, leptin levels are typically elevated; however, a state of leptin resistance develops, impairing its physiological action. Leptin also exerts pro-inflammatory and profibrotic effects on hepatic stellate cells, thereby contributing to liver fibrosis progression [7-9].

In contrast, ghrelin, primarily secreted by gastric endocrine cells, is an orexigenic hormone that stimulates appetite and regulates energy balance. Beyond its role in hunger regulation, ghrelin has been shown to exert anti-inflammatory and hepatoprotective effects in experimental models. Reduced ghrelin levels have been associated with increased insulin resistance, hepatic steatosis, and metabolic dysfunction, suggesting its protective role in liver metabolism [10-12].

The interaction between leptin and ghrelin represents a critical regulatory axis in metabolic homeostasis. The imbalance between these two hormones may contribute significantly to NAFLD progression by influencing energy intake, fat deposition, and inflammatory signaling pathways. Recent evidence suggests that the leptin-to-ghrelin ratio may serve as a more reliable biomarker than either hormone alone in reflecting metabolic imbalance and disease severity [13-15].

Despite advances in understanding NAFLD pathophysiology, there remains a lack of reliable non-invasive biomarkers for early disease stratification and progression monitoring. Liver biopsy, although considered the gold standard, is invasive and unsuitable for routine screening. Imaging techniques such as ultrasonography have limitations in detecting early fibrosis and subtle histological changes. Therefore, there is a growing need for serum-based biomarkers that can accurately reflect disease severity and metabolic dysfunction [16-18].

Recent research has increasingly focused on hormonal and inflammatory mediators as potential diagnostic tools in NAFLD. However, most studies have evaluated ghrelin and leptin independently, with limited data on their combined predictive value across different disease stages. Furthermore, variability in study populations and methodologies has limited the reproducibility of findings. This highlights the necessity for well-structured experimental studies evaluating both hormones simultaneously in a stratified disease model [19-20].

Methodology

This experimental observational study included 180 participants recruited from Department of Pathology, King Edward Medical University a tertiary care hospital setting. Subjects were divided equally into four groups: healthy controls, mild NAFLD, moderate NAFLD, and severe NAFLD, with 45 participants in each group. Diagnosis and staging of NAFLD were performed using abdominal ultrasonography supported by clinical assessment and fibrosis scoring indices. Participants aged 30 to 65 years were included to ensure metabolic homogeneity across groups.

Sample size estimation was performed using Epi Info software based on expected differences in leptin levels between NAFLD and control groups derived from previous literature. With a confidence interval of 95%, power of 80%, and standard deviation of 1.2, the minimum required sample size was calculated to be 164. To account for potential

attrition and incomplete data, a total of 180 participants were enrolled.

Inclusion criteria consisted of adults diagnosed with NAFLD based on imaging, with no history of significant alcohol intake, viral hepatitis, autoimmune liver disease, or other chronic liver conditions. Exclusion criteria included pregnancy, malignancy, chronic kidney disease, endocrine disorders affecting metabolism, and use of medications influencing lipid or hormonal profiles. Written verbal informed consent was obtained from all participants prior to enrollment.

Baseline demographic data including age, sex, body mass index, waist circumference, fasting blood glucose, and lipid profile were recorded. After overnight fasting, venous blood samples were collected under sterile conditions. Serum ghrelin and leptin levels were measured using standardized enzyme-linked immunosorbent assay kits according to manufacturer protocols. All samples were processed in duplicate to ensure accuracy.

Statistical analysis was performed using SPSS software. Continuous variables were expressed as mean ± standard deviation. Intergroup comparisons were analyzed using one-way ANOVA, while correlations between variables were assessed using Pearson's correlation coefficient. A p-value of less than 0.05 was considered statistically significant

.**Results**

Table 1: Demographic and metabolic profile of study participants

Parameter	**Control**	**Mild NAFLD**	**Moderate NAFLD**	**Severe NAFLD**	**p-value**
Age (years)	41.2 ± 8.3	43.5 ± 7.9	45.8 ± 8.1	47.6 ± 7.5	0.08
BMI (kg/m²)	23.1 ± 2.4	27.5 ± 2.8	30.2 ± 3.1	33.4 ± 3.6	<0.001
Waist circumference (cm)	82.3 ± 6.2	92.5 ± 7.1	101.4 ± 8.3	110.6 ± 9.0	<0.001
Fasting glucose (mg/dL)	89.4 ± 9.1	102.3 ± 10.4	116.8 ± 12.6	128.7 ± 13.9	<0.001

Table 2: Serum ghrelin, leptin, and leptin/ghrelin ratio across groups

Group	Ghrelin (pg/mL)	Leptin (ng/mL)	L/G Ratio
Control	210.5 ± 25.4	6.2 ± 1.1	0.029
Mild NAFLD	180.3 ± 22.1	10.5 ± 2.3	0.058
Moderate NAFLD	150.6 ± 20.7	16.8 ± 3.2	0.111
Severe NAFLD	120.4 ± 18.9	24.6 ± 4.5	0.204
p-value <0.001			

Table 3: Correlation of biomarkers with disease severity

Marker	Correlation (r)	p-value
Ghrelin	-0.72	<0.001
Leptin	0.78	<0.001
L/G Ratio	0.85	<0.001

Table 1 demonstrates a progressive increase in BMI, waist circumference, and fasting glucose with worsening NAFLD severity, indicating strong metabolic deterioration across groups. Table 2 shows a significant decline in ghrelin levels and a progressive rise in leptin levels, with the leptin-to-ghrelin ratio showing the strongest gradient across disease stages. Table 3 confirms a statistically significant correlation between hormonal markers and NAFLD severity, with the leptin-to-ghrelin ratio exhibiting the highest predictive strength.

Discussion

The present study demonstrates a significant alteration in ghrelin and leptin levels across different stages of NAFLD, highlighting their potential role as metabolic biomarkers of disease severity [11-12]. The observed decline in ghrelin levels with disease progression supports its proposed protective role in hepatic metabolism, as reduced ghrelin may contribute to increased lipogenesis and insulin resistance. Similar findings have been reported in recent metabolic studies where ghrelin deficiency was associated with worsening hepatic steatosis [13-14].

Elevated leptin levels observed in the current study align with the concept of leptin resistance in obesity-related liver disease. Despite high circulating leptin concentrations, its physiological signaling is impaired, leading to persistent appetite dysregulation and pro-inflammatory hepatic effects. Leptin-mediated activation of inflammatory pathways and hepatic stellate cells may explain its strong association with fibrosis severity observed in NAFLD patients [15-16].

The leptin-to-ghrelin ratio demonstrated the highest correlation with disease severity, suggesting that combined hormonal profiling may provide superior diagnostic accuracy compared to individual biomarkers. This finding supports emerging evidence that metabolic diseases are better characterized by hormonal balance indices rather than single-marker evaluation [17].

The progressive metabolic deterioration observed in anthropometric parameters further strengthens the association between hormonal imbalance and NAFLD severity. Increasing BMI, waist circumference, and fasting glucose reflect worsening insulin resistance, which may act synergistically with adipokine dysregulation to accelerate hepatic injury [18].

Inflammatory pathways also play a central role in linking hormonal changes to liver pathology. Ghrelin has been shown to suppress pro-inflammatory cytokines, whereas leptin enhances inflammatory signaling, creating a biochemical environment favoring hepatic fibrosis. This opposing mechanism may explain the strong inverse relationship observed between ghrelin and disease severity [19].

The findings of this study also highlight the potential clinical utility of ghrelin and leptin as non-invasive biomarkers. Current diagnostic approaches for NAFLD are limited by invasiveness or low sensitivity in early disease stages. Hormonal profiling could provide a cost-effective and scalable alternative for early detection and monitoring [20].

Overall, the results suggest that NAFLD progression is closely linked to hormonal dysregulation involving ghrelin and leptin. These findings support the concept of a gut-adipose-liver axis in metabolic liver disease and underscore the importance of integrated biomarker approaches for improved disease stratification.

Conclusion

Serum ghrelin decreases progressively with NAFLD severity while leptin increases significantly across disease stages.

The leptin-to-ghrelin ratio demonstrates the strongest predictive accuracy for disease stratification.

Hormonal profiling of ghrelin and leptin may serve as a valuable non-invasive tool for early diagnosis and risk assessment in NAFLD.

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