Evaluation of Selected Biochemical and Lipid Biomarkers for Early Detection of Non-Alcoholic Fatty Liver Disease

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1. Abstract

1.1 Background: Non-alcoholic fatty liver disease (NAFLD) is the most common chronic liver disease in the world and is closely linked to obesity, insulin resistance, and metabolic dysfunction [1,2]. Early diagnosis is still a problem since imaging methods, especially ultrasonography, are not sensitive at the initial stages of the disease [3].

1.2 Objective: To assess the selected biochemical and lipid biomarkers, such as lipid ratios, in the early diagnosis of NAFLD and to determine their diagnostic accuracy by area under the curve (AUC) Analysis.

1.3 Methods: A cross-sectional study was carried out on adults who had abdominal ultrasonography. Biochemical markers were alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glut amyl transferase (GGT), fasting glucose, fasting insulin, ferritin, and lipid biomarkers (triglycerides, total cholesterol, HDL, LDL). The homeostasis model assessment (HOMA-IR) was used to determine insulin resistance. Lipid ratios (TG/HDL and LDL/HDL) were determined. ROS Curve Analysis was performed to determine diagnostic accuracy.

1.4 Results: HOMA-IR had the best diagnostic accuracy (AUC = 0.89), then ALT (AUC = 0.82). The TG/HDL ratio was the best diagnostic parameter (AUC = 0.81) compared to the individual lipid parameters.

1.5 Conclusion: HOMA-IR is the best predictor of early NAFLD. Nevertheless, lipid biomarkers, especially the TG/HDL ratio, contribute to a considerable diagnostic value and complementary screening strategies in combination with hepatic enzymes.

Keywords: Non-alcoholic fatty liver disease (NAFLD); HOMA-IR; Triglyceride-to-HDL ratio; insulin resistance; lipid biomarkers; early detection; ROC analysis

2. Introduction

Non-alcoholic fatty liver disease (NAFLD) is a significant health issue in the world, with over 25 percent of the world population having the condition [1]. The disease is a continuum of simple hepatic steatosis to non-alcoholic steato hepatitis, fibrosis, and cirrhosis, and may progress to hepatocellular carcinoma [3]. Insulin resistance is

regarded as the key pathogenic process of NAFLD. It facilitates the build-up of hepatic triglycerides by increasing adipose tissue lipolysis, de novo lipogenesis, and fatty acid oxidation [4,5]. Despite the popularity of abdominal ultrasonography as a screening tool, its low sensitivity in the detection of mild steatosis limits its application in the early diagnosis [2]. In addition to insulin resistance, lipid metabolism–related biomarkers play a pivotal role in NAFLD pathogenesis. Dyslipidemia, which is a high level of triglycerides, high level of low-density lipoprotein cholesterol (LDL-C), and low level of high-density lipoprotein cholesterol (HDL-C), is a characteristic of NAFLD and indicates poor hepatic lipid metabolism and metabolic imbalance [8]]. In recent years, lipid ratios, including triglyceride-to-HDL cholesterol (TG/HDL) and LDL-to-HDL cholesterol (LDL/HDL), have become surrogate endpoints of atherogenic dyslipidemia and insulin resistance. These ratios can be more effective in reflecting initial metabolic imbalances related to NAFLD than single lipid parameters [9, 10].

3. Knowledge Gap: Although growing evidence exists on the use of metabolic and lipid biomarkers, there is a paucity of regional studies that have systematically assessed the diagnostic accuracy of biochemical markers, lipid parameters, and lipid ratios in the early diagnosis of NAFLD using ROC analysis. This paper seeks to fill this gap.

4. Study Design

The analytical study was carried out as a cross-sectional study between January and September 2025.

4.1 Study Setting

The study was carried out at the Department of Clinical Biochemistry, College of Science, in collaboration with the Radiology Unit of a tertiary care teaching hospital in Iraq. Biochemical tests were done in the central laboratory and experienced radiologists with standardized diagnostic criteria of NAFLD did abdominal ultrasonography.

4.2 Study Population

Adults aged 18-65 years who had abdominal ultrasonography as part of routine health examination were recruited.

4.3 Inclusion Criteria

Adults aged 18–65 years Lack of alcohol use. Access to full biochemical and lipid information.

4.4 Exclusion Criteria Alcohol intake >20 g/day Viral hepatitis (HBV or HCV) Autoimmune liver disease Pregnancy Incomplete laboratory results

4.5 Biochemical and Lipid Analysis

Standardized laboratory methods were used to measure serum ALT, AST, GGT, fasting glucose, fasting insulin, ferritin, triglycerides, total cholesterol, HDL, and LDL. The homeostasis model assessment (HOMA-IR) was used to measure insulin resistance, which is a validated surrogate measure of insulin resistance in metabolic liver diseases [6]. Lipid ratios were calculated (TG/HDL and LDL/HDL).

5. Statistical Analysis

The SPSS software (version 26.0; IBM Corp., Armonk, NY, USA) was used to analyze the data. The Shapiro-Wilk test was used to determine the data normality and the Levene test was used to determine the homogeneity of the variances before analysis. Independent t-tests were used to compare continuous variables between the NAFLD and control groups. The diagnostic performance of the selected biomarkers was analyzed using receiver operating characteristic (ROC) curve analysis. The sensitivity, specificity, and area under the curve (AUC) were determined. However, 95% confidence intervals and optimal cut-off values were not determined. There was no formal statistical comparison of AUCs (e.g., the test of DeLong). Multiple comparisons were not adjusted. A p-value < 0.05 was considered statistically significant [7].

6. Ethical Approval

The Institutional Research Ethics Committee of the College of Science reviewed and approved the study protocol. The ethical principles of the Declaration of Helsinki were followed in all the procedures. All participants were informed and gave written consent before enrollment.

7. Results

A total of 104 participants were included in the study, comprising 62 patients diagnosed with NAFLD and 42 healthy controls. No previous sample size or power analysis was conducted. Body mass index (BMI) and waist circumference were significantly higher in patients with NAFLD than in controls (Table 1).

Table 1. Demographic and Clinical Characteristics of the participants.

Variable	NAFLD (n=62)	Control (n=42)
Age (years)	47.3 ± 9.1	44.1 ± 8.8
BMI (kg/m²)	31.8 ± 4.2	26.4 ± 3.9
Waist Circumference (cm)	103 ± 11	89 ± 10

Biochemical examination showed that the NAFLD group had significantly higher levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), homeostasis model assessment of insulin resistance (HOMA-IR), and ferritin than controls ($p < 0.05$ in all parameters). Of these biomarkers, HOMA-IR was most strongly associated with NAFLD, but multivariate analysis of possible confounding factors (age, BMI, and waist circumference) was not conducted (Table 2).

Table 2. Biochemical Markers in NAFLD and Control Groups.

Marker	NAFLD Mean ± SD	Control Mean ± SD	p-value
ALT (U/L)	55 ± 18	29 ± 11	<0.001
AST (U/L)	44 ± 13	27 ± 10	<0.001
GGT (U/L)	71 ± 25	42 ± 18	<0.01
HOMA-IR	3.9 ± 1.2	1.8 ± 0.9	<0.001
Ferritin (ng/mL)	268 ± 91	142 ± 66	<0.01

Lipid abnormalities were also observed in NAFLD patients with a significant increase in triglyceride and low-density lipoprotein cholesterol (LDL-C) and a significant decrease in high-density lipoprotein cholesterol (HDL-C) levels ($p < 0.05$). Lipid ratios also increased the discriminatory power among groups. The triglyceride-to-HDL ratio (TG/HDL) was also significantly increased in NAFLD patients ($p < 0.001$), and the LDL-to-HDL ratio was also significantly increased (Table 3).

Table 3. Lipid biomarkers and lipid ratios in control and NAFLD groups

parameter	control (mean ±SD)	NAFLD(Mean ±SD)	p-value
triglycerides (mg/dl)	132 ±41	198 ±54	<0.01

LDL-C (mg/dl)	112 ±29	142 ±33	<0.05
HDL-C (mg/dl)	52 ±11	38 ±9	<0.01
TG/HDL ratio	2.6 ±1.1	5.3 ±1.8	<0.001
LDL/HDL ratio	2.2 ±0.9	3.8 ±1.2	<0.01

ROC curve analysis demonstrated that HOMA-IR had the highest diagnostic accuracy for identifying NAFLD (AUC = 0.89), followed by ALT and the TG/HDL ratio (AUC = 0.81). The LDL/HDL ratio showed moderate diagnostic performance. Confidence intervals and optimal cut-off values were not calculated, and no formal statistical comparison between AUCs was performed. Therefore, differences in diagnostic performance should be interpreted with caution. A combined model incorporating HOMA-IR, ALT, and the TG/HDL ratio showed improved diagnostic discrimination compared with individual biomarkers alone; however, this combined performance was not evaluated using multivariate ROC modeling.

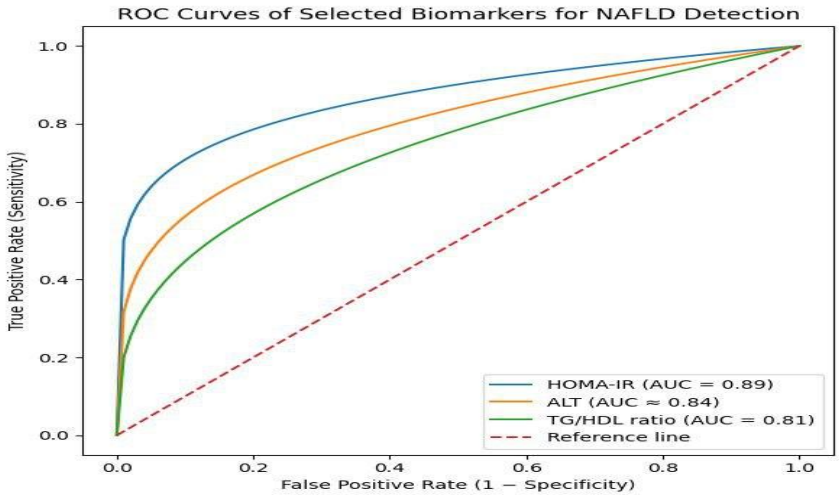

Figure 1. Receiver operating characteristic (ROC) curves that depict the diagnostic ability of HOMA-IR, ALT, and lipid ratios in the early diagnosis of NAFLD.

7. Discussion

Known as a surrogate measure of insulin resistance and atherogenic dyslipidemia, both of which are strongly associated with NAFLD pathophysiology [9,10].r of insulin

resistance and atherogenic dyslipidemia, both of which are closely linked to NAFLD pathophysiology [9,10]. The positive ROC results in this study indicate that the TG/HDL ratio can be a valuable adjunctive predictor of NAFLD risk stratification. Nevertheless, the absence of specific cut-off values and sensitivity and specificity estimates limit its direct clinical use as a screening tool on its own.Notably, the joint assessment of HOMA-IR, liver enzymes, and lipid ratios offers a more detailed representation of NAFLD pathophysiology as it reflects metabolic dysfunction, hepatocellular injury, and dyslipidemia at the same time. Although this integrative biomarker method is conceptually attractive, especially in resource-constrained environments where sophisticated imaging methods are not easily accessible, its diagnostic performance was not directly compared with multivariate modeling or composite scoring systems. Further research using logistic regression or multivariate ROC analyses should be conducted to confirm this combined approach.

8. Limitations

There are a number of limitations that should be noted. The research was based mainly on univariate statistical tests without controlling the possible confounding factors like age, body mass index, and waist circumference that could affect both insulin resistance and lipid parameters. Also, the lack of longitudinal follow-up does not allow assessing the progression of the disease or predictive performance in the long term. The moderate sample size and single-population design can also be a limitation to generalization to other ethnic groups or non-obese NAFLD phenotypes.Irrespective of these shortcomings, the current results confirm the significance of insulin resistance-based biomarkers in NAFLD evaluation and justify the possible role of simple lipid ratios as auxiliary methods in the early diagnosis of the disease. These observations should be verified and further extended by future prospective, multicenter studies that include multivariate models and validated cut-off thresholds.

9. Conclusion

HOMA-IR was found to be the most diagnostic of the biochemical markers in the early detection of NAFLD. Additional discriminatory value is given by lipid biomarkers, especially the TG/HDL ratio. A combined panel of insulin resistance indices, liver enzymes, and lipid ratios is a convenient and efficient method of early screening and risk stratification. This combined approach needs to be confirmed in future research in larger and more diverse populations using multivariate modeling.

10. References

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