Research Article

IMPACT OF ANTI-HYPERLIPIDEMIC TREATMENT ON LIVER FUNCTIONS AND CK18 AND TGF-B IN NAFLD

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Abstract

Background: Non-alcoholic fatty liver disease (NAFLD) is defined by lipid accumulation in liver cells, with steatosis in the absence of alcohol consumption. The disease may develop into fibrosis, cirrhosis, and hepatocellular carcinoma (HCC).

Aim: Evaluate the effect of anti-hyperlipidemic treatments on liver function disease, on Liver function, and serum Cytokeratin-18 (CK18) and Transforming growth factor-Beta (TGF-β) in patients with NAFLD.

Material and Methods: This research is a case-control study (January–August 2025). It included 180 participants, divided into three groups: group I consist of 60 patients diagnosed with NAFLD who were evaluated before the start of the anti-hyperlipidemic agent. Group II 60 patients diagnosed with NAFLD who received a designated antihyperlipidemic medication for 3 months. and 60 healthy controls, matched for age and sex.

Results: This study showed that non-alcoholic fatty liver disease (NAFLD)patients had significantly elevated mean blood CK-18 and TGF-β levels compared with healthy controls before treatment. After three months of antihyperlipidemic treatment, both TGF-β and CK-18 levels were markedly decreased. NAFLD patients had elevated liver enzyme levels, ALT, AST, ALP, TSB, and GGT pre-treatment relative to the control group and showed improvement following therapy. ROC analysis found the highest diagnostic accuracy for the CK-18 (AUC = 0.962, 95% sensitivity, 86.7 %specificity, 93.3% at cutoff (6.0435) and TGF-β revealed strong differentiated performance (AUC = 0.89), sensitivity 83.3 %, specificity90 % at cutoff (358.9)

Conclusion: Patients with NAFLD showed increased fibrotic (TGF-β) and apoptotic (CK-18). Anti-hyperlipidemic medication was associated with improvement in liver enzymes and reduction of fibrosis-related biomarkers. significantly decreased liver enzyme levels.

Keywords: NAFLD, CK-18, TGF-β, Antihyperlipidemic therapy.

Introduction

Non-alcoholic fatty liver disease (NAFLD) is the most common chronic liver disease worldwide, a condition of liver disorders identified by hepatic steatosis, impacting over 5% of hepatic cells, in the absence of alcohol intake. NAFLD affects 10–30% of people globally and 30% of people in developed countries. [1,2]. NAFLD is acknowledged as a hepatic disease of metabolic syndrome, progressing from simple steatosis to nonalcoholic steatohepatitis (NASH). It may result in fibrosis, cirrhosis, and an elevated risk of HCC [3,4]. In 2020, NAFLD was reclassified as metabolic-associated fatty liver disease (MAFLD), diagnosed by hepatic steatosis and one additional criterion: obesity, type 2 diabetes mellitus (T2DM), or metabolic syndrome, including factors such as increased abdominal obesity, hyperglycemia, hypertriglyceridemia, dyslipidemia, and hypertension [5].

It consists of a range from mild steatosis to non-alcoholic steatohepatitis (NASH), potentially advancing to severe issues such as inflammation, fibrosis, cirrhosis, and HCC [4]. In NAFLD, hepatic fat accumulation results from an imbalance across multiple pathways, including impaired lipid absorption. These factors simulated hepatic de novo lipogenesis (DNL), inadequate improvement of fatty acid catabolism, and alterations to lipid export as VLDL particles [6]. The "multiple parallel hits hypothesis" explains how NAFLD turns into NASH. It says that insulin resistance, genetic effects, mitochondrial impairment, chronic low-grade inflammation, and adipose tissue dysfunction all work together to hurt the liver [7]. Biopsies may be less necessary if non-invasive biomarkers are used to evaluate the severity of NAFLD and the effectiveness of a treatment. The liver enzymes, cytokeratin 18, TGF β, and inflammatory cytokines, become more significant in statins, which could decrease NASH and lower the risk of liver fibrosis [8]. Fibrates like fenofibrate and bezafibrate could help individuals who have NASH improve their liver function and dyslipidemia. They are connected to low levels of CK18, a potential non-invasive biomarker for the early identification of NAFLD, since higher blood levels are detected in Patients with NAFLD in comparison to healthy individuals, therefore assisting in monitoring and diagnosis [9]. Research found that plasma levels of TGF-β are positively correlated with abnormal ultrasound results for steatosis. This suggests that TGF-β might be a useful biomarker for liver problems linked to obesity [10].

Materials and methods

This study was a hospital-based case-control study involving 180 participants, divided into three groups: group I consist of 60 patients diagnosed with NAFLD who were evaluated before the start of the anti-hyperlipidemic agent. Group II 60 patients diagnosed with NAFLD who received a designated antihyperlipidemic medication (e.g., statin, fibrate) for 3 months. and 60 healthy controls, matched for age and sex. This study in Kirkuk City, Iraq, from January 2025 to the end of August 2025.

Exclusion Criteria:

1- Excessive alcohol consumption.
2- Other chronic liver diseases were present.
3- Use of specific medications known to affect liver function, such as corticosteroids, Amiodarone, methotrexate, tamoxifen, valproic acid, allopurinol, antifungal medications, and synthetic estrogens
4- Pregnancy or breastfeeding.

Data and Sample Collection

Data were collected using patient interviews, clinical exams, structured questionnaires, and laboratory tests. Five milliliters of fasting venous blood were drawn from each participant in gel tubes and centrifuged at 4000 rpm for 10 minutes to separate serum. The serum from both patients and the control group had been separated and divided into two Eppendorf tubes. Liver function tests (ALT, AST, GGT, ALP, TSB) were performed immediately post serum separation, and the remaining serum was stored in a deep freezer at -20°C for further analysis of CK-18 and TGF-β. Liver function tests, including ALT, AST, TSB, ALP, and GGT, were assessed using the Cobas C311-analyzer (Roche-Germany). Serum levels of CK-18 and TGF-β were also assessed using an enzyme-linked immunosorbent assay.

Results

Table 1 summarizes a comparative analysis of the demographic, clinical, and biochemical characteristics of NAFLD patients and age-matched healthy controls. The NAFLD group showed significantly higher BMI and increased liver function parameters, including ALT, AST, GGT, ALP, and TSB, compared with the controls ($p < 0.001$). In addition, biomarkers associated with hepatocellular injury and fibrosis, CK-18, and TGF-β. ($p < 0.001$, $p < 0.05$).

Following anti-hyperlipidemic therapy, a reduction in liver function tests was observed, as well as in CK-18 and TGF-β levels, when compared to pre-treatment values.

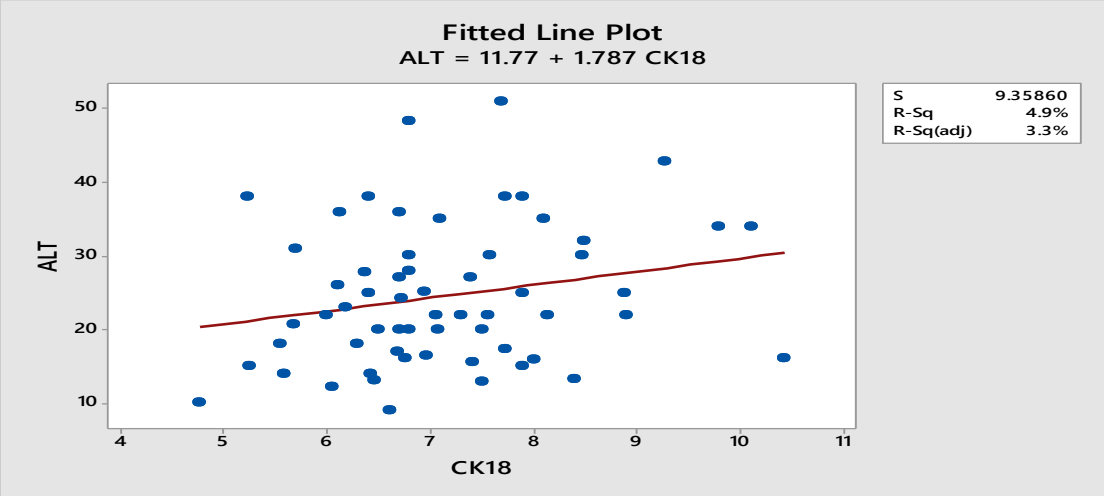
Correlation analysis revealed several statistically significant associations among the studied variables. CK-18 was positively correlated with ALT ($r = 0.221$, $p = 0.089$), both pre- and post-treatment, suggesting that elevated CK-18 levels are often associated with raised ALT levels, as illustrated by figures 1 and 2. No significant correlation between CK-18 and AST ($r = 0.02$, $p = 0.85$) before treatment. After treatment, CK-18 showed a weak positive correlation, as shown in Figure 3,4.

Receiver Operating Characteristic (ROC) curve analysis of CK -18 demonstrated the highest diagnostic accuracy with an Area Under the Curve (AUC =0.962) along with high sensitivity and specificity, indicating its potential as a non-invasive biomarker reflecting hepatocellular injury. TGF-β revealed strong differentiated performance (AUC = 0.89), and TGF-β exhibited a balanced sensitivity–specificity profile ideal for both screening and diagnostic purposes, as shown in Table 3, Figures 5 and 6.

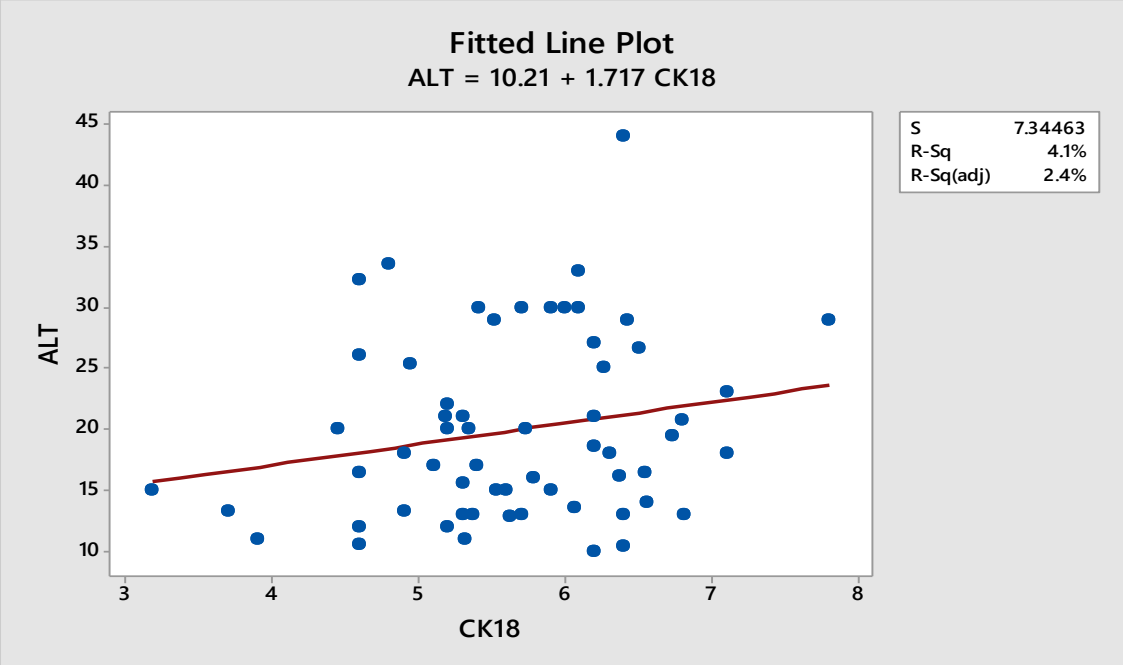
Table 1: Comparison of demographic, clinical, and biomarker profiles between patients with NAFLD and the control group.

	Patients Pre-Treatment n = (60)	Patients Post-Treatment n = (60)	Control n = (60)	P-Value
Gender				
Male	26 (43.3%)		30 (50%)	0.923
Female	34 (56.7%)		30 (50%)	

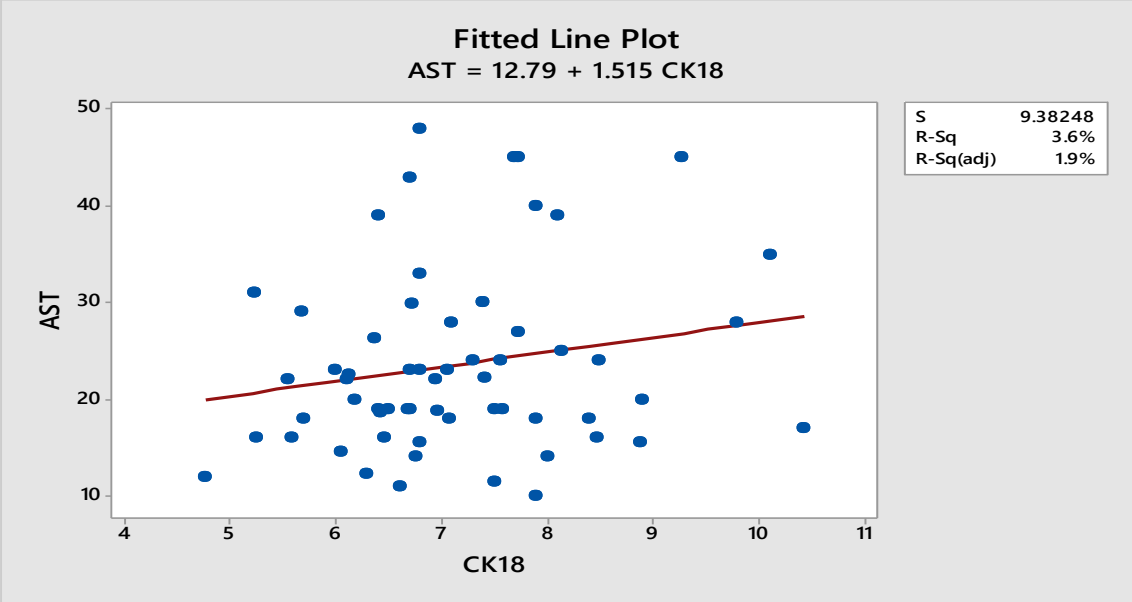
Chi-square				0.143
Age groups				
20 – 40 years	34.7± 4.9		31.8 ± 6.5	< 0.0001
40 – 60 years	48.8 ± 5.5		45.9 ± 5.1	
BMI (kg\m^2)	32.19 ± 7.98	30.3 ± 7.13	22.58 ± 2	< 0.001
ALT (U/L)	24.52 ±5.52	19.9±4.43 b	14.5 ± 2.32	< 0.001
AST (U/L)	23.60 ± 9.47	9.38 ± 7.06	14.60 ± 4.48	< 0.001
ALP(U/L)	85.2 ± 14.33	81.54 ± 12.03	78.62 ± 9.55	< 0.05
GGT (U/L)	37.08 ± 8.69	32.3 ± 6.8	30.4 ± 6.41	< 0.001
TSB (mg/dl)	0.43 ± 0.23	0.37 ± 0.18	0.32 ± 0.15	< 0.001
CK-18 ng/ml	6.1 ± 1.17	5.6 ± 0.87	4.7 ± 0.92	< 0.05
TGFβ(pg/ml)	422.2±77.4	367.6±64.3	311.7±62.6	< 0.001



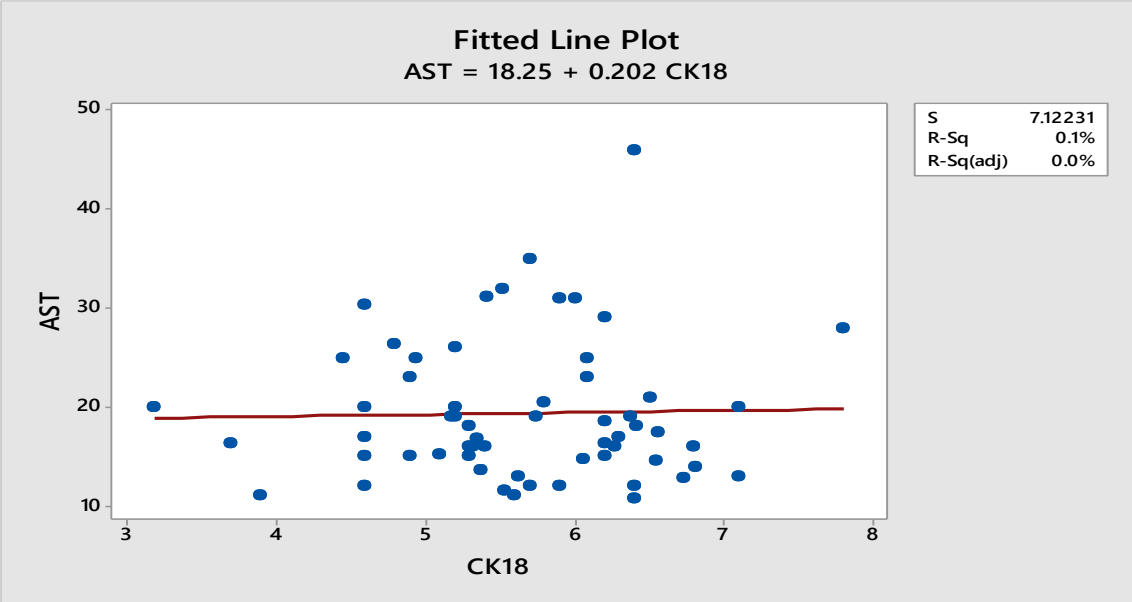
Figures (1) Correlation between CK-18 and ALT before treatment



Figures (2) Correlation between CK-18 and ALT after treatment



Figures (3) Correlation between CK-18 and AST before treatment.



Figures (4) Correlation between CK-18 and AST after treatment.

2-Effects of Anti-Hyperlipidemic Therapy on Biomarker Levels after

3 months of treatment.

Cytokeratin 18 (CK 18) levels, a marker of hepatocellular injury, showed a significant reduction in all treatment groups. The most improvement was observed with the combination group. (TGF-β) showed a significant decrease after therapy. This finding reflects a potential reduction in fibrotic and inflammatory signaling, as shown in Table 2

2: Effects of Anti-Hyperlipidemic Therapy on Biomarker Levels after treatment.

Parameter	Treatment	Pre-Treatment	Post-Treatment	Change%	P-value
TGF-β	Rosuvastatin	422.2 ± 47.4	359.6 ± 59.4	- 14.82	0.01

	Rosuvastatin/Ezetimibe	422.2 ± 47.4	370.7 ± 46.9	- 12.2	
	Fenofibrate/Rosuvastatin	422.2 ± 47.4	342.7 ± 45.7	- 18.8	
CK18	Rosuvastatin	7.135 ± 1.179	5.74 ± 0.94	- 19.5	0.01
	Rosuvastatin/Ezetimibe	7.135 ± 1.179	5.54 ± 0.91	- 22.2	
	Fenofibrate/Rosuvastatin	7.135 ± 1.179	5.36 ± 0.83	- 24.8	

3-Diagnostic Performance of Novel Biomarkers in NAFLD

Receiver Operating Characteristic curve analysis showed CK-18 showed the highest diagnostic accuracy with a cut-off of 6.0435, with a high sensitivity of 86.7 % with a specificity 93.3%, and an Area Under the Curve (AUC = 0.962), indicating its potential as a non-invasive biomarker reflecting hepatocellular injury. TGF-β also revealed strong differentiated performance with a cut-off of 358.9, and TGF-β exhibited a balanced sensitivity 83.3 %, specificity 90%, and an Area Under the Curve (AUC = 0.89), a profile ideal for both screening and diagnostic purposes. These results are summarized in Table 3 and shown in Figures 5 and 6.

Table 3: Diagnostic Performance of Novel Biomarkers in NAFLD

Marker	Cut-off	Sensitivity	Specificity	AUC	Std	95 %	p-value
TGF-β	358.9 (pg/ml)	83.3 %	90%	0.892	0.031	0.830-0.953	< 0.001
CK-18	6.0435 (ng/ml)	86.7 %	93.3%	0.962	0.015	0.932-0.991	< 0.001

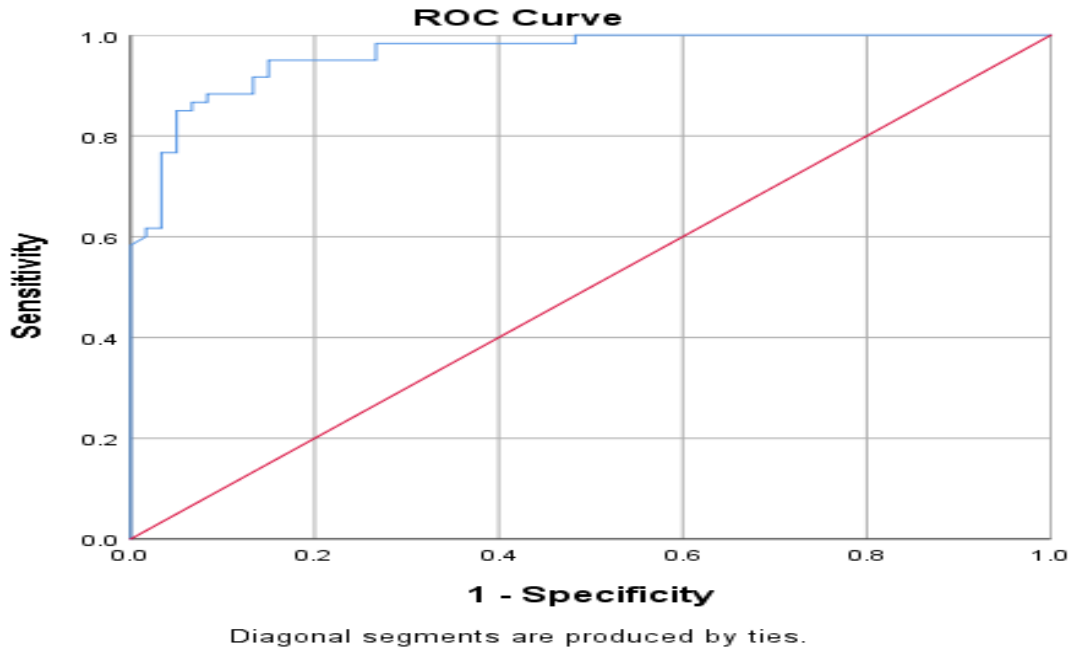


Figure 5: ROC Curve Analysis of Novel Biomarkers for NAFLD Diagnosis. CK-18 distinguishes between the pre-treatment patient from control.

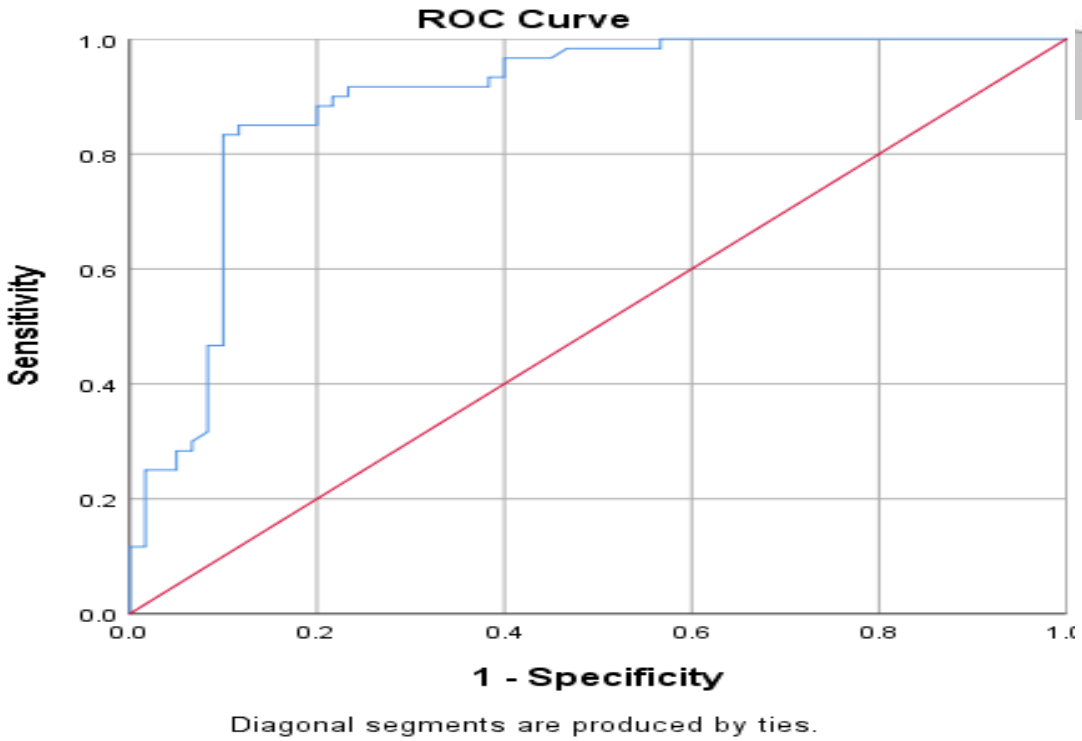


Figure 6: ROC Curve Analysis of Novel Biomarkers for NAFLD Diagnosis. TGF-β distinguishes between the pre-treatment patient from control.

Discussion

Patients with NAFLD exhibited significantly higher levels of liver function tests in comparison to the control group before treatment. Indicating hepatocellular injury and inflammation. After anti-hyperlipidemic therapy, both ALT and AST levels decreased compared to pre-treatment values [(11).] Elevated transaminase levels are correlated with hepatic lipid accumulation, lipotoxicity, oxidative stress, and mitochondrial dysfunction. cause liver cell damage and the release of transaminases into the bloodstream [(12).]

Elevated serum GGT levels in NAFLD patients are consistent with studies that demonstrated a significant association between increased GGT and increased degree of NAFLD, supporting the role of GGT as a marker of disease progression. [(13,14)]

The CK18 level is significantly elevated in NAFLD patients in comparison with controls. These results corroborate a prior study indicating that serum CK18 levels are markedly elevated in NASH. There is a strong connection between CK18, liver enzymes, and NAFLD severity. CK18 is excellent at diagnosing NASH because it is very accurate, sensitive, and specific [(15)]. CK18 is a crucial biomarker for NAFLD, especially in the detection of nonalcoholic steatohepatitis (NASH). CK18, when used with other markets, supports the assessment of treatment outcomes and the prediction of NAFLD-related complications [(16).]

.CK-18 showed a positive correlation with ALT and AST, both before and following treatment. Case-control NAFLD work shows CK-18 positively correlated with ALT, AST, and other metabolic markers; higher CK-18 accompanies higher transaminases and more severe disease [(17)]. The patients with NAFLD have markedly increased serum TGF-β levels in comparison to healthy controls, with a partial decrease seen after anti-hyperlipidemic treatment. Increased TGF-β levels are associated with lipotoxicity, leading to fat and cholesterol accumulation in hepatocytes. causing oxidative stress and disrupting cellular functions, ultimately resulting in mitochondrial and endoplasmic reticulum stress [(18)]. Recent years have seen a rise in the use of drug combinations for treating NAFLD, as clinicians fear that monotherapy may lack adequate efficacy. Key therapeutic goals include improving liver enzyme levels (ALT, AST), reducing hepatic fat accumulation, optimizing lipid profiles, and ensuring liver safety [(19).]

The study demonstrates that administering rosuvastatin to NAFLD patients, either as monotherapy or in combination with ezetimibe or fenofibrate, diminishes inflammation-related biomarkers. These findings are in agreement with prior studies that show statins lower cholesterol and also have anti-inflammatory and antioxidant effects. These effects could help treat and prevent NAFLD/NASH [(20,21)]. Ezetimibe inhibits intestinal

cholesterol absorption, reducing the cholesterol transport to the liver. Although considered safe for individuals with NAFLD, its efficacy for improving NAFLD remains debatable [22,23]. Combination therapy showed a slight improvement in all markers. The use of statins in combination with ezetimibe may contribute to the improvement of NAFLD through several mechanisms [24].

CONCLUSION

Both measured novel biomarkers (TGF-β and CK-18) were much higher in pre-treatment NAFLD patients than in healthy individuals, suggesting they may contribute to the development of NAFLD, particularly in inflammation and oxidative stress. Anti-hyperlipidemic agents markedly decreased the levels of TGF-β and CK-18. However, they remained elevated relative to controls, indicating an ongoing underlying disease after treatment. Cytokeratin-18 showed a weak positive correlation with ALT both before and after treatment.

Ethical approval

The scientific committee at the Faculty of Medicine, Tikrit University, officially approved the research protocol. Kirkuk Teaching Hospital in Kirkuk City also approved the study method and allowed the collection of patient samples. (Approval Letter Code MIDTUH11 on 12 April 2026).

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