

Biochemical and Genetic Determinants of Early Cardiovascular and Hepatic Dysfunction: A Focus on *ADIPOQ* Expression

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ABSTRACT

OBJECTIVE Non-alcoholic fatty liver disease (NAFLD) and cardiovascular disease (CVD) frequently coexist due to common metabolic dysfunctions such as insulin resistance, chronic inflammation, and dyslipidemia. Adiponectin, encoded by the *ADIPOQ* gene, is an anti-inflammatory adipokine vital for glucose and lipid regulation. Reduced adiponectin levels and certain *ADIPOQ* gene variants (e.g., rs266729, rs2241766) have been linked to both NAFLD and CVD progression. This study aims to evaluate the association between *ADIPOQ* gene expression and early biochemical markers of hepatic and cardiovascular dysfunction.

METHODS A case-control study (n = 300) was conducted in Thiruvananthapuram, Kerala, India (2022-2024), analyzing blood samples for fasting blood sugar (FBS), lipid profile, malondialdehyde (MDA), interleukin-6 (IL-6), and *ADIPOQ* expression (RT-PCR). Statistical analyses included receiver operating characteristic (ROC) curves and quantile regression using Stata 17.0.

RESULTS Cases exhibited significantly elevated FBS, triglycerides (TG), low-density lipoprotein (LDL), MDA, and IL-6, along with reduced high-density lipoprotein (HDL) and adiponectin levels ($p < 0.05$). *ADIPOQ* gene expression was markedly downregulated, particularly in individuals with both NAFLD and CVD. A positive correlation was observed between *ADIPOQ* expression and HDL, and a negative correlation with IL-6. Reduced *ADIPOQ* expression showed an association with risk in unadjusted analysis, but this association was not statistically significant after adjustment (OR = 0.0220). Nevertheless, ROC analysis indicated potential diagnostic utility.

CONCLUSIONS Reduced *ADIPOQ* expression is significantly associated with early metabolic and inflammatory markers in NAFLD and CVD. These findings highlight the potential utility of *ADIPOQ* as a biomarker for early detection and targeted prevention strategies in at-risk populations.

KEYWORDS *ADIPOQ* gene expression, adiponectin, non-alcoholic fatty liver disease, cardiovascular disease, inflammation

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INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) and cardiovascular disease (CVD) are among the leading chronic health burdens globally; both are closely linked to impaired metabolic balance. They frequently coexist due to shared pathophysiological drivers such as insulin resistance, low-grade chronic inflammation, obesity, and dyslipidemia (1). Early detection of molecular disruptions underlying both hepatic and cardiovascular pathology is critical to preventing progression to irreversible stages, including myocardial infarction or cirrhosis.

Adiponectin is a key anti-inflammatory and insulin-sensitizing adipokine encoded by the *ADIPOQ* gene, playing a central role in linking NAFLD and CVD. It regulates crucial metabolic processes such as glucose and lipid metabolism and supports vascular endothelial function. Notably, low circulating adiponectin levels are frequently observed in individuals with NAFLD and CVD, sometimes even before overt symptoms appear, suggesting a role in the early onset of these diseases (2). The *ADIPOQ* gene, located on chromosome 3q27, is tightly regulated at both transcriptional and post-transcriptional levels. Genetic variants, particularly single nucleotide polymorphisms (SNPs) such as rs266729 and rs2241766, have been associated with altered adiponectin levels and an increased risk of metabolic dysfunction. These SNPs may influence gene expression or protein activity, thereby diminishing adiponectin's protective effects and facilitating the development of NAFLD and CVD (3).

Biochemically, reduced adiponectin levels are closely linked to a pro-atherogenic lipid profile, heightened systemic inflammation, and oxidative stress, all of which synergistically contribute to endothelial dysfunction, hepatic fat accumulation, and fibrosis. One of the key indicators of metabolic disruption, FBS provides a direct measure of glucose regulation. Elevated FBS often reflects insulin resistance, a common pathophysiological driver of both NAFLD and CVD. Persistent hyperglycemia promotes endothelial damage and hepatic steatosis by enhancing *de novo* lipogenesis and impairing lipid oxidation (4). Dyslipidemia, manifested as elevated triglycerides (TG), total cholesterol (TC), and low-density lipoprotein (LDL) levels, coupled with decreased high-density li-

poprotein (HDL), is a hallmark of both CVD and NAFLD. Notably, oxidized LDL (ox-LDL) serves as a potent inflammatory stimulus, contributing to atherogenesis and vascular dysfunction (5). Malondialdehyde (MDA), a well-established biomarker of lipid peroxidation and oxidative stress, is significantly elevated in patients with NAFLD and CVD. High MDA levels reflect an increased oxidative burden and indicate ongoing endothelial injury and hepatic dysfunction (6). IL-6, a key pro-inflammatory cytokine, acts at the intersection of immune dysregulation and metabolic stress. Elevated IL-6 levels are associated with exacerbated insulin resistance, hepatic inflammation, and adverse cardiovascular outcomes, underscoring its central role in the pathogenesis of both NAFLD and CVD (7).

The complex interplay between biochemical markers and genetic determinants is pivotal in the early development of cardiovascular and hepatic dysfunction. Gaining insights into these relationships is essential for improving risk assessment and advancing personalized treatment strategies. This study investigates the association between *ADIPOQ* gene expression and early biochemical markers of hepatic and cardiovascular dysfunction. By unravelling these molecular and metabolic connections, the study seeks to contribute to the development of targeted, precision-based interventions for the prevention and effective management of these closely linked metabolic disorders.

METHODS

Study design and ethical considerations

This case-control study was conducted over a period of two years, from December 2022 to December 2024, adhering strictly to the ethical principles outlined in the Declaration of Helsinki (1964) and its subsequent revisions. Ethical approval was obtained from the Institutional Ethics Committee of Genetika, Centre for Advanced Genetic Studies (Reference No: 09/2022/IECG). Written informed consent was obtained from all participants before recruitment, ensuring full voluntary participation and confidentiality.

Study setting and participant recruitment

Participants were recruited from multiple sources, including the Hridayalaya Heart and the Robotic Research Centre, Lords Hospital, World

Heart Day Medical Camp, and several affiliated blood collection centers across Thiruvananthapuram, Kerala, India. All laboratory investigations, including biochemical and molecular analyses, were conducted at the Genetika, Centre for Advanced Genetic Studies, ensuring methodological consistency.

Participant characteristics

A total of 300 individuals (age 18–45 years) were enrolled: 150 individuals with NAFLD and/or cardiovascular risk factors (cases), and 150 healthy controls, age- and sex-matched. Recruitment from the same geographic region minimized selection bias (Table 1).

Data collection and sample processing

Detailed demographic, lifestyle, and clinical data were collected using pre-validated structured questionnaires. Approximately 6–8 mL of fasting venous blood was drawn from each participant under aseptic conditions into both plain and EDTA-coated tubes for downstream biochemical and molecular assessments.

Biochemical analyses

Fasting blood glucose levels were estimated using the glucose oxidase-peroxidase (GOD-POD) enzymatic method. The serum lipid profile, including TG, TC, HDL, and LDL, was analyzed using standard enzymatic colorimetric assays with commercially available diagnostic kits. Lipid peroxidation was quantified by measuring serum MDA levels using a competitive enzyme-linked

immunosorbent assay (ELISA). Additionally, systemic inflammation was assessed by measuring serum IL-6 concentrations using a high-sensitivity ELISA kit according to the manufacturer's protocol.

Gene expression analysis

Total RNA was extracted from whole blood using a commercially available RNA extraction kit, and its quantity and purity were assessed using bio spectrophotometry. Complementary DNA (cDNA) was synthesized from 50 ng of RNA using reverse transcriptase. Real-time quantitative PCR (qPCR) was conducted using the CFX Opus 96 System (Bio-Rad, Hercules, CA, USA) and SYBR Green Master Mix (Lot No: 103024 RMM). The expression of the *ADIPOQ* gene was evaluated using gene-specific primers:

Forward: 5'-CAGGCCGTGATGGCAGAGATG-3'

Reverse: 5'-GGTTTCACCGATGTCTCCCTTAG-3'

Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was employed as the internal house-keeping gene. The primer sequences were as follows:

Forward: 5'-CCATGGAGAAGGCTGGGG-3'

Reverse: 5'-CAAAGTTGTCATGGATGACC-3'

Relative gene expression levels were calculated using the $2^{-\Delta\Delta CT}$ method.

Sample size determination

The minimum sample size was estimated using the formula for comparing two independent means:

$$N = \frac{(Z\alpha/2 + Z\beta)^2 \times (S_1^2 + S_2^2)}{(\mu d)^2}$$

Table 1. Participant characteristics and eligibility criteria

Item	Case group (NAFLD and/or CV risk, n = 150)	Control group (healthy, n = 150)
Age range	18–45 years	18–45 years
Sex matching	Matched with controls	Matched with cases
Clinical condition	Diagnosed NAFLD and/or established CV risk factors	No known chronic/systemic illnesses
Inclusion criteria	Confirmed NAFLD Presence of CV risk conditions	Healthy individuals without chronic/systemic disease
Exclusion criteria	- Age < 18 or > 45 - Chronic systemic illnesses - Malignancies - Long-term pharmacological treatment - Prior exposure to ionizing radiation, chemotherapy, or mutagenic agents	Same as case group

NAFLD, non-alcoholic fatty liver disease; CV risk, cardiovascular risk factors

Where:

- S1 = Standard deviation in group 1 (NAFLD)
- S2 = Standard deviation in group 2 (controls)
- μd = Expected mean difference between the

two groups

- α = significance level (type I error)
- β = type II error (1 - Power)

Based on available prevalence estimates and to ensure adequate statistical power, the final sample size was fixed at 150 cases and 150 controls.

Statistical analysis

Descriptive statistics were used to summarize demographic, clinical, and laboratory data. Independent t-tests were applied to compare mean values between case and control groups for variables with normal distribution. Receiver operating characteristic (ROC) curve analysis was employed to assess diagnostic performance metrics, including the area under the curve (AUC), sensitivity, and specificity. Quantile regression was used to evaluate the associations between *ADIPOQ* gene expression ($2^{-\Delta\Delta CT}$) and clinical parameters, while accounting for potential heteroscedasticity in the data. For variables violating the assumption of normality, bootstrap methods were applied to derive robust *p*-values. All statistical analyses were performed using Stata version 17.0 (StataCorp LLC, College Station, TX, USA).

RESULTS

Participant characteristics

The study enrolled 300 young adults aged 18-45, including 150 individuals with clinically diagnosed CVD and/or NAFLD and 150 age- and sex-matched healthy controls. Comprehensive

demographic, anthropometric, physiological, clinical, and lifestyle data were collected, as well as biochemical and genetic analyses.

Biochemical profiles

Table 2 summarizes the descriptive statistics of biochemical parameters. FBS levels were significantly higher in cases (mean: 129.1 mg/dL) compared to controls (90.3 mg/dL), with a broader range suggesting higher prevalence of hyperglycemia. Serum TG were also elevated in cases (147.1 mg/dL vs. 114.1 mg/dL in controls), with greater variability indicating frequent dyslipidemia. TC levels were markedly higher in cases (232.8 mg/dL) than in controls (182.2 mg/dL), while HDL cholesterol levels were substantially lower in cases (38.2 mg/dL) compared to controls (54.3 mg/dL). LDL cholesterol followed a similar pattern, with elevated mean levels in cases (165.1 mg/dL) versus controls (105.1 mg/dL).

Inflammatory and oxidative stress markers

Cases exhibited significantly higher MDA levels (4.3 mg/L vs. 1.7 mg/L in controls), indicating increased oxidative stress. IL-6 levels were also elevated in cases (7.9 pg/mL) compared to controls (3.8 pg/mL), highlighting systemic inflammation. Conversely, adiponectin gene (*ADIPOQ*) expression was markedly reduced in cases (0.59 μ g/mL) relative to controls (1.01 μ g/mL), suggesting impaired protective metabolic regulation.

Comparative statistical analysis

Table 3 presents the results of bootstrap t-tests with Levene's test for variance. Significant differences were observed for fasting blood

Table 2. Descriptive statistics of the biochemical parameters for cases and controls

Variable name	Cases		Controls	
	Min - Max	Mean $\pm$ SD	Min - Max	Mean $\pm$ SD
Fasting blood sugar (mg/dL)	75.0-261.0	129.1 $\pm$ 30.5	70.7-134.0	90.3 $\pm$ 13.2
Serum triglycerides (mg/dL)	90.6-220.0	147.1 $\pm$ 30.5	80.6-156.1	114.1 $\pm$ 21.1
Serum total cholesterol (mg/dL)	148.0-358.0	232.8 $\pm$ 35.1	147.1-245.0	182.2 $\pm$ 20.5
Serum HDL cholesterol (mg/dL)	24.0-68.0	38.2 $\pm$ 7.1	30.2-79.0	54.3 $\pm$ 8.3
Serum LDL cholesterol (mg/dL)	57.6-286.4	165.1 $\pm$ 33.2	61.6-179.8	105.1 $\pm$ 24.1
Malondialdehyde (nmol/mL)	1.24-6.2	4.3 $\pm$ 1.2	0.5-4.0	1.7 $\pm$ 0.6
Interleukin-6 (pg/mL)	0.126-15.2	7.9 $\pm$ 2.8	0.126-12.1	3.8 $\pm$ 2.6
Adiponectin encoding gene (<i>ADIPOQ</i>) ($2^{-\Delta\Delta CT}$)	0.066-1.60	0.59 $\pm$ 0.31	0.350-1.66	1.01 $\pm$ 0.2

All values are presented as mean $\pm$ standard deviation (SD). Min-max represents the range of values.

Statistical comparison between cases and controls was performed using Student's t-test for normally distributed variables or Mann-Whitney U test for non-normally distributed variables.

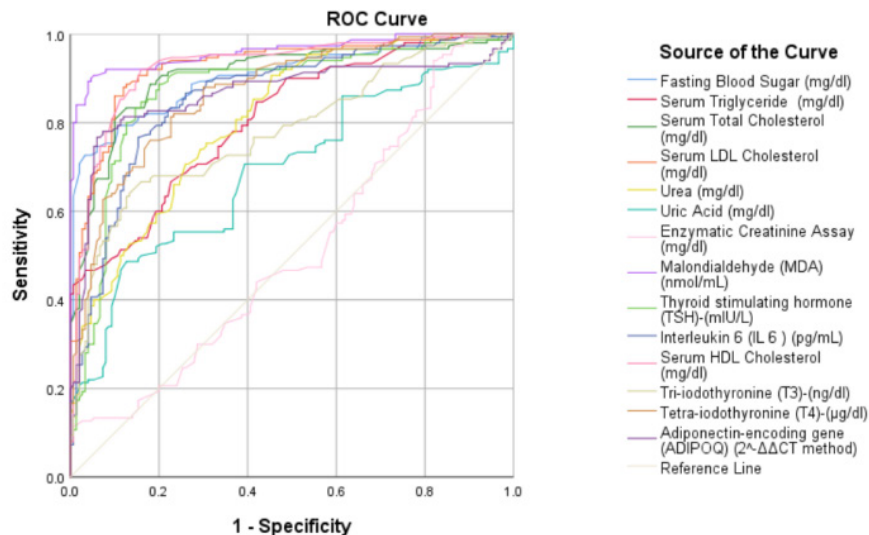
HDL, high-density lipoprotein; LDL, low-density lipoprotein

Table 3. Comparative analysis of clinical investigation parameters between cases and control (Levene's test with Bootstrap t-test)

Variable name	Levene's Test p-value	Bootstrap t-test p-value
Fasting blood sugar (mg/dL)	$p < 0.01$	$p < 0.01$
Serum triglyceride (mg/dL)	$p < 0.01$	$p < 0.01$
Serum total cholesterol (mg/dL)	$p < 0.01$	$p < 0.01$
Serum HDL cholesterol (mg/dL)	0.155	$p < 0.01$
Serum LDL cholesterol (mg/dL)	0.002	$p < 0.01$
Malondialdehyde (MDA) (nmol/mL)	$p < 0.01$	$p < 0.01$
Interleukin 6 (IL 6) (pg/mL)	0.802	$p < 0.01$
Adiponectin-encoding gene (ADIPOQ) ($2^{-\Delta\Delta CT}$)	$p < 0.01$	$p < 0.01$

Data presentation: All biochemical values in the study are presented as mean $\pm$ standard deviation (SD). Levene's Test was used to assess the equality of variances between cases and controls. Bootstrap p -values were calculated to assess robust significance, accounting for non-normality where applicable. A p -value < 0.05 was considered statistically significant

HDL, high-density lipoprotein; LDL, low-density lipoprotein; MDA, malondialdehyde

**Figure 1.** Receiver operating characteristic (ROC) curve for various clinical parameters

sugar, TG, TC, HDL cholesterol, LDL cholesterol, MDA, IL-6, and ADIPOQ expression (all $p < 0.01$). HDL cholesterol remained consistently lower in cases despite equal variance, while LDL cholesterol showed significance with unequal variance. These findings reinforce the roles of hyperglycemia, dyslipidemia, oxidative stress, and inflammation in disease status.

Diagnostic utility of biomarkers

Figure 1 illustrates ROC curves for the biochemical and genetic markers. MDA demonstrated the highest discriminative power (AUC = 0.9605), followed by LDL cholesterol (AUC = 0.9262), HDL cholesterol (AUC = 0.9259), TC (AUC = 0.9063), and FBS (AUC = 0.9046). IL-6 (AUC =

0.8589) and ADIPOQ expression (AUC = 0.8654) showed strong diagnostic performance, while TG (AUC = 0.8087) demonstrated moderate predictive capacity.

Correlation analyses

Scatter plots (Figures 2-8) illustrate associations between ADIPOQ expression and biochemical markers. Negative correlations were observed with fasting blood sugar, TG, TC, LDL cholesterol, MDA, and IL-6, indicating reduced ADIPOQ expression alongside metabolic and inflammatory disturbances. In contrast, a positive correlation was observed with HDL cholesterol, suggesting its protective role in maintaining adiponectin activity.

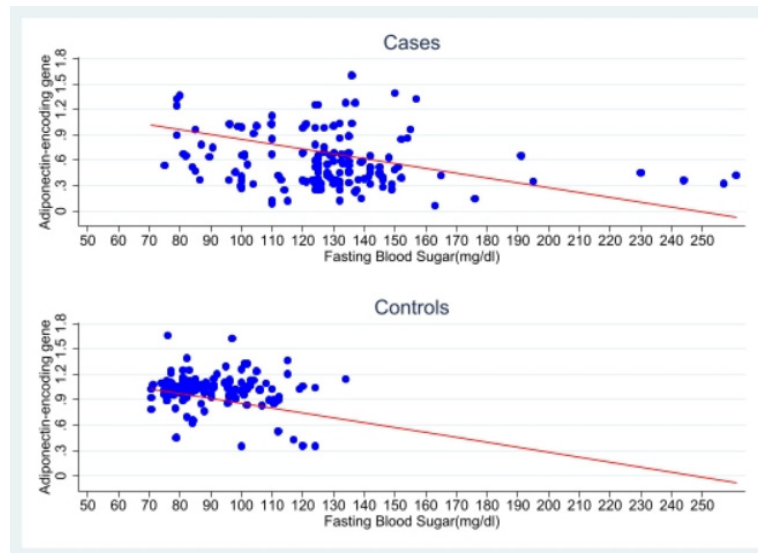


Figure 2. Scatterplot of adiponectin-encoding gene expression and fasting blood sugar in cases and controls

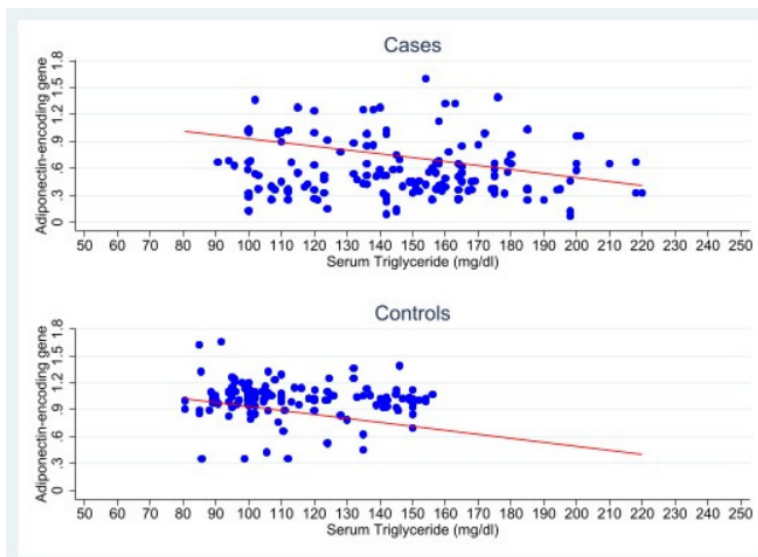


Figure 3. Scatterplot of adiponectin-encoding gene expression and serum triglyceride in cases and controls

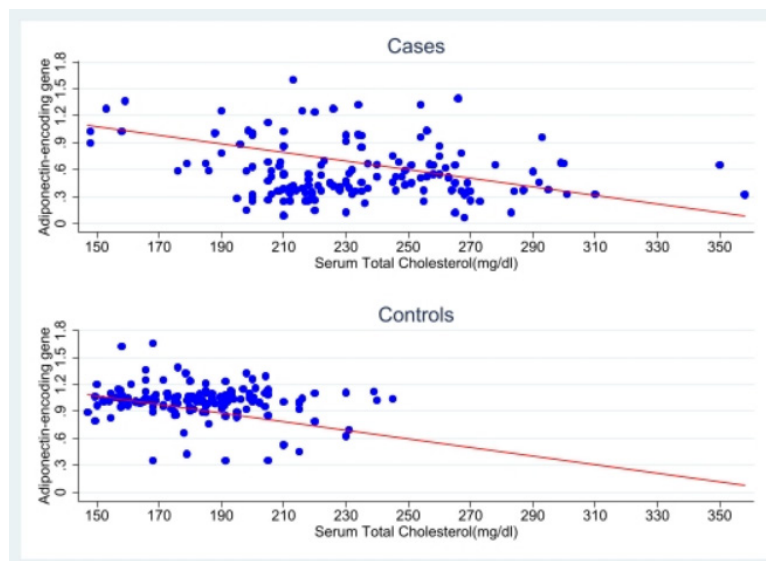


Figure 4. Scatterplot of adiponectin-encoding gene expression and serum total cholesterol in cases and controls

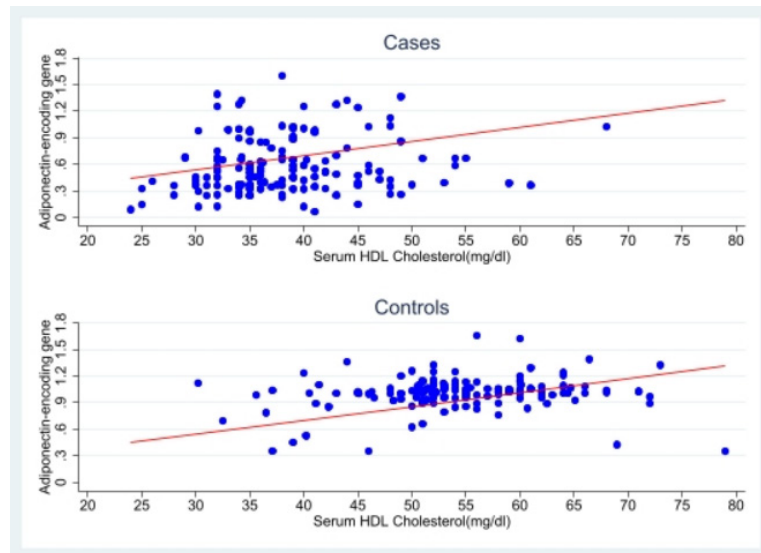


Figure 5. Scatterplot of Adiponectin-encoding gene expression and serum HDL cholesterol in cases and controls

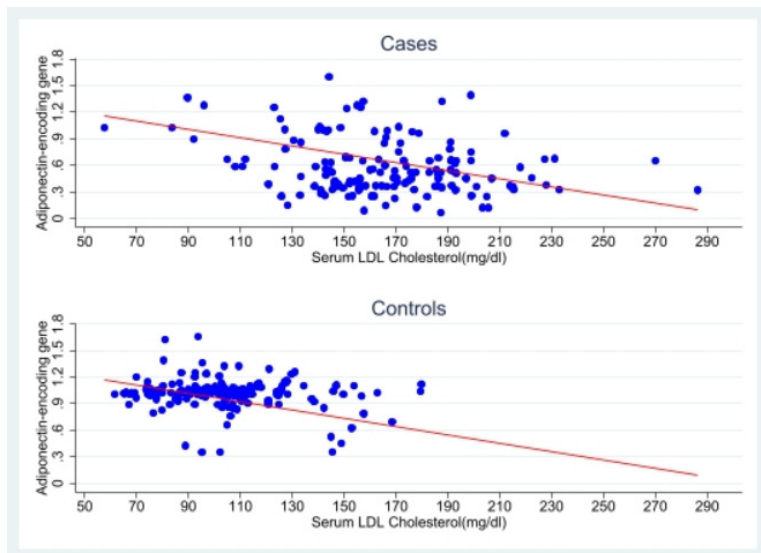


Figure 6. Scatterplot of adiponectin-encoding gene expression and serum LDL cholesterol in cases and controls

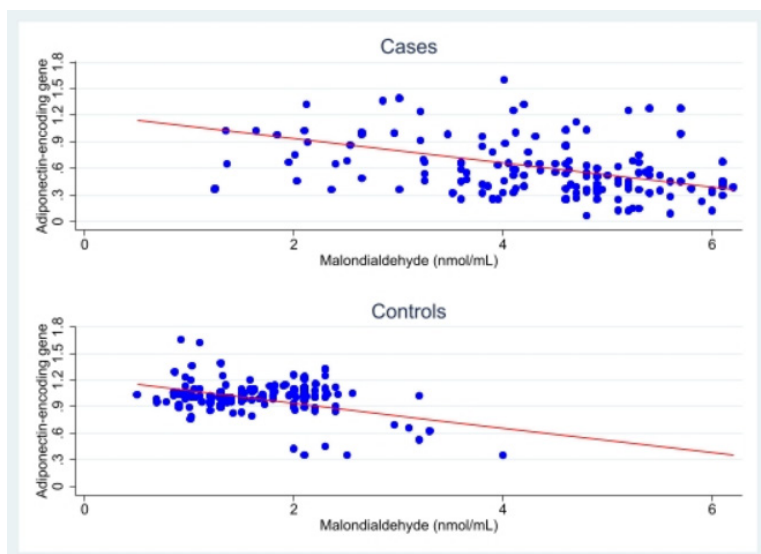


Figure 7. Scatterplot of adiponectin-encoding gene expression and malondialdehyde in cases and controls

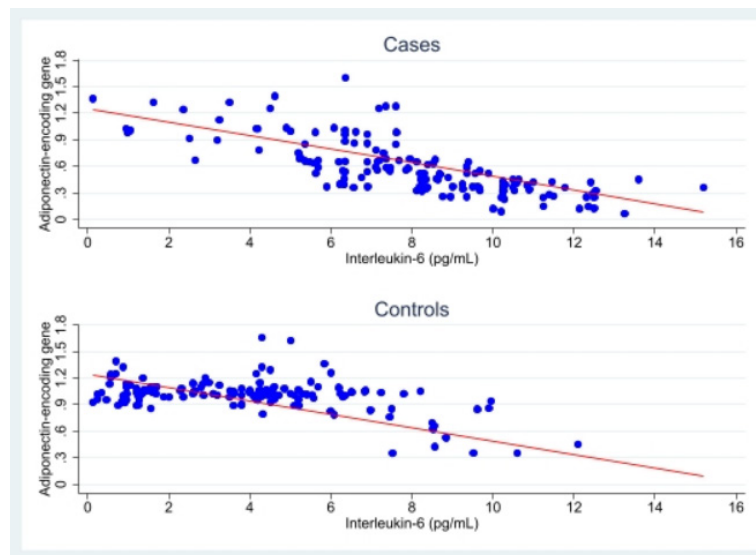


Figure 8. Scatterplot of adiponectin-encoding gene expression and interleukin 6 in cases and controls

Table 4. Comparative analysis of clinical investigation parameters between cases and controls

Variable	Model 1 OR [95%CI]	Model 2 OR [95%CI]
Adiponectin-encoding gene (<i>ADIPOQ</i>)	0.0034 [0.0010-0.0114]***	0.0220 [0.00004-11.6542]
Fasting blood sugar (mg/dL)	1.0995 [1.0781-1.1214]***	1.2089 [1.0667-1.3701]***
Serum triglycerides (mg/dL)	1.0473 [1.0358-1.0590]***	1.0185 [0.9769-1.0618]
Serum HDL cholesterol (mg/dL)	0.7861 [0.7480-0.8263]***	0.7674 [0.6369-0.9248]***
Serum LDL cholesterol (mg/dL)	1.0712 [1.0558-1.0868]***	1.0002 [0.9619-1.0401]
Malondialdehyde (MDA) (nmol/mL)	11.5491 [6.4634-20.6362]***	29.1660 [3.5853-237.2580]***
Interleukin-6 (pg/mL)	1.6907 [1.5034-1.9014]***	0.2859 [0.1122-0.7284]***

Model 1: Unadjusted logistic regression; Model 2: Adjusted for relevant covariates.

Significance indicators: *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$. All biochemical variables were presented as mean $\pm$ SD in descriptive analyses.

OR, odds ratio, 95%CI, 95% confidence interval; HDL, high-density lipoprotein; LDL, low-density lipoprotein; MDA, malondialdehyde

Logistic regression models

Table 4 summarizes binary logistic regression results. In unadjusted models, *ADIPOQ* expression showed a strong inverse association with disease status (OR = 0.0034), but this lost statistical significance after adjustment (OR = 0.0220). FBS remained an independent predictor in both models (OR = 1.0995 unadjusted; OR = 1.2089 adjusted). HDL cholesterol consistently exhibited a protective effect (OR = 0.7861 and 0.7674). TG and LDL cholesterol were significant in unadjusted models but lost significance after adjustment, suggesting confounding.

Notably, MDA was a powerful independent predictor, with odds ratios of 11.5491 (unadjusted) and 29.1660 (adjusted), both highly significant. Interestingly, IL-6 showed a positive association in the unadjusted model (OR = 1.6907) but reversed

to a significant inverse association after adjustment

(OR = 0.2859), indicating complex interactions with other predictors.

DISCUSSION

CVDs continue to be a significant global health concern, contributing significantly to morbidity and mortality rates across populations. In recent years, adiponectin, an adipocyte-derived cytokine encoded by the *ADIPOQ* gene, has garnered attention for its pivotal role in regulating metabolic homeostasis, insulin sensitivity, and inflammatory responses, all of which are crucial to the pathogenesis of both CVD and metabolic disorders. In parallel, NAFLD has emerged as a prevalent liver condition, particularly among younger individuals, driven by increasing rates of obesity, sedentary

lifestyles, and insulin resistance. NAFLD is not only a hepatic disorder but also a significant risk factor for cardiovascular complications, given the shared pathophysiological mechanisms, including systemic inflammation, oxidative stress, and dysregulated lipid metabolism.

This study was designed to explore the differential expression of the *ADIPOQ* gene and its correlation with biochemical and clinical parameters in individuals affected by NAFLD and/or CVD. By focusing on young adults, this research highlights an at-risk population where early genetic and biochemical profiling could inform targeted preventive strategies.

Previous literature has reported associations between alterations in the *ADIPOQ* gene and specific metabolic diseases. For example, Saki et al. and Gruzdeva et al. evaluated *ADIPOQ* gene expression in the context of either liver-related or cardiovascular conditions, demonstrating that altered adiponectin levels are linked to disease severity and progression (8, 9). However, integrated studies examining *ADIPOQ* expression in individuals simultaneously exhibiting both NAFLD and CVD remain scarce.

Błażejewska et al. contributed to this field by investigating the epigenetic regulation of *ADIPOQ* via DNA methylation in patients with NAFLD, revealing its role in suppressing adiponectin expression and promoting liver fat accumulation (10). De et al. identified a genetic protective factor, the G allele of rs17300539, in the *ADIPOQ* gene, which was associated with reduced risk of NAFLD complications, likely due to its influence on adiponectin transcriptional activity (11). Furthermore, the rs1501299 polymorphism, another well-studied variant in the *ADIPOQ* gene, has been consistently associated with an increased risk of NAFLD, suggesting a genetic predisposition to disease susceptibility.

Together, these findings underscore the need for comprehensive studies examining the expression and regulation of the *ADIPOQ* gene across overlapping metabolic conditions, such as NAFLD and CVD. The current research aims to bridge this gap by offering insights into how *ADIPOQ* expression interacts with inflammatory and metabolic biomarkers in dual-pathology cases, potentially guiding future diagnostic and therapeutic approaches.

The present study revealed significant differences in biochemical and molecular parameters between cases and healthy controls, emphasizing the metabolic and inflammatory derangements associated with cardiovascular and hepatic dysfunction. FBS levels were markedly elevated in cases, with a mean of 129.1 mg/dL compared to 90.3 mg/dL in controls. The broader range observed among cases (75.0–261.0 mg/dL) indicates a higher prevalence of hyperglycemia, a known contributor to endothelial dysfunction and the development of atherosclerosis (Table 2). This finding aligns with previous studies that have identified impaired glucose regulation as a significant metabolic abnormality in individuals with cardiovascular pathology as well as its role in hepatic steatosis by promoting insulin resistance (12).

Serum TG levels were considerably higher in cases, with a mean of 147.1 mg/dL compared to 114.1 mg/dL in controls. The broader range observed in cases (90.6–220.0 mg/dL) reflects increased variability and the presence of dyslipidemia, a well-established risk factor for both cardiovascular complications and fatty liver disease (Table 2). Elevated TG has been linked to impaired hepatic lipid metabolism and increased risk of atherosclerosis, supporting the hypothesis of overlapping mechanisms in early cardiohepatic dysfunction (13).

TC was significantly elevated in cases (mean: 232.8 mg/dL) compared to controls (182.2 mg/dL), with values in cases reaching as high as 358.0 mg/dL (Table 2). Such elevated levels are indicative of systemic lipid imbalance and contribute to the formation of plaques and liver fat accumulation (14). In contrast, serum HDL cholesterol levels, which offer protective effects against both vascular and hepatic injury, were considerably lower in cases (38.2 mg/dL) compared to controls (54.3 mg/dL). The reduced HDL levels further emphasize a loss of protective lipid regulation in individuals at risk (Table 2).

LDL cholesterol levels were also significantly increased in cases, with a mean of 165.1 mg/dL versus 105.1 mg/dL in controls. The upper limit of LDL in cases reached 286.4 mg/dL, reinforcing its critical role in atherogenesis and possibly in hepatic inflammation through lipotoxicity (Table 2). This dyslipidemic profile, in conjunction with elevated glucose levels, suggests a synergistic

burden on both the cardiovascular and hepatic systems.

Oxidative stress and inflammation—two central processes in the pathogenesis of both CVD and liver dysfunction—were reflected by elevated levels of MDA and IL-6 in cases. MDA levels averaged 4.3 mg/L in cases compared to 1.7 mg/L in controls, indicating enhanced lipid peroxidation and oxidative damage (Table 2). Similarly, IL-6 levels were significantly higher in cases (7.9 pg/mL) compared to controls (3.8 pg/mL), indicating persistent low-grade systemic inflammation (Table 2). These biochemical perturbations are consistent with previous findings that link oxidative and inflammatory stress to endothelial damage and hepatocellular injury (15, 16).

Significantly, the expression of the *ADIPOQ* gene, which encodes adiponectin, a key regulator of metabolic balance and anti-inflammatory protection, was significantly reduced in the case group (mean: 0.59 µg/mL) relative to controls (1.01 µg/mL) (Table 2). Adiponectin deficiency has been strongly associated with insulin resistance, dyslipidemia, and inflammatory conditions affecting both the heart and liver. The inverse relationship observed between adiponectin levels and other metabolic and inflammatory markers in this study supports its role as a protective factor in cardiohepatic health (17, 18). Its reduced expression in cases highlights a potential early genetic signal of metabolic distress.

Collectively, these results underscore the interrelated nature of metabolic dysfunction, systemic inflammation, oxidative stress, and genetic regulation via *ADIPOQ* expression in the development of early cardiovascular and hepatic diseases. These findings not only validate adiponectin as a crucial biomarker of dual-organ dysfunction but also underscore the importance of integrating biochemical and molecular parameters to predict disease onset and progression.

The comparative analysis of clinical investigation parameters (Table 3) revealed significant biochemical and molecular alterations between cases and controls, underscoring their relevance in the early stages of cardiovascular and hepatic dysfunction. FBS levels were significantly higher in cases ($p < 0.01$), reinforcing the association between hyperglycemia and disease risk. Similarly, serum TG, TC, and LDL cholesterol were markedly

elevated (all $p < 0.01$). In contrast, HDL cholesterol levels were significantly reduced ($p < 0.01$), reflecting atherogenic dyslipidemia commonly linked to cardiovascular and hepatic pathologies. Metabolic indicators such as oxidative stress, as evidenced by increased MDA levels ($p < 0.01$), further highlight the pathophysiological burden in affected individuals.

Elevated IL-6 levels in cases ($p < 0.01$) confirmed heightened systemic inflammation. Most notably, *ADIPOQ* gene expression was significantly downregulated in cases ($p < 0.01$), emphasizing the diminished protective role of adiponectin in metabolic homeostasis and its potential as a key molecular marker for early cardiohepatic dysfunction.

The present study introduces a novel diagnostic perspective by evaluating the ROC performance of biochemical and genetic markers (Figure 1) in distinguishing young adults with NAFLD and/or CVD. MDA showed the highest accuracy (AUC = 0.9605), followed by LDL, HDL, TC, and FBS (all AUC > 0.90), indicating excellent diagnostic potential. Notably, *ADIPOQ* gene expression (AUC = 0.8654) and IL-6 (AUC = 0.8589) also demonstrated strong discriminatory power, highlighting their roles in metabolic and inflammatory pathways. These findings underscore the novelty of integrating oxidative, metabolic, and genetic markers for early risk identification.

In the present study, we observed a consistent and statistically significant negative correlation between adiponectin-encoding gene (*ADIPOQ*) expression and various metabolic, oxidative, and inflammatory markers, including FBS, serum TG, TC, LDL cholesterol, MDA, and IL-6. In contrast, a positive correlation was noted with HDL cholesterol levels. These findings, as illustrated in Figures 2–8, suggest that diminished *ADIPOQ* expression is associated with worsening metabolic and inflammatory profiles in both cases and controls, with a more pronounced pattern observed in affected individuals. The inverse relationship between *ADIPOQ* and FBS, TG, and LDL supports the role of adiponectin in enhancing insulin sensitivity and regulating lipid metabolism, consistent with prior evidence indicating its protective effect against metabolic syndrome and atherosclerosis (19). Moreover, the observed decline in *ADIPOQ* expression with increasing MDA and IL-6 levels

emphasizes its antioxidative and anti-inflammatory functions, which may be compromised in individuals at higher cardiometabolic risk.

The logistic regression analysis highlights key biochemical predictors of disease status (Table 4). *ADIPOQ* expression showed a strong inverse association in the unadjusted model, although it lost significance after adjustment. FBS remained a significant independent risk factor, while HDL cholesterol consistently demonstrated a protective effect. TG and LDL were significant only before adjustment, suggesting potential confounding. Notably, MDA emerged as a strong independent predictor, emphasizing the role of oxidative stress. Interestingly, IL-6 exhibited a reversed association after adjustment, indicating complex interactions with other variables. These findings underscore the multifactorial nature of disease risk, involving metabolic, oxidative, and inflammatory pathways.

The novelty of our findings lies in the comprehensive gene-level analysis of *ADIPOQ* in relation to a broad panel of clinical parameters among young adults at risk for CVD and NAFLD. While previous studies have focused on circulating adiponectin levels, only limited research has explored its gene expression profile in this demographic, particularly in the context of combined metabolic and inflammatory dysregulation. Our study is among the first to highlight the potential of *ADIPOQ* gene expression as a molecular biomarker reflecting early changes in glucose metabolism, lipid imbalance, oxidative stress, and inflammation in a high-risk population. The observed associations reinforce the hypothesis that reduced adiponectin activity may be a central mechanism linking metabolic dysfunction and systemic inflammation to early cardiovascular and hepatic pathogenesis. These insights underscore the relevance of *ADIPOQ* expression not only as a marker of disease presence but also as a potential target for early intervention strategies aimed at mitigating long-term cardiometabolic complications.

CONCLUSIONS

This study highlights significant biochemical and genetic differences between individuals with NAFLD and/or cardiovascular risk and healthy controls in a young adult population. Elevated levels of fasting blood sugar, TG, TC, LDL, MDA,

and IL-6, alongside reduced HDL and adiponectin gene (*ADIPOQ*) expression, underscore the metabolic and inflammatory disturbances commonly associated with these conditions, notably, the downregulation of *ADIPOQ* gene expression in cases suggests a diminished protective role of adiponectin, which may contribute to the development and progression of metabolic and inflammatory pathologies. The study emphasizes the relevance of integrating biochemical and molecular profiling for early identification and risk stratification in young adults at risk for NAFLD and/or cardiovascular diseases.

This study has certain limitations that should be acknowledged. First, its cross-sectional design restricts the ability to establish causal relationships between *ADIPOQ* expression, biochemical parameters, and disease outcomes. Second, the relatively modest sample size, although adequately powered for the primary analyses, may limit the generalizability of findings to broader or more diverse populations. Third, as the study population was restricted to young adults aged 18–40, the results may not fully capture the effects of *ADIPOQ* dysregulation across different age groups. Additionally, unmeasured confounding factors, such as dietary intake, physical activity, and genetic variations beyond *ADIPOQ*, could have influenced the observed associations. Future longitudinal and multi-center studies incorporating larger, ethnically diverse cohorts and broader genetic profiling are warranted to validate and extend these findings.

Future research should explore longitudinal designs to validate adiponectin as a predictive biomarker and investigate potential therapeutic interventions aimed at restoring its expression and function. Early screening and targeted lifestyle modifications may prove pivotal in mitigating long-term complications in this vulnerable population.

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CONFLICTS OF INTEREST

There was no conflict of interest in this manuscript.

AUTHOR CONTRIBUTIONS

R.R.: conceptualization, study design, data collection, experimental work, statistical analysis, interpretation of results, and manuscript drafting; A.J.: Supervision, guidance in study design and methodology, critical revision of the manuscript, and corresponding author responsibilities; V.S.: Support in experimental design, validation of laboratory techniques, and manuscript review; P.K.: Contributed to quality control measures; V.M.G., S.k.K.S.: participant recruitment, clinical data collection, and data curation; S.S., M.K, S. J.P., J.P.J., R.K.S., S.P.: technical support in sample processing, laboratory work, and data management; D.R.D.: senior supervision, statistical validation, manuscript refinement, and corresponding author responsibilities.

All authors read and approved the final manuscript.

DATA AVAILABILITY STATEMENT

The data supporting the findings of this study are available from the corresponding author upon reasonable request. All datasets have been anonymized to ensure participant privacy and confidentiality.

INSTITUTIONAL REVIEW BOARD STATEMENT,

Ethics approval (09/2022/IECG) was secured from the Institutional Ethics Committee of Genetika.

INFORMED CONSENT STATEMENT

All participants were informed about the purpose and procedures of the study in detail. They were told about any possible risks and benefits before taking part. Each participant gave written consent voluntarily after understanding the information provided. Their personal details and data were kept confidential and used only for research purposes.

REFERENCES

1. Younossi ZM, Zelber-Sagi S, Henry L, Gerber LH. Lifestyle interventions in nonalcoholic fatty liver disease. *Nat Rev Gastroenterol Hepatol*. 2023;20:708-22.
2. Polyzos SA, Mousiolis A, Mintziori G, Goulis DG. Non-alcoholic fatty liver disease in males with low testosterone concentrations. *Diabetes Metab Syndr*. 2020; 14:1571-7.
3. Pehlivan S, Senkal N, Oyaci Y, Kose M, Pehlivan M, Hasanoglu Sayin S, Medetalibeyoglu A, Serin I, Tukek T. Metabolic syndrome and effect of gene polymorphisms: ADIPOQ, AGT, AGTR1, AGTR2, ApoC-III, NR3C1 and GNB3 gene polymorphisms. *Mol Biol Rep*. 2025;52:422. PubMed PMID: 40272664
4. Zheng H, Sechi LA, Navarese EP, Casu G, Vidili G. Metabolic dysfunction-associated steatotic liver disease and cardiovascular risk: a comprehensive review. *Cardiovasc Diabetol*. 2024;23:346. PubMed PMID: 39342178
5. Chait A, Den Hartigh LJ. Adipose tissue distribution, inflammation and its metabolic consequences, including diabetes and cardiovascular disease. *Front Cardiovasc Med*. 2020;7:522637. PubMed PMID: 32158768
6. Lee TB Jr, Kueh MT, Jain V, Razavi AC, Alebna P, Chew NW, Mehta A. Biomarkers of hepatic dysfunction and cardiovascular risk. *Curr Cardiol Rep*. 2023;25:1783-95.
7. Wang MJ, Zhang HL, Chen F, Guo XJ, Liu QG, Hou J. The double-edged effects of IL-6 in liver regeneration, aging, inflammation, and diseases. *Exp Hematol Oncol*. 2024;13:62. PubMed PMID: 38890694
8. Saki S, Saki N, Poustchi H, Malekzadeh R. Assessment of genetic aspects of non-alcoholic fatty liver and premature cardiovascular events. *Middle East J Dig Dis*. 2020;12:65-88.
9. Gruzdeva OV, Dyleva YA, Belik EV, Sinitsky MY, Kozyrin KA, Barbarash OL. Characteristics of adipocytokine expression by local fat depots of the heart: Relationship with the main risk factors for cardiovascular diseases. *Front Endocrinol (Lausanne)*. 2022;13:991902. PubMed PMID: 36157437
10. Błażejewska W, Dąbrowska J, Michałowska J, Bogdański P. The Role of Adiponectin and ADIPOQ Variation in Metabolic Syndrome: A Narrative Review. *Genes*. 2025;16:699. PubMed PMID: 40565591
11. de Luis Roman D, Izaola Jauregui O, Primo Martin D. The polymorphism rs17300539 in the adiponectin promoter gene is related to metabolic syndrome, insulin resistance, and adiponectin levels in Caucasian patients with obesity. *Nutrients*. 2023;15:5028. PubMed PMID: 38140287
12. Meshkani R, Adeli K. Hepatic insulin resistance, metabolic syndrome, and cardiovascular disease. *Clin Biochem*. 2009;42:1331-46.
13. Zhang S, Hong F, Ma C, Yang S. Hepatic lipid metabolism disorder and atherosclerosis. *Endocr Metab Immune Disord Drug Targets*. 2022;22:590-600.

14. Kim EJ, Kim BH, Seo HS, Lee YJ, Kim HH, Son HH, Choi MH. Cholesterol-induced non-alcoholic fatty liver disease and atherosclerosis aggravated by systemic inflammation. *PLoS One*. 2014;9:e97841. PubMed PMID: 24901254
15. Banerjee P, Gaddam N, Chandler V, Chakraborty S. Oxidative stress-induced liver damage and remodeling of the liver vasculature. *Am J Pathol*. 2023;193: 1400-14.
16. Vairappan B. Endothelial dysfunction in cirrhosis: Role of inflammation and oxidative stress. *World J Hepatol*. 2015;7:443-59.
17. Méndez-Sánchez N, Chávez-Tapia NC, Villa AR, Sánchez-Lara K, Zamora-Valdés D, Ramos MH, Uribe M. Adiponectin as a protective factor in hepatic steatosis. *World J Gastroenterol*. 2005;11(12):1737. PubMed PMID: 15793855
18. Matsuda M, Shimomura I. Roles of adiponectin and oxidative stress in obesity-associated metabolic and cardiovascular diseases. *Rev Endocr Metab Disord*. 2014;15:1-10. PubMed PMID: PMID: 24026768
19. Okamoto Y. Adiponectin provides cardiovascular protection in metabolic syndrome. *Cardiol Res Pract*. 2011;2011:313179. PubMed PMID: PMID: 21318102