

Exploring the Role of Pnpla3 Protein Level Expression in Fatty Liver Disease: Links to Insulin Resistance and Liver Disease Severity

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ABSTRACT

Background: Metabolic dysfunction-associated steatotic liver disease (MASLD), formerly termed non-alcoholic fatty liver disease (NAFLD), is associated with metabolic dysfunction and progressive hepatic injury. Patatin-like phospholipase domain-containing protein 3 (PNPLA3) is involved in hepatic lipid metabolism, but the clinical significance of circulating PNPLA3 protein concentrations remains insufficiently characterized.

Objective: To assess the association of serum PNPLA3 protein expression with insulin resistance, liver function parameters, fibrosis indices, and serum lipid profile in patients with fatty liver disease.

Materials and Methods: This cross-sectional analytical study was conducted at ASIAN HOSPITAL, Hyderabad, Pakistan, from March to August 2025. A total of 120 patients with ultrasound-confirmed fatty liver disease were recruited using convenience sampling. Serum PNPLA3 concentrations were measured using enzyme-linked immunosorbent assay (ELISA). Participants were categorized into low, moderate, and high PNPLA3 expression groups. Insulin resistance was assessed using the homeostatic model assessment of insulin resistance (HOMA-IR), while fibrosis risk was evaluated using the fibrosis-4 (FIB-4) index. Statistical analysis was performed using IBM SPSS Statistics version 26.0.

Results: Higher PNPLA3 expression was associated with increased HOMA-IR, alanine aminotransferase, aspartate aminotransferase, and FIB-4 values across expression groups. Spearman correlation analysis demonstrated positive associations between PNPLA3 concentrations and HOMA-IR ($r=0.45$), ALT ($r=0.30$), and FIB-4 ($r=0.28$). Associations with serum lipid parameters were not statistically significant.

Conclusion: Serum PNPLA3 protein concentrations were associated with insulin resistance and biochemical indicators of hepatic injury and fibrosis risk. Further multicenter prospective studies using standardized assays and validated fibrosis assessments are needed to establish its clinical utility.

Keywords: PNPLA3; fatty liver disease; insulin resistance; liver fibrosis; liver enzymes

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1. INTRODUCTION

The following introduction is tailored to your manuscript on serum PNPLA3 protein expression in fatty liver disease, with epidemiological evidence and recent PubMed-indexed literature. It distinguishes evidence on the PNPLA3 genetic variant from evidence on circulating PNPLA3 protein levels, because these are not interchangeable measures.

Metabolic dysfunction-associated steatotic liver disease (MASLD), previously termed non-alcoholic fatty liver disease (NAFLD), is a major cause of chronic liver disease worldwide. A systematic review and meta-analysis of 92 studies involving 9.36 million individuals estimated the global prevalence of NAFLD at 30.05%, with prevalence increasing from 25.26% during 1990–2006 to approximately 38% during 2016–2019¹. The disease ranges from hepatic steatosis to steatohepatitis, progressive fibrosis, cirrhosis, and hepatocellular carcinoma. Its development and progression are

associated with obesity, insulin resistance, type 2 diabetes, and dyslipidemia ²

The patatin-like phospholipase domain-containing protein 3 (PNPLA3) gene is an important genetic determinant of hepatic fat accumulation and liver disease severity. A 2025 systematic review and meta-analysis of 109 studies involving 118,302 individuals with MASLD reported a global PNPLA3 I148M risk-allele frequency of 45%, with substantial regional variation. Carriers of the risk variant exhibited more severe hepatic injury and histological abnormalities ³. Experimental and clinical evidence further supports the involvement of PNPLA3 in hepatic lipid-droplet metabolism and fibrotic disease progression ⁴. Although the association between PNPLA3 genetic variation and fatty liver disease has been extensively investigated, the clinical significance of circulating PNPLA3 protein concentrations remains less clearly established. Insulin resistance, assessed using the homeostatic model assessment of insulin resistance (HOMA-IR), liver enzymes, the fibrosis-4 (FIB-4) index, and serum lipid parameters provide complementary measures of metabolic dysfunction and hepatic injury. Evaluating their relationships with serum PNPLA3 protein expression may help clarify its potential relevance as a biomarker.

Evidence concerning circulating PNPLA3 protein levels in Pakistani patients with fatty liver disease remains limited. Therefore, this study aimed to assess serum PNPLA3 protein expression in patients with fatty liver disease attending ASIAN HOSPITAL, Hyderabad, Pakistan, and determine its associations with insulin resistance, liver function parameters, FIB-4 index, and serum lipid profile.

2. MATERIALS AND METHODS

A cross-sectional analytical study was conducted at ASIAN HOSPITAL, Hyderabad, Pakistan, over six months, from March 2025 to August 2025, after obtaining ethical approval from the Ethical Review Committee of Isra University and approval from the Isra University Postgraduate Committee. The study population comprised adult patients with ultrasound-confirmed fatty liver disease who attended the hospital's outpatient and inpatient departments.

The sample size was calculated using OpenEpi version 3.01 and the formula $n = N/[1 + N(e)^2]$, where N represented the estimated population of 180 and e represented the margin of error of 0.05. The calculated sample size was approximately 124 participants; however, 120 participants were enrolled based on feasibility. A non-probability convenience sampling technique was used to recruit eligible participants. Individuals aged 18–55 years with ultrasound-confirmed fatty liver disease were included. Patients with alcohol-related liver disease, viral hepatitis, diabetes mellitus, pregnancy, malignancy, chronic liver diseases, or other conditions likely to influence liver function were excluded.

After obtaining written informed consent, demographic and clinical information was recorded using a structured data collection proforma. Height and weight were measured, and body mass index (BMI) was calculated. Venous blood samples were collected after an overnight fast of at least 12 hours. Serum was separated and stored at -20°C until biochemical analysis.

Serum patatin-like phospholipase domain-containing protein 3 (PNPLA3) concentrations were measured using a commercially available enzyme-linked immunosorbent assay (ELISA) kit according to the manufacturer's instructions. Standards, controls, and serum samples were added to pre-coated 96-well microplates, followed by incubation, washing, addition of horseradish peroxidase-conjugated detection antibody, substrate development, and termination of the reaction. Optical density was measured at 450 nm using a microplate reader, and concentrations were calculated from a four-parameter logistic calibration curve. All samples were analyzed in duplicate, and the mean concentration was used for statistical analysis.

Fasting serum insulin was measured using a sandwich ELISA technique, while fasting plasma glucose was determined using the glucose oxidase–peroxidase method. Insulin resistance was assessed using the homeostatic model assessment of insulin resistance (HOMA-IR), calculated as $\text{fasting insulin } (\mu\text{IU/mL}) \times \text{fasting glucose } (\text{mg/dL})/405$. Liver function parameters and platelet counts were measured using standard automated laboratory techniques. The fibrosis-4 (FIB-4) index was calculated as $\text{age (years)} \times \text{aspartate aminotransferase (AST, U/L)} / [\text{platelet count } (10^9/\text{L}) \times \sqrt{\text{alanine aminotransferase (ALT, U/L)}}]$. The serum lipid profile included triglycerides, total cholesterol, high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C). LDL-C was calculated using the Friedewald equation when triglyceride concentrations were below 400 mg/dL.

The principal study variable was serum PNPLA3 protein concentration. Participants were categorized into low (≤ 250 pg/mL), moderate (251–480 pg/mL), and high (> 480 pg/mL) PNPLA3 expression groups. The assessed variables included age, sex, BMI, HOMA-IR, liver function parameters, platelet count, FIB-4 index, and serum lipid profile.

Data were entered and analyzed using IBM SPSS Statistics version 26.0. Continuous variables were summarized as means and standard deviations, while categorical variables were expressed as frequencies and percentages. Differences in continuous variables across the three PNPLA3 expression groups were assessed using one-way analysis of variance (ANOVA), and categorical variables were compared using the chi-square test. Spearman's rank correlation coefficient was used to assess associations between PNPLA3 protein concentrations and metabolic or hepatic parameters. A two-tailed p-value of less than 0.05 was considered statistically significant.

Ethical approval was obtained before commencement of the study. Written informed consent was obtained from all participants before enrollment, and participant confidentiality was maintained throughout data collection and analysis.

3. RESULTS

A total of 120 patients with fatty liver disease (FLD) were enrolled in the study. The mean age of the participants was 42.3 ± 9.4 years (range, 18–70 years), with males comprising 61.7% (n = 74) and females 38.3% (n = 46). The mean body mass index (BMI) was 31.2 ± 9.0 kg/m², and more than half of the participants (55.0%) were classified as obese. A positive family history of FLD was present in 28.3% of the study population.

The biochemical profile demonstrated elevated liver enzymes and features of dyslipidemia. Mean serum ALT, AST, and GGT levels were 46.9 ± 26.4 U/L, 38.6 ± 21.5 U/L, and 54.8 ± 37.4 U/L, respectively. Mean serum triglycerides and LDL cholesterol were 201.6 ± 96.4 mg/dL and 112.3 ± 42.0 mg/dL, whereas HDL cholesterol was relatively low (39.4 ± 11.8 mg/dL). Additional baseline demographic and biochemical characteristics are presented in Table 1.

Table 1. Baseline Demographic, Clinical, and Biochemical Characteristics of the Study Population (n = 120)

Variable	Mean $\pm$ SD / n (%)	Range
Demographic characteristics		
Patients Age (years)	42.3 ± 9.4	18–55
Male - Female	74 (61.7%) – 46 (38.3%)	–
BMI (kg/m ²)	31.2 ± 9.0	18–68
Underweight (<18.5)	4 (3.3%)	–
Normal (18.5–24.9)	18 (15.0%)	–
Overweight (25–29.9)	32 (26.7%)	–
Obese (≥ 30)	66 (55.0%)	–
Family history of FLD	34 (28.3%)	–
Liver Function Test		
ALT (U/L)	46.9 ± 26.4	14–130
AST (U/L)	38.6 ± 21.5	15.6–100.1
GGT (U/L)	54.8 ± 37.4	4–201
Alkaline phosphatase (U/L)	124.3 ± 47.8	65–285
Total bilirubin (mg/dL)	0.92 ± 0.36	0.3–2.1
Albumin (g/dL)	4.06 ± 0.58	2.8–5.3
Platelets ($\times 10^9$ /L)	248.9 ± 96.4	113–550
Lipid Profile		
Triglycerides (mg/dL)	201.6 ± 96.4	63–553
Total cholesterol (mg/dL)	266.7 ± 203.4	75–1142
HDL (mg/dL)	39.4 ± 11.8	2.6–66
LDL (mg/dL)	112.3 ± 42.0	12–222
Total lipids (mg/dL)	771.7 ± 246.2	371–1770

Patients with higher PNPLA3 expression demonstrated significantly higher HOMA-IR, ALT, AST, and FIB-4 values compared with the lower PNPLA3 tertiles (p<0.05). No statistically significant differences were observed for LDL-C or HDL-C among the three groups (Table 2).

Table 2. Comparison of HOMA-IR, ALT, AST, LDL-C, HDL-C and FIB-4 values across PNPLA3 expression groups

Parameters	Low PNPLA3 (<251pg/ml) (N=40 (33.3%))	Moderate PNPLA3 (251-480pg/ml) (Mean $\pm$ SD) (N=40 (33.3%))	High PNPLA3 (>480pg/ml) (Mean $\pm$ SD) (N=40 (33.3%))	Test used	p-value
HOMA-IR	2.1 ± 1.1	2.7 ± 1.3	3.6 ± 1.8	F _{value} = 10.9	0.000**
ALT(U/L)	38 ± 16	47 ± 20	56 ± 25	F _{value} = 6.1	0.003*
AST (U/L)	34 ± 12	42 ± 18	50 ± 22	F _{value} = 5.5	0.006*
FIB-4 Score	0.9 ± 0.4	1.1 ± 0.5	1.4 ± 0.7	F _{value} = 4.6	0.012*
LDL-C (mg/dL)	135.38 ± 71.33	133.94 ± 64.14	135.60 ± 42.33	Spearman p P = 0.137	0.885
HDL-C(mg/dl)	37.89 ± 14.88	33.38 ± 10.74	30.50 ± 14.20	Spearman p P = 0.221	0.084

Spearman's correlation analysis showed that PNPLA3 protein expression was positively correlated with HOMA-IR

($r=0.45$, $p<0.01$), ALT ($r=0.30$, $p<0.05$), and FIB-4 ($r=0.28$, $p<0.05$). Weak positive correlations were observed with triglycerides, total cholesterol, and LDL-C, while HDL-C displayed a weak negative correlation; however, these lipid correlations were not statistically significant.

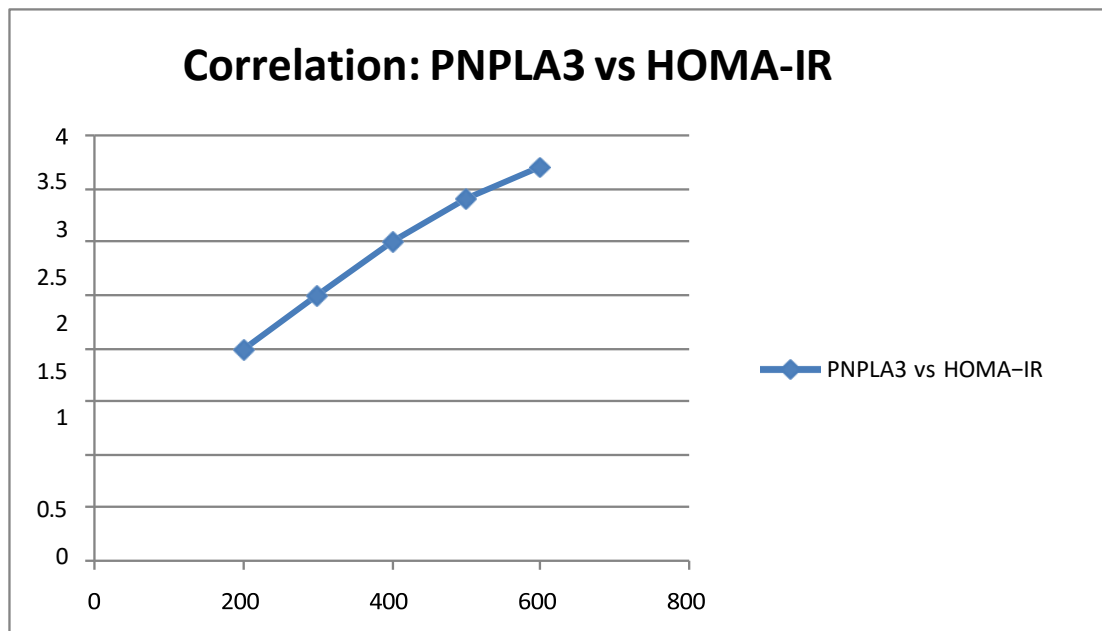


Figure IV- 3. Correlation between PNPLA3 levels and HOMA-IR.

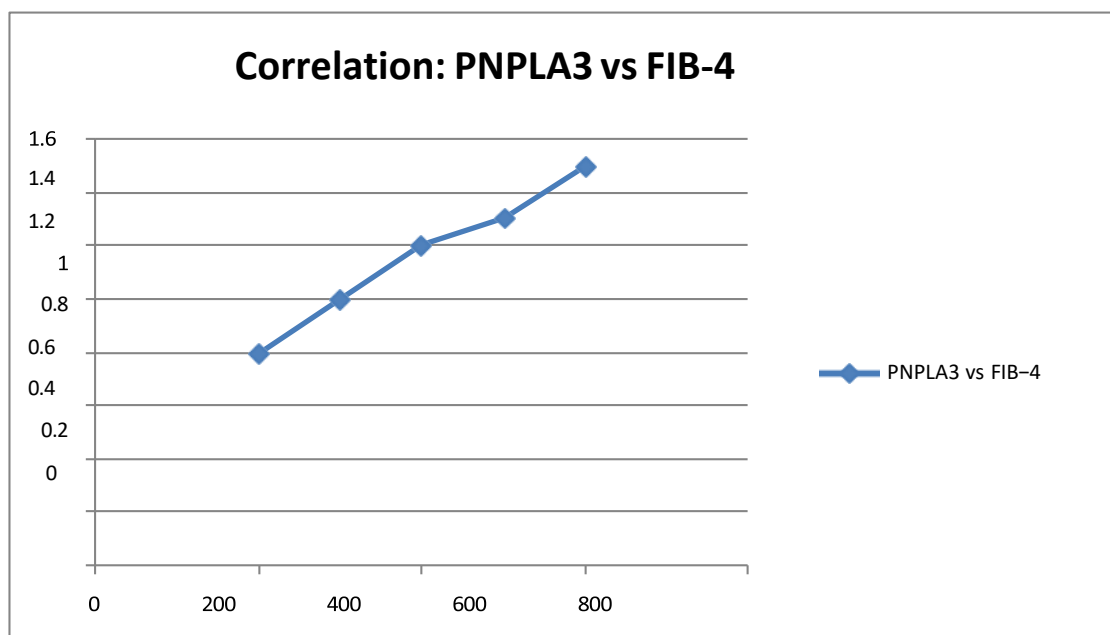


Figure IV-4: Correlation between PNPLA3 levels and FIB-4 index.

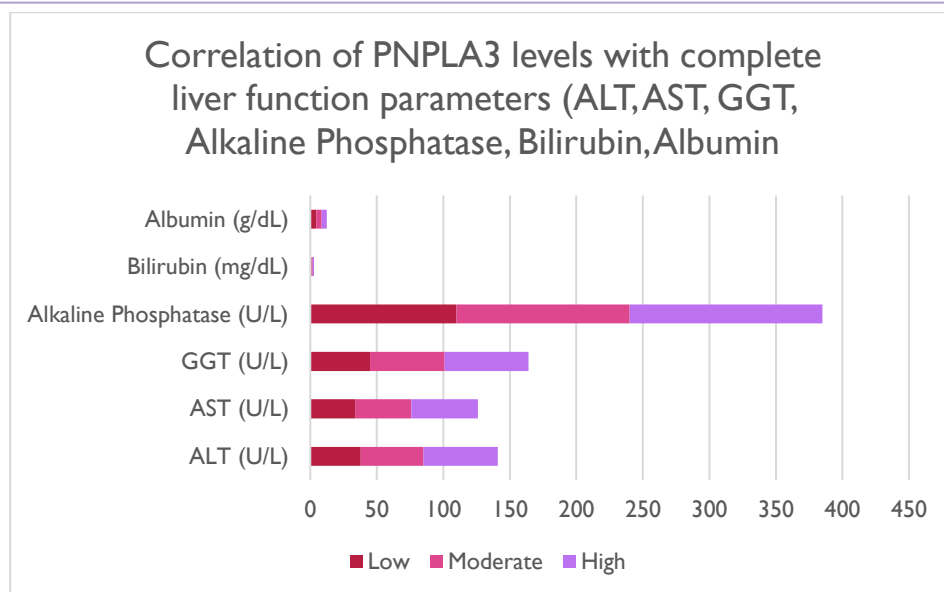


Figure IV-5 Correlation of PNPLA3 levels with LFT

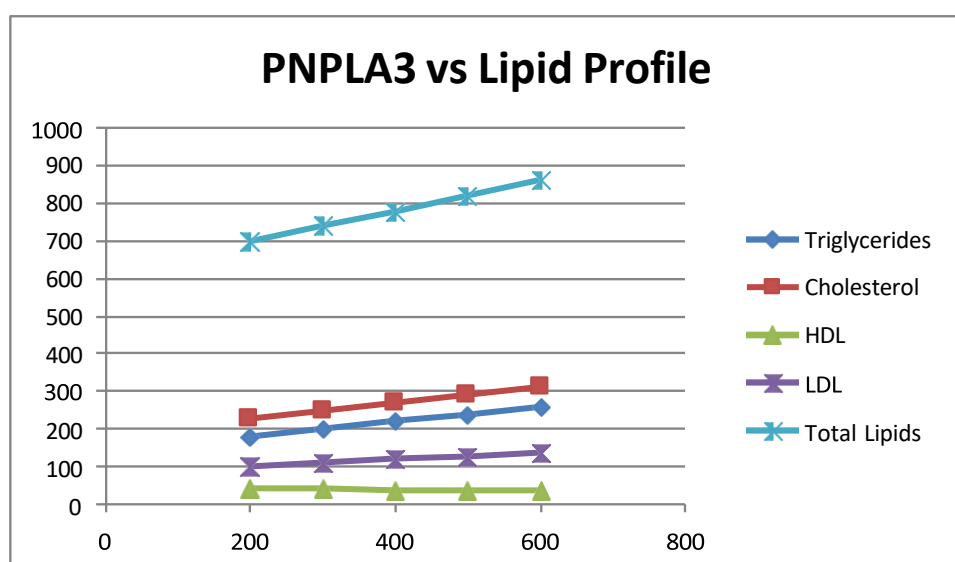


Figure IV- 6. Correlation of PNPLA3 levels with lipid profile parameters

4. DISCUSSION

The present study examined the relationship between serum PNPLA3 protein concentrations and metabolic and hepatic parameters in patients with fatty liver disease. The observed associations with insulin resistance, aminotransferases, and the FIB-4 index suggest that circulating PNPLA3 concentrations may be related to metabolic dysfunction and biochemical indicators of liver injury. However, because the study was cross-sectional, these relationships cannot establish causality or demonstrate that PNPLA3 protein concentrations independently predict disease progression.

The association between PNPLA3 expression and insulin resistance is biologically plausible. PNPLA3 participates in hepatic lipid-droplet metabolism, while insulin resistance promotes adipose tissue lipolysis, increases free fatty acid delivery to the liver, and stimulates hepatic de novo lipogenesis. A clinical study published in 2024 demonstrated that insulin resistance remained an important feature of metabolic dysfunction-associated steatotic liver disease (MASLD), irrespective of PNPLA3 I148M genotype⁵. These findings support the importance of metabolic dysfunction in patients with PNPLA3-associated disease, although the genetic evidence cannot directly establish the metabolic effects of circulating PNPLA3 protein concentrations.

Experimental research has further clarified the biological mechanisms linking PNPLA3 to hepatic lipid accumulation. Sherman et al. demonstrated that the PNPLA3-I148M variant altered lipid-droplet–Golgi interactions and induced cellular changes associated with liver disease⁶. Similarly, Gou et al. reported that the variant promoted free cholesterol

accumulation and mitochondrial dysfunction in hepatic stellate cells, potentially contributing to fibrogenesis⁷. Research on PNPLA3 interactions with perilipin 5 and adipose triglyceride lipase has also suggested impaired lipid-droplet lipolysis as a mechanism of hepatic lipid retention⁸. These findings provide a mechanistic basis for investigating PNPLA3-related pathways in liver disease; nevertheless, they concern genetic variation and intracellular protein function rather than the clinical effects of elevated circulating protein levels.

The association between serum PNPLA3 concentrations and liver enzymes observed in this study is also consistent with the broader literature linking PNPLA3 genetic susceptibility to hepatic injury. A systematic review and meta-analysis published in 2025 found that carriers of the PNPLA3 I148M risk variant generally exhibited more severe hepatic histological abnormalities, although substantial heterogeneity existed between studies⁹. Furthermore, research using three-dimensional extracellular matrix models of human hepatic stellate cells has demonstrated that PNPLA3 I148M may influence cellular pathways involved in fibrotic progression¹⁰. A recent review also emphasized the interactions between hepatocytes, hepatic stellate cells, and inflammatory cells in PNPLA3-associated liver injury¹¹. These findings support further investigation of PNPLA3-related pathways, but the present study did not include liver biopsy or direct histological assessment to confirm fibrosis.

The relationship between PNPLA3 expression and the FIB-4 index warrants cautious interpretation. FIB-4 is a non-invasive score calculated from age, AST, ALT, and platelet count and is commonly used for initial assessment of fibrosis risk. An association between serum PNPLA3 concentrations and FIB-4 may indicate a relationship with biochemical or clinical features linked to liver disease severity. However, FIB-4 is not a direct measurement of hepatic fibrosis, and its association with PNPLA3 does not establish that circulating PNPLA3 can accurately identify histological fibrosis. Future studies should compare serum PNPLA3 concentrations with validated fibrosis assessments, including transient elastography and, where clinically indicated, liver histology.

The absence of statistically significant associations between PNPLA3 concentrations and individual lipid parameters also merits consideration. Although PNPLA3 is involved in hepatic lipid-droplet biology, circulating lipid concentrations reflect multiple processes, including intestinal lipid absorption, hepatic lipoprotein production, peripheral lipid clearance, diet, and metabolic status. Moreover, genetic studies have reported that the effects of PNPLA3 I148M on circulating lipid profiles may differ from its effects on intrahepatic fat accumulation¹². Therefore, serum PNPLA3 protein concentrations should not be assumed to reflect circulating lipid abnormalities directly. The findings suggest that further research should distinguish between hepatic lipid handling, circulating lipid concentrations, and serum PNPLA3 protein abundance.

The study's strengths included the direct measurement of serum PNPLA3 protein using ELISA, the concurrent assessment of metabolic and hepatic parameters, and the use of a standardized data collection approach. The inclusion of participants from a hospital in Hyderabad also provides locally relevant preliminary evidence from a population in which direct measurements of circulating PNPLA3 protein remain insufficiently characterized. Nevertheless, several limitations should be acknowledged. The single-center, cross-sectional design and convenience sampling limited generalizability and prevented assessment of temporal relationships. The absence of PNPLA3 genotyping meant that genetic variation could not be separated from protein-level associations. Furthermore, fibrosis was evaluated using FIB-4 rather than histology or elastography, and potential confounding factors, including dietary habits and physical activity, were not assessed. The analytical characteristics of the ELISA assay also require further documentation and independent validation.

Future multicenter prospective studies should investigate whether serum PNPLA3 concentrations are reproducibly associated with disease severity and progression. Such studies should incorporate PNPLA3 genotyping, validated fibrosis assessments, appropriate adjustment for metabolic and clinical confounders, and standardized laboratory procedures. Diagnostic performance should also be evaluated before considering PNPLA3 protein measurement for routine clinical risk stratification. Until these findings are independently validated, circulating PNPLA3 should be regarded as an investigational biomarker rather than an established clinical tool.

5. CONCLUSION

Serum PNPLA3 protein expression was associated with insulin resistance, elevated liver enzymes, and higher FIB-4 scores among patients with fatty liver disease. However, its associations with serum lipid parameters were not statistically significant. These findings suggest that circulating PNPLA3 protein concentrations may be related to metabolic dysfunction and biochemical indicators of hepatic disease severity. Nevertheless, the cross-sectional design and single-center setting limit causal inference and generalizability. Further multicenter prospective studies incorporating standardized PNPLA3 assays, genetic analysis, and validated fibrosis assessments are required to determine its potential clinical utility as a biomarker for disease severity and risk stratification.

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