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Association of Remnant Lipoprotein Cholesterol with Non-Alcoholic Fatty Liver Disease and Hepatic Steatosis Severity

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ABSTRACT

Background: Non-Alcoholic Fatty Liver Disease (NAFLD) is a prevalent chronic liver disorder closely linked to obesity, diabetes, metabolic syndrome, and dyslipidaemia. Remnant lipoprotein cholesterol (RLP-C) is associated with cardiovascular risk and may be elevated in NAFLD patients. To assess the diagnostic utility of RLP-C in NAFLD and its correlation with biochemical and anthropometric parameters.

Methodology: A total of 100 subjects (50 NAFLD, 50 controls) were enrolled. Clinical history, anthropometric measurements (BMI, waist circumference), and biochemical analyses were analysed, including Fasting blood glucose and lipid profiles. Serum RLP-C levels were calculated and analyzed with NAFLD grading and the ROC curve.

Results: NAFLD patients showed significantly higher RLP-C levels (26.47±2.08 mg/dl) than controls (18.2±1.40 mg/dl, p=0.005). RLP-C was positively correlated with TC, TG, LDL, and VLDL. ROC curve yielded an AUC of 0.663, with 54% sensitivity and 78% specificity.

Conclusion: RLP-C is significantly associated with NAFLD and lipid abnormalities but shows moderate diagnostic accuracy.

Keywords: *Non-Alcoholic Fatty Liver Disease (NAFLD), Lipid Profile, Fasting Blood Glucose, Remnant Lipoprotein Cholesterol (RLP-C).*

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1. INTRODUCTION

NAFLD encompasses a spectrum of diseases from simple hepatic steatosis or accumulation of fat (non- alcoholic fatty liver, NAFL) to steatosis with inflammatory changes and progressive fibrosis (non-alcoholic steatohepatitis, NASH), further progressing to cirrhosis, and end-stage liver disease in the absence of excessive alcohol consumption (1). According

to a recent study that used the Markov prediction model, NAFLD is expected to rise by 21% from 83 million in 2015 to 101 million in 2030, and NASH is expected to increase by 63% between 2015 and 2030, from 17 million to 27 million (2). The risk of developing NAFLD has increased with the presence of conditions like metabolic syndrome (MetS), diabetes mellitus type II (T2DM), hypertension, and obesity (3). According to various clinical and laboratory data, approximately. 90% of NAFLD individuals have at least one risk factor of MetS, 30-100% of subjects with obesity, and 33% of individuals with type 2 diabetes mellitus (4). The pathogenesis of NAFLD can be explained by the "two-hit hypothesis" model where the 'first-hit" explains the development of hepatic steatosis due to the accumulation of lipids in the hepatocytes and insulin resistance, and the "second-hit" explains the development of hepatocyte injury, inflammation, and fibrosis due to initiating factors such as oxidative stress due to subsequent lipid peroxidation, mitochondrial dysfunction, adipokines, cytokines, and proinflammatory molecules (5).Remnant Lipoprotein Cholesterol (RLP-C), an atherogenic lipid profile emerging as a novel cardiovascular risk factor. In fasting conditions, RLP-C is the cholesterol content of TG-rich lipoproteins of very low-density lipoprotein (VLDL) and intermediate-density lipoprotein (IDL). VLDL originates in the liver and increases more in the case of dyslipidemia. In Jackson Heart and Framingham Offspring Cohort studies (USA), remnant lipoprotein cholesterol was associated with incident coronary heart disease, and it is also predicted to be significantly higher in NAFLD patients (6).

In India, limited data exist on the association of GGT/HDL-C ratio and RLP-C levels with NAFLD. This study aims to evaluate variations in RLP-C levels among NAFLD patients, assess their correlation with lipid profile and anthropometric measures, and determine their diagnostic value. Early identification using these markers may improve NAFLD diagnosis and help prevent progression to NASH and hepatocellular carcinoma.

2. MATERIAL AND METHODS

A case-control study was conducted at the Departments of Biochemistry and Medicine, Hamdard Institute of Medical Sciences and Research (HIMSR) and associated HAHC Hospital, New Delhi. Institutional ethical approval was obtained (Ref: EC/NEW/INST/2020/961), and written informed consent was secured from all participants.

Participants

100 individuals were enrolled, comprising 50 ultrasound-confirmed NAFLD patients and 50 clinically healthy controls. Inclusion criteria included adults over 18 years of age, both male and female, with NAFLD diagnosed by ultrasound. Exclusion criteria included a history of alcohol consumption, smoking, pregnancy, drug abuse, use of steatogenic drugs, viral hepatitis, other liver diseases, and comorbidities that could affect liver enzyme levels. Control subjects were free from any known illness or comorbidities.

Clinical and Biochemical Assessment

All participants underwent a detailed medical history and physical examination. Anthropometric parameters (BMI and waist circumference) and blood pressure (SBP and DBP measured in sitting position using a sphygmomanometer) were recorded. BMI was calculated as weight (kg) divided by height squared (m^2); a BMI $\geq$25 kg/m^2 was classified as obese based on Indian guidelines.

Venous blood samples (3 mL) were collected after 8–12 hours of fasting. Fasting glucose was collected in sodium fluoride vials, while other parameters were drawn into serum separator tubes. Biochemical parameters- lipid profile, and fasting glucose were analyzed using a fully automated clinical chemistry analyzer (Beckman Coulter AU 480). Remnant Lipoprotein Cholesterol (RLP-C) was calculated using the formula:

RLP-C = Total Cholesterol – (HDL-C + LDL-C).

Statistical Analysis

Data analysis was performed using SPSS version 28.0. Normality of the data was assessed using Shapiro-Wilk and Kolmogorov-Smirnov tests. Results are expressed as mean ± SD for normally distributed data, and as median (interquartile range) for non-parametric data. Group comparisons were made using the Student's t-test or Mann–Whitney U test for continuous variables, and the chi-square test for categorical variables. Spearman's correlation was used to examine associations between RLP-C and other variables. Diagnostic performance was evaluated using the Receiver Operating Characteristic (ROC) curve analysis. A p-value < 0.05 was considered statistically significant.

Result: The study comprised 50 NAFLD patients and 50 healthy control subjects. The age and sex distribution of participants is summarized in Table 1, Fig. 1. Among the healthy controls, 44% were male and 56% were female, while in the NAFLD group, 46% were male and 54% were female. The mean age of NAFLD patients was 49.1 ± 14.16 years, compared to 48.52 ± 13.3 years in the control group, with no statistically significant difference between the two groups.

Table 1. Age and sex distribution of the study population.

	NAFLD (n=50)	Control (n=50)	p-value
Age (years) (Mean ± SD)	49.1±14.16	48.52±13.3	0.617

Data are expressed as mean ± SD and categorical variables as count, n (%). $p < 0.05$ is considered a statistically significant difference.

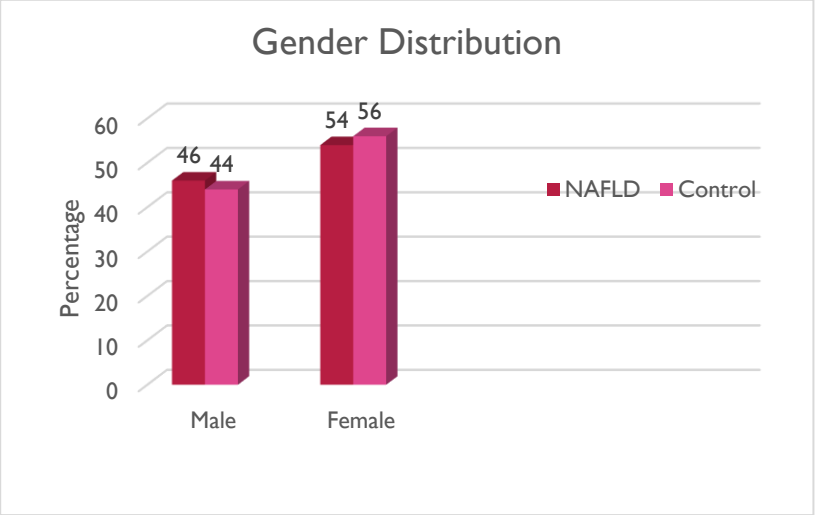

Figure 1: Gender distribution of the study population.

The study subjects' anthropometric variables and blood pressure are given in "**Table 2".** The BMI of NAFLD patients was significantly higher than that of the control subjects (p = 0.01). There was a significant difference between the SBP of the NAFLD and control group (p =0.001). No significant difference was observed between WC and DBP in the NAFLD group (p > 0.05) compared to the control group.

Table 2: Anthropometric and Blood pressure characteristics of the study population.

Variable	NAFLD	Control	p-value
	(Mean ± SD)	(Mean ± SD)	
Weight (kg)	71.4 ± 9.6	68.26±7.2	0.034
Height (m)	1.60±0.77	1.68±0.686	<0.001
BMI (kg/m^2)	27.7±4.25	24.1±1.34	0.01
WC (cm)	94.1±1.51	91.3±0.87	0.097
SBP (mmHg)	129.48±15.9	120.38±7.83	0.001
DBP (mmHg)	82.7±10.7	83.9±17.30	0.928

BMI, body mass index; WC, waist circumference; SBP, systolic blood pressure; DBP, diastolic blood pressure;$p < 0.05$ considered as a statistically significant difference.

The biochemical parameters of NAFLD patients and healthy controls are summarized in **Table 3**. Serum triglyceride (TG) and VLDL levels were significantly elevated in NAFLD patients compared to healthy controls (**p < 0.001**), while serum HDL-C levels were significantly reduced in the NAFLD group (**p = 0.004**). The levels of RLP-C levels were significantly higher in the NAFLD group (26.47± 2.08 mg/dl) and had a p-value of 0.005 compared to the control group (18.2±1.40

mg/dl)

Table 3: Biochemical profile of the study population

Variable	NAFLD	Control	p-value
FG (mg/dl)	117.8 ± 32.3	92.3 ±9.37	<0.001#
AST (IU/L)	37 (39)	26 (15)	0.001*
ALT (IU/L)	33.5(52.2)	27(25.25)	0.001*
ALP (IU/L)	33.5(52.55)	98.5(47.25)	0.215*
GGT (IU/L)	81.6±11.3	30.0±2.50	<0.001
LDL-C(mg/dl)	99.8±37.38	101.5±28.7	0.692 #
VLDL (mg/dl)	36.50±17.36	21.3±6.62	<0.001#
RLP-C (mg/dl)	26.47± 2.08	18.2±1.40	0.005

FG, fasting glucose; TG, triglyceride; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; VLDL, very low-density lipoprotein. Continuous variables were presented as mean ± SD or median (interquartile range). The statistical significance of differences between the control and NAFLD group was analyzed using the Student's t-test (#) and the Mann-Whitney U test (*).

The prevalence of metabolic syndrome (MetS) and obesity among the study participants is presented in **Table 4**. MetS was significantly more common in the NAFLD group (73.8%) compared to the control group (26.2%) (**p < 0.001**). Similarly, obesity was observed in 76% of NAFLD patients, which was significantly higher than the 24% seen in controls (**p < 0.001**).

Table 4. Prevalence of Metabolic syndrome (MetS) and obesity in the study population.

Characteristics	NAFLD	Controls	p-Value
MetS	73.8 %	26.2%	<0.001
Obesity	76 %	24%	<0.001

The statistical significance of differences was analyzed by chi-square test.

The NAFLD group was further classified into 3 grades based on the degree of steatosis illustrated in the ultrasound: grade 1 (mild), grade 2 (moderate), and grade 3 (severe). Serum RLP-C levels were significantly higher in grade 3(50±33) than in grade 2 (27±19) with a p-value of 0.014 and in grade 1(24 ± 19) with a p-value of 0.005 **(Figure 3).** No significant rise in RLP-C levels was seen between Grade 2 and Grade 1.

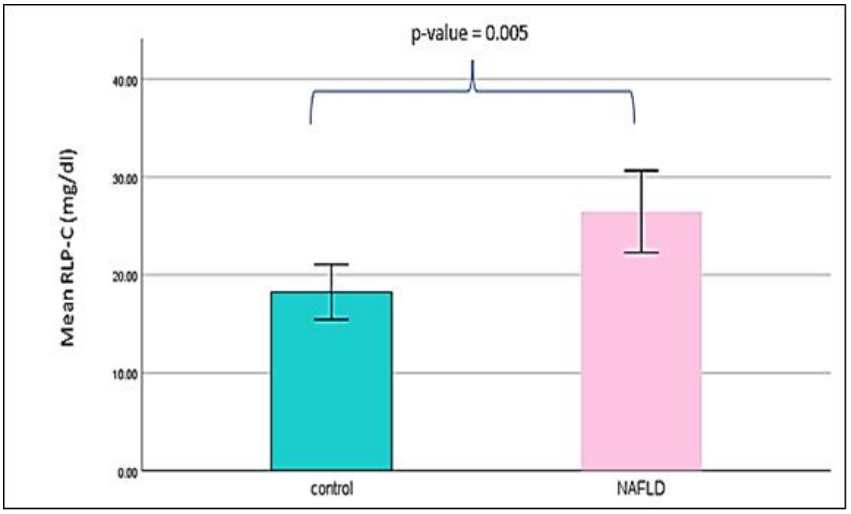

Fig 2. Bar graph of RLP-C (Mean±SD) levels in NAFLD and control group.

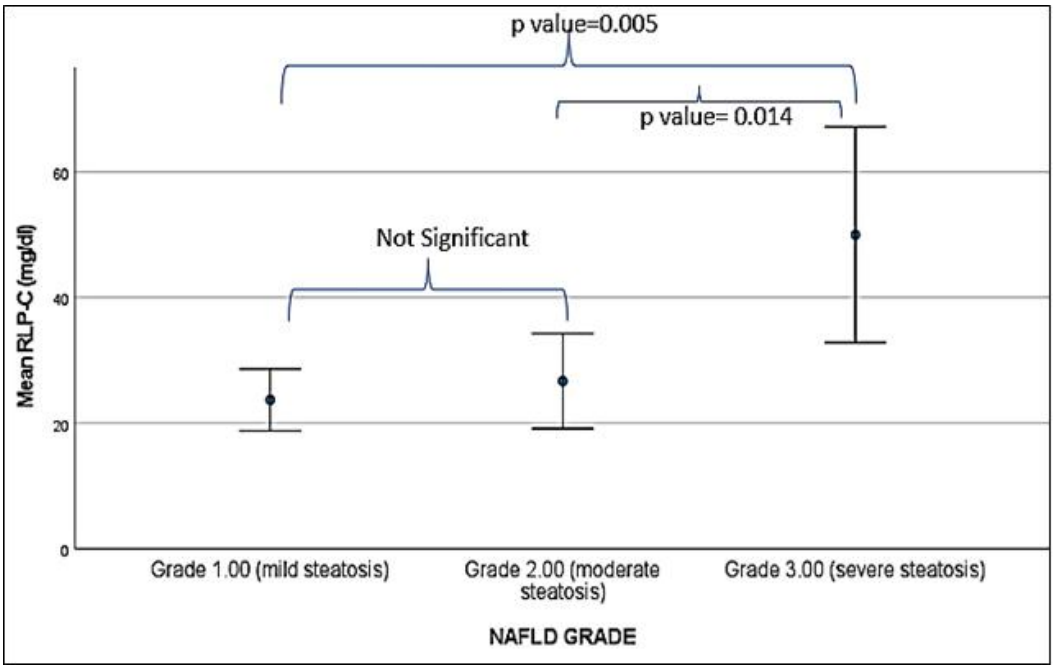

Figure 3: Association between serum RLP-C levels with NAFLD grading based on ultrasonography

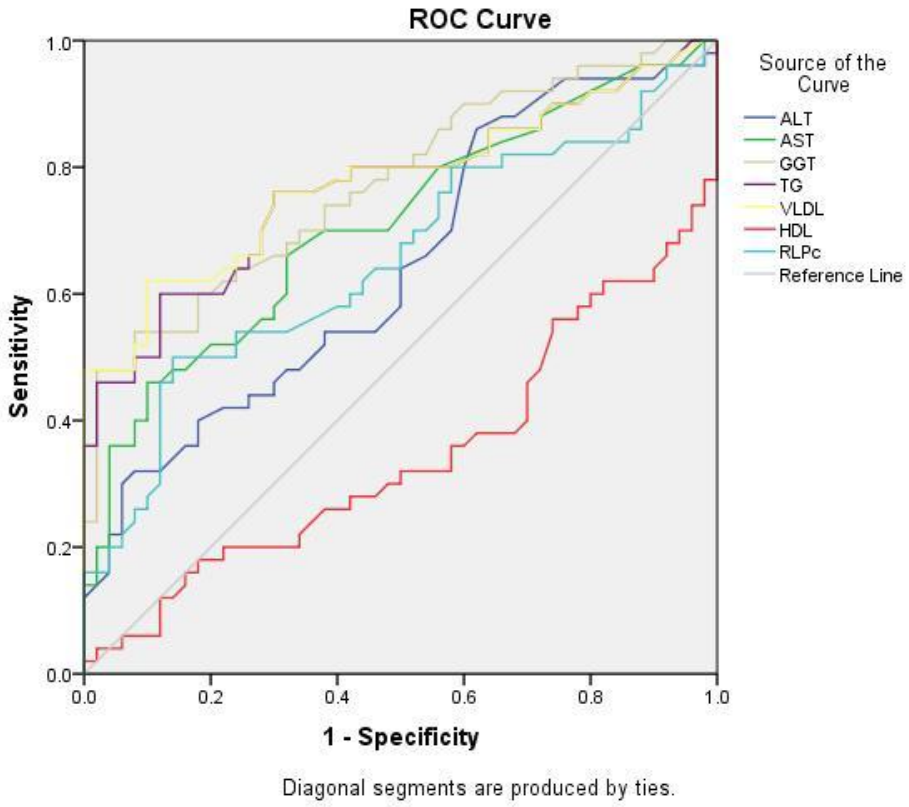

Fig 4: Analysis of Receiver-operating characteristic (ROC) curves.

Correlation analysis of RLP-C with other variables in NAFLD subjects was analysed**:** RLP-C was seen to be negatively correlated with BMI and positively correlated with WC, SBP, and DBP. However, no statistically significant correlation was seen between RLP-C and these variables**.** There was a significant positive correlation of RLP-C with TC, TG, LDL, and VLDL as shown in "**Table 5"**.

Table 5 Spearman correlation of RLP-C with other biochemical variables.

Variable	r value	p-value
BMI	-0.148	0.303
WC	0.141	0.327
SBP	0.160	0.267
DBP	0.092	0.526
TC	0.679	<0.001
TG	0.677	<0.001
LDL-C	0.482	<0.001
HDL-C	0.160	0.268
VLDL	0.580	<0.001
FG	0.009	0.952

3. DISCUSSION

In this hospital-based case-control study, serum remnant lipoprotein cholesterol levels were significantly elevated in NAFLD patients compared to healthy controls. The findings suggest a strong association between elevated RLP-C levels and the presence as well as the severity of hepatic steatosis. As shown in Table 2, fasting serum RLP-C levels increased progressively with the grade of hepatic steatosis, supporting its potential as a marker for disease progression in NAFLD. The positive correlation of RLP-C with other lipid parameters—total cholesterol, triglycerides, VLDL, and LDL-C—in NAFLD patients (Table 5) underscores its link with atherogenic dyslipidemia. These findings are in line with previous studies, including the Jackson Heart and Framingham Offspring Cohort studies (7), which reported elevated RLP-C levels in individuals at increased cardiovascular risk. Similarly, studies by Pastori et al. and Chin et al. also demonstrated elevated RLP-C in NAFLD and its association with hepatic fat content (8). NAFLD is frequently accompanied by dyslipidemia, particularly increased TG-rich lipoproteins such as VLDL and LDL-C. RLP-C, being a cholesterol-rich remnant of these particles, has the potential to infiltrate the arterial wall, deliver large amounts of cholesterol, and promote foam cell formation, thereby contributing to atherosclerosis (9). This mechanistic pathway supports the hypothesis that elevated RLP-C in NAFLD patients may be an indicator of heightened cardiovascular disease (CVD) risk. ROC curve analysis further evaluated the diagnostic performance of RLP-C in identifying NAFLD (Figure 1). The area under the curve (AUC) for RLP-C was 0.663, indicating moderate diagnostic value. Although lower than, it still outperformed some traditional lipid markers and showed good specificity (78%) at the optimal cut-off value of 23.34 mg/dL. These results reinforce its role as a potential biochemical marker for NAFLD. This study highlights the elevated serum RLP-C levels in NAFLD patients, their association with disease severity, and their correlation with atherogenic lipid parameters. These findings suggest that RLP-C may serve as a valuable, low-cost, and accessible marker for identifying individuals with NAFLD and for assessing cardiovascular risk in this population. However, larger multicenter studies are needed to validate these findings and establish standardized reference values and clinical cut-offs.

Conclusion: RLP-C levels are elevated in NAFLD and show a positive correlation with atherogenic lipid profiles. Although its diagnostic accuracy is moderate, RLP-C may serve as a complementary marker alongside other biochemical parameters to aid in identifying individuals at risk for NAFLD.

Conflict of interest: None

Funding: None

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