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Original Article

Biochemistry Section

Assessment of Remnant Lipoprotein Cholesterol (RLP-C) Levels and its Correlation with Carotid Intima Media Thickness in Insulin Resistant Type 2 Diabetes Mellitus Patients

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ABSTRACT

Introduction: Remnant lipoproteins are Triglyceride (TG) rich lipoproteins products of partially metabolised chylomicrons and Very Low Density Lipoproteins (VLDL). Remnant Lipoprotein Cholesterol (RLP-C) levels are related to the risk of ischaemic heart disease. It has been hypothesized that Carotid Intima Media Thickness (CIMT) measurements could aid in the prediction of Cardiovascular Disease (CVD).

Aim: To evaluate the levels of RLP-C in Insulin Resistant (IR) Type-2 Diabetes Mellitus (T2DM) patients and correlate the levels of RLP-C with CIMT, IR, TG and High Density Lipoprotein (HDL).

Materials and Methods: The present cross-sectional study was conducted in the Department of Internal Medicine, SRM Medical College and Research Centre, Tamil Nadu, India. A total of 80 (aged 30 to 65 years) T2DM, men and women, aged 30-65 years, were included in the study. Serum lipids (Total Cholesterol (TC), TG, HDL-C and LDL-C), and serum insulin were measured. Insulin Resistance (IR) was estimated by the Homeostatic Model Assessment-Insulin Resistance (HOMA-IR). Based on HOMA-IR values, the individuals with HOMA-IR >2.5 were categorised as IR and HOMA-IR<2.5 as Insulin sensitive. RLP-C was calculated by using the formula: RLP-C=TC-(HDL-C+LDL-C). CIMT was measured in patients who had IR by using B Mode Ultrasonography (USG). Statistical analysis was done using Student's t-test and Pearson correlation analysis.

Results: Among the insulin resistant diabetic patients, a positive correlation was observed between IR and serum TG (r=0.0448). In these subjects, RLP-C was positively correlated with serum TG (r=0.5191) and negatively correlated with HDL-C (r=-0.0542). A positive correlation was also observed between RLP-C and CIMT (r=0.513) in these patients with IR.

Conclusion: Type 2 diabetic patients with IR are characterised by high RLP-C and TG levels and low HDL-C levels. Estimation of RLP-C may help to identify individuals at high risk of atherosclerosis. The calculation of RLP-C can be easily done from the already existing conventional lipid profile at no additional cost.

Keywords: Atherosclerosis, Dyslipidaemia, Triglycerides

INTRODUCTION

RLP-C is the cholesterol which present in the TG-rich lipoproteins namely VLDL and Intermediate Density Lipoprotein (IDL) in the fasting state and VLDL, IDL and chylomicron remnants in the non-fasting state. Remnant cholesterol is a powerful contributor towards Coronary Artery Disease (CAD) and stroke risk. Remnant cholesterol irrespective of the fasting status is also causally related to the risk of CAD and low grade inflammation independent of HDL-C levels [1]. Cholesterol in atherogenic particles other than the LDL-C is currently emerging as a major risk factor for ischaemic heart disease and is mostly found in fasting and non-fasting Triglyceride Rich Lipoproteins (TRLs) [2]. Literature suggests that lowering the levels of the atherogenic remnant particles reduce the residual risk in patients even after they have achieved an optimal response with statins [3]. Taniguchi A et al., in multiple studies reported that elevated RLP-C levels were associated with IR in T2DM patients [4], and elevated RPL-C are an independent risk of CAD in T2DM [5]. Nakamura T et al., reported that high levels of RLP-C predicts ischaemic stroke in patients with metabolic syndrome and mild carotid atherosclerosis [6]. CIMT is being widely used as a surrogate marker of atherosclerosis [7]. Considering these finding, the present study was designed to evaluate the levels of RLP-C in IR T2DM patients and assess the correlation of RLP-C with CIMT, IR, TG and HDL-C.

MATERIALS AND METHODS

This cross-sectional study was conducted from July 2017 to June 2018, at SRM Medical Hospital and Research Centre, Chennai, Tamil Nadu, India. The study protocol was approved by the Institutional Ethics Committee (ECN: 1375/IEC/2018) and informed written consent was taken from all the subjects.

A total of 80 patients diagnosed with T2DM (>5 years duration of the disease) and on treatment for the same, attending the Diabetology Out Patient Department of SRM MCH and RC for routine follow-up. Diagnosis of type 2 diabetes was based on World Health Organisation Criteria [8].

Pregnant women, patients with history of recent infections/surgery, alcoholism, hypothyroidism or patients on corticosteroids, oestrogens, antiretroviral drugs, psychotropic medications were excluded from the study.

After an overnight fasting, five millilitres of venous blood sample was collected from all the participants. Estimation of TC was done by cholesterol oxidase method, TG by glycerol peroxidase, LDL-C and HDL-C by direct method using Beckman Coulter auto-analyser [9,10]. The RLP-C was calculated using the formula: RLP-C=TC-(HDL-C+LDL-C) [11].

Insulin was estimated by enhanced chemiluminescence immunoassay (CLIA) VITROS immunoanalyser by Ortho Care Diagnostics. Out of 80 patients, 46 (19 males and 27 females) were found to be insulin resistant by using HOMA-IR formula. (HOMA-IR: fasting serum insulin (U/mL)×fasting plasma glucose (mmol/L)÷22.5, value more than 2.5 indicates IR). CIMT was measured in 24 patients who had IR by using B Mode Ultrasonography.

STATISTICAL ANALYSIS

Data was analysed using Statistical Package for Scientific Studies (SPSS) version 16. The results were represented as mean±standard deviation (SD). Student's t-test was used to analyse the difference between the mean levels of various parameters. Correlation between various variables was assessed using Pearsons correlation equation. The p-value <0.05 was considered statistically significant.

RESULTS

Among the 80 T2DM patients, 46 (19 males and 27 females) patients were insulin resistant, with average age 44.58±4.75 years and 34 (12 males and 22 females) were insulin sensitive, with average age of 45.61±4.43.

The mean levels of insulin, insulin resistance (HOMA-IR), total cholesterol, triglycerides, LDL-C and RLP-C were found to be significantly elevated in the insulin resistant group compared to insulin sensitive group. However, no significant difference was observed between the HDL-C levels between the two groups [Table/Fig-1].

Parameters	Insulin resistance (46)	Insulin sensitive (34)	p-value
Insulin (µ/mL)	17.82±1.90	5.30±1.90	<0.01*
Insulin Resistance (HOMA-IR)	5.33±2.77	1.53±0.54	<0.001*
Total cholesterol (mg/dL)	181.34±53.88	157.67±42.63	<0.001*
Triglycerides (mg/dL)	142±51.99	112.58±44.33	<0.001*
HDL-C (mg/dL)	45.29±12.88	42.64±12.91	0.3662
LDL-C (mg/dL)	105.78±44.56	98.44±36.55	<0.001*
RLP-C (mg/dL)	25.51±19.64	23.602±15.87	0.0499*

[Table/Fig-1]: Biochemical parameters of the insulin resistant and insulin sensitive T2DM patients.
Values expressed as Mean±SD. Student's t-test, *p-value <0.05 statistically significant.

In the insulin resistant diabetic patients, a positive correlation was observed between IR and TG (r=0.0448). RLP-C was positively correlated with TG (r=0.5191) and CIMT (r=0.513) and negatively correlated with HDL-C (r=-0.0542) [Table/Fig-2].

Parameters	No. of individuals	Mean±SD	r - value
RLP-C (mg/dL) Triglycerides (mg/dL)	46	25.51±19.64 142±51.99	r=0.5191 (p<0.001)*
Triglycerides (mg/dL) Insulin Resistance	46	142±51.99 5.04±3.58	r=0.0448 (p <0.001)*
RLP-C (mg/dL) HDL-C (mg/dL)	46	25.51±19.64 45.29±12.88	r= -0.0542 (p=0.8603)
RLP-C (mg/dL) CIMT	24	23.37±22.26 0.32±1.17	r=0.513 (p=0.0097)*

[Table/Fig-2]: Pearson's correlation analysis between various biochemical parameters in insulin resistant T2DM patients (n=46).

DISCUSSION

IR causes hypertriglyceridemia [12]. RLP-C is the cholesterol content of TG rich lipoprotein composed of VLDL and IDL in fasting and of these of two lipoprotein together and chylomicrons in non-fasting state [13]. Elevated remnant cholesterol is a marker of elevated non-fasting plasma TG and is associated with increased risk for cardiovascular disease [14]. Remnant lipoproteins get entrapped in the intima of the arterial wall and cause accumulation of cholesterol [15].

In our study, we have observed a positive correlation between IR &TG (r=0.0448). Using a Mendelian randomisation approach Jorgensen et al., observed a causal association between elevated levels of remnant cholesterol in hypertriglyceridaemic patients and an increased risk of CHD [13]. Atherosclerosis appears to be caused mainly by the cholesterol content of remnants as cells are capable of degrading TG. RLP carries 5 to 20 times more cholesterol per particle when compared to LDL. Unlike native LDL-C, remnant cholesterol may be involved in the upregulation of scavenger receptors and thus promote the foam cell formation [16].

In our study we have observed a positive correlation between RLP-C and CIMT (r=0.513). IMT is a surrogate marker for subclinical atherosclerosis [17].

Elevated levels of both calculated and measured remnant cholesterol have been reported to be associated with increased all-cause mortality in patients with IHD, however no such association was observed with increasing concentrations of LDL cholesterol [18]. Mechanistic studies have shown that remnant cholesterol can accumulate and infiltrate the endothelial barrier, producing inflammatory reaction and thereby causing atherogenic process in the arterial wall. Experimental studies have indicated that remnants are found to be associated with impaired endothelial function and enhanced inflammatory response [19].

RLP-C levels of the insulin resistant DM patients of this study were correlated positively with TG (r=0.5191) and negatively correlated with HDL-C (r=-0.0542). Other researchers have also found elevations in RLP-C levels to be associated with increased TG levels and decreased HDL-C levels [20]. There is a strong correlation between TG content and remnant cholesterol. In T2DM, excess availability of free fatty acids in the myocardium, shifts the substrate for metabolism to depend more on oxidation of free fatty acids which may lead on to diabetic cardiomyopathy [21].

It has been proposed that reduction of plasma remnant lipoprotein should be the target for patients with metabolic syndrome rather than lowering of LDL-C alone [22]. Hence apart from lowering the LDL-C levels, measures to reduce RLP-C levels should also be taken.

Elevated levels of remnant cholesterol can be lowered by adopting lifestyle modifications and by pharmaco-therapy. Life style changes which may help to lower remnant cholesterol levels are weight reduction, decreased alcohol intake, reduction in the intake of saturated fat, avoidance of smoking and increased physical activity. These lifestyle changes may reduce remnant cholesterol levels by decreasing the hepatic secretion of VLDL particles and by enhancing their clearance. Statins, niacin and fibrates play a role in lowering of remnant cholesterol levels [23].

Calculation of RLP-C is not being widely practiced and not much information is available in the Asian population. Assessment of RLP-C levels can be included for the better risk stratification. Optimal standardization of procedures to estimate RLP-C is desirable. Large scale studies may help to understand the role of RLP-C in the prediction of cardiovascular events.

LIMITATION

Small sample size of the group of diabetic patients with IR and the measurement of RLP-C was calculated instead of direct measurement.

CONCLUSION

Our study concludes that IR diabetic patients have elevated levels of RLP-C which correlates with CIMT, a marker of cardiovascular disease. These RLP-C levels could be measured, in addition to the standard lipid profile, for assessing the propensity for developing atherosclerosis, without any additional financial burden.

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