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Research Article

Association of cardiovascular risk factors with insulin & insulin resistance in type-2 Diabetes Mellitus patients

Momin AA[1*], Bankar MP[2], Bhoite GM[3], Shaheen B[1], Yadav RD[4]

[1]Assistant Professor, Department of Biochemistry, Institute of Medical Sciences & Research, Mayani- 415102, Maharashtra, India

[2]Professor, Department of Biochemistry, B. J. Government Medical College, Pune- 411001, Maharashtra, India

[3]Assistant Professor, Department of Physiology & Biochemistry, BVDU Dental College, Pune- 411043, Maharashtra, India

[4]Assistant Professor, Department of Biochemistry, Dr. Ulhas Patil Medical College, Jalgaon- 425309 Maharashtra, India

***Corresponding author**
Abdulrahaman A. Momin
Email: rahamanmomin@gmail.com

Abstract: Cardiovascular diseases are the most common complications of type 2 diabetes patients with presence of dyslipidemia. Dyslipidemia is characterized by increased triglycerides and decreased HDL cholesterol. The ratios calculated from the lipids and lipoproteins may be useful to know the status of the CVD complications in type 2. In the present study, the concentrations of total cholesterol, triglycerides, HDL cholesterol were estimated from fasting serum samples, followed by the calculations of LDL & VLDL cholesterol and lipoprotein ratios including TC/HDL-C, TG/HDL-C, LDL-C/HDL-C, CRF and non HDL cholesterol. Also estimated insulin level, and homeostasis model assessment for insulin resistance (HOMA-IR) was calculated as discriminator of insulin resistance, from fasting glucose and insulin levels, and association of insulin & insulin resistance with cardiovascular risk factors was evaluated. The present study had included 150 type 2 diabetic subjects and 150 age and sex matched healthy controls. Significant increase in the lipid and lipoprotein levels, except HDL cholesterol which were significantly decreased in type 2 diabetics than controls. BMI, Fasting blood glucose, TC, TG, LDL-C, VLDL-C, TC/HDL-C, TG/HDL-C, LDL-C/HDL-C, Non HDL-C and CRF were positively associated with insulin level and HOMA IR, while HDL-C was negatively associated with insulin and insulin resistance. Therefore, the lipoprotein ratios along with the lipid and lipoprotein levels may be used as markers for insulin resistance and for cardiovascular risk.

Keywords: type 2 diabetes mellitus, cardiovascular diseases, Lipoproteins, Triglyceride, total Cholesterol, insulin resistance

INTRODUCTION

Type 2 diabetes mellitus is a heterogeneous condition characterized by the presence of both impaired insulin secretion and insulin resistance. Type 2 diabetes mellitus makes up about 90% of the diabetic population. It is chronic disease and usually irreversible, so, the diabetic patients have to consult health care providers on a regular basis throughout their lives [1-3]. Type 2 diabetics are prone to certain complications, which might be due to abnormalities in lipid and lipoprotein metabolism [4, 5].

Diabetes can lead to severe complications such as obesity, neuropathy, nephropathy, retinopathy, cardiopathy, dyslipidemia, osteoporosis and coma leading to death [6, 7]. Dyslipidemia which includes both quantitative and qualitative abnormalities of lipoproteins, is one of the pathophysiologic mechanism known to play a significant role in the pathogenesis of vascular complications in individuals with insulin resistance and diabetes mellitus [8, 9].

Characteristic abnormalities of dyslipidemia in type 2 diabetes mellitus include elevated triglycerides (TG) levels, and increased levels of low density lipoprotein cholesterol (LDL-C). (2) Hypertriglyceridemias often accompanied by low levels of high-density lipoprotein cholesterol (HDL-C) and modification of low-density lipoprotein (LDL) into atherogenic small dense LDL particles [10]. This affects the insulin mediated glucose disposal and thereby hyperinsulinemia, with high levels of fasting glucose. Thus, dyslipidemia shows the association with insulin resistance and increased risk for cardiovascular diseases (CVD) [11].

The literature is inconsistent regarding the relationship between baseline insulin levels, insulin resistance and risk of cardiovascular diseases. The aim

of this study was to investigate the association between insulin resistance and levels of various CVD risk factors. The lipids and lipoprotein levels were estimated and the ratios of total cholesterol TC/HDL-C, TG/HDL-C, LDL-C/HDL-C, cholesterol retention fraction (CRF), and the levels of non-HDL-C were calculated. The association of insulin resistance and cardiovascular disease risk factors in type 2 diabetic patients was studied.

MATERIAL AND METHODS

Subjects

In present case-control study, 150 type 2 diabetes mellitus patients within the age 40-60 years were included randomly, from Department of Medicine, B. J. Government Medical College, Pune. Diabetic patients with cardiovascular diseases, pulmonary diseases and patients on insulin treatment were excluded from the study. In addition to diabetic patients, age and sex matched 150 healthy subjects were included as controls. The control subjects were non-diabetic and with no history of cardiovascular disorders. The study protocol was approved by institutional Ethics Committee and written informed consent was obtained from each participant after complete explanation of the procedure. Blood pressure of the patients and controls was measured in the sitting position after 5 minutes rest with a mercury sphygmomanometer. Body Mass Index (BMI) was calculated using formula weight in KGs/Height in m^2, and Waist to hip ratio (WHR) was calculated by dividing length of waist to the length of hip.

Laboratory measurements

The blood samples were collected by venipuncture in a sitting position after fasting of 12 hours. Fasting plasma glucose was measured by enzymatic Glucose Oxidase Peroxidase method [12] and Insulin concentrations were detected using chemiluminescence immunoassay after the serum samples were thawed at room temperature. Insulin resistance was calculated by means of HOMA-IR, [6, 13] by using the following formula.

HOMA IR= Fasting glucose (mmol/l) x fasting insulin (µU/ml) / 22.5

Total cholesterol [14] and triglyceride [15] levels were measured by enzymatic methods. HDL-C was estimated after precipitation of chylomicrons, very low density lipoproteins (VLDL) and LDL fractions of cholesterol from serum with phosphotungstic acid and magnesium chloride. After centrifugation the cholesterol in the HDL fraction, which remains in the supernatant is assayed with enzymatic Cholesterol Oxidase Peroxidase method [16]. All the estimations were done on fully auto analyser. LDL-C and VLDL-C were calculated with Friedwald's formula [17] and TC/HDL-C ratio, TG/HDL-C ratio, the CRF and non-HDL-C were calculated.

Statistical analysis

Continuous variables were presented as mean ± standard deviation (SD) and differences in means between diabetic subjects and controls were compared using 'z' test. Associations between insulin level & insulin resistance with cardiovascular risk factors viz. TC, TG, HDL-C, LDL-C, VLDL-C, ratios of TC/HDL-C, TG/HDL-C, LDL-C/HDL-C, CRF and non-HDL-C were tested in type 2 diabetic patients using Pearson's correlation coefficient.

RESULTS

Out of 150 type 2 diabetes mellitus patients, 41 had hypertension of which 21 were females and 20 males. Total number of 17 patients had only cholesterol level >200 mg/dl (hypercholesterolemia), 7 had only hypertriglyceridemia (TG ≥ 150), and 8 females had HDL-C ≤50 mg/dl, while 13 males had HDL-C ≤40 mg/dl. 23 of the diabetic women and 11 men had hypercholesterolemia as well as hypertriglyceridemia, while 17 females were with hypercholesterolemia, hypertriglyceridemia, and HDL-C level≤50 mg/dl, and 03 males had had hypercholesterolemia, hypertriglyceridemia, and HDL-C level ≤40 mg/dl. 16 of diabetic women had hypertriglyceridemia, and HDL-C level ≤50 mg/dl, and 3 men were with hypertriglyceridemia, and HDL-C level ≤40 mg/dl

Blood glucose, systolic BP, Diastolic BP, BMI, WHR, TC, TG, LDL-C, TC/HDL-C, TG/HDL-C, LDL-C/HDL-C, CRF, non-HDL-C, HOMA-IR and insulin levels were significantly ($p<0.05$) higher in type 2 diabetes mellitus patients when compared with control subjects, and HDL-C was significantly ($p<0.05$) lower in type 2 diabetes mellitus (T2DM) patients than control subjects. The results of demographic and cardiovascular risk factors in type 2 diabetic patients and controls are shown in table no. 1 & 2.

Table 1: Comparison of demographic characteristics between type 2 diabetic patients and healthy control subjects

Variables	T2DM Patients (n=150)	Healthy Controls (n=150)
Age	56.1 ± 10.73	54.5 ± 11.65
Systolic BP (mmHg)	132.7 ± 16.15	121.8 ± 8.50*
Diastolic BP (mmHg)	85.2 ± 8.21	78.8 ± 6.35*
BMI (Kg/m2)	27.2 ± 2.58	24.3 ± 2.70*
WHR	1.0 ± 0.08	0.9 ± 0.08*

* Statistically significant

Table 2: Comparison of biochemical characteristics between type 2 diabetic patients and healthy control subjects

Variables	T2DM Patients (n=150)	Healthy Controls (n=150)
Glucose (mg/dl)	132.0 ± 16.06	97.2 ± 15.71*
Glycated hemoglobin	7.9 ± 1.91	3.2 ± 1.38*
Total cholesterol (mg/dl)	204.8 ± 31.12	171.8 ± 23.71*
Triglycerides (mg/dl)	160.5 ± 25.29	130.5 ± 34.98*
HDL-C (mg/dl)	43.7 ± 5.69	47.8 ± 3.93*
LDL-C (mg/dl)	128.9 ± 30.47	97.9 ± 23.20*
VLDL (mg/dl)	32.1 ± 5.06	26.1 ± 6.99*
TC/HDL-C ratio	4.8 ± 0.93	3.6 ± 0.56*
TG/HDL-C ratio	3.7 ± 0.82	2.8 ± 0.77*
LDL-C/HDL-C ratio	3.0 ± 0.85	2.1 ± 0.53*
CRF	0.6 ± 0.11	0.5 ± 0.16*
Non-HDL-C (mg/dl)	161.0 ± 31.56	124.1 ± 23.74*
Insulin level (μU/ml)	10.0 ± 3.83	7.7 ± 1.60*
Insulin resistance (HOMA-IR)	3.3 ± 1.46	1.8 ± 0.43*

* Statistically significant

The correlation of serum insulin & HOMA IR with cardiovascular risk factors was evaluated Pearson's correlation coefficient. It was observed, BMI, Fasting blood glucose, total cholesterol, triglycerides, LDL cholesterol, VLDL cholesterol, ratio of TC/HDL cholesterol, TG/HDL cholesterol, LDL/HDL cholesterol, Non HDL cholesterol and CRF were positively associated with insulin level and HOMA IR, while HDL-C was negatively associated with insulin and insulin resistance. The results of Pearson's correlation of cardiovascular risk factors with insulin & insulin resistance are depicted in table no. 3, the value of 'r' denotes the correlation coefficient.

Table 3: Correlation of TC, TG, HDL-C, LDL-C, TC/HDL-C ratio, TG/HDL-C ratio, LDL-C/HDL-C ratio, non-HDL-C and CRF with insulin level and insulin resistance (HOMA-IR) in T2DM patients

Variables	HOMA IR		INSULIN	
	r	*P*	r	*P*
BMI	0.197	<0.0157*	0.206	0.0116*
WHR	0.083	0.3139	0.107	0.1905
Fasting blood glucose	0.587	<0.0001*	0.337	<0.0001*
Total cholesterol	0.231	0.0044*	0.255	0.0017*
Triglycerides	0.436	<0.0001*	0.471	<0.0001*
HDL cholesterol	-0.216	0.0080*	-0.171	0.0.0362*
LDL cholesterol	0.204	0.0122*	0.214	0.0086*
VLDL cholesterol	0.436	<0.0001*	0.472	<0.0001*
TC/HDL cholesterol	0.325	<0.0001*	0.314	0.0001*
TG/HDL cholesterol	0.440	<0.0001*	0.439	<0.0001*
LDL cholesterol /HDL cholesterol	0.269	0.0009*	0.259	0.0014*
Non HDL cholesterol	0.267	0.0010*	0.282	0.0005*
CRF	0.271	0.0008*	0.265	0.0011*

* Statistically significant

DISCUSSION

Insulin resistance is a common phenomenon and recently has gained importance because of its possible etiopathogenic role in the various clinical conditions [18]. It is one of the pathogenic factor for type 2 diabetes and is associated with diverse cardiovascular disease risk states, including obesity, hypertension, hypertriglyceridemia, and low HDL cholesterol [19]. Insulin-resistant individuals show the features like glucose intolerance, a high plasma triglyceride and low HDL cholesterol concentration, and elevated blood pressure [20]. All of these characteristics have been individually identified as risk factors for contribution to coronary heart disease.

In the present study lipids and lipoprotein cholesterol levels were estimated and also derived the lipoprotein ratios. The levels of fasting blood glucose, systolic & diastolic blood pressure, BMI, WHR, TC, TG, LDL-C, and TC/HDL-C, TG/HDL-C, LDL-C/HDL-C, CRF,

non-HDL-C, insulin and HOMA-IR (insulin resistance) levels were elevated significantly in type 2 diabetic patients than the healthy controls, while HDL-C was significantly decreased in type 2 diabetes patients than controls. Similar results were found by Tangvarasittichai S *et al.* [21], significant increase in the lipids and lipoprotein levels along with significant difference in their ratios was found in their study.

The type 2 diabetic patients are at increased risk of cardiovascular diseases which may be due to the increased levels of lipids and lipoproteins [4, 5] or it may be seen due to increased insulin resistance [7, 8]. The insulin resistance syndrome is most quantitatively associated with elevation in TG related lipoproteins, and decreased levels of HDL-C. [22] Many studies revealed the cause of increase in VLDL production rates in subjects of insulin resistance [23, 24]. Insulin has found to be profound effects on VLDL metabolism, the patients with insulin resistance show hypertriglyceridemia as the most common lipoprotein abnormality. Basically, it is due to an elevation in the synthesis of hepatic VLDL triglyceride. Production as well as secretion of Hepatic apoB-100 also increases, but the increase is less than VLDL triglycerides [25].

It is observed that acute insulin infusion reduces production of VLDL, apolipoprotein B, particularly in the large triglyceride-rich VLDL1 fraction, in healthy non-obese adults; however, this suppressive effect of insulin is attenuated or lost in the insulin resistant conditions of obesity or type 2 diabetes [26].

Insulin resistance clusters with elevated blood pressure, obesity, central obesity, elevated levels of total triglycerides, low levels of HDL cholesterol, and hemostatic abnormalities. This clustering of CVD risk factors exists in non-diabetic individuals and patients with type 2 diabetes and predicts CHD [27].

Association between lipids, lipoproteins and lipoprotein ratios with insulin and insulin resistance by Pearson's correlation coefficient was found. BMI, Fasting blood glucose, TC, TG, LDL-C, VLDL-C, TC/HDL-C, TG/HDL-C, LDL-C/HDL-C, Non HDL-C and CRF were positively associated with insulin and HOMA IR, while HDL-C was negatively associated with insulin and insulin resistance.

Tangvarasittichai S. *et al.* [21] also studied the correlation between lipids, lipoproteins and their ratios with insulin resistance, our results are consistent with this study. J. Vangipurapu *et al.* [28] studied the correlation of liver IR index and Matsuda ISI in non-diabetic subjects, they found significant correlation with all CVD risk factors examined, except LDL cholesterol for both indices and total cholesterol fir Matsuda ISI. In a meta-analysis Ruige *et al.* [29] shown, a weak positive association between high insulin levels and CVD events.

CONCLUSION

From the above results it can be concluded that the lipid levels along with the lipoprotein ratios can be useful for the prediction of insulin level and degree of insulin resistance and thereby the cardiovascular risk in type 2 diabetic patients and therefore lipoprotein ratios should be made available to the clinician with every lipid profiles ordered, this will eliminate any additional costs to the laboratories or to the patients, and it will help in the management of the dyslipidemia in type 2 diabetic patients.

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