ORIGINAL RESEARCH

Association of insulin and HOMA-IR with lipid profile and LCAT activity in type 2 diabetes

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Received: 10 February, 2023 Accepted: 15 March, 2023

ABSTRACT
Background: Diabetes mellitus type 2 is a metabolic disorder characterized by hyperglycemia, hyperinsulinemia and insulin resistance. Insulin activity is essential for maintaining glucose homeostasis and insulin resistance leads to impaired glucose tolerance which in turns cause development of diabetes and also effects serum lipids. Quantification of insulin and its association with insulin resistance and serum lipids is essential in guiding treatment options and predicting diabetes risk.
Methods: A case-control was conducted, recruiting 45 type 2 diabetes patients and 45 age/sex matched healthy controls.FBS, HbA1c, lipid profile, insulin, LCAT and Apolipoprotein A-1 was estimated and HOMA-IR was calculated. All study parameters were compared between both the study groups and the association of insulin and HOMA-IR with these parameters were examined. **Results:** Diabetic cases have significant higher level of FBS, HbA1c, total cholesterol, TG, LDL and VLDL than controls, whereas HDL, LCAT and Apolipoprotein A-1 levels were significantly higher in controls than diabetic cases.Insulin levels in patients (28.49 ± 11.31) was significantly higher than controls (5.96 ± 1.78). Calculated HOMA-IR was significantly increased in diabetic group. Insulin had significant positive correlation with HOMA-IR.
Conclusion: Insulin and HOMA-IR activity is significantly increased in patients with type 2 diabetes and had non-significant positive association with LCAT, Apolipoprotein or lipid profile A-1in type 2 diabetic cases.
Key words: Diabetes mellitus, insulin, HOMA-IR, LCAT, Lipid profile

1. INTRODUCTION

Diabetes mellitus is a metabolic disorder characterized by high blood glucose levels and have a high risk of developing various serious life-threatening health issues. The global prevalence of diabetes has been increased over the recent years due to rapid urbanization and sedentary lifestyle. Estimated data projected by International Diabetes Federation (IDF) shows that by 2045, 629 million people, equaling 9.9% of population would be living with diabetes compared to 425 million in 2017 [1]. Nearly half of all adults in India and China live with diabetes and have underlying predisposition to deficient insulin resistance [2]. Primary factors for the pathogenesis of diabetes mellitus are defective insulin secretion by pancreatic β-cells and inappropriate response of insulin-sensitive tissues to insulin (Insulin resistance). Release of insulin and its activity is essential in maintaining glucose homeostasis and defects in any of the mechanism involved in these processes can lead to development of the disease [3].

Insulin is a peptide hormone secreted by β-cells of islets of Langerhans and controls the metabolism of various biomolecules like carbohydrates, proteins and fats. Insulin stimulates the absorption of glucose from blood to various tissues such as skeletal muscle cell, liver etc. Insulin resistance is triggered by reduction in insulin signaling in the insulin receptor substrate (ISR)/Phosphoinisitide-3-kinase (PI3K)/Protein Kinase B (PKB)/AKT pathway and is associated with

inflammation, hyperinsulinemia and lipotoxicity[4]. Due to decrease in insulin activity, not only hyperglycemia occurs but serum lipids are also strongly affected. Dyslipidemia is commonly observed in diabetic patients irrespective of insulin deficiency or resistance. Insulin exhibits anti-lipolytic action, promotes storage of TG in adipose tissue and inhibits VLDL production in Liver. Insulin also acts on HDL through activation of LCAT and hepatic lipase [5].

Hypertriglyceridemia and low levels of high density lipoproteins (HDL) was frequently observed in diabetic subjects rather than hypercholesterolemia. Triglycerides (TG) are elevated along with the degree of hyperglycemia and hyperinsulinemia (in compensation of insulin resistance).In patients with impaired glucose tolerance, loss of normal insulin sensitivity leads to compensatory hyperinsulinemia and increased Very low density lipoprotein (VLDL)/TG secretion [6].The homeostasis model assessment (HOMA) is widely used clinical tool to estimate insulin resistance and β-cell function. The HOMA of insulin resistance (HOMA-IR) index, is considered as a simple, inexpensiveand reliable alternate measure of insulin resistance [7].

Lecithin cholesterol acyltransferase (LCAT) enzyme helps in the production of HDL by transferring 2-acyl group from lecithin to unesterified cholesterol and is believed to play a vital role in macrophage reverse cholesterol transport. Alteration in its activity significantly contributes to metabolic syndrome and cardiovascular diseases [8].

Association of insulin and HOMA-IR with dyslipidemia is well established but its association with LCAT and apolipoprotein A-1 is not yet well known. Therefore purpose of this study was to determine the differences in clinical parameters among diabetes and healthy subjects and to examine the association of serum insulin and HOMA-IR with lipid profile, LCAT and Apolipoprotein A-1.

2. MATERIALS AND METHODS

2.1 SOURCES OF THE DATA

45 Diabetic patients, both male and female between the age group of 20-65, attending medicine outpatient department (OPD) of MGM Hospital, Navi Mumbai, during the period between February 2015 to February 2016 and 45 age/sex matched controls was recruited. Laboratory investigations and data analysis was performed in the Department of Biochemistry of Mahatma Gandhi Mission Medical College, Navi Mumbai. Maharashtra, India. The study was approved by the Institutional Ethics Committee. [Approval No. 2015/SC/97]

2.2 INCLUSION AND EXCLUSION CRITERIA

Both diabetic cases (as per WHO criteria) and healthy controls within the age group of 20-65 were included in the study after taking the informed written consent. Patients with hypothyroidism, Type 1 diabetes, Cushing's syndrome, renal or hepatic diseases were excluded from the study. Patients with known/suspected pregnancy were also excluded.

2.3 SAMPLE COLLECTION

6.0 ml of blood after 12 hours of overnight fasting was collected from each subject by venipuncture with standard blood collection technique in a plane vial for serum separation, sodium fluoride vial for plasma and EDTA vial for HbA1c estimation. Plasma was collected again after two hours of post meal for the postprandial glucose estimation.

Serum FBS was measured by GOD-POD method with the help of Randox RX, a fully automatic analyzer. Estimation of serum Cholesterol, serum Triglyceride& serum HDL was done by CHOD-POD method, GPO-POD Method & CHO-POD Method respectively. VLDL and LDL were calculated by Friedewald's Formula. HbA1c was measured by turbidimetric immunoassay with the help of HPLC Method. The assays were performed according to the manufacturers' instructions. Serum insulin was measured using ELISA kit, according to manufacturer's protocol and HOMA-IR was calculated as: Glucose (mg/dl)x Insulin (mIU/L)/405

Plasma LCAT activity was estimated initially by following the removal of one substrate, the reduction of free cholesterol, after incubation of the plasma at 37 'C. Free cholesterol was estimated after Digitonin-precipitation by the colorimetric method (1960) [9].

Quantitative determination of APO-A1 was done by an Immunoturbidimetric method, by semi-automatic analyzers using Ben - Biochemical Enterprise S.R.L. - via Toselli, Milano, Italy. This method was based on the reaction of a sample containing human Apo AI and specific antiserum to form an insoluble complex which can be measured turbidimetrically at 340nm. By constructing a standard curve from the absorbance of standards, the concentration of Apo AI can be determined [10].

2.4 STATISTICAL ANALYSIS

Data were analyzed using 'GraphPadQuickCals t-test calculator'. The results were further subjected to students 't' test for Comparisons between the groups and further expressed as mean ± SD. A 'p' value of less than 0.05 was considered significant.

Ethical clearance was obtained from Institutional Ethics Review Committee (IERC). All patients and healthy volunteers were enrolled and a written informed consent was taken. The proforma included, name, age, sex, dietary habit, personal history of disease (if any), smoking habit, drinking habit, socioeconomic status and occupation.

3. RESULTS

The study had 90 participants between the age groups of 20-65 years. The diabetic group included 28 males and 17 females whereas the control group included 26 males and 19 females (Table 1).

Table1:Gender wise distribution of subjects in control group (normal Healthy individuals) and Study group (Type -2 Diabetes Patients)

Groups	Male	Female	Total
Healthy Control	26(57.78%)	19 (42.22%)	45 (100%)
Type 2 Diabetes	28 (62.22%)	17 (37.78%)	45 (100%)
Total	54 (60%)	36 (40%)	90 (100%)

The mean age of the patients in the case and control group were 56.75± 9.63 and 34.4± 10.1 respectively. A non-significant increase in BMI was observed in the case group compared to controls. Total cholesterol, TG, VLDL, Fasting Blood Sugar (FBS), Post-prandial Blood sugar (PPBS) and HbA1c were significantly elevated in diabetic patients compared to controls. However LDL shows non-significant difference in the case and control group. HDL, LCAT and Apo-lipoprotein A-I was significantly deceased in the diabetic group compared to controls(Table 2).Serum insulin levels and insulin resistance (evaluated by HOMA-IR) was markedly increased in diabetic patients compared to controls (Figure 1).

Table 2:Clinical and biochemical parameters of the enrolled cases and controls showing mean ± SD

Parameters	Case mean ± SD	Control mean ± SD	p- value
Age(years)	56.75 ± 9.63	34.4 ± 10.1	<0.0001**
BMI (m/Kg$^{2)}$	21.66 ± 2.21	21.28 ± 3.81	0.5642
FBS(mg/dl)	161.23 ± 52.95	91.65 ± 8.95	<0.0001**
PPBS (mg/dl)	259.97 ± 77.11	128.02 ± 10.52	<0.0001**
HbA1c (%)	9.26 ± 1.56	5.14 ± 0.21	<0.0001**
T. Cholesterol (mg/dl)	199.8 ±54.94	179.8±29.14	0.0337*
TG (mg/dl)	160.21 ±71.77	133.18±27.09	0.0204*
HDL (mg/dl)	34.22 ±10.07	42.1±6.76	<0.0001**
LDL (mg/dl)	127.62 ±47.81	111.66±27.97	0.0565
VLDL (mg/dl)	35.48 ± 24.38	26.52± 5.76	0.0185*
Insulin (mIU/L)	28.49± 11.31	5.96± 1.78	<0.0001**
HOMA-IR	11.2± 6.76	1.35± 0.43	<0.0001**
LCAT (mg/dl)	57.37 ± 10.01	93.56 ± 4.9	<0.0001**
Apolipoprotein A-I (mg/dl)	57.98 ± 12.8	124.25±17.53	<0.0001**

*$p\leq0.05$ Significant, **$p\leq0.001$ Highly significant

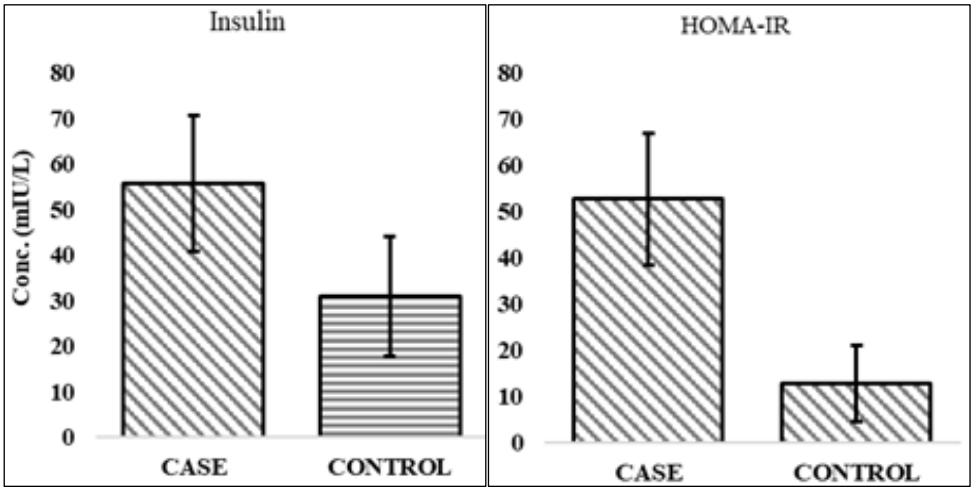

Figure1:Comparison between Insulin and HOMA-IR showing mean± SD

Pearson's correlation analysis of insulin and HOMA-IR with various biochemical parametersin diabetic cases was performed. Both insulin and HOMA-IR is positively correlated with FBS, HbA1c, lipid profile, LCAT and apolipopreotein A-1. However the correlation is non-significant (Table 3). Insulin was

positively associated with HOMA-IR in both, diabetic and healthy groups. The correlation between insulin and HOMA-IR was highly significant (Figure 2).

Table 3:Pearson's correlation of Insulin and HOMA-IR with various parameters in diabetic patients

Parameter	Insulin		HOMA-IR	
	r value	p value	r value	p value
BMI	0.01	0.945	0.04	0.800
FBS	0.13	0.400	0.55	0.0001**
PPBS	0.27	0.076	0.48	0.0008**
HbA1c	0.25	0.099	0.40	0.006*
T. Cholesterol	0.22	0.153	0.31	0.036*
TG	0.03	0.854	0.20	0.192
HDL	0.41	0.005*	0.41	0.005*
LDL	0.21	0.158	0.22	0.151
VLDL	-0.03	0.860	0.11	0.462
LCAT	0.23	0.124	0.13	0.390
Apolipoprotein A-I	0.15	0.331	0.15	0.321

*p≤0.05 Significant, **p≤0.001 highly significant

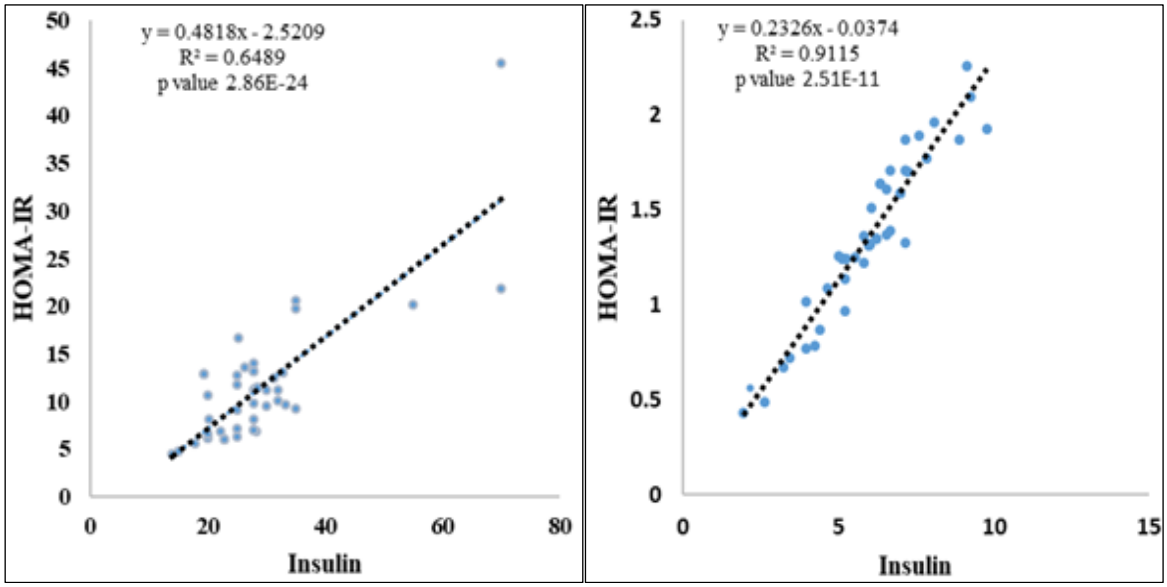

Figure2: Scatter plot showing correlatio between Insulin and HOMA-IR in (A) Diabetic patients and (b) Healthy controls

4. DISCUSSION

Diabetes and pre-diabetes are major public health burden globally. Identifying and controlling the risk factors such as obesity, dyslipidemia and insulin resistance before the occurrence of diabetes are vital to control this metabolic disorder. Insulin resistance and HOMA-IR index are good predictors of diabetes pathogenesis even in individuals with normal glucose tolerance. Basal hepatic glucose production and hepatic insulin sensitivity is reflected by HOMA-IR[11].

The aim of our study was to find an association between serum insulin, insulin resistance and lipid profile, BMI, LCAT and apolipoprotein A-1 in type 2 diabetic cases and healthy controls. Ethical clearance was granted by Scientific and Ethical committee of MGM Medical College and patients were recruited from Medicine OPD after taking the written consent. Our study includes 45 diabetic patients and 45 age/sex matched healthy control subjects.

In the present study, we estimated fasting and post prandial blood glucose and HbA1c. We found we found significant elevation in their levels in diabetic group compared to control group. We also estimated fasting, post-prandial blood glucose and HbA1c levels and found significantly increased levels in diabetic group.Ghanei A *et al.*Also reported significantly higher levels of FBS, HbA1c, HOMA-IR and TG in type 2 diabetic patients [12].

Hyperinsulinemia is one of the characteristic of type 2 diabetes. Results of our study showed increased serum insulin and HOMA-IR levels in diabetes cases compared to healthy controls suggesting β-cell dysfunction and insulin resistance in diabetes. Our analysis also provides evidence for a significant positive association between serum insulin and

HOMA-IR in diabetic group. Previous studies have also reported the association between insulin and HOMA-IR in type 2 diabetes. So *et al.* [13] and Khalil D *et al*. [14]reported HOMA-IR as a suitable marker of insulin resistance and good predictors of pre-diabetes and diabetes. Another study by Adiga U *et al.* also reported a positive correlation between insulin and HOMA-IR (both insulin and C-peptide), C-peptide based HOMA being more sensitive marker of insulin resistance [15].

Dyslipidaemia is commonly observed in type 2 diabetes and is major risk factor for diabetic microvascular complications and cardiovascular diseases. In the present study, we compared the mean levels of total cholesterol, TG, LDL, VLDL and HDL in diabetic cases and healthy controls and observed a significant elevation of Total cholesterol, TG and VLDL in diabetic cases. Serum HDL levels was significantly reduced in diabetic cases.Our study also observed a positive correlation of insulin and HOMA-IR with lipid profile. However the correlation was non-significant. Our findings are concurrent withPourfarzam, *et al*.Who studied the association of HOMA-IR with TG/HDL-C ratio in diabetic patients with and without metabolic syndrome and reported a positive correlation between HOMA-IR and TG/HDL-C ratio suggesting their vital role in increased CVD risk in type 2 diabetes patients [16].

LCAT is regulated by insulin and controls HDL-C levels. In our study, levels of LCAT and apolipoprotein A-1 was significantly reduced in diabetic group compared to controls that might be due to decreased insulin activity and increased insulin resistance. However we found no significant correlation of insulin and HOMA-IR with LCAT or apolipoprotein A-1.Our findings are concurrent with Leança*et al*.,who reported low LCAT activity and diminished LCAT mediated conversion of precursor pre-beta1-HDL into the larger HDL. They had also reported decreased TG/HDL-C ratio in hyper-alphalipoproteinemia that is believed to be critically dependent on insulin activity [17].Ghanei A *et al*.also estimated the activity of LCAT and its association with various factors in patients with diabetes and reported significantly.Decreased activity of LCAT in type 2 diabetes with no significant association with HOMA-IR [12]. In contrast, Afandi MR *et al*.Found a strong correlation between HOMA-IR and Apoprotein B/Apoprotein A-1 ratio[18].

Our study may have some limitations like bias from daily dietary habits and exercise may exist and the pathophysiological basis of hyperglycaemia and hyperinsulinemia may vary among different ethnicity.

5. CONCLUSION

Control of BMI and WC (Waise Circumference) is vital to avoid metabolic diseases. In conclusion, diabetic patients had different lipid profiles and BMI. Diabetic patients had elevated serum insulin levels and insulin resistance as evaluated by HOMA-IR and is correlated positively with lipid profile parameters. LCAT and apolipoprotein A-1 activity was decreased in diabetes cases.

ACKNOWLEDGMENTS

The authors would like to express their sincere gratitude to all the patients who agreed to participate in this study.

FUNDING

The authors received no funding from any government or private organizations for this work.

COMPETING INTERESTS

The authors declare that we have no conflict of interests to declare.

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