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ASSESSMENT OF INSULIN RESISTANCE AND Apo B / Apo A1 RATIO IN TYPE II DIABETES PATIENTS

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Background: Insulin resistance (IR) is known to play an important role in the development of coronary artery disease (CAD). Some recent studies have reported that the serum Apo B/Apo A_1 ratio is a predictive index of coronary artery disease.

Aims and Objectives:

1. To determine the serum Apo B and Apo B/Apo A_1 ratio.

2. To assess the association of the serum Apo B and Apo B/Apo A_1 ratio with insulin resistance in diabetic patients and healthy controls.

Materials and Methods: This study was a case control study which included 40 diabetic patients and 40 healthy controls. Fasting blood sugar, Post prandial blood sugar, fasting serum Insulin level, lipid profile, Apo A_1 and Apo B_{100} were analyzed. The insulin resistance was determined using the homeostasis model assessment of insulin resistance index (HOMA-IR).

Results: Apo B/ApoA_1 ratio was found to be significantly high in diabetic patients when compared to the normal subjects. The Apo B, total cholesterol, low-density lipoprotein-cholesterol (LDL-C) and Apo B/Apo A_1 ratio showed positive correlation with insulin resistance. The Apo A_1 showed negative correlation with insulin resistance.

Conclusion: Insulin resistance plays a significant role in raising the serum Apo B/Apo A_1 ratio in diabetic patients, which is a candidate risk factor for coronary artery disease and its severity.

Key Words: Type 2 diabetes, Apo B, Apo A, Insulin Resistance

INTRODUCTION

Type 2 diabetes is a metabolic disorder, characterized by chronic hyperglycemia resulting from defects of insulin secretion, insulin action or both.[1] It is estimated that there are currently 285 million people with diabetes worldwide and this number is set to increase to 438 million by the year 2030.[2] The pathogenesis of type 2 diabetes are complicated by factors like insulin resistance, obesity, lack of physical activity and stress.[3] Resistance to insulin is found to be the major contributor to the atherogenic dyslipidemia like increased level of LDL and VLDL and decreased level of HDL-C. However, these conventional risk factors explain only a portion (25%) of the excess of cardiovascular risk in Type II Diabetes Mellitus(Type II DM). New datas are accumulating in favour of apoproteins as more informative lipid risk factors than conventional one.[4] ApoB which indicates the number of potentially atherogenic lipoprotein particles and Apo A_1, which reflects antiatherogenic HDL particles, indicate more accurate cardiovascular (CV) risk factor than LDL C and other lipids.[4] So the present study was designed to determine the serum Apo B and Apo B/Apo A_1 ratio and to assess the association of ApoB/Apo A_1 ratio with insulin resistance in diabetic patients and controls.

MATERIALS AND METHODS

Study group consisted of 40 diagnosed Type II DM subjects (18 females and 22 males) and 40 age and sex matched control subjects in the age group of 30-65 years. The study protocol was approved by the Ethical Committee of Vinayaka Missions Kirupanandha Variyar Medical College, Salem and the present case control study was conducted at the Department of Biochemistry of the same college. From each patient, their medical history was obtained through a structured questionnaire and an informed consent was obtained. The inclusion criteria included diagnosed patients who were on oral

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hypoglycemic drugs and the patients excluded were those diagnosed to have infectious diseases, fever, hepatic or renal disease, malignancies, lipid lowering medication or history of cardio vascular disease.

Venous blood sample was collected after an overnight fast of 12 hours and the serum was used for the estimation of Fasting Blood Glucose (FBG) by enzymatic GOD-POD method, Total Cholesterol (TC) by enzymatic 'CHOP-PAP' method, Triglyceride (TG) by enzymatic GPO-POD method, High Density Lipoprotein cholesterol (HDL-C) by direct enzymatic colorimetric method and LDL-C by direct enzymatic colorimetric method. VLDL-C was calculated using the Friedewald's formula.[5] Apo A_1 and Apo B was measured by immunoturbidimetric method on the Cobas INTEGRA.[6] Fasting insulin was detected by ELISA method.

HOMA IR an index of IR was calculated using the formula

$$\text{HOMA-IR}^{7} = \frac{\text{Fasting Plasma Insulin (}\mu\text{U/ml) X Fasting Plasma Glucose (mmol/L)}}{22.5}$$

STATISTICAL ANALYSIS

The data were expressed as means ± SD. Statistical comparisons were performed by one way analysis of variance (ANOVA). The results were considered statistically significant if the p values were 0.05 or less.

RESULTS

Table I indicates the prevalence of clinically observed variables like age, sex distribution and duration of diabetes mellitus. No statistical difference was observed between the groups. This table also depicts the age, number of male and female subjects, duration of diabetes and HOMA IR level in patients with Type II DM when compared to the control. The mean HOMA IR level in Type II DM subjects was 3.21 ± 2.15 when compared to 0.971 ± 0.34 in control group. It was significantly elevated in diabetic patients when compared to controls ($p<0.001$). BMI was also found to be significantly high in diabetic patients.

Table II depicts the level of Triglyceride, Total cholesterol, HDL and LDL levels in DM patients and control. HDL-C level was found to be significantly low ($p>0.05$) and triglyceride level was found to be high in patients with Type II DM when compared to the control. No significant difference was observed in other parameters between the groups.

Table III represents the mean values of Apo A_1, Apo B and Apo B/Apo A_1 ratio in Type II DM patients and control subjects. Significantly low level of Apo A_1 and significantly high levels of Apo B and Apo B/Apo A_1 ratio was observed in Type II DM patients compared to control.

As per Table IV, in our study, highly significant positive correlation between Insulin Resistance and Body Mass Index (BMI), Waist Hip Ratio (WHR), Triglycerides (TGL), Apo B and ApoB/A_1 ratio and significantly negative correlation with Apo A_1 was observed.

Table I : Characteristics of the study group.

VARIABLES	CONTROL (n=40)	DIABETIC PATIENTS (n=40)
Age (in years)	48.6 ± 10.66	55.52 ± 6.5
Male	23	22
Female	17	18
Duration of DM (in years)	–	9.2 ±6.1
HOMA IR	0.971 ± 0.34	3.21 ± 2.15**
BMI	22.7 ± 2.1	27.4 ± 3.2*

* differ significantly from the control at $p<0.05$

** differ significantly from the control at $p<0.001$.

Table II. Lipid and lipoprotein values (mean±S.D.) in subjects with Type II DM and controls.

PARAMETERS	CONTROL	DIABETIC PATIENTS
Total cholesterol (mg/dl)	179±39.43	195 ± 37.7
Triglycerides (mg/dl)	104 ± 22.3	161 ± 18*
LDL (mg/dl)	126.27 ± 34.4	135±22.7
HDL-C (mg/dl)	50.08 ± 5	44.98 ± 7*

Values are expressed as mean ± SD

*differ significantly from the control at $p<0.05$.

Table III. Apolipoprotein values in subjects with Type II DM and control group		
Parameters	Control **(mean ±S.D.)**	Diabetic patients **(mean ±S.D.)**
Apolipoprotein A_1 (g/L)	2.02 ± 0.22**	1.2 ± 0.2
Apolipoprotein B (g/L)	1.02 ± 0.18	1.46 ± 0.33**
ApoB/$ApoA_1$	0.51 ± 0.1	1.26 ± 0.35**

Values are expressed as mean ± SD .

** differ significantly from the control at p<0.001.

Table IV: Correlation Analysis on study subjects		
	HOMA IR	
	r value	p value
BMI	0.434	0.000
WHR	0.297	0.007
TGL	0.295	0.008
LDL	0.122	0.011
HDL	0.115	0.022
Apo A_1	-0.719	0.000
Apo B	0.501	0.000
ApoB/A ratio	0.682	0.000

DISCUSSION

In our study insulin resistance was found to be significantly high in patients when compared to the control. Insulin resistance has been defined as a smaller than normal response to a given dose of insulin. The gold standard for assessing insulin resistance and insulin sensitivity is the hyperinsulinemic - euglycemic clamp technique; however, this test was found to be too labor intensive, time consuming, and costly for routine clinical practice. The Homeostasis Model Assessment (HOMA) may be used alternatively as it is minimally invasive, easy to apply in a standard office setting and provide reasonable indices of insulin action in pre diabetes and diseases of recent onset.[8] The main locus of Insulin resistance are liver cells which fails to recognize insulin signal as a suppressor of Phosphoenol pyruvate carboxy kinase (PEPCK) and also muscle and fat cells. There is also resistance to Insulin induced suppression of plasma Free fatty acids (FFA) in Type II DM and hence hyperinsulinemia is required to prevent elevation of circulating FFA. When islet beta cells are unable to sustain the hypersecretion of insulin, elevated levels of blood glucose and circulatory FFA were observed.[9]

In our study the BMI was found to be significantly high in diabetic subjects (Table I). This increase in BMI might be a consequence of Insulin resistance, the major causative factor for Type II DM.[10] IR greatly reduces the sensitivity of cell walls to insulin. So the vital process whereby glucose passes through the cell wall via insulin to be converted into energy gets greatly impaired. As a result, excess glucose remains in the blood stream, causing elevated levels of blood sugar, which are sent to the liver. Once it reaches there, the sugar gets converted into fat and carried via the blood stream throughout the body. This process can lead to weight gain and obesity. Goossens has **revealed that normal function of Adipose issue is disturbed during obesity and** adipose tissue dysfunction plays a prominent role in the development and/or progression of insulin resistance.[11] Henceforth increase in BMI might be due to insulin resistance which in turn causes hyperglycemia and adipose tissue dysfunction which can aggravate insulin resistance.

In the study, plasma triglyceride and VLDL were found to be significantly high and HDL-C level was significantly low in diabetic subjects when compared to control (Table II). Hyper triglyceridemia has been found to be one of the most consistent finding in Type II DM.[8] Evidence from both animal and human studies implied that insulin resistance which causes increased synthesis and/or decreased clearance of VLDL has been an important underlying cause of hypertriglyceridemia in subjects with Type II DM.[9]

Resistance to insulin may contribute to atherogenic

dyslipidemia of diabetes by increasing the hepatic secretion of very low density lipoprotein. This insulin resistance may be at the level of the regulation of Apo B degradation or inhibition of microsomal triglyceride transfer protein activity. Increased endogenous secretion of Apo B- containing lipoprotein particles, the increased plasma level of triglyceride drive a metabolic process that result in reduced HDL cholesterol.

Level of Apolipoproteins overwhelms the lipids because $ApoA_1$ are under more genetic control than lipid components and hence depicts the number of lipoprotein particles more accurately.[12] The present study has shown that the level of Apo A_1 was significantly low ($p<0.001$) in diabetic subjects when compared to controls. This might be due to the presence of high level of Apo E which cause the catabolism of Apo A_1 and HDL. Apo A_1 not only initiates the reverse cholesterol transport by activating the Lecithin Cholesterol Acyl Transferase(LCAT) but also manifests antioxidant and anti inflammatory effects.[12] Furthermore, Apo A_1 is the ligand for the ATP-binding cassette (ABC) protein, $ABCA_1$, and hence is involved in the docking procedure by which excess cholesterol in peripheral cells is externalized to HDL.[12]

The total value of ApoB indicates the number of potentially atherogenic lipoproteins. Apo B is essential for the binding of LDL particles to the LDL receptor, allowing cells to internalize LDL and thus absorb cholesterol.[12] Thus Apo B has been found to be a better predictor of risk than LDL-C, VLDL and chylomicrons. In most conditions, more than 90% of all ApoB in blood is found in LDL.

The ratio between the concentrations of ApoB and $ApoA_1$ (henceforth ApoB/A) reflect the balance between the opposing processes of arterial internalization of cholesterol and the reverse transport of cholesterol back to the liver.[12] The reason for improved predictive effect of ApoB/A ratio might be due to the fact that the ratio reflects and integrates the "cholesterol balance" between potentially atherogenic lipoprotein particles (ApoB) in relation to all antiatherogenic particles ($ApoA_1$).

Our study showed positive correlation of IR with BMI, WHR and triglycerides. Yajnik et al has reported a similar result on a study on characteristic of Type II DM and IGT (Impaired Glucose Tolerance) in an urban diabetic clinic in western India.[13] In insulin resistant and insulin deficient states the FFAs released from adipocytes increase hepatic VLDL production leading to hypertriglyceridemia. The IR states also decrease the lipoprotein lipase activity leading to increased triglyceride level. Studies have shown that Asian Indians, at any value of BMI tend to have an abnormal WHR, which may be considered as an independent risk factor for DM.[14]

In the study, IR was found to have a more significant correlation with Apo B, Apo A_1 and ApoB/Apo A_1 ratio than with conventional risk factors like LDL-C and HDL-C. This might be due to the fact that IR has an influence over the expression of Apo B and Apo A_1 at the transcriptional level.[8]

The ratio between the concentrations of ApoB and $ApoA_1$ (henceforth ApoB/A) reflect the balance between cholesterol transport and can be expressed as one number. The reason for improved predictive effect of $ApoB/A_1$ ratio might be due to the fact that the ratio reflects and integrates the "cholesterol balance" between potentially atherogenic lipoprotein particles (ApoB) in relation to all antiatherogenic particles($ApoA_1$). It has been found to be better than one single lipid fraction or the LDL-C/HDL-C ratio. Another contributing explanation is that the methodological errors of the apolipoproteins are smaller than those for lipids.[4]

CONCLUSION

Our study has shown that insulin resistance is positively

correlated with ApoB/A_1 ratio and may be considered as a causative factor for increased risk of coronary artery disease in Type II DM. Hence their inclusion in further clinical guidelines should not be discarded.

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