APO B/APO A1 RATIO AND ITS CORRELATION WITH CARDIOMETABOLIC RISK FACTORS AND OXIDATIVE STRESS IN SUBJECTS WITH METABOLIC SYNDROME

Biochemistry

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ABSTRACT

Objective: This study was designed to assess the apolipoprotein B (Apo-B), apolipoprotein A (Apo A) and Apo B/Apo A-I ratio in subjects with and without metabolic syndrome and to evaluate the correlation of Apo B/Apo A-I ratio with oxidative stress marker and cardiovascular disease risk.

Methods: A total of 308 subjects including one hundred and fifty- five cases and one hundred and fifty- three controls were recruited for this study. All the subjects were classified according to the NCEP ATP III (National cholesterol education program – Adult treatment panel III) criteria for MetS. Anthropometric and clinical characteristics were recorded using clinical Proforma. Blood samples were collected for doing plasma glucose, Lipid profile analysis [Total Cholesterol (TC), Triglyceride (TG), high-density lipoprotein (HDL)], ApoA1, Apo B and oxidative stress marker - Malondialdehyde (MDA). Serum low-density lipoprotein (LDL), very-low-density lipoprotein (VLDL) and Apo B/Apo A-I ratio were calculated. Comparison of data between the two groups was done by t test. Correlation coefficient of Apo B/Apo A1 ratio with cardiometabolic risk factors were calculated.

Result: We found that cardiometabolic risk factors like abdominal obesity, systolic and diastolic blood pressure, fasting plasma glucose, TG, atherogenic lipoproteins LDL, Apo B, Apo B/Apo A-I ratio and MDA were significantly high in subjects with MetS whereas anti-atherogenic factor Apo A1 was significantly low. We also observed that Apo B/AI ratio was positively related to cardiometabolic risk factors and with oxidative stress marker.

Conclusion: Apo B/AI ratio was related to metabolic syndrome and was found to be a reliable indicator of cardiovascular risk in MetS.

KEYWORDS

Apo lipoproteins, Apo B/Apo A1 ratio, cardiovascular disease, Cardiometabolic risk factors, Metabolic syndrome, Oxidative stress.

INTRODUCTION

Metabolic syndrome (MetS), also known as syndrome X, Insulin resistance, etc. in the literature, is really not a single disease but a constellation of cardiovascular disease risk factors and is now considered as a major worldwide public health problem (1). MetS is a collection of cardiometabolic risk factors that includes abdominal obesity, impaired fasting glucose level, and hypertension and is also related to certain lipid disturbances, including low levels of HDL, high fasting and postprandial levels of triglyceride -rich lipoproteins [mainly VLDL], and increased levels of small, dense LDL particles all of which increase the risk of cardiovascular disease incidence and mortality. MetS is often characterized by oxidative stress, a condition in which an imbalance exists between the production and inactivation of reactive oxygen species (2). Oxidative stress is thought to play a major role in the pathogenesis of a variety of human diseases, including atherosclerosis. Malondialdehyde (MDA) is the product of oxidative stress and lipid peroxidation and the extent of lipid peroxidation can be estimated by the level of malondialdehyde (MDA) which is considered to be the oxidative stress marker.

Apolipoproteins are important structural and functional proteins in lipoprotein particles. Apo B and Apo A1, main constituents of atherogenic and anti-atherogenic lipoproteins, play important roles in cholesterol and lipid transportation. The level of apolipoprotein B (Apo-B) reflects the total number of potentially atherogenic particles, including VLDL, intermediate density lipoproteins, large buoyant LDL, and small dense -LDL, and the level of apolipoprotein A-I (Apo A-I) represents the number of HDL particles (3). The major HDL apoprotein, apoA-I involved in the antioxidant ability HDL to neutralize one-electron free-radical oxidants. Thus, the ApoB/ApoA1 ratio could represent the balance between atherogenic and anti-atherogenic lipoproteins and may be an ideal indicator of atherogenic lipid disturbances and cardiovascular risk. In the clinical setting, the Apo B/Apo A1 ratio can be measured at any time without fasting. It

implies that the Apo B/Apo A1 ratio may be a more convenient biomarker in predicting MetS (4).

Present study was undertaken to assess and compare the levels of Apo B, Apo A1 & Apo B/Apo A-I ratio in subjects with and without metabolic syndrome and also to evaluate the correlation of Apo B/Apo A-I ratio with cardiometabolic risk factors and oxidative stress marker.

MATERIALS AND METHODS

This study was conducted at Department of Biochemistry, SGT Medical College, Gurugram for a period of 9 months. A total of 308 samples were collected from subjects visiting the outpatient department (OPD) for normal health check up. All the subjects were classified according to the NCEP ATP III (National cholesterol education program – Adult treatment panel III) criteria for MetS (5). One hundred and fifty- five cases and one hundred and fifty- three controls were recruited for the study.

After obtaining Institutional Ethics Committee (IEC) approval, anthropometric characteristics of all subjects were recorded using clinical Proforma. Blood samples were collected after taking informed written consent from subjects. Plasma glucose was analyzed by glucose oxidase/peroxidase (GOD/POD) method, Lipid profile - Total Cholesterol (TC), Triglyceride (TG), HDL-C were done based on enzymatic principle using commercially available kits obtained from ERBA diagnostics (Transasia Bio-Medicals Ltd, Germany). Serum LDL and VLDL levels were calculated. ApoA1 and Apo B were measured by immunoturbidimetric method (Agappe Diagnostics) and Apo B/Apo A-I ratio was calculated. MDA was analyzed colorimetrically by using Lipid Peroxidation Assay Kit.

Statistical Analysis

Student -'t' test was performed to estimate the significance in difference for comparing various cardiovascular risk factors, biochemical

characteristics such as total cholesterol, LDL cholesterol, HDL cholesterol and malondialdehyde (MDA) in study subjects and in the control subjects. The correlation of various risk/lifestyle factors and biochemical characteristics with ApoB/A1 ratio was studied by Pearson's correlation. Correlation coefficient (r) and its corresponding p-values were estimated. SPSS (windows version 17.0), Graph pad prism 6 software were used for the statistical analysis.

RESULT

Participant's characteristics

Table 1: Characteristics of participants

Parameters	Controls (n= 153)	MetS subjects (n=155)	p-value
Age(years)	41.24±18	45.01±18.6	<0.01**
Male n%	88(56)	112(72)	<0.01**
Female n%	65(42)	43(28)	<0.01**
Height(cm)	166.01±5.41	169.19±5.85	<0.01**
Weight(Kg)	68.3 ± 8.25	80.55 ± 8.49	<0.01**
WC(cm)	83.52 ± 5.77	92.89 ± 5.28	<0.01**
BMI(kg/M ²)	24.72 ± 2.03	28.13 ± 2.46	<0.01**
Hypertension ¹ n%	14(9)	119(76.8)	<0.01**
Diabetes ² n%	15(10)	39(25.2)	<0.01**
Dyslipidemia ³ n%	83(53.5)	149(96.1)	<0.01**

Values are mean ± SD. P value ** considered to be highly significant

Data are expressed as mean±SD, median (minimum-maximum) or total percentage. MetS – Metabolic syndrome, BMI-Body mass index, WC – waist circumference

¹Hypertension is defined as an average BP ≥ 140/90 mm of Hg.

² Diabetes is defined as per the standard diagnostic criteria proposed by IDF FPG <100mg/dl (<6.1mmol/L) is normoglycemia, 100-125 mg/dl (6.1-7.0 mmol/L) is intermediate hyperglycemia (Pre-diabetic) and >125 (>7.0 mmol/L) is diabetic level.

³ Dyslipidemia is defined by the presence of one or more abnormal serum lipid concentration. Subjects were considered normolipidemic or dyslipidemic according to the cutoff points recommended by National Cholesterol Education Programme (NCEP). Cutoff values for diagnosing dyslipidemia are: TC >200mg/dl, LDL-C >100mg/dl, TG >150mg/dl and HDL-C <40mg/dl.

The characteristics of subjects are summarized in **Table 1**. The test population comprised of 112 (72%) males and 43 (28%) females with a mean age of 45.01±18.6. Control group consisted of 88 (58%) males and 65 (42%) females with a mean age of 41.24±18. All these differences were statistically significant. Mean values of height, weight, WC and BMI of the MetS subjects were compared with that of control subjects and the results were statistically significant (p<0.01). BMI were significantly high (p <0.01) in subjects with metabolic syndrome than the control group. 76.8% subjects were hypertensive in test group whereas only 9% were hypertensive in control group (p<0.01). 25.2% subjects were diabetic and 96.1% were having dyslipidemia in the test group whereas this was 10% and 53.5% in the control group respectively (p<0.01)

Among the MetS subjects maximum number of patients (70%, n=108) were in the overweight category with BMI >30 kg/m² (mean 27.19±1.40 kg/m²). Only 5% (n=8) had normal BMI (mean 24.38 ± 0.36 kg/m²), and the remaining 25% (n=39) were obese (mean 31.48±1.39 kg/m²) as shown in the **Figure 1**

In case of control subjects 58% (n=88) had normal BMI (mean 23.46±0.97 kg/m²) whereas 42% (n=64) were overweight (mean 26.54±1.08 kg/m²) and the remaining one person was obese (mean 32.35 kg/m²) as shown in the **Figure 1**.

Figure 1: Distribution of subjects in MetS and in control groups based on BMI

Table 2. Levels of Fasting plasma glucose and Lipid profile values in study subjects

Parameters	Category		t test	p value
	Control(n=153)	Test (n=155)		
TC (mg/dL)	178.61 ± 36.53	209.61 ± 39.81	-7.12	<0.01**
HDL (mg/dL)	49.48 ± 9.48	48.25 ± 9.64	1.13	0.262
LDL (mg/dL)	107.88 ± 35.22	122.04 ± 35.06	-3.54	<0.01**
TG (mg/dL)	103.77 ± 35.53	205.77 ± 91.97	-12.81	<0.01**
VLDL (mg/dL)	20.37 ± 7.17	40.85 ± 18.53	-12.75	<0.01**
Non-HDL (mg/dL)	129.28 ± 34.92	160.78 ± 38.36	-7.53	<0.01**
TG/HDL Ratio	2.2 ± 0.93	4.48 ± 2.41	-10.95	<0.01**
FPG (mg/dl)	96.48 ± 27.2	114.74 ± 26.04	-6.02	<0.01**
MDA (nmol/L)	3 ± 0.75	3.88 ± 0.55	-11.72	<0.01**

Values are mean ± SD. P value ** considered to be highly significant

Figure 2: Comparison of Lipid Profile in Test and control subjects

The comparison of biochemical parameters such as serum levels of fasting blood sugar (FBS), total cholesterol (TC), triglyceride (TG), low density lipoprotein (LDL), high density lipoprotein (HDL) and very low density lipoprotein (VLDL) are given in Table 2 and Figure 2. The serum levels of total cholesterol, triglyceride, LDL cholesterol and VLDL cholesterol were significantly higher in the MetS subjects compared to the normal healthy controls, while HDL levels were not significantly lower in the MetS group. Level of non-HDL is significantly high in test population. The ratio of TG/HDL is also significantly high in subjects with metabolic syndrome.

Analysis of Apolipoproteins in Controls and MetS subjects

The main objective of this study was to analyze and compare the major apolipoproteins in the HDL molecule and to find out the correlation with the cardiometabolic factors. For this we have analyzed the major apolipoproteins, ApoA1 and ApoB by immunoturbidimetric assay in both control and MetS subjects.

Comparison between the serum levels of Apo B, Apo A and ApoB/ApoA-I ratio has been given in the Table 3. Serum ApoB and Apo B/ApoA-I ratio were found to be higher in the MetS group than in the Control group whereas the antiatherogenic protein, ApoA1 was found to be lower in MetS subjects compared to normal healthy subjects (Table 3 & Figure 3). All these differences were statistically significant (p<0.01).

Table 3: Serum levels of Apo B, Apo A and ApoB/ApoA-I ratio in study subjects

Parameters	Category		t test	p value
	Control(n=153)	Test (n=155)		
Apo A1(g/dl)	1.5 ± 0.24	1.22 ± 0.72	4.58	<0.01**
Apo B(g/dl)	1.10±0.29	1.52±0.24	4.38	<0.01**
Apo B/A1	0.76 ± 0.23	1.33 ± 0.31	-18.1	<0.01**

Values are mean ± SD. P value ** considered to be highly significant

Figure 3: Serum levels of Apo B, Apo A and ApoB/ApoA-I ratio in study subjects

ApoB/ApoA1 ratio and its correlation with cardiometabolic risk factors

ApoB/ApoA1 ratio was found to be higher in the MetS subjects than the control subjects. In the present study, we compared cardiometabolic risk factors like abdominal obesity, systolic and diastolic blood pressure, fasting plasma glucose, TG, HDL-C and MDA with Apo B/Apo A1 ratio in subjects with and without MetS. We observed that Apo B/Apo A-I ratio was positively related to cardiometabolic risk factors (Table 4).

Table 4: Correlation analysis of ApoB/ApoA1 ratio with cardiometabolic risk factors

Parameters	r value	p value
WC	0.502	<.0001**
Systolic Blood Pressure	0.383	<.0001**
Diastolic Blood Pressure	0.354	<.0001**
FPG	0.225	<.0001**
TG	0.405	<.0001**
HDL	-0.069	0.113
MDA	0.380	<.0001**

Values are mean $\pm$ SD. P value ** considered to be highly significant

DISCUSSION

Abdominal obesity is reported to be associated with metabolic abnormalities and increased risk of cardio vascular disease (CVD) (6). Several authors reported that all cardiometabolic risk factors had a positive correlation with increasing waist, and the overall abdominal adiposity is a strong and independent risk factor for MetS. BMI and WC are widely accepted as the anthropometric indices of obesity. In the present study majority of the subjects with MetS had elevated measurements of WC and BMI which is in agreement with the previous studies (7). Studies unequivocally showed that serum TG, LDL and VLDL are elevated while HDL is decreased in persons with MetS. Obesity, an invariable component of MetS, itself has been reported to reduce HDL levels and obese patients with MetS almost always have low HDL levels (6). The observation in the present study of elevations in TC, TG, LDL and VLDL in patients with MetS is in agreement with previous reports (8).

Apolipoproteins regulate the synthesis and metabolism of lipoprotein particles and stabilise their structures. Hence, it is not unexpected that the Apo B/Apo A-I ratio was closely related with TG, TC, HDL-C and LDL-C. Apolipoprotein B and A-I are the major protein moieties of LDL and HDL, respectively. The level of Apo B reflects the number of potentially atherogenic particles (9). Meanwhile, Apo A-I plays an important role in removing excess cholesterol from tissues and incorporating it into HDL for reverse transport to the liver, forming the basis for atheroprotective events (10). Thus, the Apo B/Apo A-I ratio reflects the balance of atherogenic and atheroprotective particles, so the higher the level, the higher the tendency of cholesterol deposition, and consequently the higher the risk of CVD (11). Oxidative stress plays critical role in the pathogenesis of various diseases. Furukawa et al. report that increased oxidative stress in accumulated fat is an important pathogenic mechanism of obesity-associated metabolic syndrome (12). In the present study, malondialdehyde (MDA), oxidative stress marker was found to be high in metabolic syndrome subjects which is in agreement with the previous studies.

A number of prospective studies have shown that high Apo B/Apo A-I ratio may be a promising marker for predicting the occurrence of future cardiovascular events, such as myocardial infarction and stroke (13). In addition, Apo B concentration and Apo B/Apo A-I ratio were found to be associated with risk of MetS and its components and were independent of conventional risk factors in several previous studies (14). Our results were consistent with these findings. We found a significant correlation between Apo B/Apo A-I ratio and measures of MetS and thus CVD risk.

The main strength of this study is the careful selection of truly healthy controls and MetS subjects and the equality in the number of controls and MetS subjects and the main limitation is that the two groups being compared were not age and gender matched.

In conclusion, the present study demonstrated that atherogenic and antiatherogenic lipoprotein particles are associated with metabolic syndrome associated variables. In that context, use of Apo B/Apo A-I ratio, a reliable indicator of cardiovascular risk is useful in predicting MetS.

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