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Assessment of Apo-B and TG/HDL-C Ratio as Indicators of Insulin Resistance in Patients with Metabolic Syndrome

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Authors' contributions

This work was carried out in collaboration between all authors. Authors PS and UM managed the laboratory analysis. Author PS also did statistical analysis. Authors SR and PS designed the study. Author PC wrote the protocol and wrote the first draft of the manuscript All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Objectives: The aim of the present study was to assess Apolipoprotein B (Apo-B) and Triglyceride/High Density Lipoprotein Cholesterol (TG/HDL-C) ratio as indicators of insulin resistance (IR) with Homeostasis Model of assessment of insulin resistance (HOMA IR)) in metabolic syndrome patients .
Study Design: Observational and prospective.
Place and Duration of Study: The study was carried out in Department of Biochemistry and Department of Medicine, MGM Medical College, Navi-Mumbai from March 2012 to June 2013.
Methodology: Total 110 normal subjects and patients were recruited in the study after obtaining informed written consent. They were divided in to two groups. Group I was healthy controls (n=50) and Group II included subjects with MS (n=60) as per NCEP ATP III criteria. Anthropometric measurements & biochemical analysis was performed in all subjects. IR was defined by HOMA IR. Simple & multiple regression analysis were used to

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obtain relationship between IR (HOMA IR) using TG/HDL-C (model -1) and Apo-B (Model-2) as independent variables.
Result: There were statistically significant differences in anthropometric, glycemic and lipid parameters between the control and study group ($p<0.0001$).The regression model between HOMA IR and TG/HDL-C ratio showed a positive correlation, ($r=0.29$, $p < 0.05$). HOMA IR & Apo-B also showed a significantly positive correlation (0. 41, $p < 0.001$). But combined multivariate analysis indicated that Apo-B is a better predictor of IR compared to TG/HDL-C ratio.
Conclusion: We concluded in our study that Apo-B may be a better predictor of IR than TG/HDL-C and hence could be adopted in routine laboratory practice as a lipid marker for prediction of insulin resistance (IR) in metabolic syndrome patients at an early stage.

Keywords: Insulin resistance; Apo B; metabolic syndrome; Insulin resistance indicators; lipid markers.

1. INTRODUCTION

Insulin resistance (IR) is a major finding in the metabolic syndrome (MS) [1] and a contributing factor for risk of development of type 2 diabetes and cardiovascular diseases (CVD).Therefore, a reliable measure of insulin resistance is important for investigating its link with metabolic syndrome (MS) [2,3]. For this, the homeostasis model assessment of insulin resistance (HOMA-IR) [4] is a widely accepted method as an alternative to the glucose clamp which is laborious. HOMA-IR is comparable to the glucose clamp technique, in terms of precision but not accuracy and hence it is possible to study a large number of subjects using a single measurement of glucose and insulin in the fasting state [5]. However, as it is empirical, additional tests have been used for assessment of IR [6].

Another alternative proposed for the identification of IR is the determination of fasting triglycerides (TG) and glucose [7]. Since elevated triglycerides is one of the NCEP ATP III criteria for MS, the hypertriglycerdemic state which may accompany either normal and impaired fasting glucose should contribute to promoting surveillance for IR [8,9].

Recently Triglycerides/High density lipoprotein cholesterol (HDL-C) ratio has been proposed as one of the indices to evaluate the atherogenic state due to the association between dyslipidemia and IR [10,11]. IR significantly impacts lipoprotein metabolism and is associated with increased TG levels and decreased HDL-C levels [12,13].

But TG/HDL-C ratio is known to be affected by gender and ethnic variabilities. Besides, TG itself may also be influenced by biological variability such as lifestyle, medications and metabolic abnormalities. Hence TG/HDL-C ratio may not be a stable predictor of IR [14]. Recent studies have suggested the use of Apo-B as a direct marker of atherogenic state & IR [14,19].

Apo-B is a structural protein of atherogenic lipoproteins such as very-low-density lipoprotein [VLDL], intermediate-density lipoprotein [IDL] as well as both large, buoyant, low density lipoprotein cholesterol (LDL-C) and small, dense LDL[15,16]. Cardiovascular risk is more directly related to the number and size of circulating atherogenic particles than to the concentration of cholesterol in these particles [17,18]. In hypertriglyceridemic patients,

plasma Apo-B is strongly associated with LDL-C, making Apo-B an effective surrogate for LDL particle concentration [19].

Apo-B may be particularly relevant in the assessment of insulin resistant states, such as diabetes and metabolic syndrome, as patients with these disorders often manifest normal LDL-C values but have a preponderance of small, dense LDL-C particles and higher Apo-B levels.

Hence this study was undertaken to compare the existing model of HOMA IR with both TG/HDL-C ratio and Apo-B levels for assessment of insulin resistance in MS patients.

2. MATERIALS AND METHODS

This was an observational, prospective study approved by Institutional ethics review committee. A total of 110 subjects between age group of 35-65 years were recruited for the study after obtaining their informed consent. The subjects were divided into two groups. Group I (n=50) included healthy controls and Group II (n=60) included subjects with metabolic syndrome. The diagnosis of metabolic syndrome was based on NCEP ATP III criteria which is based on the presence of three or more of the risk factors such as: Waist circumference (WC): men > 102 cm (40 in); women > 88 cm (35 in); Triglycerides ≥150 mg/dl; HDL-C: men < 40 mg/dl; women < 50 mg/dl; Blood pressure ≥130/≥ 85 mmHg; Fasting glucose ≥110 mg/dl. All the subjects were matched for age, gender and were non smokers, non alcoholic and of same socioeconomic status. Subjects with chronic diseases of kidney, liver, patients of cancer and diabetes mellitus were excluded from the study.

Measurements of height and weight were done with the subjects standing, without shoes and with light clothing. BMI was calculated as weight in kg divided by height in meter squared. WC was measured at the level of the umbilicus with a tape in centimeter scale.

Venous blood samples were collected after 12 hrs of fasting by closed collection system. For fasting plasma glucose, samples were collected in fluoride vacutainer and for serum lipid profile and Apo-B, samples were collected in plain vacutainer. All the samples were processed on the same day for biochemical analysis. Fasting blood glucose, TG, total cholesterol and HDL-C levels were measured by enzymatic methods using kits supplied by Beckman Coulter. All the assays were performed on fully automated analyzer, AU 480 of Beckman Coulter. Subjects selected in Group-II had TG values between 150 to 192 mg/dl.

LDL-C and VLDL values were obtained using Friedewald's formula [20]. Serum Apo-B levels were estimated by immunoturbidometry method using kits supplied by Beckman Coulter. Fasting plasma insulin was measured by ELISA technique using kits supplied by Immunoshop India Pvt Ltd. Insulin Resistance was determined by means of homeostasis model assessment index (Homeostasis Model Assessment−Insulin Resistance, HOMA−IR) using the formula,

HOMA−IR = fasting insulin (μIU/ml) x fasting glucose (mg/dl)/405 [21]. The TG/HDL-C ratio was calculated using the formula: Fasting TG (mg/dl)/HDL-cholesterol (mg/dl).

2.1 Statistical Analysis

Data are presented as Mean ± SD, student *t*-test was used to compare age, BMI, W/H ratio, SBP, DBP, FPG, FPI, Lipid profile and Apo-B levels between patients and controls. The correlation of HOMA IR with both TG/HDL-C ratio and Apo-B was determined by Pearson correlation coefficient. Multiple regression analysis was performed wherever deemed appropriate. Statistical Package for Social Sciences (version 17.0) was used for all statistical analysis. P values < 0.05 were considered statistically significant.

3. RESULTS

Table 1 shows anthropometric and clinical characteristics of control and study group (mean and standard deviation).

Table 1. Descriptive and comparative statistics for different groups by student t-test

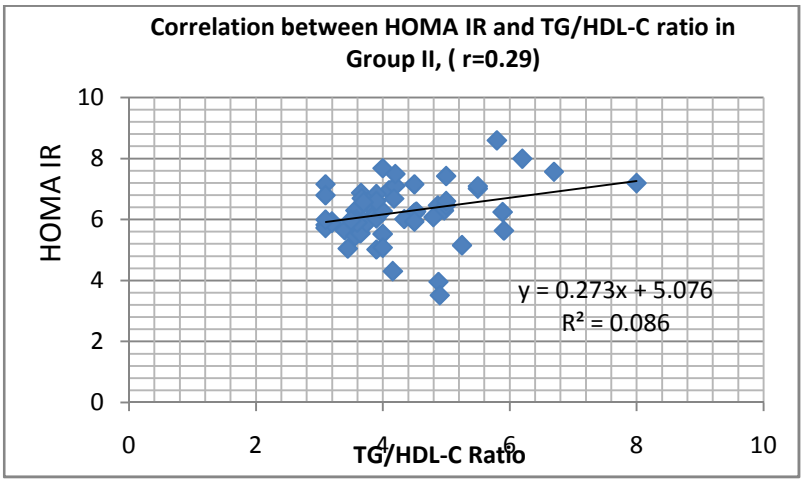
Parameters	Controls	Patients	P Value
SBP(mm/Hg)	115±6.98	134±2.86	≤ 0.0001
DBP (mm/Hg)	76.7±4.98	96±0.60	≤ 0.0001
BMI (Kg/m^2)	23.3 ±1.39	29.6±0.53	≤ 0.0001
W/H ratio	0.802±0.03	0.99±0.01	≤ 0.0001
Fasting Plasma Glucose mg/dl	88.1±5.06	113±1.19	≤ 0.0001
Fasting Plasma insulin µIU/ml	10.2±3.2	22±0.11	≤ 0.0001
HOMA IR	2.23±0.69	6.14±0.09	≤ 0.0001
Cholesterol mg/dl	146.3±10.59	174±15	≤ 0.0001
Triglycerides (mg/dl)	110±17.2	189±7.5	≤ 0.0001
HDL Cholestrol mg/dl	44.5±3.58	40±0.6	≤ 0.0001
VLDL mg/dl	21.59±2.72	36±2.1	≤ 0.0001
LDL cholesterol mg/dl	83.92±14.2	103±22.2	≤ 0.0001
Apo-B mg/dl	82.615±15.34	108.96±23.06	≤ 0.0001
TG/HDL-C ratio	2.4653±0.2094	4.554±0.152	≤ 0.0001

Data is expressed as mean ± SD

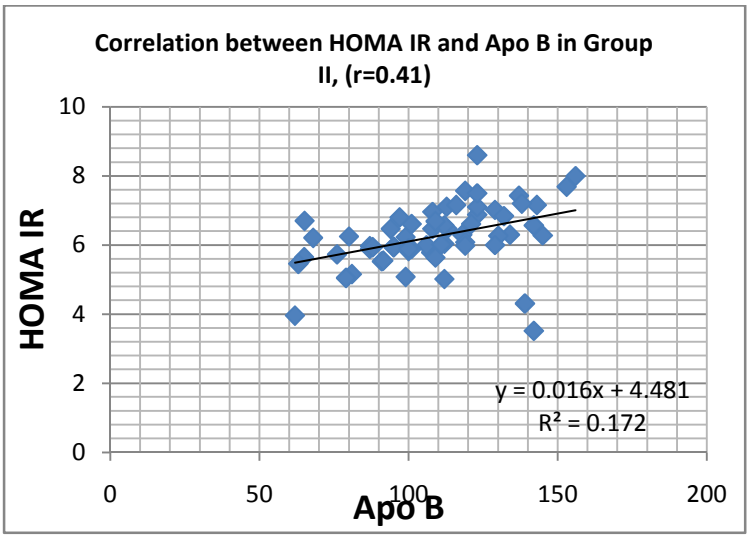
There were significant differences in the values of anthropometric parameters ($p< 0.0001$) between control and patients of metabolic syndrome. The BMI of MS subjects was between 25 to 30 kg/m^2. The average W/H ratio for the patients exceeded the NCEP ATP III criteria in both the genders. The difference in fasting plasma glucose and LDL- C was statistically significant between the control and study group ($p< 0.0001$). HOMA IR was elevated in the metabolic syndrome group compared to controls. The mean high density lipoprotein-cholesterol (HDL-C) level was significantly lower in the patients ($p<0.0001$), whereas Apo-B levels were significantly higher ($p< 0.0001$). Regression analysis was performed for patients using HOMA IR as dependent variable and TG/HDL-C as an independent variable in first model. The model showed positive correlation $r=0.29$, ($p<0.05$) as shown in Graph 1. In Model 2, Apo-B was used as independent variable with HOMA IR which also showed positive correlation ($r=0.41$, $P<0.001$) as depicted in Graph 2. The combined multivariate analysis of HOMA IR with TG/HDL-C ratio and Apo-B which is represnted in Table 2 showed that Apo-B variable is statistically significant ($p<0.005$) while TG/HDL-C ratio was not significant. The regression model of control group did not reveal any statistical significance for both the measured independent parameters as predictors of IR.

Table 2. Linear regression model dependent variable: HOMA IR

Variable	Reg. coefficient	SE	P-value	Correlation coefficient	R-square
TG/HDL-C	0.180	0.114	0.120	0.46	0.21
APO-B	0.014	0.005	0.005		



Graph 1. Showing positive correlation between HOMA IR (Y-axis) and TG/HDL-C (X-axis) ratio in subjects with metabolic syndrome (r=0.29; P<0.05)



Graph 2. Showing positive correlation between HOMA IR (Y-axis) and Apo-B (X-axis) ratio in subjects with metabolic syndrome (r=0.41; P<0.001)

4. DISCUSSION

In our study we have used HOMA IR, the widely accepted model for assessment of IR and compared the lipid profile markers such as TG/HDL-C and Apo-B, as predictors of IR.

The TG/HDL-C ratio was calculated in metabolic syndrome patients and control group and we found that TG/HDL-C ratio was higher in the study group. There was a significant positive correlation between HOMA IR and TG/HDL-C ratio ($r=0.29$; $p<0.05$) in the study group, refer to Graph 1. The regression model showed R^2 value of 0.087, indicating 8.7 percent variation in the HOMA IR due to TG/HDL-C ratio. We also performed regression analysis for control group and we did not get any significant correlation between HOMA IR and TG/HDL-C or Apo-B.

Our results are in accordance with Marotta et al. [22] and Brehm et al. [23] who demonstrated positive correlation between TG/HDL-C ratio and insulin resistance, confirming that TG/HDL-C ratio predicts insulin resistance in metabolic syndrome. But an independent study by Knight et al. [24] stated that, TG/HDL-C ratio fails to predict insulin resistance in African American women.

The predictive level and significance of correlation between HOMA IR and Apo-B was assessed by regression analysis taking HOMA IR as dependent variable. As shown in Graph 2, a significant positive correlation ($r=0.41$; $p < 0.001$) was obtained between HOMA IR and Apo-B in metabolic syndrome patients. The R^2 value showed by regression model was 0.17 showing 17 percent variation in HOMA IR due to Apo-B levels.

When combined multivariate analysis of TG/HDL-C and Apo-B with HOMA IR was performed, the regression model indicated that TG/HDL-C ratio is a non significant variable ($p>0.05$) whereas Apo-B was statistically significant ($p<0.05$) as shown in Table 2. Most of the studies have compared HOMA IR with either TG alone or with TG/HDL-C ratio [25,21]. Some studies have estimated LDL and Apo-B levels or Apo-B to Apo AI ratio in metabolic syndrome or diabetic patients. We have performed multivariate regression analysis of HOMA IR with both TG/HDL-C and Apo-B. The results of our study support that measurement of Apo-B is superior to TG/HDL-C ratio as a predictor of IR. Although the TG/HDL-C ratio is economic and easy to calculate [26] and is also a good predictor of LDL size, it does not necessarily identify patients with hyper Apo-B. Moreover, in addition to the requirement of a fasting state, it shows high biological variability inherent to triglycerides [27], as reflected by the wide range of recommended cutoff points. On the other hand, Apo-B reflects the total number of atherogenic particles and its determination can also be made in a non fasting state [28,29]. Its biological variability is known to be lower than other lipid components.

Thus, our study shows that Apo-B levels may be used as a more stable, atherogenic biomarker and IR predictor in metabolic syndrome patients compared to TG/HDL-C. Miller et al. [14] in a recent review compared the predictive value of Apo-B with non–HDL-C for CVD outcomes and reported that Apo-B has been commonly identified as either superior marker or equivalent to non–HDL-C. While non–HDL-C alone has been more predictive only in limited cases [30]. Yet, in studies that demonstrated statistically significant differences between Apo-B and non–HDL-C, the differences in point estimates were often quite small and therefore unlikely to have a major impact in day-to-day clinical practice [31].

As per NCEP ATP III final guidelines of 2002 Apo-B is recommended as a potential marker for MS and it has been proposed as an alternative to LDL cholesterol. Thus, Apo-B may be

linked to metabolic Apo-B risk factor clustering phenomena. As non-HDL-C is significantly correlated with Apo-B, it can serve as a “surrogate” for metabolic syndrome [32].

This study is limited by the fact that HOMA IR index was not correlated with insulin sensitivity by the gold standard method such as euglycemic clamp.

5. CONCLUSION

In conclusion, we propose that Apo-B estimation be used to predict long term lipid control for diagnosing IR in line with the current use of HbA1C as the long term glycemic control marker.

CONSENT

Authors declare that written informed consents were obtained from patients before their participation in the study.

ETHICAL APPROVAL

All authors hereby declare that approval from Institutional ethics review committee was obtained and study was carried out as per standards.

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COMPETING INTERESTS

The authors have no competing interests exist.

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