Original Research Article

Assessment of Apo B as an indicator of Insulin resistance in comparison with TG/HDL-C ratio and HOMA IR

Objectives:

The aim of the present study was to evaluate the Triglyceride/High Density Lipoprotein Cholesterol (TG/HDL-C) ratio and Apolipoprotein B (Apo B) levels in metabolic syndrome patients to predict Insulin Resistance and compare them with HOMA IR.

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 was 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 was used to obtain relationship between IR (HOMA IR) using TG/HDL-C(model -1) and Apo B (Model-2) as variables.

Result

There was statistically significant difference in anthropometric, glycemic and lipid parameters in control and study group($p<0.0001$).The regression model between HOMA IR and TG/HDL-C ratio was positive correlation ,($r=0.29$,$p< 0.05$) while between HOMA IR & Apo B also showed significantly positive correlation(0. 41,$p< 0.001$). But combined multivariate analysis indicated Apo B as a better predictor of IR as compared to TG/HDL-C ratio.

Conclusion.We report in our study that ApoB is more significant and a better predictor of IR than TG/HDL-C and hence can be adopted in routine laboratory practice as a lipogenic marker for prediction of insulin resistance (IR). So that patients with metabolic syndrome may be identified at an early stage.

1. INTRODUCTION

Insulin resistance (IR), is a major finding in 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 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 large number of

subjects using a single measurement of glucose and insulin in fasting state [5]. Since it is empirical, additional tests have been used for assessment of IR.[6]

Another alternative proposed for the identification of IR is determination of fasting triglycerides (TG) and glucose.[7] Since elevated triglyceride is one of the NCEP ATP III criteria for MS, hypertriglycerdemic state which may accompany either normal and impaired fasting glucose should promote 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 depressed HDL levels.[12,13]

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

ApoB 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 lipoproteins (LDL-C) cholesterol 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, the vast majority of total plasma Apo B is associated with LDL-C, making Apo B100 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.

Hence the 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 in to 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 presence of three or more of the risk determinants stated further. Waist circumference: men > 102 cm (40 in); women > 88 cm (35 in), Triglycerides ≥150 mg/dl, HDL cholesterol: 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. The subjects with chronic diseases of kidney, liver, cancer and diabetes meliitus were excluded from 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. Waist circumference (WC) was measured at the level of the umbilicus with a tape in centimeter scale.

Venous blood samples were collected under 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 and HDL cholesterol levels were measured by enzymatic methods using kits supplied by Beckman and Coulter. All the assays were performed on fully automated analyzer, AU 480 of Beckman and Coulter. TG values of Group-II subjects were between 150 to 192 mg/dl.

LDL-C and VLDL values were obtained by 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).

3. Statistical analysis

Data are presented as means ± s.d. student *t*-test was used to compare age, BMI, W/H ratio, SBP, DBP, FPG,FPI, Lipid profile and Apo B levels. HOMA IR was correlated with both TG/HDL-C ratio and Apo B and Pearson correlation coefficient was found. Multiple regression analysis was performed when appropriate. Statistical Package for Social Sciences (version 17.0) was used for calculations.P values < 0.05 were considered statistically significant.

4.Result:

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

5.DISCUSSION:

In our study we have used HOMA IR, the widely accepted Model for assessment of IR and compared with both TG/HDL and Apo B , an atherogenic marker of IR.

The TG/HDL ratio was calculated in metabolic syndrome patients and control group and we found that TG/HDL ratio was higher in the study group. There was significant positive correlation between HOMA IR and TG/HDL 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 ratio. We also performed regression analysis for control group and we did not get any significant correlation between HOMA IR and TG/HDL or Apo B.

Our results are in accordance with Salazar et al [22] Marotta et al [23] and Brehm et al [24] 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 [25] stated that, triglyceride/high-density lipoprotein cholesterol 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 ($p > 0.05$) variable 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.[26,21] Some studies have estimated LDL and Apo B levels or Apo B to Apo AI ratio in metabolic syndrome or diabetic patients. But combined model of HOMA IR with either TG/HDL-C or Apo B studied by multivariate analysis has not been reported yet. The results of our study support that measurement of Apo B is superior to TG/HDL cholesterol ratio. Although the TG/HDL C ratio is cheap and easy to calculate [27] and is also a good predictor of LDL size. However, it does not identify most of the patients with hyper ApoB. Moreover, in addition to requirement of fasting state it shows high biological variability inherent to triglycerides[28], as reflected by the wide range of recommended cutoff points and it necessitates fasting state. On the other hand, Apo B reflects the total number of atherogenic particles and its determination can also be made in non fasting state.[29,30] Its biological variability is also known to be lower than other lipidic components.

Thus, our study shows that measurement of Apo B levels is a stable, predictive atherogenic biomarker than TG/HDL-C ratio in metabolic syndrome patients with insulin resistance. Miller et al [14] in his review has 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.[31] 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 of day-to-day clinical practice.[32]

As per NCEP ATP III final guidelines of 2002 apolipoprotein 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 risk factor clustering phenomena. As Non-HDL cholesterol is significantly correlated with apolipoprotein B, it can serve as a “surrogate” for metabolic syndrome.[33]

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.

6.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 long term glycemic control marker.

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COMPETING INTERESTS

The authors have no competing interests.

CONSENT

Authors declare that written informed consent was obtained from subjects (normal controls) and 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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Table.1 Showing descriptive and comparative statistics for different groups by student t test

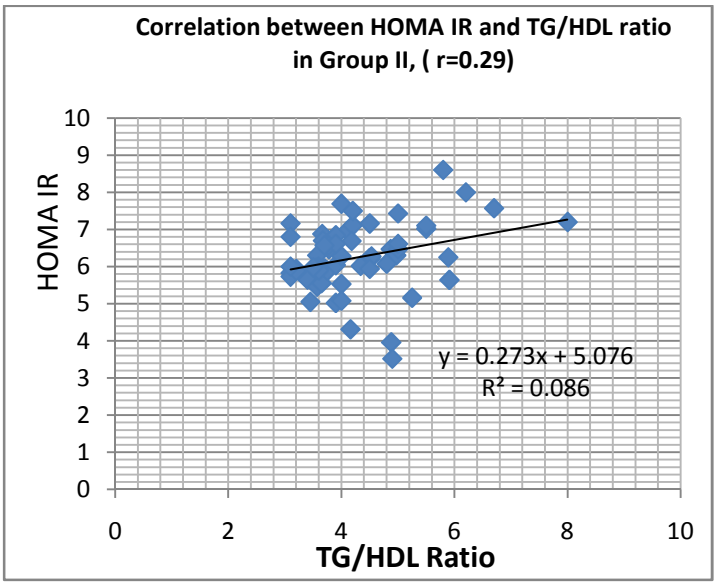
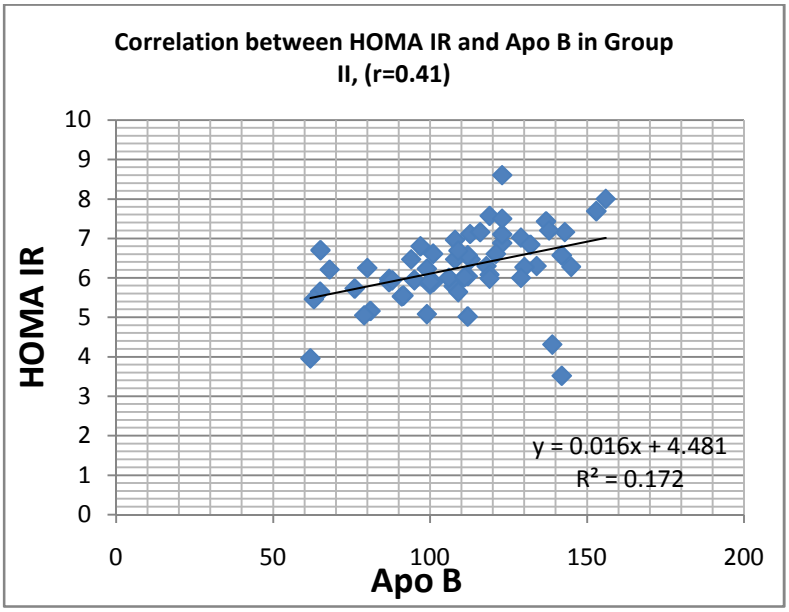
Parameters	Group I	Group II	P Value
Blood Pressure (Systolic) mm/Hg	115 ± 6.98	134 ± 2.86	0.0001
Blood Pressure (Diastolic) mm/Hg	76.7 ± 4.98	96 ± 0.60	0.0001
Body Mass Index in kg/m2	23.3 ± 1.39	29.6 ± 0.53	0.0001
Waist/Hip ratio	0.802 ±0.03	0.99±0.01	0.0001
Fasting Blood Glucose mg/dl	88.1 ± 5.06	113. ± 1.19	0.0001
Fasting plasma insulin µIU/ml	10.2 ± 3.2	22 ± 0.11	0.0001
Homeostasis Model Assessment of Insulin Resistance	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
High Density Lipoprotein Cholestrol mg/dl	44.5±3.58	40±0.6	0.0001
Very Low Density Lipoprotein Cholestrol mg/dl	21.59±2.72	36±2.1	0.0001
Low Density Lipoprotein Cholestrol mg/dl	83.92±14.2	103±22.2	0.0001
Apolipoproteins B mg/dl	82.615±15.34	108.96±23.06	0.0001
Triglycerides/High Density Lipoproteins cholesterol Ratio	2.4653± 0.2094	4.554± 0.152	0.0001

Table 2 Linear Regression Model

Dependent Variable: HOMA IR

Variable	Reg. Coefficient	SE	P-value	Correlation Coefficient	R-square
Tg/HDL	.180	.114	.120	.455	.207
APO B	.014	.005	.005		

Graph 1 Correlation between HOMA IR and TG/HDL ratio (r=0.29)



Graph 2 Correlation between HOMA IR and Apo B (r=0.41)