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Serum lipoprotein ratios as markers of insulin resistance: A study among non-diabetic acute coronary syndrome patients with impaired fasting glucose

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***Background & objectives*: Recent data suggest that insulin resistance can predict cardiovascular disease independently of the other risk factors, such as hypertension, visceral obesity or dyslipidaemia. However, the majority of available methods to evaluate insulin resistance are complicated to operate, expensive, and time consuming. This study was undertaken to assess whether serum lipoprotein ratios could predict insulin resistance in non-diabetic acute coronary syndrome (ACS) patients.**

***Methods*: Ninety non-diabetic patients with impaired fasting glucose admitted with a diagnosis of ACS were included in the study. At the time of admission fasting glucose and insulin concentrations were measured. The homeostatic model assessment-insulin resistance (HOMA-IR) was used for insulin resistance. The fasting serum total cholesterol (TC), triglycerides (TG) and high density lipoprotein cholesterol (HDL-C) levels were checked, and then TC/HDL-C and TG/HDL-C ratios were calculated. The areas under the curves (AUC) of the receiver operating characteristic (ROC) curves were used to compare the power of these serum lipoprotein ratios as markers.**

***Results*: Lipoprotein ratios were significantly higher in patients with HOMA-IR index > 2.5 as compared to patients with index <2.5 ($P < 0.05$). Both TG/HDL-C and TC/HDL-C ratios were significantly correlated with HOMA-IR ($P<0.05$). The area under the ROC curve of the TG/HDL-C and TC/HDL-C ratio for predicting insulin resistance was 0.80 (95% CI, 0.67 to 0.93), 0.78 (95% CI, 0.65 to 0.91), respectively.**

***Interpretation & conclusions*: The findings of this study demonstrate that serum lipoprotein ratios can provide a simple means of identifying insulin resistance and can be used as markers of insulin resistance and cardiovascular diseases risk in adult non-diabetic patients.**

Key words Acute coronary syndrome - fasting glucose - HDL-C - HOMA-IR - insulin resistance - lipoprotein ratios - non-diabetic - triglycerides

The condition of insulin resistance plays a primary role both in the pathogenesis of metabolic syndrome and in the prediction of cardiovascular events; thus elevated fasting glucose and insulin resistance may be correlated positively with the incidence of cardiac events in patients with CAD[1], with or without a previous diagnosis of diabetes[2]. Several studies have suggested that although insulin resistance is associated with traditional risk factors, it may influence independently the progression of coronary atherosclerotic plaques in asymptomatic patients[3], also in virtue of the correlation with the endothelial dysfunction[4]. Plasma triglyceride (TG), high density lipoprotein cholesterol (HDL-C), and total cholesterol (TC) are independently associated with insulin resistance, insulin level, and are independent predictors of CVD[5,6]. The majority of available methods to evaluate insulin resistance are complicated to operate, expensive, and time consuming. The present study was, therefore, aimed to assess how well insulin resistance could be predicted from serum lipoprotein ratios (TG/HDL and TC/HDL) in a group of non-diabetic patients with acute coronary syndrome (ACS).

Material & Methods

From April 2011 to March 2012, a total of 90 consecutive patients admitted with a diagnosis of acute coronary syndromes [ST elevation myocardial infarction (STEMI) or unstable angina/Non ST elevation myocardial infarction (UA/NSTEMI)] without any previous history of diabetes mellitus in the Cardiology department of Medical College & Hospital, Kolkata, West Bengal, India, were enrolled in this study. Eligibility criteria were a clinical history of ACS accompanied by at least one of the following: electrocardiographic changes consistent with ACS, serial increases in biochemical markers of cardiac necrosis (creatine kinase MB, creatine phosphokinase, or troponin T). The study protocol was approved by the Institutional Ethics Committee. Informed written consent was obtained from all patients. Fasting blood samples were collected for the analysis of all routine investigations including blood sugar, serum insulin levels, lipid profiles and high sensitivity C-reactive protein (hsCRP) levels. Patients with fasting blood sugar level between 100-126 mg/dl were included in the study. All participants who had a positive history of diabetes or currently had a fasting glucose concentration ≥ 126 mg/dl were excluded.

Blood samples (10 ml) were collected without anticoagulant for insulin and lipid profiles, and in sodium fluoride tubes for glucose. Serum was separated and stored in a -80°C freezer. Fasting plasma glucose was measured by glucose oxidize test using glucose reagent and glucose standard. Total cholesterol, triglyceride and HDL cholesterol were estimated on the same day using commercial kits on the Erba Transasia XL-600 auto analyzer. TG/HDL-C ratio > 3.5 and TC/HDL-C ratio > 4.5 were used as cut-off points to identify patients with insulin resistance[7]. Serum fasting insulin levels were measured by Monobind Insulin Microplate ELISA test (accubind ELISA Microwells, Monobind, Inc.). The assay sensitivity was found to be 0.75 μIU/ml and intra- and inter-assay precisions were <5 per cent and <10 per cent, respectively. As per the system guidelines the normal insulin value for non-diabetic adult is 0.7-9 μIU/ml. Insulin resistance by this method is defined as fasting insulin concentration of > 9 μIU/ml. Though proinsulin cross-reacts with most competitive insulin assays, there is less than 1 per cent cross-reaction found with proinsulin using Monobind Insulin Microplate ELISA.

Insulin resistance was defined from the homeostasis model assessment of insulin resistance (HOMA-IR) according to the following formula: HOMA-IR = [fasting insulin concentration (μU/ml) x fasting glucose concentration (mg/dl)/405][8]. Haemolysed blood samples were excluded because of the presence of inactivating insulin enzymes. Patients with HOMA-IR > 2.5 were defined as having insulin resistance. Subjects with HOMA-IR < 2.5 were defined as insulin sensitivity group[8-10].

Statistical analysis was carried out using the SPSS 15.0 software (SPSS Inc., USA). All non-parametric data like hypertension, dyslipidaemia, and smoking were analyzed by the Chi-square test. All parametric data like BMI, lipid parameters, and serum insulin were compared across the two groups using unpaired t test. Pearson correlation was used to evaluate the correlation between lipid parameters and HOMA-IR. Evaluation of serum lipoprotein ratios with receiver operating characteristic (ROC) curve was used to examine the predictive value of TG/HDL-C, and TC/ HDL-C ratios for insulin resistance (HOMA> 2.5). Values for the area under the ROC curve of 0.5, ≥ 0.7 but < 0.8, ≥0.8 but < 0.9, and ≥ 0.9 have been suggested as reflecting the following levels of discrimination: none, acceptable, excellent, and outstanding[11].

Results

Of the 90 non-diabetic patients with ACS and impaired fasting glucose (IFG), 60 had STEMI and 30

Table I. Basic parameters of study population

	HOMA< 2.5	HOMA> 2.5
n	58	32
Age (yr)	53.38 ± 6.58	56.65 ± 11.19
Sex (M)	76.9%	78.4%
BMI (kg/m^2)	22.88 ± 0.95	23.84 ± 1.18*
Hypertension	38.5%	35.1%
Insulin (µIU/ml)	3.91 ± 2.58	12.45 ± 5.97*
TC (mg/dl)	161.15 ± 28.37	191.54 ± 28.20*
TG (mg/dl)	112.38 ± 21.48	146.38 ± 24.43*
HDL (mg/dl)	38.23 ± 6.46	38.54 ± 5.63
LDL (mg/dl)	99.08 ± 22.33	123.81 ± 21.51*
TG/HDL-C ratio	2.96 ± 0.64	3.82 ± 0.55*
TC/HDL-C ratio	4.23 ± 0.45	4.99 ± 0.70*

BMI, body mass index; TC, total cholesterol; TG, triglyceride; HDL, high density lipoprotein; LDL, low density lipoprotein, hsCRP high sensitivity C-reactive protein
*P<0.05 compared with HOMA<2.5 group

had UA/NSTEMI. There were 68 male and 22 female patients. The mean was 56.3 ± 9.95 yr. The mean age of male and female patients were 56 ± 9.84 and 57.39 ± 10.24 yr, respectively.

The index admission was retrospectively classified into two cohorts, HOMA-IR >2.5 (n= 32 patients) and HOMA-IR <2.5 (n= 58 patients). The baseline demographic variables for these two groups are shown in Table I. The relationship between the lipid ratios and the surrogate measure of insulin resistance (HOMA) was assessed within the two groups; elevated HOMA, and at low HOMA (HOMA<2). Lipid ratios were significantly (P<0.05) higher in individuals with HOMA > 2.5 as compared to subjects with HOMA <2.5 (Table I).

There was a significant positive correlation between HOMA-IR, TG/HDL-C (r=0.368, P=0.009) TC/HDL (r=0.305, P<0.03), respectively (Fig. 1). Significant correlation was also observed between fasting insulin and TG/HDL-C ratio (r=0.35, P=0.01).

ROC curve analysis showed that TG/HDL-C and TC/HDL-C were effective diagnostic markers for persons with insulin resistance taking HOMA IR as gold standard (>2.5 was taken as positive for insulin resistance). The AUC of the ROC curves were used for prediction of lipid ratios as markers for insulin resistance (HOMA-IR) in non-diabetic ACS patients. The area under the ROC curve of TG/HDL-C (>3.5 was taken as positive for insulin resistance) was larger (Table II, Fig. 2) than the AUC for TC/HDL-C (> 4.5 was taken as positive for insulin resistance). So TG/HDL-C was found to have better screening ability for insulin resistance as compared to TC/HDL-C as higher sensitivity was achieved with similar 1-specificity level. To further strengthen the validity of the results, the sensitivity and specificty were calculated for various values of HOMA-IR starting from 2.2 to 2.7. After analyzing the results it is found that cut-off value of 2.5 for HOMA-IR provided the best combination of sensitivity, specificity and area under the ROC curve.

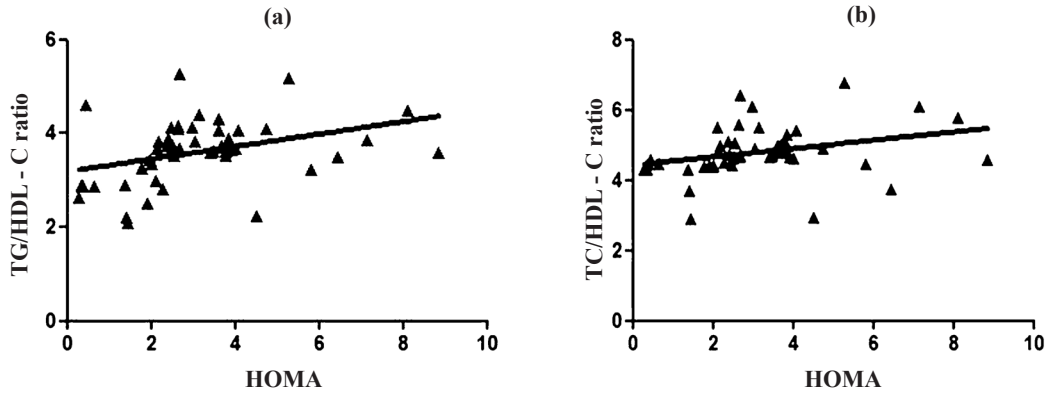

Fig. 1 (a). Scatter diagram showing significant positive correlation between HOMA-IR index and TG/HDL-C ratio as per Pearson's correlation analysis; **(b)** Scatter diagram showing significant positive correlation between HOMA-IR index and TC/HDL-C ratio as per Pearson's correlation analysis.

Table II. Serum lipoprotein ratios and the areas under ROC (receiver operating characteristic) curve for the detection of insulin resistance (HOMA-IR)

Serum lipoprotein ratios	Area under the ROC curve	95% Confidence interval	*P* value
TG/HDL-C ratio	0.803 ± 0.06	0.672 - 0.934	<0.001
TC/HDL-C ratio	0.783 ± 0.06	0.651 - 0.914	0.001

Discussion

This study was conducted to investigate the association between serum lipoprotein ratios and insulin resistance in non-diabetic patients admitted with ACS. HOMA-IR is a useful surrogate index of insulin resistance in both healthy and patients with diabetes. The HOMA-IR method requires measuring a single fasting plasma glucose and the corresponding fasting plasma insulin level. Although HOMA-IR has been widely used, yet there is no consensus on the cut-off points for classification of insulin resistance. Radikova *et al*[10] and Lee *et al*[12] selected the 75th percentile of non-diabetic population for cut-off point of IR which corresponded with HOMA-IR values of 2.29 and 3.04, respectively. In a study by Kim-Dorner *et al*[13], insulin resistance was defined by HOMA-IR of at least 2.5. In our study, HOMA-IR cut-off value of > 2.5 was used to classify patients as insulin resistant without regard to gender. Patients with HOMA-IR < 2.5 were defined as insulin sensitive. To implement the HOMA-IR method successfully, it is important to define specific cut-points for the race or age of the studied population. However, we used fasting insulin concentration and HOMA-IR to demonstrate the practical usage of insulin resistance in clinical settings.

In our study, lipoprotein ratios (TG/HDL-C and TC/HDL-C) were significantly higher in individuals with HOMA > 2.5 as compared to subjects with HOMA <2.5. Both TG/HDL-C and TC/HDL-C ratios were significantly correlated with HOMA-IR.

Though several methods for evaluation of insulin resistance are available, but majority of these are expensive and time consuming. The TG/HDL-C concentration ratio has been reported to be closely related to insulin resistance[7,14]. Thus, it seems possible that use of the TG/HDL-C ratio, based on commonly available and standardized measurements, may help clinicians identify persons who are insulin resistant. An elevation in the TG/HDL-C ratio could be a novel marker for hyperinsulinaemia among people with normal weight in routine clinical practice[15]. In a study in an East African population, the TG/HDL-C ratio was found to be significantly associated with insulin

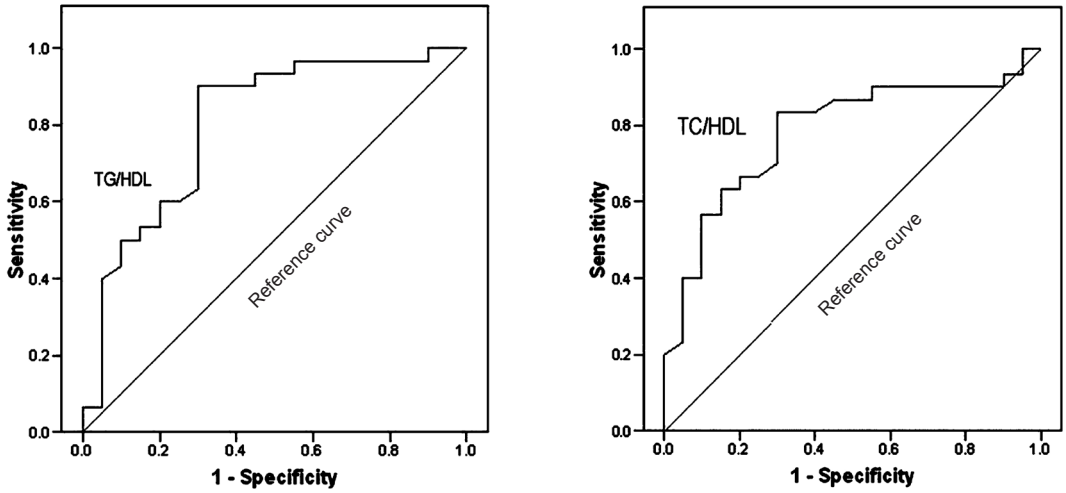

Fig. 2. ROC (receiver operating characteristic) curve showing high sensitivity and specificity of serum lipoprotein ratios for the detection of patients with insulin resistance.

resistance as measured by HOMA[16]. Brehm *et al*[17] demonstrated that TG/HDL-C ratio also had significant positive correlation with insulin resistance in non-diabetic obese individuals. McLaughlin *et al*[7] proposed TG/HDL-C ratio ≥ 3.5 as a cut-off value to predict the presence of insulin resistance. They found that this cut-off had high sensitivity (79%) and specificity (85%) in their study population and concluded that a plasma TG/HDL-C concentration ratio might provide a simple means of identifying insulin resistant, and dyslipidaemic patients who were likely to be at increased risk of cardiovascular disease. Another study on use of metabolic markers to identify overweight individuals who were insulin resistant found optimal cut-off values for TG/HDL-C ratio to be 3 with the sensitivity and specificity of 57 and 71 per cent[14]. These studies have also shown that TG/HDL-C is as close as fasting plasma insulin concentration with IR and may be used as an indicator of IR in clinical setting; as TG/HDL > 3.5 is a strong indicator of the presence of IR. Hadaegh *et al*[18] showed that prevalence of metabolic syndrome in Iranian men with TG/HDL-C level of 2.8-4.4 was five-fold higher than those with TG/HDL-C < 2.8.

A cross-sectional study among normal and overweight non-diabetic individuals showed that those with high value of TC/HDL-C were more resistant to insulin-stimulated glucose disposal, and had higher blood pressure, increased TG concentrations, and hyperinsulinaemia, rendering these individuals at increased risk of coronary heart disease and possibly diabetes for reasons unrelated to cholesterol metabolism[19]. In this study a ratio of 4.6 in the high TC/HDL-C group represented an approximate two-fold increase in risk for CHD as compared to the low TC/HDL-C patients. Kinosian *et al*[20] observed that the total cholesterol/HDL ratio was a superior measure of risk for coronary heart disease compared with either total cholesterol or LDL cholesterol levels.

In a large, population-based study by Ingelsson *et al*[21], the overall performance of apo B: apo A-I ratio for prediction of CHD was comparable with that of traditional lipid ratios but did not offer incremental utility over total cholesterol: HDL-C. They supported total cholesterol and HDL-C measurements instead of apo B or apo A-I in clinical practice. Hadaegh *et al*[22] assessed lipid ratios for prediction of diabetes and also searched for appropriate cut-off values in a large population of non-diabetic subjects during six years follow up. The TC/HDL-C and TG/HDL-C ratios showed similar performance for diabetes prediction in men population; however, among women TG/HDL-C highlighted higher risk than did TC/HDL-C, although there was no difference in discriminatory power. In this study, TC/HDL-C cut-off value of 5.3 in both genders was used to predict incident diabetes.

Although the use of TG and TG/HDL-C as surrogates for insulin resistance has been recommended, but it is important to note that the relationship between TG and TG/HDL-C with insulin may differ by ethnicity; hence using TG and TG/HDL-C to predict insulin resistance would not be appropriate in certain populations[11,16,23]. Several epidemiologic studies have demonstrated that the TC/HDL-C and the LDL-C/HDL-C ratios are better predictors of atherosclerosis and cardiovascular disease than any other single lipid marker[20,24]. Likewise, the TG/HDL-C ratio was demonstrated to be as significant a predictor of cardiovascular disease as the two other lipid ratios[25].

Our study had certain limitations. First, the number of patients per group was rather small, and multiple linear regression analyses were not performed to exclude possible effects of age, sex and body mass index on the relationship between the lipid ratios and HOMA. Secondly, there could be a selection bias affecting the sensitivity and specificity of the test as only high risk population was selected in our study.

In conclusion, our findings showed that in clinical settings, the lipid ratio could be used as an indicator of insulin resistance. In large health surveys, it could be used to monitor trends in cardiovascular health in diverse populations. The TC/HDL-C and TG/HDL-C ratios are easily available, thus eliminating any additional costs. Future studies should include a larger number of participants with a wide range of body mass index and diverse ethnic backgrounds.

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