

Original Research Article

Comparison of Apo B and TG/HDL ratio with HOMA IR model to predict insulin resistance in patients with metabolic syndrome

Objectives:

The aim of the present study was to evaluate the Triglyceride/High Density Lipoprotein Cholesterol (TG/HDL) ratio and Apolipoproteins B (Apo B) levels in metabolic syndrome patients to predict Insulin Resistance.

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 patients were recruited in the study after obtaining informed written consent. They were divided into two groups. Group I was healthy controls (n=50) and Group II was subjects with metabolic syndrome (n=60) as per NCEP ATP III criteria. Anthropometric measurements & biochemical analysis was performed in all subjects.

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 showed was positive correlation, ($r = 0.29, p < 0.02$) while between HOMA IR & Apo B also showed significantly positive correlation ($0.041, p < 0.0009$). But combined multivariate analysis indicated TG/HDL ratio to be non significant variable whereas Apo B is strongly significant.

Conclusion

We report in our study that ApoB is more significant and a better predictor of IR than TG/HDL 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 beneficial 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, hypertriglyceridemic state which may accompany either normal and impaired fasting glucose should promote surveillance for IR.[8,9]

Recently Triglycerides/High density lipoproteins (HDL) 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 in TG levels and depressed HDL levels.[12,13]

But TG/HDL 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 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.

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 LDL 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, 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 LDLC values, but have a preponderance of small, dense LDL particles and higher Apo B.

Hence the study was undertaken to compare the existing model of HOMA IR with both TG/HDL 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 120 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 included healthy controls and Group II

included subjects with metabolic syndrome. All the subjects were matched for age, gender and were excluded for chronic diseases of kidney and liver as well as for cancer and diabetes mellitus. The diagnosis of metabolic syndrome was based on NCEP ATP III criteria. 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 obtained from the antecubital vein under conditions of 12 h of fasting. Fasting blood glucose, TG, Total and HDL cholesterol levels were measured by enzymatic techniques on fully automated analyzer. LDL and VLDL values were obtained by using Friedewald's formula.[20] Fasting plasma insulin was measured by ELISA technique. Insulin Resistance was determined by means of homeostasis model assessment index (Homeostasis Model Assessment–Insulin Resistance, HOMA–IR) using the formula,

$$\text{HOMA-IR} = \text{fasting insulin } (\mu\text{IU/ml}) \times \text{fasting glucose (mg/dl)} / 405. \text{ [21]}$$

The TG/HDL ratio was calculated using the formula: Fasting TG (mg/dL)/HDL-cholesterol (mg/dl).

3.Result:

Table 1 shows anthropometric and clinical characteristics of control and study group.

There was significant differences in the values of body mass index, waist circumference, systolic blood pressure, diastolic blood pressure in control and study group ($p < 0.0001$). The difference in fasting blood glucose and LDL cholesterol 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 ($p < 0.0001$). The correlation coefficient between HOMA IR & TG/HDL was 0.029 where as between HOMA IR & Apo B it was 0.041.

4.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 ($r=0.29$; $p<0.02$) in the study group. The regression model showed R^2 value of 0.087, indicating 8.7 % variation in the HOMA IR due to TG/HDL ratio.

Our results are in accordance with Salazar et al [22] Morato et al [23] and Brehm et al [24] who demonstrated positive correlation between TG/HDL ratio and insulin resistance, confirming that TG/HDL ratio predicts insulin resistance in metabolic syndrome. But an independent study by Knights 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. A significant positive correlation ($r=0.41$; $p < 0.0009$) 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 and Apo B with HOMA IR was performed, the regression model indicated that TG/HDL ratio is a non significant variable whereas Apo B is strongly significant. Most of the studies have compared HOMA IR with either TG alone or with TG/HDL 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 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 cholesterol ratio is cheap and easy to calculate [27] and is also a good predictor of LDL size but 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 the 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 ratio in metabolic syndrome patients with insulin resistance. Miller et al 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 lipemic control for diagnosing IR in line with the current use of HbA1C as long term glycemic control marker.

ACKNOWLEDGEMENT

This work was supported by MGM Medical College and Hoapital, Kamothe, Navi-Mumbai, India. We acknowledge for performing FMD, and Mr. Pandurang Thatkar for helping with data analysis.

COMPETING INTERESTS

The authors have no competing interests.

CONSENT

Authors declare that written informed consent was obtained from the patients before their participation in the study.

British Journal of Medicine & Medical Research, 4(1): 441-450, 2014

449

ETHICAL APPROVAL

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

REFERENCES:

- 1.Martin BC, Warram JH, Krolewski AS, Bergman RN, Soeldner JS, Kahn CR: Role of glucose and insulin resistance in development of type 2 diabetes mellitus: results of a 25-year follow-up study. *Lancet*. 1992; 340:925-929.
2. Hanley AJ, Williams K, Stern MP, Haffner SM: Homeostasis model assessment of insulin resistance in relation to the incidence of cardiovascular disease: the San Antonio Heart Study. *Diabetes Care*. 2002; 25:1177-1184.

- 184
- 185 3. Rutter MK, Meigs JB, Sullivan LM, D'Agostino RB Sr, Wilson PW: Insulin resistance, the metabolic
186 syndrome, and incident cardiovascular events in the Framingham Offspring Study. *Diabetes*. 2005;
187 54:3252-3257.
- 188
- 189 4. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC: Turner, Homeostasis
190 model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin
191 concentrations in man. *Diabetologia*. 1985; 28:412-419.
- 192
- 193 5. Bonora E, Targher G, Alberiche M, Bonadonna RC, Saggiani F, Zenere MB, Monauni T, Muggeo M:
194 Homeostasis model assessment closely mirrors the glucose clamp technique in the assessment of insulin
195 sensitivity: studies in subjects with various degrees of glucose tolerance and insulin sensitivity. *Diabetes*
196 *Care*. 2000; 23(1):57-63.
- 197
- 198 6. Wallace TM, Levy JC, Matthews DR: Use and abuse of HOMA modeling. *Diabetes Care*.2004;
199 27:1487-1495.
- 200
- 201 7. Simental-Mendía LE, Rodríguez-Morán M, Guerrero-Romero F. The product of fasting glucose and
202 triglycerides as surrogate for identifying insulin resistance in apparently healthy subjects. *Metab Syndr*
203 *Relat Disord*. 2008;6:299-304
- 204
- 205 8 Haffner SM, Stern MP, Hazuda HP, Mitchell BD, Patterson JK. Cardiovascular
206 risk factors in confirmed prediabetic individuals: does the clock for coronary heart disease start ticking
207 before the onset of clinical diabetes? *JAMA*. 1990;263:2893–2898
- 208
- 209 9. D'Agostino RB Jr, Hamman RF, Karter AJ, Mykkanen L, Wagenknecht
210 LE, Haffner SM; Insulin Resistance Atherosclerosis Study Investigators. Cardiovascular disease risk
211 factors predict the development of type 2 diabetes: the Insulin Resistance Atherosclerosis Study.
212 *Diabetes Care*. 2004;27:2234 –2240.
- 213
- 214 10. McLaughlin T, Abbasi F, Cheal K, Chu J, Lamendola C, Reaven G: Use of metabolic markers to
215 identify overweight individuals who are insulin resistant. *Ann Intern Med*. 2003; 139:802-809.
- 216
- 217 11. McLaughlin T, Reaven G, Abbasi F, Lamendola C, Saad M, Waters D, Simon J, Krauss RM: Is there a
218 simple way to identify insulin-resistant individuals at increased risk of cardiovascular disease? *Am J*
219 *Cardiol*. 2005;96:399-404.
- 220

- 221
- 222 12. M. Laakso and E. Barrett-Connor, "Asymptomatic hyperglycemia is associated with lipid and
- 223 lipoprotein changes favoring atherosclerosis," *Arteriosclerosis*.1989;9(5):665–672.
- 224
- 225 13. B. V. Howard, "Insulin resistance and lipid metabolism," *American Journal of Cardiology*. 1999; 84:
- 226 28J–32J.
- 227
- 228 14. Michael Miller, Neil J. Stone, Christie Ballantyne, Vera Bittner, Michael H. Criqui, Henry N. Ginsberg,
- 229 Anne Carol Goldberg Triglycerides and Cardiovascular Disease: A Scientific Statement From the
- 230 American Heart Association. *Circulation*, 2013;2293-2305.
- 231
- 232 15. Marcovina S, Packard CJ. Measurement and meaning of apolipoprotein AI and apolipoprotein B
- 233 plasma levels. *J Intern Med*. 2006;259:437–446.
- 234
- 235 16. Walldius G, Jungner I. The apoB/apoA-I ratio: a strong, new risk factor for cardiovascular disease and
- 236 a target for lipid-lowering therapy—a review of the evidence. *J InternMed*. 2006;259:493–519.
- 237
- 238 17. Barter PJ, Ballantyne CM, Carmena R, et al. Apo B versus cholesterol in estimating cardiovascular
- 239 risk and in guiding therapy: report of the thirty-person/ten-country panel. *J Intern*
- 240 *Med*. 2006;259:247–258
- 241
- 242 18. Kathiresan S, Otvos JD, Sullivan LM, et al. Increased small lowdensitylipoprotein particle number: a
- 243 prominent feature of the metabolic syndrome in the Framingham Heart Study. *Circulation*. 2006;113:20–
- 244 29.
- 245
- 246 19. Michael H. Davidson. Apolipoprotein Measurements: Is More Widespread Use Clinically Indicated?
- 247 *Clin. Cardiol*. 2009;32, 9, 482–486.
- 248
- 249 20. Friedewald, W.T., Levy, R.I., and Fredrickson, D.S.: Estimation of the concentration of
- 250 low-density Lipoprotein cholesterol in plasma, without use of the preparative
- 251 ultracentrifuge. *Clin. Chem*. 1972;18:499–502.
- 252
- 253 21. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC: Turner, Homeostasis
- 254 model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin
- 255 concentrations in man. *Diabetologia* 1985, 28:412-419
- 256

- 257 22.Martin R Salazar, Horacio A Carbajal, Walter G Espeche, Carlos E Leiva Sisnieguez, Carlos E March,
258 Eduardo Balbín. Comparison of the abilities of the plasma triglyceride/high-density lipoprotein cholesterol
259 ratio and the metabolic syndrome to identify insulin resistance. Diabetes & Vascular Disease Research.
260 2013;0(0): 1–7
261
- 262 23.Teodoro Marotta, Barbara F. Russo² and L. Aldo Ferrara. Triglyceride-to-HDL-cholesterol Ratio and
263 Metabolic Syndrome as Contributors to Cardiovascular Risk in Overweight Patients. Obesity.2010;
264 18:1608–1613
265
- 266 24.Attila Brehm, Georg Pfeiler, Giovanni Pacini,Heinrich Vierhapper, and
267 Michael Roden. Relationship between Serum Lipoprotein Ratios and Insulin Resistance in Obesity.
268 Clinical Chemistry. 2004; 50(12):2316–2322
269
270
- 271 25.Michael G. Knight, Julia H. Goedecke, Madia Ricks, Juliet Evans, Naomi S. Levitt,
272 Marshall K. Tulloch-Reid,Anne E. Sumner. The TG/HDL ratio does not predict insulin resistance in
273 overweight women of African descent: A study of south African, African Americans and West African
274 Women.
275
- 276 26.Chávez A.G, Simental-Mendía L.E, Elizondo-Argueta S. Elevated triglycerides/HDL-cholesterol ratio
277 associated with insulin resistance.Cir Cir. 2011;79:115-119
278
- 279 27. Wanger A.M, Perez A.P.,Sanchez-Quesada J.L., Ordonez-Linos J.Triglycerides-to-HDL Cholesterol
280 ratio in the Dyslipidemic state classification of type 2 diabetes mellitus. Diabetic Care. 2005;28(7):1798-
281 1800.
282
283
- 284 28. Ortola´ J, Castineiras MJ, Fuentes-Arderiu X: Biological variation data applied to the
285 selection of serum lipid ratios used as risk markers of coronary heart disease. Clin Chem 38:56 –59, 1992
286
- 287 29. Sniderman AD, Furberg CD, Keech A,Roeters van Lennep JE, Frohlich J, Jungner I, Walldius G:
288 Apolipoproteins versus lipids as indices of coronary risk and as targets for statin treatment. Lancet
289 361:777–780, 2003
290
- 291 30. Walldius G, Jungner I, Holme I, Aastveit AH, Kolar W, Steiner E: High apolipoprotein

292 B, low apolipoprotein A-I, and improvement in the prediction of fatal myocardial infarction (AMORIS
293 study): a prospective study. Lancet 358:2026–2033,
294 2001

295 31. Wiesbauer F, Blessberger H, Azar D, Goliasch G, Wagner O, Gerhold L, Huber K, Widhalm K,
296 Abdolvahab F, Sodeck G, Maurer G, Schillinger M. Familial-combined hyperlipidaemia in very young
297 myocardial infarction survivors (< or = 40 years of age). Eur Heart J. 2009;30:1073–1079
298

299 32. De Graaf J, Couture P, Sniderman A. A diagnostic algorithm for the atherogenic apolipoprotein B
300 dyslipoproteinemias. Nat Clin Pract Endocrinol Metab. 2008;4:608–618.

301

302 33. National Cholesterol Education Program. National Heart, Lung, and Blood Institute
303 National Institutes of Health NIH Publication No. 02-5215. September 2002.

304

305

306

307

308

309

310

311

312 **Table.1 Showing descriptive and comparative statistics for different groups by Tukey's POST-**
313 **HOC 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/m ²	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	2.23 ± 0.69	6.14 ± 0.09	0.0001

Insulin Resistance			
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

314

315

316

317

318

319

320

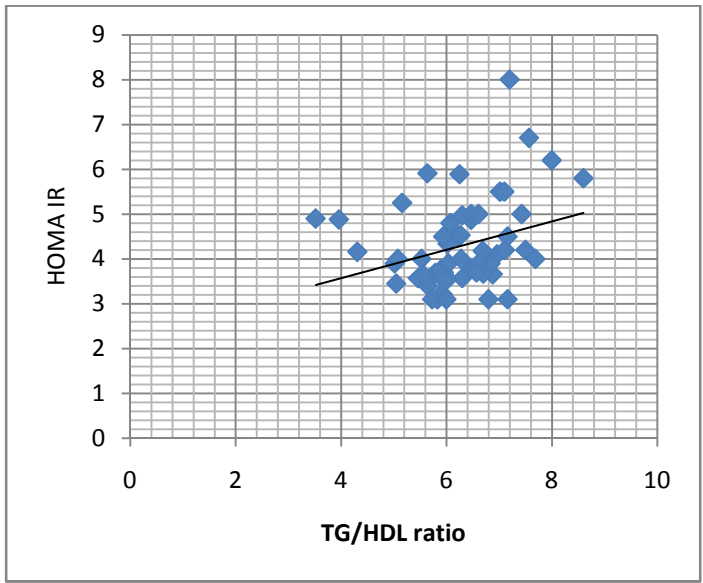
321

322

323

324

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



Graph 2 Correlation between Apo B and HOMA IR (r=0.410)

