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RISK PREDICTION OF CORONARY ARTERY DISEASE WITH OR WITHOUT DIABETES MELLITUS: APOLIPOPROTEINS SCORE OVER LIPID FRACTIONS.

NOORJAHAN MOHAMMED*[1], AMBIKA DEVI.K[2], RAMARAO JANYAVULA[3], BHASKAR RAJU.D.S[4] AND SAI BABA SS KOMPELLA[1]

[1]Nizam's Institute of Medical Sciences, Hyderabad.
[2]Alluri Sitarama Raju Academy of Medical Sciences, Eluru.
[3]Mallareddy Medial College for Women, Hyderabad.
[4]Star Hospitals, Hyderabad.

ABSTRACT

To evaluate the association of lipoproteins & apolipoproteins with Coronary artery disease (CAD) in patients with (CAD-DM$^+$) & without Type II Diabetes mellitus (CAD-DM$^-$) and to determine better risk predictor, we conducted a case-control study. 40 patients with clinical and angiographic evidence of CAD (20 diabetics, 20 non-diabetics) and 20 healthy controls were drawn for the study. Significantly high total cholesterol(TC), Triglycerides(TG), low density cholesterol(LDL-C), TC/HDL-C were observed in CAD-DM$^+$ patients but no significant differences were found in CAD-DM$^-$ patients when compared with controls. On the other hand, levels of ApoB/A-I(apolipoproteinB/ apolipoproteinA-I) and apoB were significantly high in both groups. When comparison was done between two CAD groups, significantly higher($p<0.001$) levels of ApoB/A-I and apoB were observed in diabetics compared to non-diabetics. In ROC analysis between CAD-DM$^+$ vs. controls, maximum AUC was found for apoB/A-I(0.9975) and apoB(0.9925) compared to LDL-C(0.8425), TC/HDL-C(0.7913). Between CAD-DM$^-$ and controls, AUC for apoB/A-I(0.81) was maximum compared to apoB(0.6575), LDL-C(0.6313) and TC/HDL-C(0.5613). These findings suggest that ApoB/A-I is a better predictor of risk for CAD whether associated with DM or not, and is superior to traditional lipid markers in patients without DM.

KEY WORDS: **ApolipoproteinA-I; ApolipoproteinB; ApolipoproteinB/ApolipoproteinA-I ratio; Coronary artery disease (CAD); Diabetes mellitus (DM)**

NOORJAHAN MOHAMMED
Nizam's Institute of Medical Sciences, Hyderabad.

INTRODUCTION

Coronary artery disease (CAD) in Indians often occurs in prime of life and in many cases the initial event is often fatal. In others the disease is so advanced and diffuse that little can be done to reduce the risk of further coronary event[1]. With recognition of many risk factors in the causation of coronary heart disease, prevention has been found to be the best solution to tackle this problem at present[2]. The only major risk factor which seems to be more prevalent in Asian Indians is diabetes mellitus[3]. According to the International Journal of Diabetes in developing countries, India is labeled as the diabetic capital of the world[4]. Patients with type II diabetes have 2 - 4 times higher risk of experiencing cardiovascular disease than adults without diabetes[5]. The pathogenesis of CAD is complex. Distinguishing the factors contributing to the development of CAD in diabetic and non-diabetic population helps to develop specific preventive and therapeutic strategies in these populations. A major finding of the Framingham study is that most cases of premature vascular disease occurred in individuals with levels of total and LDL-C that were indistinguishable from those of individuals who did not develop premature disease[6]. Apolipoproteins are important components of lipoprotein particles, and there is accumulating evidence that measurement of various forms of apolipoproteins may improve the prediction of the risk of cardiovascular disease[7]. The involvement of apolipoproteins in regulating the synthesis and metabolism of lipoprotein particles is gradually being defined[8]. Elevated levels of apolipoprotein-B (apoB), a constituent of atherogenic lipoproteins, and reduced levels of apoA-I, a component of anti-atherogenic HDL, are associated with increased cardiac events. ApoB, apoA-I and the apoB/A-I ratio have been reported as better predictors of cardiovascular events than LDL-C and they even retain their predictive power in patients receiving lipid-modifying therapy. Measurement of these apolipoproteins could improve cardiovascular risk prediction[8].

Apolipoproteins, lipoprotein metabolism and atherogenesis

Lipoprotein particles are made up of an insoluble lipid core surrounded by a coat of phospholipid, free cholesterol and apolipoproteins. Each class of lipoprotein particles is associated with distinctive apolipoproteins that, in addition to stabilizing lipoprotein structure, play an essential role in regulating metabolism. ApoB-100 is synthesized in liver and is present in LDL, IDL and VLDL particles. Only one apoB molecule is present in each of these lipoprotein particles and therefore the total apoB value indicates the total number of potentially atherogenic lipoproteins. ApoB is essential for the binding of LDL-particles to the LDL receptor, allowing cells to internalize LDL and thus absorb cholesterol. An excess of apoB-containing particles is a main trigger in the atherogenic process. Target levels for apoB have now been included in a table on treatment goals in an update of the NCEP ATP III guidelines. ApolipoproteinA-I is the major apolipoprotein associated with HDL-C and largely responsible for determining the plasma level of HDL. ApoA-I acts as a cofactor for lecithin cholesterol acyl transferase(LCAT), which is important in removing excess cholesterol from tissues and incorporating it into HDL for reverse transport to the liver. Using apoB and apoA-I, expressed as the apoB/apoA-I ratio, seems to be a very effective way of characterizing cardiovascular risk in any patient irrespective of their lipoprotein abnormality[8]. This study was undertaken with the dual aim of (i) elucidating the specific lipids and apolipoproteins that contribute to the development of CAD and (ii) identifying the parameters that help in the risk prediction of CAD in diabetic and non-diabetic subjects.

MATERIALS AND METHODS

The study was conducted in 60 subjects of Hyderabad population between age group of 30 – 65yrs, in Osmania General Hospital. The subjects were divided into 3 groups of 20 patients each.

Group I (CAD-DM$^+$): The study group consisted of 20 patients with CAD and type II DM; between age of 30 – 65yrs.

Group II (CAD-DM$^-$): Comprised of 20 patients of similar age group with CAD without DM. Group III: Consisted of 20 normal adults as control with no history of either CAD or DM or hypertension. They are normal symptomatically, fasting glucose and electrocardiographically. CAD patients recruited for the study are those who were undergoing elective coronary artery bypass surgery based on angiographic evidence (significant stenosis >70% stenosis in the left anterior descending, left circumflex, or right coronary artery and >50% stenosis in the left main coronary artery)[9]. American Diabetes Association, 2000criteria was used for diagnosis of Type II DM[10]. Diagnosed cases of DM on regular use of diabetes medication were also included. Patients with recent myocardial infarction (<6weeks), chronic liver and kidney disease and acute or chronic infections were not included in the study. An informed consent was taken from the patients. Hospital ethics committee approval was taken.

Blood samples were drawn following overnight fast and the assays were performed adhering to the standard protocols and quality control procedures. Reagent kits employed for performing the test were as follows: (a) Total cholesterol using CHOD-PAP enzymatic procedure by autopak kit, Bayer Diagnostics, (b) HDL-Cholesterol where apoB containing lipoproteins(LDL+VLDL) were precipitated with phosphotungstic acid and Mg^2using ERBA KIT, (c) Triglyceride by GPO-PAP enzymatic procedure, using autopak kit, (d) ApoA-I and ApoB-100 by immunoturbidimetric method by using Human kit. All the samples were analysed on RA-50 semi auto-analyzer. (e) LDL-Cwas measured indirectly by calculation using Friedwald's equation [LDL-C=TC-{HDL-C + (TG/5)}] [11].

STATISTICAL ANALYSIS

The data was analyzed in order to assess the significance of analyses using SAS (version 1.2).

RESULTS

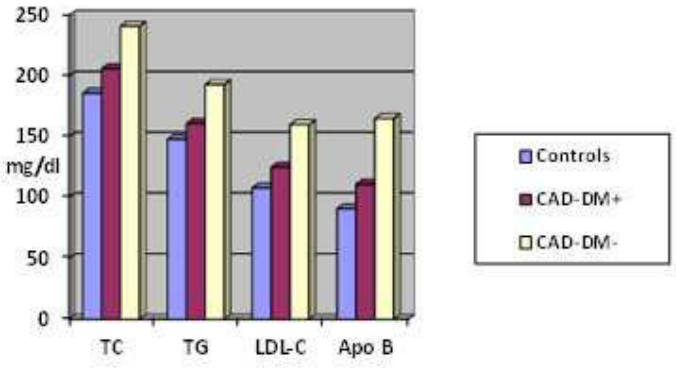
Table 1
Mean & S.D. Values of analyzed parameters in 3 the groups

Parameters	CAD-DM$^+$ (Group-I)	CAD-DM$^-$ (Group-II)	CONTROLS (Group-III)
Total cholesterol (TC)(mg/dl)	240.75 ± 43.35**	205.7 ± 35.6†	186.35 ± 23.9
Triglycerides (TG) (mg/dl)	193.35 ± 50.43**	161.45 ± 43.45†	148.15 ± 26.10
HDL-C (mg/dl)	42.45 ± 8.27	47.8 ± 10.36	47.5 ± 9.8
LDL-C (mg/dl)	159.9 ± 45.63**	125.57 ± 38.16†	108.15 ± 25.97
TC/HDL-C	6.01 ± 2.1**	4.3±1.96†	3.92±1.25
Apolipoprotein B (mg/dl)	164.65 ± 27.4**	110.85 ± 28.5**‡	91.35 ± 21.22
Apolipoprotein A-I(mg/dl)	98.95 ± 15.3**	101.25 ± 26.02*	120.3 ± 14.05
ApoB/A-I	1.7 ± 0.38**	1.13 ± 0.32**‡	0.76 ± 0.17

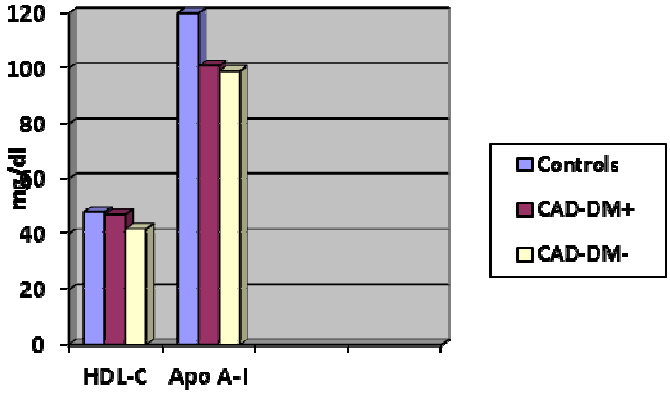
Values are expressed as mean ± SD, values that are marked with * differ significantly from the controls at p<0.05 and values that are shown as ** differ significantly at p<0.001. Mean ± SD values that are marked with † differ significantly from the CAD-DM$^+$ at p<0.05 and values that are shown as ‡ differ significantly at p<0.001. (Analyzed by Student t test).

The serum levels of TC(240.75 ± 43.35mg/dl), TG(193.35 ± 50.43 mg/dl), LDL-C(159.9 ± 45.63 mg/dl) and TC/HDL-C(6.01 ± 2.1) in CAD-DM$^+$ patients are significantly higher when compared to levels in controls (186.35 ± 23.9 mg/dl, 148.15 ± 26.10 mg/dl, 108.15 ± 25.97 mg/dl and 3.92±1.25 respectively) with p <0.001(Table1). In case of apolipoproteins, apoB (164.65 ± 27.4 mg/dl) and apoB/A-I ratio (1.7 ± 0.38) increased significantly with p of <0.001 in CAD-DM$^+$ patients when compared to controls (91.35 ± 21.22 mg/dl and 0.76 ± 0.17 respectively). Atherogenic parameters showed significant increase in CAD-DM$^+$ patients when compared with controls (Graph1). In these patients anti-atherogenic parameters (Graph2) like HDL-C (42.45 ± 8.27 mg/dl) and apoA-I(98.95 ± 15.3 mg/dl) showed decreased values when compared with controls (47.5 ± 9.8 mg/dl and 120.3 ± 14.05 mg/dl respectively), but only the decrease in apoA-I is statistically significant(p <0.001).

Graph 1
Atherogenic parameters in Controls & CAD subjects with and without DM.

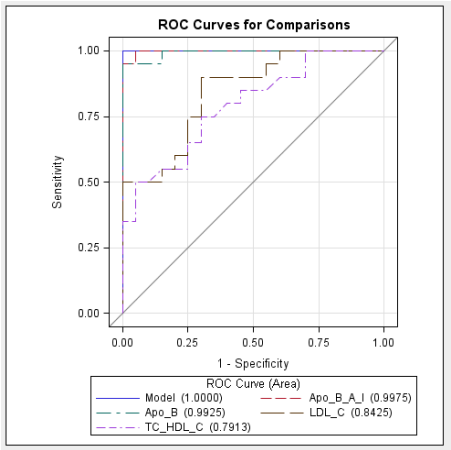


Graph 2
Anti-atherogenic parameters in Controls & CAD subjects with and without DM.

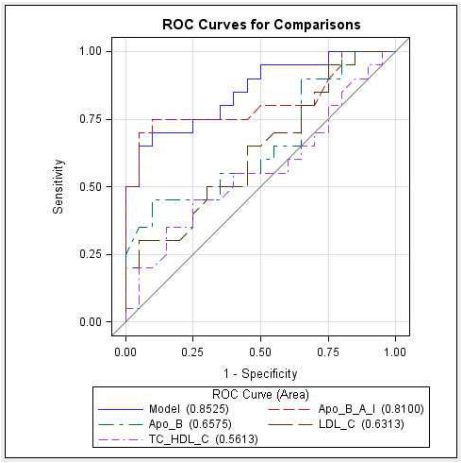


The serum levels of TC (205.7 ± 35.6 mg/dl), TG (161.45 ± 43.45 mg/dl), HDL-C (47.8 ± 10.36 mg/dl), LDL-C (125.57 ± 38.16 mg/dl) and TC/HDL-C(4.3±1.96) in CAD-DM$^-$ patients, did not show statistically significant difference when compared to controls. In case of Apolipoproteins, apoB (110.85 ± 28.5 mg/dl) and apoB/A-I ratio (1.13 ± 0.32) increased significantly with p of <0.001, whereas apoA-I (101.25 ± 26.02 mg/dl) decreased significantly with p of <0.05. When the two CAD groups are compared, atherogenic parameters showed statistically significant increase in CAD-DM$^+$ patients with p of <0.05 for TC, TG, LDL-C and TC/HDL-C, and p of <0.001 for apoB and apoB/A-I ratio. Whereas antiatherogenic parameters like HDL-C and ApoA-I did not show any statistical significant difference. In ROC analysis between CAD-DM$^+$ vs. controls (Graph3) the maximum area under the curve was for apoB/A-I(0.9975) and for apoB(0.9925) compared to LDL-C (0.8425), TC/HDL-C (0.7913). Between CAD-DM$^-$ (Graph4) and controls, AUC for the apoB/A-I(0.81) was maximum compared to apoB(0.6575), LDL-C(0.6313) and TC/HDL-C(0.5613).

Graph 3
ROC between CAD-DM^{+} vs Controls



Graph 4
ROC between CAD-DM^{-} vs Controls.



DISCUSSION

Coronary artery disease (CAD) is the leading cause of mortality and morbidity in developed and developing countries. The knowledge of aetiopathogenesis and progressive studies indicated that this disease is amenable for prevention. Hence, early identification of high risk for CAD is important. In spite of the use of various biochemical markers, till to date no single specific and sensitive marker is available to identify the high risk group population. So the search is on for biochemical marker that can enhance the predictability of CAD. Initial clinical and epidemiological studies established a relation between plasma total cholesterol and CAD[12]. Later on growing evidence (in this field) indicated that this risk is mediated through the major cholesterol carrying lipoproteins. This led the NCEP to identify LDL-C as the major atherogenic lipoprotein and as the primary target for cholesterol lowering treatment[13, 14,15,16,17 &18]. As a consequence of better understanding of lipoprotein composition and metabolism in relation to CAD, attention is being increasingly focused on protein components of lipoproteins[19]. Current clinical practice in the diagnosis and treatment of lipid disorders is undeniably complex and confusing. Five parameters — total cholesterol, plasma triglycerides, HDL cholesterol, LDL cholesterol and the total cholesterol–HDL-C ratio- are reported. All 5 must be interpreted by the physician and understood by the patient[20]. On the other hand, in the routine clinical setting LDL-C is most often calculated from multiple

parameters, usually using the Friedwald's formula. This approach is inherently subject to compound errors for the LDL-C values and is especially problematic in the low range of LDL-C[21]. According to Scharnagl et al.[22]LDL-C calculations are frequently inaccurate at levels <3 mmol/L (116mg/dl) and can result in clinical misjudgment. Furthermore, measurements are not standardized and LDL-C cannot be calculated in subjects with TG levels above 4.5 mmol/L (399 mg/dl). Given that the present system is far from ideal, should we not consider modifying it? Requiring the necessity for more complicated analysis, several authors showed correlation between apoproteins and corresponding lipoproteins which are shown to be atherogenic and also suggested that apoproteins are better markers over corresponding lipoproteins. The other advantages described in using the precision with which they can be determined and the fact that they are not influenced by a meal and are especially useful during analysis of a patient whose fasting status is in question[23].

CAD without DM (CAD-DM$^-$)

In our study CAD patients without DM in comparison to controls showed significant increase in levels of apo B/A-I & apoB and decrease in apoA-I, whereas TC, LDL-C, TG, TC/HDL-C showed increased values that are not statistically significant. Several authors have reported increases in apo B concentrations in CAD patients[24, 25, 26 & 27]. Some studies have shown that increases in small dense LDL concentrations (more atherogenic and contain less cholesterol than normal LDL particles) are reflected by a more pronounced elevation of apoB rather than LDL-C levels[28]. In our study also statistically significant increase in apoB was seen in CAD subjects rather than increase in LDL-c, indicating that there is only one apoB molecule per LDL particle and which explains the role of apolipoproteinB in pathogenesis and was a better index of the high risk phenotype for CAD. The measurement of apolipoproteinB and A-I would provide an early indication of the risk of CAD which otherwise may be missed if the concentrations of LDL-C and HDL-C only are measured. Therefore, estimates of these apolipoproteins may be superior in identifying the high risk for CAD.

Our results also showed that apoB/A-I ratio has shown more significant increase in CAD than any other parameters tested. From the literature available some authors have shown significant increases of apoB/A-I ratio in CAD patients and that it is the better marker of all the markers tested for association with CAD[29, 30 & 31].

CAD with DM (CAD-DM$^+$)

In our study, traditional lipid parameters (i.e. TC, TG and LDL-C) and apoB were significantly increased in CAD with patients as compared to controls ($p<0.001$) [Table1], which is in accordance with previous studies byWagner AM *et al* (1999)[32], Walldius G *et al.* (2001)[33] and John HC *et al* (2009)[34]. In our study apoB was found to be significantly higher in CAD with DM when compared to those without DM and control subjects. These observations coincide with the findings of several studies related to coronary artery disease[35, 36 & 37].The elevated CAD risk affecting patients with Type II diabetes may be attributed to a combined dyslipidemia characterized by elevated triglycerides, elevated triglyceride rich remnant lipoproteins(TGRLP), elevated apolipoprotein (ApoB) and low levels of HDL cholesterol, with a predominance of small, dense low density lipoprotein(sLDL) particles[38]. The present study has shown that LDL-C level was significantly elevated in subjects with CAD & diabetes when compared to the CAD & non-diabetics and normal controls. Clinical studies have shown an increased level of AGEs on LDL obtained from diabetics compared with normal individuals[39 & 40]. Glycosylation of ApoB results in a significant impairment of LDL-receptor-mediated uptake decreasingthe in vivo clearance of LDL[41]. In diabetes, in addition to hypercholesterolemia, low HDL-C, hypertriglyceridemia, hyper apoB appeared to be better index for evaluating CAD risk[36]. The prospective Quebec study also showed a strong association of apoB levels with CAD risk than the levels of TG or cholesterol. It was also seen that diabetes per se increased the apoB concentrations[42]. However it was also observed that decrease in apoA-I levels was more significant than the decrease in HDL level. The decrease in HDL and apoA-I is due

to impaired VLDL lipolysis, increased activity of hepatic lipase which increases the rate of HDL-C clearance and altered composition of HDL-C which includes non-enzymatic glycosylation[43]. Glycation modification of apoA-I contributes to the development and progression of atherosclerotic lesions in diabetic patients, besides low apoA-I levels[44].

Risk predictors for CAD in subjects with and without DM

ROC analysis between CAD-DM$^+$ and controls (Figure III) for the lipid fractions and apolipoproteins, showed maximum AUC for apoB/A-I (0.9975) and apoB (0.9925). The AUC for LDL-C (0.8425), TC/HDL-C (0.7913) are lesser than apo B/A-I. AUC (Figure IV) between CAD-DM$^-$ and controls for the apoB/A-I (0.81) is maximum compared to apo B (0.6575), LDL-C (0.6313) and TC/HDL-C (0.5613). This suggests that apoB/A-I is a better predictor of risk assessment in both the groups of patients whereas TC/HDL-C is a poor predictor. ROC analysis of these data indicates that apoB/A-I ratio has high accuracy in identifying clinically important degrees of CAD in patients. Accuracy, as reflected in the areasunder the ROC curves, was higher for apolipoproteinsthan for lipids. When compared with conventional lipid measurements, apolipoprotein determinations seem to yield better ROC curves with larger areas under the curves. The diagnostic accuracy is the highest for apoB/A-I ratio. As apoB and apoA-I appear to have opposing effects on atherogenic risk, the ratio between the two, indicating the balance between potentially atherogenic versus athero-protective cholesterol-rich particles, may be a more useful measure of risk than either parameter alone.

CONCLUSION

In this study we report the association of plasma apoA-I, apo-B and ratio of apoB /A-I with CAD. Ratio of apoB/A-I and apoB are superior to the "traditional lipids" like TC, LDL-C, HDL-C, and TGs in predicting the presence or absence of CAD. Apolipoproteins ratio gave additional information for normolipidemic patients. It has been suggested that measurement of Apo B and Apo A-I could significantly improve the assessment of cardiovascular risk, especially in patients without elevated LDL-C, and these markers should be included in revisions to the international guidelines for lipid-modifying treatment. Thus, instead of having to measure TC, TG, LDL-C, HDL-C and lipid ratios as recommended by ATP III guidelines, it may suffice to measure apoB/A-I, to effectively evaluate cardiac risk. In addition, apoB/A-I was found to be superior to traditional lipid markers in CAD not associated with DM.

Conflict of Interest

Conflict of interest declared none.

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