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APO B/APOA1 RATIO IS STATISTICALLY THE BEST PREDICTOR OF MYOCARDIAL INFARCTION COMPARED TO OTHER LIPID RATIOS

DR. RAJNI DAWAR*[1], DR. ANIL GURTOO[2] AND DR. RITU SINGH[1]

[1]Department of Biochemistry, Lady Hardinge Medical College & Assoc. SSK Hospital, New Delhi.
[2]Department of Medicine, Lady Hardinge Medical College & Assoc. SSK Hospital, New Delhi.

Corresponding Author rajni.dawar@yahoo.com/ drrajni_dawar@rediffmail.com

ABSTRACT

The aim of our work was to study the predictive value of various lipid ratios in patients with Myocardial Infarction (MI). Study group with 60 patients of documented myocardial infarction and 60 healthy controls were enrolled. Extended lipid profile with Serum Apolipoprotein A and Apolipoprotein B was undertaken for each subject. Lipid ratios of LDL/HDL, TC – HDL/HDL, TG/HDL-C and Apo B/Apo A1were calculated and statistically evaluated to determine the best predictor of MI amongst them. All the above ratios were significantly higher in cases compared to the controls ($p<0.0001$for all). On multiple regression analysis, the best predictor for MI was Apo B/Apo A1 ratio. The coefficient Beta for ApoB/Apo A1 was 0.691 with t value of 9.916 showing highly significant prediction of MI ($p<0.000$). Based on our findings we advocate the use of ApoB/Apo A1 ratio in Indian Population for assessing risk of MI.

KEY WORDS

Myocardial infarction, Apolipoprotein A1, Apolipoprotein B

INTRODUCTION

Coronary Artery Disease (CAD) is the leading cause of morbidity and mortality in both developed and developing countries[1]. The increase of CAD among Indians has been observed throughout the country, as well as among Indian immigrants in different parts of the world. Coronary Artery Disease epidemic in India has entered into an epidemiological transition phase. It has been projected that mortality attributable to "circulatory system diseases" in India would rise by 103% in men and by 90% in women during the period 1985 to 2015 [2]. By 2015, these diseases are expected to account for 34% of all male deaths and 32% of all female deaths in India [2]. Myocardial Infarction (MI) is a manifestation of Coronary artery disease (CAD) due to atherosclerotic plaque deposits undergoing dynamic changes. Pathogenesis actually involves interplay of dyslipidemia with oxidative damage and inflammation leading to atherosclerosis [3]. Lipids are involved in the pathogenesis of atherosclerosis, and hence lipid profile is a basic investigation done in cases of MI. The lipoprotein transport system is central to the mechanism by which genes, diet and hormones interact to regulate the cholesterol and triglyceride plasma levels and their tissue distribution [4]. For over three decades it has been recognised that a high level of total cholesterol (TC), particularly low density lipoprotein cholesterol (LDL-C), is a major risk factor for developing MI but a considerable

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proportion of patients with MI have levels of LDL-C and total cholesterol within the recommended range[5,6,7].The other lipid parameters are also associated with elevated cardiovascular risk and it has been suggested that TC and LDL-C may not be the best discriminants for the presence of CAD. Elevated levels of intermediate density lipoprotein (IDL) and very low density lipoprotein (VLDL) are also associated with increased Cardiovascular risk as are low levels of high density lipoprotein (HDL) and high levels of plasma triglycerides [8, 9,10].

In the last decade, mounting evidence also implicates apolipoprotein (apo) B and apolipoprotein A-I levels in the pathogenesis of CAD [11-17]. Indeed, several recent reports have raised the possibility that these measures might be superior to traditional lipid measures for CAD risk prediction[10,11,12,16] based on the premise that apo B levels better reflect the number of atherogenic lipoprotein particles in a given volume of plasma. However, the published data are not entirely consistent because in some other studies apo B and apo A-I did not perform better than traditional lipid measures for the purpose of risk prediction fuelling an intense debate [13,14,15,17,18,19]. Though many researchers have studied ApoB/Apo A ratio and compared it with other lipid parameters, comparison with conventional lipid ratios have not been done for prediction of risk of MI.

Thus, we carried out a research project in an effort to determine that among different lipid ratios used (TG/HDL, TC-HDL/HDL, LDL/HDL and Apo B/Apo A1 ratio)which is the best predictor of myocardial Infarction.

MATERIALS AND METHODS

The study was carried out in the Department of Biochemistry in collaboration with the Department of Medicine, Lady Hardinge Medical College and Smt. Sucheta Kriplani Hospital, New Delhi during the period of May 2008 to April 2009.The study was approved by Scientific Review Board and Ethics Committee of Lady Hardinge Medical College.

SUBJECTS

A total of 120 subjects were included in the study with informed consent. They were selected from the wards and OPD of medicine department, LHMC & SSK Hospital. Subjects were divided into two groups. Study Group consisted of 60 patients of documented Myocardial Infarction, of both sexes and above 40 years of age. (n=60).The selection criteria of MI cases [20] included: Typical rise and/or fall of biochemical markers of myocardial necrosis with at least one of the following (a) Ischemic symptoms ;(b) Development of pathological Q waves in the ECG;(c) ECG changes indicative of ischemia (ST segment elevation or depression);(d)Imaging evidence of new loss of viable myocardium or new regional wall motion abnormality. Control Group Consisted of 60 age and sex matched controls with no present or past history or clinical evidence suggestive of Coronary Artery Disease. (n=60).All cases and controls were subjected to detailed clinical history with special reference to cardiovascular disease risk factors followed by clinical examination.

SPECIMEN AND LABORATORY ANALYSIS

Venous blood was collected from the antecubital vein of the subjects with informed consent under sterile conditions after overnight fasting. 4 ml of blood was collected in plain vial for routine biochemical investigations and extended lipid profile. Serum sample was stored in aliquots at -20°C and not previously thawed till batch analysed for serum Apo A1 and Apo B. Analysis was done within 8 weeks as per their stability suggested in the protocol provided with kit. Lipid profile (TC, TG,

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and HDL), ApoA1, and Apo B were carried out in autoanalyser Synchron CX 9 (Beckman) using kit from Randox (UK).Serum total Cholesterol was measured by enzymatic end point method. Cholesterol formed after hydrolysis by cholesterol esterase is acted upon by cholesterol oxidase to yield hydrogen peroxide which was reacted with 4-aminoantipyrine in the presence of phenol and peroxidase to yield quinoneimine .Change in absorbance was measured at 500nm. Serum Triglycerides were determined with enzymatic end point method. Glycerol produced by hydrolysis of triglycerides with lipases is acted upon by glycerol kinase and glycerol 3 phosphate oxidase to yield hydrogen peroxide which is coupled to production of indicator Quinoneimine, from hydrogen peroxide, 4- Aminoantipyrine and 4-chlorophenol under catalytic influence of peroxidases. Quinoneimine concentration was measured by measuring absorbance at 500nm, which was proportional to the amount of triglycerides present. High Density Lipoprotein Cholesterol (HDL) was estimated by Enzymatic Direct Clearance Assay. The assay consists of two distinct reaction steps where first step involved elimination of chylomicrons, VLDL-C and LDL-C by Cholesterol esterase, Cholesterol oxidase and subsequently catalase and the second step involved specific measurement of HDL cholesterol after its release by detergent. This HDL Cholesterol is then acted upon by cholesterol esterase, cholesterol oxidase and peroxidase to yield chromogenic quinoneimine which was directly proportional to the cholesterol concentration and action of catalase was inhibited by sodium azide in the second part of assay. Serum Low Density Lipoprotein calculation is done by assuming that total cholesterol is composed primarily of cholesterol in VLDL, LDL and HDL. It was calculated by Fredrickson-Friedwald formula [21] as the value of TG in our study population was less than 400mg/dl so it could be applied. The factor TG/5 is an estimate of VLDL Cholesterol concentration and is based on average ratio of triglycerides to cholesterol in VLDL. Determination of Apolipoprotein A1 and Apolipoprotein B in serum was performed by immunoturbidometric immunoassay on SYNCHRON CX9 using Kit from Randox (U.K).Sample containing human Apo A1 and apo B were made to react with the respective specific antiserum to form an insoluble complex which was measured turbidometrically at 340 nm. Apolipoprotein A1 and B concentrations were thus determined by constructing standard curve.

STATISTICAL ANALYSIS

Statistical analysis was done by using SPSS version 13.0. Mean and standard deviation of all parameters was calculated. Student's t test was used to analyze clinical and laboratory data and chi square test was used wherever required. $p \leq 0.05$ was considered statistically significant; $p \leq 0.001$, highly significant and $p \leq 0.0001$ was considered very highly significant. Multiple regression analysis was done, Beta coefficient and t value were calculated and the significance of each lipid ratio was ascertained and compared.

RESULTS

The baseline clinical characteristics of the study sample are shown in Table 1

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Table 1.

Baseline characteristics of the two groups

Baseline Characteristics	Study Group (n=60) (Mean±S.D)	Control Group (n=60) (Mean±S.D.)	P Value
Age (in years)	47.38 ± 4.58	46.98 ± 4.60	0.477
Sex Distribution	Males= 32(53%) Females=28(47%)	Males=35 (58%) Females= 25(42%)	0.713

The study group and control group were comparable to each other with respect to age and sex distribution as indicated by p>0.05.

Table 2.

Lipid Profile In Study And Control Group

Parameter (mg/dl)	Study Group (n=60) (Mean±S.D)	Control Group (n=60) (Mean±S.D.)	P Value
S.Total Cholesterol	153.50 ± 38.50	139.00 ± 27.13	0.019*
S.Triglycerides	127.70 ± 23.84	115.1 ± 34.85	0.023*
HDL-C	24.63 ± 4.19	35.82 ± 7.81	<0.0001**
LDL-C	103.70 ± 38.84	80.84 ± 27.55	0.003*
VLDL-C	25.54 ± 4.77	23.03 ± 6.97	0.023*
S. Apo A1	73.03 ± 9.69	99.8 ± 7.71	<0.0001**
S. ApoB	96.96 ± 10.59	73.92 ± 14.25	<0.0001**

*p≤0.05; significant; **p≤ 0.0001; very highly significant

Table 2 is showing comparison of lipid profile in patients of Myocardial Infarction and controls. Total serum cholesterol, triglycerides and LDL-C were found to be significantly higher in cases compared to controls with p<0.05.Serum Apolipoprotein B was higher in cases compared to controls which was very highly significant with p<0.0001.Also, HDL-C and Apolipoprotein A1 was lower in patients of myocardial infarction as compared to controls and the difference was very highly significant p<0.0001.

Table 3.

Lipid Ratios In Study And Control Group

RATIO	Study Group (n=60) (Mean±S.D)	Control Group (n=60) (Mean±S.D.)	P Value
LDL/HDL	4.39 ± 1.95	2.37 ± 0.98	<0.0001**

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TC-HDL/HDL	5.46 ± 2.04	3.03 ± 1.10	<0.0001**
TG/HDL	5.33 ± 1.39	3.38 ± 1.39	<0.0001**
ApoB/ApoA1	1.34 ± 0.26	0.75 ± 0.17	<0.0001**

** p≤0.0001: very highly significant.

Table 3 is showing comparison of lipid ratios in patients of myocardial infarction and controls. Ratios of LDL / HDL , TC-HDL / HDL, TG/ HDL ,Apo B/Apo A1 were higher in cases of myocardial infarction compared to controls and this difference is very highly significant statistically with p<0.0001 for all.

A model was used consisting of TG/HDL, LDL/HDL,TC-HDL/HDL and ApoB/Apo A1 ratios as predictive factors for occurrence of MI.R value was 0.829 , R square was 0.687 and adjusted R^2 was 0.676 showing that the model fitted with the population well.

Table 4.

ANOVA

Model	Sum of Squares	Df	Mean Square	F	Significance
Regression	20.617	4	5.154	63.171	0.000*
Residual	9.383	115	0.082		
Total	30.000	119			

*P<0.000; highly significant

F statistics shows overall significance of our model (p 0.000) .The variables (TG/HDL, LDL/HDL,TC-HDL/HDL and ApoB/Apo A1) overall do an excellent explanation of the variation in the occurrence of MI.

Table 5.

Regression Analysis

Lipid Ratio	Unstandardized coefficients		Standardized coefficients		Significance
	B	S.Error	Beta	t	
ApoB/Apo A1	.942	.095	.691	9.916	0.000*
LDL/HDL	-.227	.160	-.834	-1.424	0.157
TC-HDL/ HDL	.252	.161	1.022	1.566	.120
TG/HDL	-.013	.040	-.045	-.336	.737

P<0.000 ;highly significant

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t value of 9.916 for ApoB/Apo A1 ratio suggests that it is the best predictor of MI among all the lipid ratios studied

DISCUSSION

In our study we found that all the four lipid ratios (TG/HDL,LDL/HDL, TC-HDL/HDL and ApoB/Apo A1)evaluated were showing significantly higher values in study group compared to control on using students t test($p<0.0001$). Using the enter method, a significant model emerged ($F=63.171$, $p<0.000$), adjusted $R^2=0.687$, which suggests that our model accounts for 68.7% of variance in the occurrence of Myocardial Infarction. ANOVA confirmed the overall significance of our model with $p<0.000$. On individual calculation of standardized beta coefficient by linear regression analysis, ApoB/Apo A1 ratio had Beta coefficient of 0.691. LDL/HDL had t value of -1.424, TC-HDL/HDL had 1.566 (t value) and TG/HDL had -.336 (t value).Out of the entire four ratios Apo B/Apo A1 ratio had t value of 9.916 with high significance (0.000) while rest three ratios were not significant $p >0.05$. This indicates that a unit change in ApoB/Apo A1 ratio has largest impact on occurrence of MI as compared to the rest three ratios. Based on our findings we conclude that ApoB/Apo A1 ratio is better than traditional lipid ratios to predict the risk of MI.

Our findings are in consensus with findings of the global INTERHEART study of risk factors for acute myocardial infarction in 52 countries which concluded that "the apo B/A-I ratio was the most important risk factor in all geographic regions. In this large study of about 30 000 individuals from 52 countries, apo B:apo A-I was the risk factor accounting for most of the risk of myocardial infarction (population-attributable risk, 49.2% when adjusting for all other risk factors [22] but they did not evaluate the other lipid ratios used in our study. Another study showed significantly higher, regression coefficient beta for Apo-B/Apo-AI ratio as compared to other lipid ratios in cases of MI[23].We have found similar results in patients with documented Myocardial Infarction. Prof. Walldius and colleagues at the Karolinska Institute originally reported in 2001 that the apo B/A-I ratio is of potentially greater value than LDL cholesterol for predicting risk for fatal MI in men and women on the basis of a study in 175,553 individuals recruited from screening programs [24] However other studies do not support this notion [13, 14, 15,17,18,19].

Regarding the availability of the assay, the measurements of apo B and apo A-I have been standardized and are easily accomplished with an automated assay [25]. Furthermore, fasting samples are not needed for assays of apolipoproteins, which clearly is an advantage over traditional lipid ratios. Another advantage of the apolipoproteins is that they are better predictors of CHD risk of patients who are taking lipid-lowering treatment (vs. traditional lipid measures) [26]. The main arguments against the routine clinical use of apolipoproteins in preference to established lipid markers is that apolipoprotein assays are not as widely available relative to standard lipid fractions and are costly but they do provide definitive risk Prediction of Myocardial Infarction as shown by our study.

CONCLUSION

In Our study of Myocardial Infarction Patients and Controls in North Indian population, the Apo B/Apo A1 ratio emerged as the best predictor of Myocardial Infarction, than any of the conventional lipid ratios (TG/HDL, LDL/HDL,TC-HDL/HDL). Hence, in spite of the increased cost of assay and instrumentation required in measurement of Apo B and Apo A1, we strongly suggest Apo B/Apo A1

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ratio assessment in Myocardial Infarction risk prediction.

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