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Apo B/Apo A-I Ratio A Better Predictor of Coronary Artery Disease in Patients with or Without Type II Diabetes Mellitus.

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ABSTRACT: The increasing disease burden due to coronary artery disease (CAD) can be minimized by an early detection using a valid risk factor. Aim of this study was to determine the level of apolipoprotein AI(ApoAI), apolipoprotein B(ApoB) and ApoB/ApoAI ratio in CAD patients with or without type II diabetes mellitus(DM) and to analyze whether these parameters can be used as a more accurate lipid risk factor than the conventional ones. Fasting blood samples were collected from angiographically determined coronary artery disease patients with or without type II DM and control subjects. Serum total cholesterol (TC), triglycerides (TG),and high density lipoprotein (HDL-C)were measured enzymatically ,low density lipoprotein (LDL-C) was determined using Friedewald's formula ,Apo AI and Apo B were analyzed by immunoturbidimetric method. Plasma values of total cholesterol, triglycerides (TG), low density lipoprotein (LDL) cholesterol, were not significantly different in the groups with normal coronary arteries and CAD. Level of ApoB was significantly higher and the level of Apo AI was significantly lower in CAD patients when compared to normal coronary artery group. In the study, ratio of Apo B to Apo AI (Apo B/ Apo AI) was found to be markedly high in CAD patients ($p<0.001$) when compared to the control . As the ratio covers both atherogenic and antiatherogenic lipid risk factor, it can be used as a better predictor than conventional risk factor.

Key words: Apolipoprotein AI, Apolipoprotein B, coronary artery disease (CAD), Diabetes Mellitus.

INTRODUCTION

Coronary artery disease is the leading cause of mortality and morbidity in the world and had become a global problem with the increasing prevalence of obesity, metabolic syndrome and diabetes (Sheriff, et.al.,1994). In India, coronary artery disease (CAD) has increased more than 6 fold in the last 5 decades to reach a prevalence of 10% among persons in the 35 to 65 years age group .It is the most frequent cause of cardiovascular disease, and is expected to account for 40% of all deaths by 2020 (Reddy KS, et.al.,2004 and Murray, et.al.,1990). An early assessment of CAD using valuable predictors can delay the onset of disease and improve the quality of life. Low density lipoprotein cholesterol (LDL- C) was recognized earlier as the primary lipid risk factor and marker. Now as proposed in major guidelines lipid related risk factors are evaluated using high-density lipoprotein cholesterol (HDL C), non-HDL-C as well as triglyceride (TG) levels, and lipid ratios such as total cholesterol (TC)/HDL-C and LDL C/HDL C.(Backer De, et.al.,2003 and Grundy SM, et.al.,2002).New datas are accumulating in favour of apoproteins as more informative lipid risk factors than conventional one(Backer, et.al.,2003). ApoB which indicates the number of potentially atherogenic lipoprotein particles and Apo A-I, which reflects antiatherogenic HDL particles, indicate more accurate cardiovascular (CV) risk factor than LDL C and other lipids . So the present study was designed to compare the plasma levels of ApoB/Apo A ratio and other lipid variables between angiographically defined CAD patients (with or without diabetes mellitus) and normal subjects , to assess their predictive power as a marker of CAD in a sample of south Indian population .

MATERIALS AND METHODS

Study group consisted of 194 subjects (36 females and 158 males) in the age group of 40-70 years who had undergone coronary angiography for evaluating their chest pain .Coronary angiography was performed in all of the subjects and were grouped into four. CAD patients with Type II diabetic mellitus(n=58), CAD patients without Type II diabetes mellitus(n=56), patients without CAD but with Type II diabetes mellitus(n=45) and normal group without CAD and Type II diabetes mellitus (n=35). The study protocol was approved by the Ethics Committee of Vinayaka Missions Medical College and Hospitals From each patient, their medical history was obtained through a structured questionnaire and an informed consent was obtained. The inclusion criteria include patients with established coronary artery disease including single vessel, double vessel and triple vessel, those who had undergone a treadmill test positive for inducible ischemia, patients with history of essential hypertension, coronary artery disease patients with essential hypertension who had border line rise in fasting blood glucose, and patients with recent onset of diabetes Type II diabetic mellitus was diagnosed according to the WHO criteria. Patients excluded were those diagnosed to have coronary artery disease with atrial fibrillation or pacemaker, history of congestive heart failure, history of stroke, transient ischemia or carotid surgery, history of coronary artery bypass graft surgery or percutaneous, transluminal coronary angioplasty, history of intermittent claudication or peripheral Vascular surgery.

Venous blood sample was collected after an overnight fast of 12 hours and the serum was used for the estimation of fasting blood glucose (FBG) by enzymatic GOD-POD method, total cholesterol (TC) by enzymatic 'CHOP-PAP' method, triglyceride (TG) by enzymatic GPO-POD method and high density lipoprotein cholesterol (HDL-C) by direct enzymatic colorimetric method. LDL-C and VLDL-C were calculated using the Friedewald's formula (Friedewald W T, et.al.,1972). Apo A1 were measured on the Cobas INTEGRA Tina-quant Apolipoprotein A-1ver.2 and Apo B on the Cobas INTEGRA Tina-quant Apolipoprotein B ver.2 following the principle of antigen-antibody reaction by immunoturbidimetric method (Rifai, et.al.,1986).

STATISTICAL ANALYSIS

The data were expressed as means ± SD. Statistical comparisons were performed by one way analysis of variance (ANOVA). The results were considered statistically significant if the p values were 0.05 or less.

RESULTS

Table 1 indicates the prevalence of clinically observed variables like duration of diabetes mellitus, smoking, sex distribution and family history of CAD .No statistical difference was observed between the groups.

Table I: Prevalence of clinical variables

Variables	Control n=35	Control with DM n=45	CAD with out DM n=56	CAD with DM n=58
Age	52 ± 10	55.2± 15	53±10	53.2±12
Male	29(82.85%)	35(77.77%)	48(85.71%)	46(79.31%)
Female	6(17.14%)	10(22.23%)	8(14.28%)	12(20.68%)
Smoking	25	28	41	44
Family history of CAD	21	34	40	45
Duration of DM(in years)	_	4.5 ±2.1	_	4.8 ±3.1

Datas are presented as mean ±SD .No significant difference was observed between the groups.

Table II depicts the level of Triglyceride, Total cholesterol, HDL and LDL levels in CAD patients and control. HDL-C level was found to be significantly low ($p>0.05$) in CAD patients with or without diabetes mellitus when compared to the control. Between the groups no significant difference was observed in other parameters

Table II : Lipid and lipoprotein values (mean±S.D.) in subjects with normal coronary group and CAD group with and without DM

Parameters	Control	Control with DM	CAD without DM	CAD with DM
Total cholesterol (mg/dl)	190 ± 22.0	192.0± 23.0	210±38	220 ±35
Triglycerides (mg/dl)	121.35 ±11.28	124.2 ± 14.32	139.58±62.19	113.13±31.69
VLDL (mg/dl)	24.27 ± 2.26	24.0 ±3.12	27.91 ±12.43	22.76±7.36
LDL-C (mg/dl)	118.32 ± 22.5	122.12 ±32	115.47 ±35	125.17±27
HDL-C (mg/dl)	40.9 ± 5.63	41.2 ± 6.52	28.13 ±5.71*	29.5 ±5.87*

Values are expressed as mean ± SD .values that are marked with astrick differ significantly from the control at $p<0.05$.

Table III represents the mean values of Apo AI, Apo B and Apo B/Apo AI ratio in patients and control subjects. Significantly low level of Apo AI and significantly high level of Apo B and Apo B/Apo AI ratio was observed in CAD patients with or without DM. But no significant difference was observed between CAD patients with DM group and CAD without DM groups.

Table III Apolipoprotein values (mean ±S.D.) in subjects with normal coronary group and CAD group with and without DM

Parameters	Control	Control with DM	CAD without DM	CAD with DM
Apolipoprotein B (g/L)	0.83 ± 0.12	0.82 ±0.16	1.54±0.304*	1.54±0.101*
Apolipoprotein A-I (g/L)	1.38 ± 0.31	1.37±0.42	40.13±8.71*	39.5±5.87*
ApoB/ApoA1	0.61 ± 0.81	0.62 ±0.79	2.02±0.96**	2.02±0.55**

Values are expressed as mean ± SD .values that are marked with astrick * differ significantly from the control at $p<0.05$ .Values that are manifested with ** differ significantly from the control at $p<0.001$

DISCUSSION

The morbidity and mortality due to coronary artery disease is increasing with the prevalence of major risk factors like obesity, metabolic syndrome and Diabetes (American Diabetes Association,1989). Worldwide, Asian Indians have the highest rate of CAD .It is 2-4 times at all ages and 5-10 times higher in those under 45 years of age. The occurrence of hyperlipidemia contributes to the high incidence of CAD and increased cardiovascular mortality in patients with type 2 diabetes mellitus (Turner, et.al.,1998).

As an early detection can reverse or reduce the worsening of condition by modification of lifestyle of patient, an establishment of robust and precise risk indicator for lipid imbalance and atherogenesis -to identify the people at high risk for CAD - will be of great practical advantage for patients and physicians. (Nam BH, 2006). LDL-C had been considered as the major atherogenic lipoprotein particles for many years and the prime predictor for CAD (Vogel RA, 1998). High concentrations of LDL-C or low levels of HDL-C promotes adhersion formation and were considered as major risk factors for atherosclerosis and CAD(Rosenson RS, et.al., 1996 and Kinosian B, et.al.,1994). In our study no significant difference was observed in the LDL-C in CAD patients when compared to normal. Hu *et al* has reported the same observation in studies on diabetic American Indians. This observation might be due to the limitation in the determination of LDL-C.(Genest ,et.al.,1992 and LaRosa, et.al., 1990) No accurately measurable standardized direct methods are available for LDL-C. Existing method of determination of LDL-C by Friedewald formula is not accurate and can have errors of about ≥5-20%. (Walldius ,et.al.,2004).This might have led to the generation of new guidelines that proposed to consider HDL-C and non HDL-C to evaluate the proper lipid related risk factors for atherogenesis (Sniderman ,et.al., 2003). Apo B-has been identified as a reliable indicator for atherogenic non HDL-C than conventional lipids. (Walldius ,et.al.,2001) In our study ApoB level was significantly higher in CAD patients with or without diabetes mellitus than normal. Total plasma ApoB indicates the total number of potentially atherogenic lipoproteins (VLDL ,IDL ,and LDL-C)and it reflects the number of cholesterol and to some degree triglyceride containing particles(Genest ,et.al., 1992 and Adult Treatment Panel III.,2001) .In most conditions more than 90% of all ApoB in blood is found in LDL. In cases where LDL C is in the normal/low range, high ApoB levels indicate an increased number of small dense LDL (sd-LDL) particles, which are the most atherogenic particles(Walldius G,et.al.,2004).This might be the reason for the observed appreciable variations in ApoB level than LDL-C in CAD patients.

HDL-C denotes antiatherogenic lipid factor as it helps in reverse cholesterol transport (Hersberger M,et.al.,2005).In our study, HDL-C level has been found to be significantly lowered in CAD patients when compared to normal. These results draw a parallel with the existing reports (Grundy SM,et.al.(2004) and Graham J, et.al.,(2007)It has been reported that ApoA1 the major apolipoprotein associated with HDL-C can be used as an indicator for antiatherogenic lipid particle(Forte TM and McCall MR ,1994)Apo A1not only initiates the reverse cholesterol transport by activating the LCAT but also manifests antioxidant and anti inflammatory effects (Walldius G and Jungner I .,2004) .Hence it can be considered as a better marker than HDL-C .Our studies has shown a significant decrease in ApoA1 activity in the CAD patients when compared to the controls.
Both ApoB and APOA1are measured directly by standardized and internationally validated methods (Walldius G, et.al., 2001and Yusuf S et.al.,2004and Walldius G,et.al.,2004) and reflects the two sides of risk equation- atherogenic Apo B and anti atherogenic ApoA 1.
To get a precise picture of both atherogenic and antiatherogenic lipid related risk , ratio of ApoB/ApoA1 was considered. In our study the ratio was found to be markedly elevated (p≤ 0.001)in CAD patients irrespective of their Diabetes status. The ratio reflects the balance of cholesterol transport and can be expressed as one number which integrates the risk associated with an imbalance between atherogenic and antiatherogenic lipoproteins. (Marcovina S, et.al.,2006 and Sniderman AD,et.al.,2003and Sniderman AD, et. A., 2006). Instead of measuring LDL ,sd-LDL,HDL, and TG it would be sufficient and easier to analyze this ratio. Further studies in a large population are required to establish a cut off value to utilize in the clinical analysis

CONCLUSION

Apolipoprotein measurement significantly predict the of coronary artery disease.The predictive ability of ApoB/ApoA1 ratio was found to better than ApoB , apoA1 values and any of the routine clinical lipids measurements. Thus ApoB/ApoA1 ratio is a better marker of cardiovascular risk and their inclusion in further clinical guidelines should not be discarded.

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