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Full Length Research Paper

Clinico-biochemical correlation with special reference to oxidized LDL and small dense LDL in Indian women with CAD

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In women with coronary artery disease (CAD), clinical presentation is different enough from men which lead to missed or delayed diagnosis of CAD. We therefore assessed the major risk factors and biomarkers in female subjects with CAD. Venous sample of control, unstable angina (UA) and myocardial infarction (MI) patients were taken. In Both UA and MI patients, predominant risk factor was menopause (76.7% UA, 86.7% MI) followed by hypertension (56.7% UA, 60% MI), central obesity (56.7% MI, 56.7% UA), dyslipidemia (50% UA, 50% MI) and diabetes mellitus (50% UA, 33.3% MI). Total serum cholesterol and LDL cholesterol were highly significant ($p<0.001$) in MI and UA as compared to controls. LDL cholesterol was significantly increased ($p<0.05$) in MI as compared to UA. Triglycerides and HDL-Cholesterol were also increased but not at the significant level ($p>0.05$). Apolipoprotein (ApoB), small dense LDL and oxidized- LDL (Ox-LDL) were highly significant ($p<0.001$) in MI and UA as compared to controls. Based on discriminate analysis ox-LDL is a potential marker to discriminate cases of UA from controls while ApoB is the reliable marker which can discriminate the cases of MI from UA and controls.

Key words: Coronary artery disease, dyslipidemia, apolipoprotein B, small dense LDL, oxidized LDL.

INTRODUCTION

The global burden of cardiovascular disease is rapidly increasing predominantly due to sharp rise in the incidence and prevalence of the same in the developing countries. India, a developing nation is undergoing in the same phase and is now in the middle of coronary artery disease (CAD) epidemic (Reddy, 2004). In the past medical research on heart disease was primarily focused on men, now CAD is considered to be the most common cause of death in women (Enas et al., 2001). Women are more likely to die after a first MI and have higher risk of recurrent MI than men. In the Framingham heart study (FHS), the one year mortality following an MI was 41% in women versus 27% in men (Kannel, 1995). The overall short term and long term CAD mortality following an MI are about 40% higher in women after adjustment for age and other risk factors (Kannel, 1995). Despite their excess risk, women are only half as likely as men to receive aspirin, beta-blockers or thrombolytic therapy or to be referred for coronary angiogram or revascularization procedures (Barakat et al., 2000).

Now researchers recognize that there are significant differences in presentation of CAD in women and men. Women having MI are more likely to present with atypical chest pain (midback pain) and atypical symptom like indigestion, nausea, vomiting and dysponea (Milner et al., 1999). More than half of sudden deaths occur within six hours of the onset of symptoms. Early diagnosis is important since two-thirds of women who experience sudden death have no previous symptoms of CAD, com-pared with about half of men (Eaker et al., 1993). Since the clinical picture in women is different enough from men, therefore the diagnosis can be missed or delayed.

Unfortunately, there have been very few studies addressing these issues from India. Cardiac biomarkers may provide earlier assessment of overall patient risk and aid in identifying women with higher risk of adverse effect. Therefore, the present study was done to evaluate the role of cardiac biomarkers in the early assessment of risk in female patients with CAD.

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MATERIALS AND METHODS

Patients

Present study was carried in 90 female subjects of age between 40-65 years comprising 30 healthy controls in group 1, 60 consecutive patients of CAD were taken which were further divided into 30 patients of unstable angina (UA) in group 2 and 30 patients of myocardial infarction (MI) in group 3, admitted in coronary care unit of University College of Medical Sciences (UCMS) and Guru Teg Bahadur (GTB) Hospital, Delhi, India. Controls were taken randomly from out patient department of Hospital who came for routine complaints unrelated to cardiovascular system. Controls group included women without hypertension, diabetes mellitus, obesity and hypothyroidism. Study was done for the period of 18 months from October- 2005 to march-2008. All subjects gave informed written consent to participate in the present study. Study was approved by the ethics committee of UCMS, Delhi, India. CAD was defined on the basis of history, clinical examination, ECG findings and elevated cardiac enzymes.

Biochemical assays

Blood samples of over-night fasted patients were allowed to clot at room temperature and were centrifuged at 2000 x g for 15 min to prepare serum samples. All samples were stored for –70°C and analyzed for the following parameters.

Blood glucose was estimated by glucose oxidase method (Burtis and Ashwood, 1976). Serum lipid profile includes total cholesterol (Allain et al., 1974), HDL-Cholesterol (Burstein et al., 1970), LDL-Cholesterol (Freidwald et al., 1972) and triglycerides (Werner and Gabrieulsen, 1981). LDL oxidation was estimated by baseline levels of diene conjugates in lipid fraction of LDL (Ahotupa, 1996). Apolipoprotein-B was estimated by immunoturbidity method (Rifai and King, 1986). Small dense LDL was measured by LDL-C/ LDL-apolipoproteinB ratio (Hattori et al., 1998).

Statistical analysis

Comparison was made between study group and control group by using one way ANOVA with Tukey's test at 5% significance level. Stepwise discriminate analysis was performed to find out the variables which are most useful for discriminating among the study groups.

RESULT

A total of 90 female patients were recruited for this study. These consisted of 30 healthy controls, 30 patients of UA, 30 patients of MI.

Table 1 shows the demographic profile of study groups. All the groups were matched for age. There was no statistically significant difference of age in UA and MI as compared to control. Body mass Index (BMI), waist circumference (WC) of UA and MI were highly significant ($p<0.001$) as compared to controls. It was also seen that BMI and WC of MI were also highly significant ($p < 0.001$) as compared to UA. Waist to hip ratio (WHR) was significantly higher ($p < 0.001$) in patients of CAD as compared to controls. A highly significant ($p < 0.001$) difference of WHR was also seen in MI as compared to UA. Systolic and diastolic blood pressure was significant in CAD patients as compared to controls.

Table 2 shows prevalence of risk factors in UA; the predominant risk factor was menopause (76.7%) while other risk factors were hypertension (56.7%), central obesity (56.7%), dyslipidemia (50%), diabetes mellitus (50%). In MI, the predominant risk factor was also menopause (86.7%) whereas other risk factors included hypertension (60%), central obesity (56.7%), dyslipidemia (50%) and diabetes mellitus (33.3%). While only 40.5% of healthy controls were in menopausal state.

Table 3 shows lipid profile of study groups. Serum total-cholesterol and LDL-C were significantly higher ($p < 0.001$) in UA and MI as compared to controls. LDL-C levels were also increased significantly ($p < 0.05$) in MI patients as compared to UA. HDL-C and TGs levels were also raised in CAD patients as compared to controls. Insignificant difference ($p > 0.05$) was found in serum HDL-C and TGs among the study groups. Levels of ox-LDL were significantly high ($p < 0.001$) in UA and MI as compared to controls. Ox-LDL was also highly significant ($p <0.001$) in MI as compared to UA. Levels of apoB were significantly high ($p<0.001$) in CAD patients as compared to controls. Apo B was also significantly high ($p < 0.001$) in MI as compared to UA. Small dense LDL was significantly decreased ($p < 0.001$) in UA (1.09 ± 1.51) and MI (1.17 ± 0.25) when compared to controls (1.35 ± 0.13).

Table 4 shows percent distribution of small dense LDL in study groups. Controls showed absence of small dense LDL whereas 96.7% patients of UA and 83.3% of MI patients showed presence of small dense LDL.

Table 5 shows the discriminate analysis, ox-LDL is an important variable to discriminate between UA and controls whereas ApoB is important variable to discriminate between MI from UA as well as controls.

DISCUSSION

Many gender differences exist among patients with CAD, including risk factor profiles, clinical presentation (Wiviott et al., 2004). Diagnosis and risk assessment of CAD in women is more difficult than in men. Various studies have shown that women have higher rates of complications and risk of death after coronary artery disease than men (Wiviott et al., 2004). Therefore, cardiac biomarkers which are considered to be associated with pathogenesis of CAD were studied. These biomarkers are released into the circulation and can be measured easily in the blood. This will help in risk stratification and treatment plan as early as possible so that the complications and mortality can be decreased by providing help at the first site.

Prevalence of risk factors is different in women. It has been seen that the major non modifiable risk factor is menopausal state (Dwivedi and Dhar, 2006). It was observed that 76.7% of UA was in menopausal state whereas 86.7% of MI patients were in menopausal state.

Table 1. Demographic profile of study groups.

Parameter	Group 1 (Control)	Group 2 (UA)	Group 3 (MI)
Age (years)	54.3 ± 6.75	55.60 ± 6.69	56.13 ± 5.00
BMI (Kg/m^2)	24.39 ±1.56	25.4 ± 2.64 [a]	26.17 ± 4.93 [b, c]
WC (cm)	75.65 ± 2.67	85.32 ± 9.65 [a]	86.89 ± 10.58[b, c]
WHR	0.69±0.04	0.75 ±0.04 [a]	0.77± 0.06 [b, c]
SBP (mmHg)	114±4.31	132.93 ±32.0 [a]	150.80±32.54[b]
DBP (mmHg)	74.7±3.64	96.97±20.73 [a]	103.90±28.47[b]

BMI, body mass index; WC, Waist Circumference; WHR, Waist to hip ratio; SBP, Systolic blood pressure; DBP, Diastolic blood pressure; RBS, Random blood sugar. Values are expressed in mean ± S.D. [a] Group1 vs. group 2($p<0.001$).[b]Group1 vs. group3 ($p < 0.001$).[c] Group 2 vs. group 3($p < 0.001$).

Table 2. Prevalence of risk factors in study groups.

Risk factors	Group 1 (Control)	Group 2 (UA)	Group 3 (MI)
Hypertension	-	56.7 %	60%
Diabetes Mellitus	-	50%	33.3%
Central obesity	-	56.7%	56.7%
Menopause	40.5%	76.7%	86.7%
Dyslipidemia	-	50%	50%

All the results are expressed in percent. Number of cases in each group is 30.

Table 3. Comparison of serum Lipid profile in study groups.

Parameter	Group 1 (Control)	Group 2 (UA)	Group 3 (MI)
TC (mg/dl)	169.77±20.58	213.67±20.40[a]	239.27±45.4[b]
HDL-C (mg/dl)	46.03±5.28	45.93±12.78	44.97±13.54
LDL-C (mg/dl)	100±17.83	124.20±33.14[a]	152.30±32.57[b, d]
TG (mg/dl)	123±44.08	186.87±82.05	193±128.71
Ox-LDL (umole/L)	31.42 ± 6.19	41.36± 7.03[a]	48.55 ±7.0[b, c]
ApoB (mg/dl)	73.84± 12.5	116.67 ±14.18[a]	129.68 ±18.20[b,c]
LDL-C/LDL-ApoB (Small dense LDL)	1.35 ±0.13	1.09± 1.51 [a]	1.17±0.25[b]

TC: Total cholesterol. HDL-C: High density lipoprotein cholesterol. LDL-C: Low density cholesterol. TG: Triglycerides.Ox-LDL: Oxidized low density lipoprotein.ApoB: Apolipoprotein B.LDL-C/LDL-ApoB: Ratio of low density cholesterol and apolipoprotein B. Values are expressed in mean ± S.D. [a]Group1 vs. group 2($p < 0.001$). [b]Group 1vs group 3($p < 0.001$). [c]Group 2 vs. group 3($p < 0.001$).[d] Group 2 vs. group 3($p < 0.05$).

Table 4. Percent distribution of large and small dense LDL.

LDL-C/LDL- ApoB ratio	Group 1 (Control)	Group 2 (UA)	Group 3 (MI)
Group3 (n=30)	≥ 1.2 (Large LDL)	100%	3.3%
16.7%			
<1.2 (Small dense LDL)	-	96.7%	83.3%

All the results are expressed in percent. LDL-C/LDL-ApoB ratio of < 1.2 represents small dense LDL and ratio of ≥1.2 represents large LDL.

Menopause is a risk factor for CAD because estrogen withdrawal has a detrimental effect on cardiovascular function and metabolism. The menopause contributes to many traditional CVD risk factors including changes in body fat distribution from gynoid to android pattern, reduced glucose tolerance, dyslipidemia, increased blood

Table 5. Discriminate analysis with respect to Ox-LDL, ApoB and LDL-C/ApoB ratio among study groups.

	Group1 (control) vs Group 2 (UA)		Group 1 (control) vs Group 3 (MI)		Group 2 (UA) vs Group 3 (MI)	
	std	**unstd**	**std**	**unstd**	**std**	**unstd**
Ox-DL(µmol/L)	0.78[a]	0.111	0.585	0.088	-	-
ApoB (mg/dl)	0.616	0.038	0.811[b]	0.052	0.485[b]	0.036
LDL-C/ApoB ratio(small dense LDL)	-	-	-	-	-0.782	5.543
Constant	-	-9.654	-	-8.789	-	1.755

Std: Standardized function. Unstd: Unstandarized function. [a]Ox-LDL, important marker to discriminate group2 from group1. [b]ApoB, reliable marker to discriminate group 3 and group 2 from group 1.

pressure, increased sympathetic tone, endothelial dysfunction and vascular inflammation (Rosano, 2007). CAD rates in women after menopause are 2 times that of premenopausal women of the same age (Akahoshi et al., 1996).

Hypertension tends to be more common in women than in men after 45 years of age (Dwivedi and Dhar, 2006). Relationship between hypertension and heart disease is well established (Greenland et al., 2003). In the present study, hypertension was seen in 56.7% of patients of UA whereas 60% of MI patients showed hypertension. Prevalence of hypertension, more with older women than their age matched male counter-parts, has been reported in several epidemiological studies (Kotchen et al., 1982). Recent data suggest that women develop high blood pressure especially systolic hypertension at an increased rate as they age (Staessen et al., 1997).

It is evident from literature that as women change from pre to post menopausal status, there is an increase in central obesity and associated risk factor for CAD (Wang et al., 2003). It has been shown that central obesity was associated with increase risk of diabetes and CAD in both men and women (Kortkiewski et al., 1993). Recently, waist circumference has been found to be a simple and better marker of central obesity. In women, the optimum waist circumference is < 80 cm. We observed that waist circumference of CAD patients was significantly high as compared to controls. Central obesity was seen in 56.7% patients of unstable angina and MI. Central obesity causes insulin resistance. The working hypothesis of many other groups (Reavan et al; 1993; Haffner et al., 1995; Tchernof; 1996) is that central obesity causes insulin resistance and elevated free fatty acid levels, with the resultant increase in hepatic apoB secretion and increased hepatic lipase activity leading to hypertriglyceridemia, small dense LDL, and decreased HDL cholesterol. Recently it has been shown that intra abdominal fat store is responsible for insulin resistance (Ash well et al., 1985) and leads to type 2 DM. It has also been shown that central obesity leads to dyslipidemia, which is the marker of abdominal obesity and better predictor of MI than BMI in both sexes and all ethnic groups studied (Yusuf et al., 2005).

Diabetes is stronger risk factor of CAD in females than males, with a 3 -7 fold higher CAD incidence and mortality as compared 2-3 fold higher risk in men (Barrett et al., 1991). In our study 50% of UA and 33.3% of MI patients showed diabetes mellitus. Diabetes increases the risk of heart failure by 8-fold in women compared to 4-fold in men. Diabetes eliminates protective effects of estrogens and removes the normal sex difference in the prevalence of CAD (Sower; 1998). Premenopausal women with diabetes face a similar risk of developing CAD as non-diabetic men of the same age (Newnham et al., 1997).

Dyslipidemia is one of the most important modifiable risk factors for CAD. In our study, 50% of UA and 50% of MI have dyslipidemia. This can be associated with other risk factors such as DM, central obesity or age itself. TC increases with age in both sexes, however, after menopause women experience an additional 10-20 mg/dl rise. Above 45 years of age a higher percentage of women than men have total blood cholesterol of ≥ 200 mg/dl (Pignone et al., 2001). However, it has been suggested that elevations in total cholesterol may be important in elderly women but not elderly men (Corti et al., 1995). Our study showed significant elevation ($p < 0.001$) of serum cholesterol in both UA and MI as compared to healthy controls. The LDL-C is a strong predictor of CAD mortality in women as well as in men. In women LDL Cholesterol increases an average of 2 mg/dl per year between ages 40 and 60, partly because of declining estrogen levels (Johnson et al., 1993). Our study showed highly significant level of LDL-C in UA and MI as compared to healthy women. However, significant ($p < 0.05$) increase in LDL-C level was also seen in MI as compared to UA.

The change in HDL-C around the time of menopause remains controversial. Some early cross sectional studies compared premenopausal with post menopausal women reported no differences in HDL-C levels as consequences of menopause (Kannel et al., 1976; Bush et al., 1984) while other described a decreased in HDL-C that occurred gradually over the 2 years preceding meno-

pause (Jensen et al., 1990). Our study showed decline in HDL-C in CAD patients as compared to controls but not at the significant level. A recent large study based on more than a thousand women showed that HDL-C was not correlated with age and does not change significantly as a consequence of menopause (Bergmann et al., 1997). In some studies, TG levels do not independently predict CAD mortality after adjustment for other cardiac risk factors, whereas others suggest an independent effect (Criqui et al., 1993), Hypertriglyceridemia appears to be a stronger risk factor in women than in men (Steinvold et al., 1993; Austin et al., 1998). In the present study, TG levels of UA and MI were high as compared to healthy controls but not at the significant levels.

LDL-C at increased concentrations in the blood stream enters the blood vessel wall. Normal LDL-C with increased LDL-apoB associated with atherosclerotic cardiovascular disease. Estimated LDL-apoB is useful for the detection of small dense LDL (Steinberg and Lewis, 1997). Small dense LDL is more atherogenic and susceptible to oxidation compared to large buoyant. Our study showed increased level of apoB in UA and MI which was highly significant as compared to controls. The levels of apoB were also highly significant in MI as compared to UA.

Lower ratio of LDL-C/LDL-apoB reflects preponderance of small dense LDL (Steinberg and Lewis, 1997). Normal values ranged from 1.2 to 2.3(90% percentile). The size and preponderance of small dense article is postulated, when the ratio falls below 1.2 (Hattori et al., 1998). In the present study, we found decreased ratio of LDL-C/LDL-apoB which was highly significant in CAD patients as compared to controls. 96.7% patients of unstable angina showed small dense LDL whereas 83.3% patients of MI showed small dense LDL. Small dense LDL was not found in any of the control (ratio ≥1.2). All controls (100%) had ratio of ≥1.2. The comparison of LDL-C/LDL-apoB ratio was significantly higher in CAD patients as compared to healthy controls (Hattori et al., 1998). Our findings correlate with the above done study. Oxidative modification of LDL is an important atherogenic factor (Holvoet et al., 2001). Our study showed significant higher value of ox-LDL in UA and MI as compared to controls. There was also highly significant difference of ox-LDL between MI as compared to UA. Recent studies have shown that CAD patients had a higher level of circulating ox-LDL as compared to controls and the sensitivity of this circulating ox-LDL was 76% for cases of CAD (Holvoet et al., 2001). This study correlates with our study which shows higher value in CAD patients as compared to controls.

Hence, menopause is the major risk factor in female patients with CAD. Levels of total cholesterol, LDL-C and ApoB were significantly high in female patients with CAD. However, small dense-LDL was highly increased in UA as compared to MI which suggest that small dense-LDL is the precursor of atherogenesis in initial stages of CAD. Ox LDL was significantly raised in patients of MI and UA as compared to controls. Based on discriminate analysis, ox-LDL is a potential marker to discriminate UA from controls while ApoB can discriminate the cases of MI from UA as well as from controls.

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