Original Article

Biomarkers for Early Detection of Risk in Female Patients with Coronary Artery Disease: Pilot Study

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Abstract

In women with coronary artery disease (CAD), clinical presentation is different enough from men which leads to missed or delayed diagnosis. Biomarkers can be used for assessment of CAD patients. In case control study, we analyzed blood samples of 30 controls, 30 cases of Unstable Angina (UA) and 30 cases of Myocardial Infarction (MI) for Pro –inflammatory markers (hs-CRP,IL-6,ICAM-1) and Pregnancy Associated Plasma Protein –A (PAPP-A). Based on discriminant analysis, hs-CRP is the potential marker to discriminate cases of UA from controls while PAPP-A is the reliable marker which can discriminate the cases of MI from UA and controls.

Introduction

The global burden of cardiovascular disease is rapidly increasing predominately due to sharp rise in incidence and prevalence of same in developing countries. India is undergoing in the same phase and is now in the middle of Coronary Artery Disease (CAD) epidemic.[1] Earlier Medical researches on heart diseases were primarily focused on men. Now CAD is the most common cause of death in women.[2] Now researchers recognize that there are significant differences in presentation of CAD in women and men. Women having myocardial infarction are more likely to present with atypical chest pain (midback pain) and atypical symptoms like indigestion, nausea, vomiting and dyspnoea. Since the clinical picture in women is different from men, therefore the diagnosis can be missed or delayed. Unfortunately, there have been very few studies addressing this issue from India. Cardiac biomarkers may provide earlier assessment of overall patient risk and aid in identifying women with higher risk of adverse effects. Therefore, the present study was done to evaluate the role of cardiac biomarkers in the identification and early assessment of risk in female patients with CAD.

Table 1: Demographic profile of study groups

	Group 1(Control)	Group 2 (UA)	Group 3(MI)
Age (years)	54.3± 6.75	55.60 ± 6.69	59.13 ± 5.00
BMI (Kg/m^2)	24.39± 1.56	25.4 ±2.63[a]	26.17 ± 4.93[b,c]
WC (cm)	75.65± 2.67	85.32 ±9.56[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 ± 32.24[b]
DBP (mmHg)	74.7 ± 3.64	96.97 ± 20.73[a]	103.90 ± 28.47[b]
RBS (mg/dl)	95.67 ± 17.21	135.90 ± 45.62[a]	135.67 ± 42.62[b]
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

BMI=Body Mass Index; WC=Waist circumference; WHR=Waist to hip ratio; SBP=Systolic Blood Pressure; DBP=Diastolic Blood Pressure; TC= Total -Cholesterol; Values are expressed in mean± S.D. a= Group 1 vs. Group 2 ($p< 0.001$); b= Group 1 vs. Group 3 ($p< 0.001$); c=Group 2 vs. Group 3 ($p< 0.001$); d=Group 2 vs.Group 3 ($p\leq 0.05$)

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Material and Methods

Study was done in 90 female subjects of age between 40-65 years comprising 30 healthy controls in Group 1, 30 patients of Unstable Angina (UA) in Group 2 and 30 patients of Myocardial Infarction (MI) in Group 3 respectively. Cases were taken from coronary care unit of University College of Medical Sciences and Guru Teg Bahadur Hospital, Delhi. Controls were taken randomly from out-patient Department of Hospital who came for routine complaints unrelated to cardiovascular system. Control group included women without hypertension, diabetes mellitus, obesity and hypothyroidism. Study was done for period of 18 months from October 2005 to march 2008. All subjects gave informed consent to participate and study was approved by the ethics committee of University College of Medical Sciences, Delhi. CAD was defined on the basis of history, clinical examination, ECG findings and elevated cardiac enzymes and troponins.

hs-CRP was estimated by using solid phase ELISA technique (Calbitech,USA),IL-6 was measured by sandwich ELISA technique (Calbitech USA), ICAM-1 was estimated using standard kit which was based on sandwich ELISA (Diaclone company, USA). PAPP-A was measured by sandwich ELISA technique using standard kit (DRG-USA).Comparison was made between study groups and control group by using one way ANOVA with two tukey's test at 5% significance level. Stepwise discriminant analysis was performed to find out the variables which are most useful for discriminating among the study groups.

Results

As shown in Table 1, there is no statistically significant difference of age in Group 2 and Group 3 as compared to Group 1. As shown in Table 1, Serum T-Cholesterol and LDL-C were significantly higher ($p<0.001$) in Group 2 and Group 3 as compared to Group 1. While LDL-C levels were increased significantly ($p<0.001$) in MI patients (Group 3) as compared to UA (Group 2). Insignificant difference ($p>0.05$) was found in serum HDL-C and serum TGs among the study Groups.

As shown in Table 2, inflammatory biomarkers (hs-CRP, IL-6, ICAM-1) and PAPP-A were highly significant (p<0.001) in Group 2 and Group 3 as compared to Group 1. However inflammatory markers and PAPP-A were also significantly higher (p<0.001) in Group 3 as compared to Group 2.

Stepwise Discriminant analysis was performed to find out the variables which are most useful for discriminating among the study groups (Table 3). Out of four biomarkers, hs-CRP showed high standardized coefficient when UA was compared to controls which shows hs-CRP is important variable to discriminate UA from controls. PAPP-A showed high standardized coefficient when MI was compared to UA and controls. This suggests that PAPP-A can discriminate cases of MI from UA and control group. Further unstandardized function of these biomarkers can be used to calculate Discrminant Score (DS) which helps in differentiating between the study groups (Table 3).

i. Calculation of Discriminant Score (DS) between control group and UA.

   DS=0.719 × hs –CRP +0.084× IL-6 + 0.004× ICAM+ 0.222× PAPP-A - 4.989 (constant).

   If DS is > 0;case belongs to UA. If DS is < 0; it belongs to Control group.

ii. Calculation of DS between control group and MI.

   DS=0.431 × hs –CRP + 0.003× ICAM+ 0.236× PAPP-A - 4.989 (constant).

   If DS is > 0; case belongs to MI. If DS is < 0; it belongs to Control group.

iii. Calculation of DS between MI and UA.

   Ds=0.347 × hs –CRP +0.017× IL-6 + 0.194 × PAPP-A - 4.989 (constant).

   If DS is > 0;case belongs to MI. If DS is < 0 ; case belongs to UA.

Discussion

Diagnosis of coronary artery disease in women is more difficult because of lower specificity of symptoms and diagnostic accuracy of non invasive cardiac biomarkers in patients with UA and MI. Multi marker approach may aid the initial risk assessment of UA and MI especially in women.[3] Finding single best marker for diagnosis can reduce the financial burden for patients and providing ease to clinician for diagnosing women with CAD who present with atypical symptoms. We studied biomarkers which are considered to be associated with pathogenesis of CAD which are released into circulation and can be measured easily in the blood.

Inflammation is believed to have role in pathogenesis of cardiovascular events. In our study, we found that pro-inflammatory marker (hs-CRP, IL-6, ICAM-1) and PAPP-A were significantly high in patients of UA and MI when compared to healthy females. These pro-inflammatory markers and PAPP-A were also significantly high in MI when compared to UA. Epidemiological and clinical studies have shown strong and consistent relationship between markers of inflammation and cardiovascular risk.[4,5] In our study, we tried to find out the clinical utility of these biomarkers which can be best used to discriminate the cases of MI, UA from healthy controls. After discriminate analysis hs-CRP was the best marker to discriminate cases of UA from controls. Elevated hs-CRP (3 mg/L) is found in <10% of normal population, 20% in patients with chronic stable or variant angina but in >65% of patients with unstable angina.[6] It was also shown that hs-CRP was the best single marker and when combined with plasma total cholesterol, HDL-C,provided more potent prediction of prospective risk.[7]

PAPP-A was found to be important biomarker to discriminate cases of MI from UA and controls after using discriminate analysis. PAPP-A is metalloproteinase which is released from fibroblast during atherosclerotic plaque rupture. Circulating PAPP-A levels are significantly higher in patients of UA or MI than in patients with stable angina and controls.[8] PAPP-A was found equally in men and women, is abundant histologically in eroded and ruptured plaques but is not expressed in stable plaques.[9] Elevated PAPP-A is found to identify patients at high risk of cardiac death or MI even in cardiac troponin negative patients. [10]

Thus we can conclude that hs-CRP is the potential biomarker to discriminate cases of UA from control whereas PAPP-A is an important biomarker to discriminate cases of MI from UA and controls.

Table 2 : Comparison of inflammatory biomarkers and Pregnancy associated plasma protein (PAPP-A) in study groups

Parameter	Group 1 (Control)	Group 2 (UA)	Group 3 (MI)
hs-CRP(mg/dl)	0.57±0.53	3.26±1.12[a]	6.71±2.07[b,c]
IL-6 (pg /ml)	2.73±1.86	18.07±8.31[a]	73.58±36.53[b,c]
ICAM-1 (ng/ml)	328.48± 93.13	598.32±87.28[a]	728.03±108.52[b,c]
PAPP-A (mIU/L)	1.41±0.68	7.56±2.60[a]	16.89±4.59[b,c]

Values are expressed in mean± S.D. a= Group 1 vs. Group 2 (p< 0.001); b= Group 1 vs. Group 3 (p< 0.001); c=Group 2 vs. Group 3 (p< 0.001).

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Table 3: Canonical discriminant functions coefficients of study groups

Parameter	Group 1 vs. Group 2		Group 1 vs. Group 3		Group 3 vs.Group 2	
	sf	nsf	sf	nsf	sf	nsf
Hs-CRP	0.636[a]	0.719	0.655	0.431	0.580	0.347
IL-6	0.505	0.084	-	-	0.445	0.017
ICAM-1	0.320	0.004	0.325	0.003	-	-
PAPP-A	0.424	0.222	0.775[b]	0.236	0.724[c]	0.194
Constant	-	-4.989	-	-5.512	-	-4.873

Sf=standardized function; nsf=non-standardized function. a= Highest standardized function between Group 1 and Group 2; b= Highest standardized function between Group 1 and Group 3; c=Highest standardized function between Group 3 and Group 2.

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