EVALUATION OF STABLE CORONARY ARTERY DISEASE BY MULTIPLE CARDIAC BIOMARKERS

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ABSTRACT

Background: Effective management of stable coronary artery disease (SCAD) relies on early detection of coronary atherosclerosis. The objective was to evaluate diagnostic accuracy and risk stratification of SCAD patients by high sensitivity C reactive protein (hs CRP), Myeloperoxidase (MPO) and Pregnancy Associated Plasma Protein-A (PAPP-A).

Methods: Validation study was conducted at Pathology Department of the Army Medical College, in collaboration with Armed Forces Institute of Cardiology (AFIC/NIHD) Rawalpindi. Total 122 subjects consisting of 61 patients of SCAD and 61 angio-negative controls were included. The levels of biomarkers were measured before angiography by using kits provided by Siemens (UK) for hs CRP and Abbott for MPO on Immulite 1000 and Architect Analyzer respectively, whereas serum PAPP-A was measured by an ELISA based method using kit provided by IBL Germany.

Results: The mean age of the patients was 56.57±8.35 years and consisted of 53 (86.9%) males and 8 (13%) females. Area under curve (AUC) and 95% CI of hs CRP 0.817 (0.736-.881) was significantly higher than that of MPO 0.685 (0.594-0.766) (p=0.018) and PAPP-A 0.565 (0.472-0.655) (p<0.001) for the diagnosis of SCAD. Patients in the highest quartile of PAPP-A were at the highest risk for adverse events as PAPP-A had the highest Hazard Ratio (HR) of 3.4 (p=0.004), as compared to hs CRP 1.124 (p=0.191) and MPO 0.998 (p=0.176).

Conclusion: hs CRP has superior diagnostic ability for detection of SCAD than MPO whereas PAPP-A is a more reliable marker for risk stratification among the cardiac biomarkers.

Keywords: Multiple Biomarker Approach, Risk Stratification, Stable Coronary Artery Disease

INTRODUCTION

Stable coronary artery disease (SCAD) is one of the earliest manifestations of coronary atherosclerosis and often remains undiagnosed for long periods of time[1,2] with increased risk of Acute Myocardial Infarction (AMI). Effective management of SCAD therefore relies on early detection and risk stratification of these patients. Since atherosclerosis is primarily an inflammatory disorder, the circulating levels of different cardiac biomarkers related to inflammation are being actively investigated for their role in its diagnosis but have yielded controversial results[3,4].

High sensitive C-reactive protein (hs-CRP) is an established proinflammatory biomarker for the detection of individuals at risk of coronary artery disease[5]. Its level correlate with the burden and inflammatory activity in the atherosclerotic plaque[6]. Yip et al, reported elevated serum hs-CRP levels in patients with acute MI of less than 6 hours duration and suggested potential use in detection of unstable plaque rupture[7]. The role of hs CRP in detection of stable plaque needs to be studied in high risk patients presenting with chest pain on exertion.

Pregnancy associated plasma protein-A (PAPP-A) is an emerging biomarker amongst the biomarkers of plaque instability. Elevated levels were demonstrated in serum as well as in the ruptured plaques from patients of AMI[8]. It has potential for use as a marker of risk stratification in patients with acute coronary syndrome[9]. The study by Bayes-Genis showed no significant difference in its circulating levels between SCAD and healthy controls, whereas Cosin-Sales et al, demonstrated increased levels in patients with SCAD which correlated with angiographically complex coronary stenosis[10].

Myeloperoxidase (MPO) is an enzyme released from the neutrophils during their activation and degranulation in the atherosclerotic plaque. Elevated levels of blood and leukocyte MPO have been associated with

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Received: 21June 2010; Accepted: 04 Nov 2010

the presence of angiographically proven CAD[3,11] and have been found to be predictive of increased risk of cardiovascular events in apparently healthy individuals[12]. However, conflicting results are seen with respect to its levels in patients of CAD and its usefulness in risk assessment across the clinical spectrum of CAD[4,13].

We sought to assess the multiple biomarker approach using MPO, PAPP-A and hs CRP for the diagnosis and risk stratification of patients with SCAD.

MATERIALS AND METHODS

The study was carried out between May-December 2009 at the Chemical Pathology Department, Army Medical College in collaboration with National Institute of Heart Disease (NIHD) Rawalpindi, Pakistan. Research protocol was approved by institutional ethics committee of Army Medical College, National University of Science and Technology, Rawalpindi, Pakistan. The study complies with the Declaration of Helsinki.

A total of 121 subjects, consisting of sixty one SCAD patients who were scheduled to undergo coronary angiography, at NIHD, were included in the study. Sixty one age and sex matched angionegative subjects were recruited as healthy controls. The diagnosis of SCAD was based on the presence of exertional angina and chest pain that did not change its pattern over the preceding two months with more than 70% stenosis in one of the main coronary arteries on angiography which is the gold standard. Patients and controls were followed up for a period of six months for the occurrence of AMI or death. Written informed consent was obtained from all the participants.

Patients with acute and chronic inflammatory diseases including CHF, myocarditis and other cardiac abnormalities, renal or hepatic diseases, pregnancy and Acute Coronary Syndromes were excluded.

Blood samples were collected in K EDTA and plain tubes (5ml each) from the patients before angiography. Total cholesterol, plasma glucose, and serum creatinine were measured on Vita Lab Selectra–E Chemistry Analyzer (Netherland) using kits provided by Pioneer Diagnostics (USA), whereas the analysis of hs-CRP was done by a chemiluminescent immunoassay on Immulite 1000 using kit provided by Siemens (UK). The coefficient of variation (CV) of the method was 3.5%.

Plasma MPO was analyzed on Architect Analyzer by using the chemiluminescent microparticle immunoassay kit of the same manufacturer (Abbott Diagnostic, USA) with a CV of 4.7%. Serum PAPP-A was measured by ultrasensitive ELISA kit provided by IBL, Germany. CV of the method was 8.1%.

Statistical analysis was performed using SPSS 16 (SPSS Inc, Chicago) and MedCalc software version 9.6.4.0. Continuous normally distributed variables were summarized as mean ±SD, while other data were expressed as median (first and third quartiles range) for variables with a skewed distribution, or percentage for categorical variables. The distribution of serum hs CRP, PAPP-A and MPO were non parametric so Mann-Whitney U test was applied. Receiver operator characteristic curves (ROC) were made using MedCalc software in order to evaluate the diagnostic values of the above mentioned biomarkers. Moreover, the curves were compared by the method of DeLong[14]. Kaplan-Meier curves constructed to assess the risk stratification of biomarkers. Differences were compared with the help of log rank (Cox-Mantel) test. A p-value of $p<0.05$ was considered significant.

RESULTS

The mean age of the patients was 56.57±8.35 years and consisted of 53 (86.9%) males and 8 (13%) females. Their baseline characteristics are shown in table 1. They were more frequently hypertensive, diabetic, and hyperlipidemic, and had significantly higher levels of serum triglyceride, creatinine, total cholesterol, MPO, and hs CRP. No difference was observed between the ages, sex distribution, BMI and levels of PAPP-A between controls and SCAD patients.

ROC analysis of biomarkers in SCAD patients and controls revealed that AUC and 95% (CI) of hs CRP 0.817 (0.736-.881) ($p<0.001$) was significantly higher than that of MPO 0.685

(0.594-0.766) (p=0.001) and PAPP-A 0.565 (0.472-0.655) (p=0.214) for the diagnosis of SCAD as shown in figure 1. No significant difference was seen in the AUC of MPO and PAPP-A.MPO and PAPP-A therefore demonstrated a weaker discriminatory power for diagnosis of SCAD.

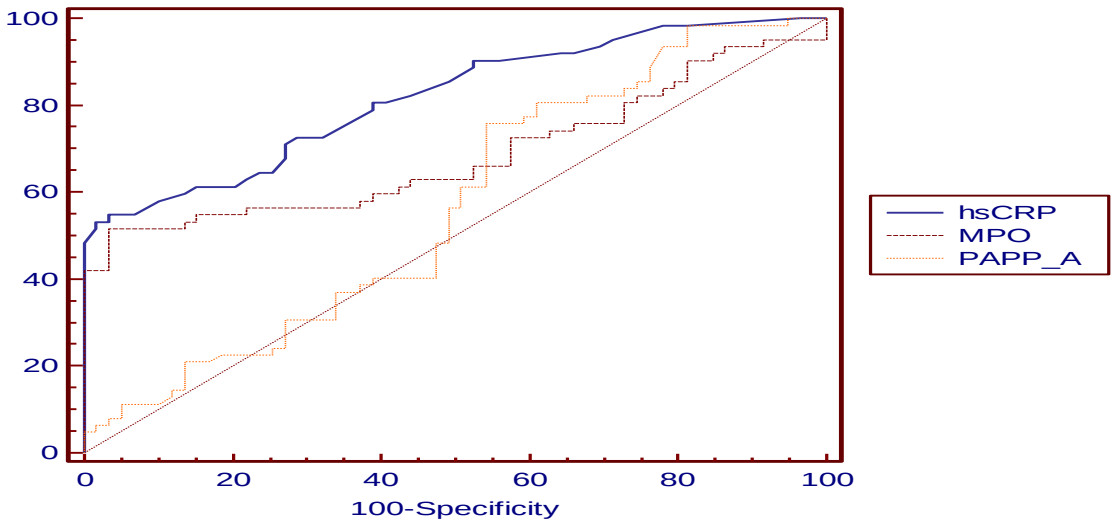
The diagnostic odds ratio (DOR) of hs CRP was highest at a cutoff level of 3.8 mg/L. The sensitivity and specificity of hs CRP at this cutoff for the diagnosis of SCAD was 53% and 98% respectively. The maximum sensitivity and specificity of MPO and PAPP-A was seen at levels of 488 pmol/L and 1.65 mIU/L respectively, their DOR's were however lower than that of hs CRP as shown in table 2.

During the follow up period, a total of 5 events occurred (4 AMI and 1 death), whereas 3 patients were lost to follow up. No adverse event was noted in the controls. Figure 2 shows

Table 1: Comparison of healthy controls and patients of SCAD (n=122).

Parameters	**Controls** (n=61) **Mean ± SD**	**SCAD** (n=61) **Mean ± SD**	**p- value**
Age (years)	57.08+10.34	56.57+8.35	0.631
Males/females, n (%)	51/10 (83.6/16.4)	53/8 (86.9/13)	0.662
BMI, (kg/m^2)	25.25+4.09	26.2+3.83	0.174
Total cholesterol (mmol/l)	4.63+.85	5.1+.97	<0.001
Triglyceride (mmol/l)	1.4+.58	1.7+.78	0.033
Creatinine(umol/L)	85.74+1.15	83.98+1.23	<0.001
Family history, n (%)	7 (12)	11 (18)	0.327
Diabetes mellitus, n (%)	1(1.7)	27(44.3)	<0.001
Hypertension, n (%)	2(3.3)	44(72.1)	<0.001
Hyperlipidemic n (%) (>5.2 mmol/L)	12(20)	40(65.5)	<0.001
Hs CRP (mg/l)	1.3(.5-2.4)	4.6(2.05-8)	0.001
Myeloperoxidase (pmol/l)	224.5 (108.12-373)	516.5 (146.5-972)	0.001
PAPP-A (mIU/l)	1.87 (0.75-3.27)	2.12 (1.75-3.45)	0.076

SCAD = patients of stable coronary artery disease; PAPP-A= Pregnancy associated plasma protein A; CAD= Coronary artery disease; BMI= Body Mass Index



AUC (95%CI): hs CRP: 0.817 (0.736-.881), MPO: 0.685 (0.594-0.766), PAPP-A: 0.565(0.472-0.655), Hs cTnT: 0.604 (0.511-0.691),

Figure-1: Receiver-Operator Characteristic (ROC) curve analyses of Myeloperoxidase (MPO), Pregnancy associated plasma protein A (PAPP-A) and **Hs** C reactive protein **(**HsCRP) for diagnosis of stable coronary artery disease (SCAD) at the time of admission (n=122).

the survival rates using quartiles of biomarkers. At six months follow up, levels of PAPP-A were associated with the end point of AMI/death. The patients in the highest quartile of PAPP-A had a cumulative mortality rate of 33.2%, whereas the cumulative mortality rate for the rest of the patients was 6% (p=0.004). Cox regression analysis showed that as compared to the rest of the biomarkers PAPP-A had the highest hazard ratio of 3.408 95% CI (1.49-7.77) (p=0.004) as compared to MPO 0.998 (0.96-1.00) (p= 0.176) and hs CRP 1.125 (0.943-1.341) and was thus the best predictor of risk.

Table-2: Diagnostic performance of hs CRP, MPO and PAPP-A at different cut offs for diagnosis of SCAD

Biomarkers	SN (%)	SP (%)	LR+	LR-	DOR
Hs CRP (mg/L) > 3.7	54.23	96.61	15	0.48	31
> 3.8*	53.00	98.00	31.40	0.48	65
> 3.9	51.34	98.25	30.45	0.49	62
MPO (pmol/L) > 434	51.67	86.38	3.81	0.56	6
> 488	51.00	96.00	10.23	0.50	20
> 539	41	96.61	8.67	0.45	19
PAPP-A (mIU/L) > 1.5	77.42	40.80	1.31	0.56	2
> 1.65	75.8	45.8	1.40	0.53	2.5
>1.87	61.2	49.1	1.21	0.79	1.5

MPO: Myeloperoxidase, PAPP-A: Pregnancy Associated Plasma Protein-A, hs CRP: high sensitive C reactive protein, SN: Sensitivity; SP: Specificity; LR+: Positive likelihood ratio; LR-: Negative likelihood ratio; DOR: Diagnostic Odds Ratio.

DISCUSSION

The results of our study show that hs CRP has better diagnostic ability for the detection of SCAD as compared to MPO and PAPP-A. Elevated levels of hs CRP seen in our patients of SCAD also suggest its causal role in atherogenesis. Anderson et al; reported two-fold higher levels of serum CRP in men with documented CAD as compared to controls[15]. These results were supported by Irfan and Ahmed in Pakistan who also found significantly elevated levels of hs CRP in patients with angiographically validated SCAD[16]. More recently, Koc et al,demonstrated that the levels of hs CRP were elevated in SCAD when compared to controls regardless of the time of sampling and also had good diagnostic power for detection of SCAD[17]. We did not observe any significant difference in PAPP-A levels between controls and SCAD patients. This is in line with the results of Bayes-Genis et al who measured PAPP-A in a small group of SCAD patients and found no significant difference in its levels as compared to controls[8]. On the other hand, levels of MPO were significantly elevated in our SCAD patients, which supports the findings of Zhang et al who associated elevated levels of MPO with the presence of SCAD OR=20.9 (95% CI 8.9-47.2)[3], but contradicts those of Kubala et al who measured MPO in patients of SCAD but did not find them to be of use in its diagnosis[4]. However, in our study this diagnostic ability of MPO for detecting the presence of SCAD was significantly lower than hs CRP as shown by its significantly lower AUC and OR. This significantly lower diagnostic performance of MPO and PAPP-A can be explained on the basis of the observation that their release is primarily related to acute inflammatory changes in the atherosclerotic plaque as a result of which they more accurately reflect plaque instability.

Our results demonstrate that in patients with SCAD circulating levels of PAPP-A are capable of predicting risk for adverse events. The role of PAPP-A in risk stratification of patients has been highlighted previously by Elesber et al who reported that plasma PAPP-A was significantly associated with the endpoint of future death with a hazard ratio (HR) of 5.29 (p=0.023) and with the future endpoint of death and ACS (HR=3.56, P=0.015)[18]. Similarly Consuegra-Sanchez et al demonstrated that increased PAPP-A concentration (>4.8 mIU/L)

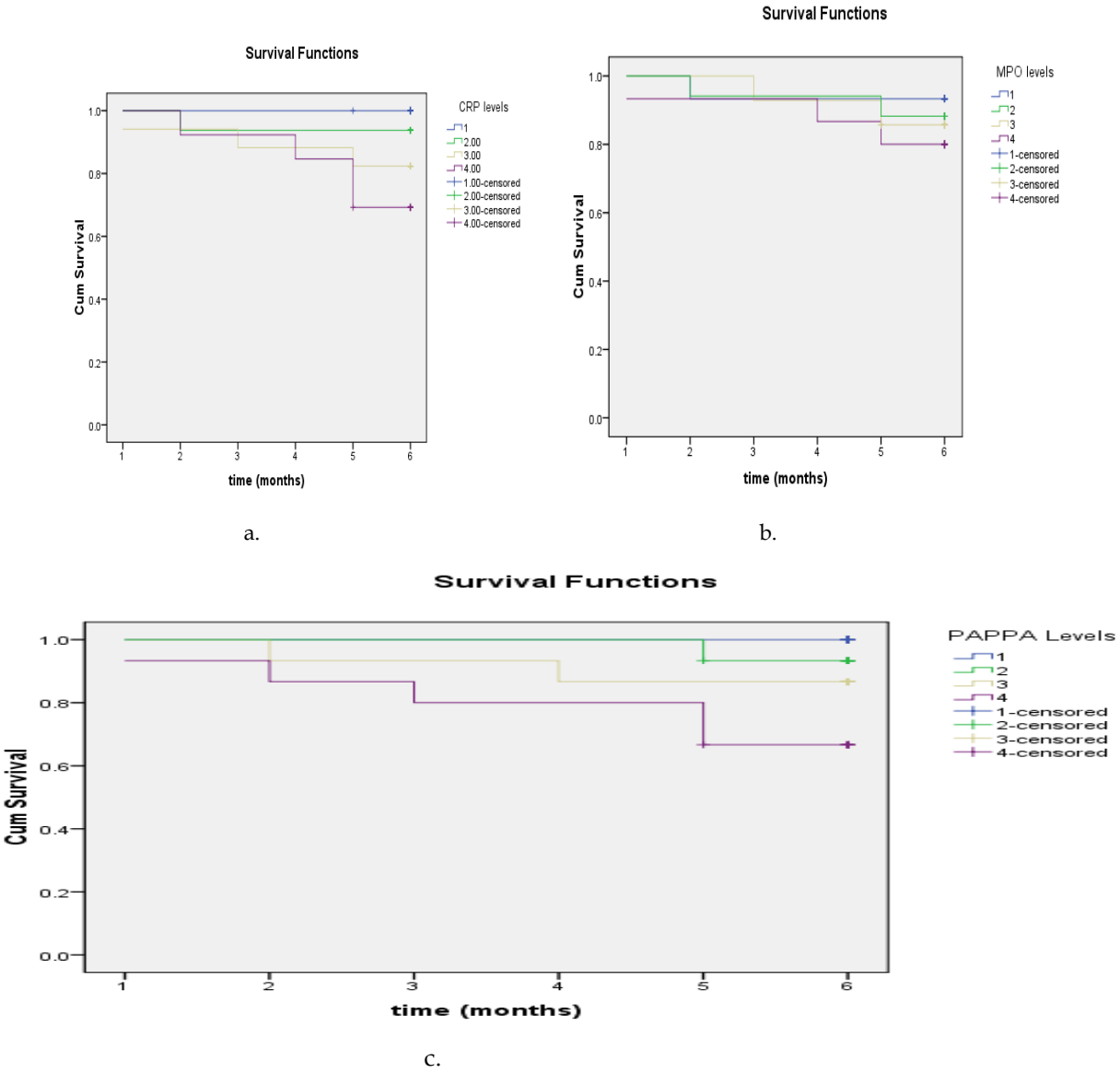


Figure 2: Kaplan Meier curves of survival according to quartiles of (a) **Hs** C reactive protein **(**HsCRP) (b) Myeloperoxidase (MPO) (c) Pregnancy associated plasma protein A (PAPP-A).

was an independent predictor of the occurrence of all-cause mortality (HR 1.953, p=.016)[19]. The levels of hs CRP and MPO did not significantly predict risk in our patients. These results are in line with the results of Stefanescu et al, who measured plasma MPO in SCAD patients but did not find it to be an independent correlate of mortality (HR=1.06, p=0.77)[20].

One of the major strengths of our study is that we have independently established the reference interval of biomarkers in our study and have included angionegative individuals as healthy controls thus allowing for a clearer comparison between patients and controls. The major limitation of our study lies in its small size. We therefore recommend that future studies be carried out in multiple centers on larger patient populations so that the patients of SCAD may be benefitted.

CONCLUSION

Our study demonstrates that hs CRP is more reliable than MPO and PAPP-A for the diagnosis of SCAD whereas PAPP-A is the most suitable marker for risk stratification in these patients.

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