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CYCLOPHILIN A - A SURROGATE BIOMARKER FOR CORONARY ARTERY DISEASE

Cardiology

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ABSTRACT

Background: Studies have reported that Cyclophilin A (CyPA) has the potential to be a valuable biomarker of coronary artery disease (CAD). It was found that plasma levels of CyPA correlated with the anatomical severity of CAD. Several issues remain regarding the role of CyPA as a biomarker of CAD. An attempt is made to study correlation of plasma CyPA levels with severity of coronary artery disease and traditional coronary risk factors. **Methods:** 404 patients aged ≥ 18 years of either gender with chest pain undergoing coronary angiography (CAG) were included. They were subjected to standard clinical evaluation, estimation of plasma CyPA by enzyme linked immunosorbent assay (ELISA) method and CAG. Plasma CyPA levels were compared with CAG and traditional coronary risk factors. Qualitative and quantitative variables were tested using Chi-Square test and Mann Whitney U test respectively. The cut off values of CyPA across the angiographic groups were calculated using Receiver Operating Characteristic (ROC) curve. The predictive significance of CyPA and risk factors in detecting significant CAD was tested using sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV). **Results:** Median CyPA levels were significantly higher in obstructive CAD (15.8 ng/ml) as compared non-obstructive CAD (7.8 ng/ml) and normal coronary arteries (6.0 ng/ml). Plasma CyPA levels of >8.0 ng/mL, >9.4 ng/mL and >13.0 ng/mL reliably predicted presence of CAD, obstructive CAD and risk of ACS respectively with excellent specificity and good sensitivity. Plasma CyPA levels were significantly higher in males, diabetic patients, patients with dyslipidemia, and smokers. **Conclusion:** Plasma CyPA is a novel biomarker for CAD

KEYWORDS

Coronary artery disease, Cyclophilin A, risk factors, Biomarker

INTRODUCTION:

It has been widely recognized that oxidative stress, generated by excessive reactive oxygen species (ROS), promotes coronary artery disease (CAD).[1] Most of the patients with a history of chest pain are asymptomatic, do not have dynamic ST-segment or T-wave changes on electrocardiogram (ECG) and have normal plasma levels of cardiac troponin T. In such cases, it becomes difficult to determine whether the patient's symptoms are cardiac or non-cardiac in origin. It is therefore helpful to consider whether such patients are at high risk for atherosclerosis. At present, there are no established biomarkers for detection of CAD. The diagnosis of CAD cannot only be based on results of imaging studies such as coronary angiography (CAG) or coronary computed tomographic (CT) angiography. Recently, many reports have shown that selected markers can be detected in the peripheral circulation during different phases of the disease progression.[2]

Studies have reported that Cyclophilin A (CyPA) has the potential to be a valuable biomarker of CAD. It was found that plasma levels of CyPA correlated with the anatomical severity of stable CAD and were independent of C-reactive protein (CRP) levels.[3] This finding is of particular significance in the clinical setting, where CRP is generally used as a marker of cardiovascular events. CRP is a marker of inflammation, whereas CyPA is a marker of both oxidative stress and inflammation. Studies also demonstrated robust CyPA expression just beneath the thin fibrous cap of atherosclerotic plaques in patients suffering from acute myocardial infarction (AMI),[3] Based on this, plasma levels of CyPA could significantly help identify patients with CAD.

Several issues remain regarding the role of CyPA as a biomarker of CAD. First, several basic science studies have demonstrated an association between CyPA and ROS, but studies showing their direct relationship in the clinical setting are lacking. Second, CyPA can be used along with traditional risk factors to predict presence of CAD. Third, CyPA may be used with other biomarkers to predict and treat acute coronary syndrome (ACS) before myocardial damage occurs.[4] Therefore, an attempt is made to study correlation of plasma CyPA levels with severity of coronary artery disease and traditional coronary risk factors.

MATERIAL AND METHODS:

This cross-sectional observational study was conducted between June 2016 and February 2018. After approval from the scientific advisory committee and institutional ethics committee, written informed consent was obtained from all patients. Patients aged ≥ 18 years of either gender with chest pain undergoing CAG were included. Patients with known CAD who have already undergone coronary catheterization in the past, patients with cardiomyopathy and with known chronic inflammatory diseases, malignancy, sepsis, viral infections, asthma, neurodegeneration and those on dialysis were excluded from this study.

Patients were subjected to standard clinical evaluation and estimation of plasma CyPA by enzyme linked immunosorbent assay (ELISA) method. For the purpose of this study, stable angina was defined as transsternal or retrosternal pressure or a choking sensation or pain that may radiate to the left arm, jaw, neck or back, brought on in a predictable manner by exertion or by emotional upset. Sublingual nitroglycerin or cessation of exertion relieved the discomfort. ACS was defined as a spectrum of unstable angina (either exertional angina of new onset or worsening exertional angina or angina at rest), non-ST elevation myocardial infarction (NSTEMI – angina at rest with elevated troponin T without ST elevation on 12 lead ECG) and ST elevation myocardial infarction (STEMI – angina at rest with elevated troponin T and ST elevation on 12 lead ECG).

The presence or absence of the following cardiovascular risk factors were assessed in each subject: Age, male gender, hypertension (systolic blood pressure >140 mm Hg or diastolic blood pressure >90 mm Hg or being on an antihypertensive medication),[5] dyslipidemia [total cholesterol (TC) >220 mg/dL or serum low density lipoprotein (LDL) cholesterol >140 mg/dL or taking lipid-lowering medication],[5] diabetes mellitus (fasting blood glucose level >126 mg/dL or treated with an oral hypoglycemic agent or insulin)[6], smoking, and family history of CAD (having ≥1 first-degree relatives with premature CAD: male before the age of 55 years and female before the age of 65 years).

Venous blood sample from each patient was collected from right cubital vein before subjecting the patient for CAG. 3 mL blood was collected in ethylene diamine tetraacetic acid (EDTA) bulb and mixed well for 10 minutes. The sample was centrifuged at 3000 rotations per minute for 20 minutes to obtain plasma. Quantitative estimation of CyPA levels in ng/ml was done using human CyPA ELISA kit (Sunred Biological Technology, Catalogue No. 201-12-0673).

CAG was performed with a Shimadzu Bransist Alexa system and routine projections were obtained for the definition of coronary anatomy. The films were analyzed by two independent interventional cardiologists who were unaware of the clinical data and plasma CyPA levels. In case of divergent opinions, the films were analyzed by a third cardiologist.

The levels of plasma CyPA were compared with CAG report according to the following groups.

a. **Group 1:** normal coronary arteries

b. **Group 2:** non-obstructive CAD (degree of stenosis <70%)
c. **Group 3:** obstructive CAD (degree of stenosis ≥70%)[7]

Plasma CyPA levels were also compared amongst the traditional risk factors for CAD. Normal values of plasma CyPA are not yet established as the molecule is still under research. On the basis of a previously published study[3], a sample size of 384 patients was calculated by a formula [8] with 80 % power and 5 % probability of Type I error to reject null hypothesis. We included 404 patients to validate the results.

Data collected were entered in Excel 2007 and analysis of data was done using Statistical Package for Social Sciences (SPSS) version 21, IBM, USA. The data on discrete variables is presented as n (% of cases). The statistical significance of difference of discrete variables was tested using Chi-Square test. Continuous variables (Plasma Cyclophilin A levels) are presented as median with interquartile range (IQR). The statistical significance of difference was tested using Mann Whitney U test. The cut off values of CyPA across the angiographic groups were calculated using Receiver Operating Characteristic (ROC) curve. The predictive significance of CyPA and risk factors in detecting significant CAD was tested using sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV). The confidence limit for significance was fixed at 95% level with p-value < 0.05.

RESULTS:

Of 404 patients 275 (68.1 %) were males and 129 (31.9 %) were females. 45 (11.1 %), 94 (23.3%) and 265 (65.6 %) patients had normal coronaries, non-obstructive CAD and obstructive CAD respectively. It is evident from table 1 that median plasma CyPA levels were significantly higher in patients with CAD (15.8 ng/ml in obstructive and 7.8 ng/ml in non-obstructive CAD) in all the patients and patients with stable angina as compared to normal coronaries (6.0 ng/ml). As evident from table 2, median CyPA levels were significantly higher in males, diabetes mellitus, dyslipidaemia, and smokers. Table 3 depicts sensitivity, specificity, PPV and NPV of CyPA levels > 8.0 ng/mL, > 9.4 ng/mL and > 13.0 ng/mL in CAD, obstructive CAD and ACS patients respectively.

Table 1: CyPA distribution and degree of stenosis of coronary vessels

Patients (n)	Median CyPA in ng/mL (IQR)			P value
	Normal	Non-Obstructive CAD (<70%)	Obstructive CAD (≥70%)	
All Patients (404)	6.00 (5.00-7.00)	7.80 (6.40-9.20)	15.80 (10.60-20.00)	0.001
Stable Angina (218)	6.00 (5.00-6.95)	7.80 (6.40-9.20)	12.70 (8.80-17.70)	0.001

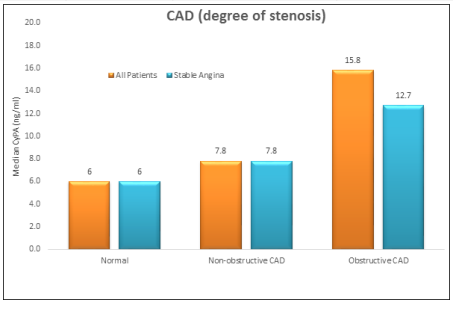

Figure 1: CyPA Distribution as per CAD severity

Table 2: Risk factors and median CyPA

Risk factors	Median CyPA in ng/mL (IQR)	P value
Age in Years		
≤ 60	9.6 (6.9-15.8)	0.250
>60	14.2 (8.0-19.0)	
Gender		
Male	12.0 (7.6-18.4)	0.048
Female	9.6 (7.1-16.5)	
Diabetes mellitus		
Present	15.8 (9.0-20.9)	0.001
Absent	9.2 (6.95-14.95)	
Hypertension		
Present	12.0 (7.28-18.45)	0.512
Absent	11.0 (7.8-16.18)	
Dyslipidemia		
Present	14.6 (9.0-20.43)	0.001
Absent	8.75 (6.3-15.3)	
Smoking		
Present	14.8 (9.0-21.1)	0.001
Absent	7.8 (6.4-15.1)	
Family history of CAD		
Present	12.6 (8.0-16.6)	0.179
Absent	10.5 (7.2-18.28)	

Table 3: Predictive values of CyPA levels

Parameters	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
CyPA > 8.0 ng/mL (Non-Obstructive CAD)	76.3	91.1	98.6	32.5
CyPA > 9.4 ng/mL (Obstructive CAD≥70%)	80.00	88.5	93.0	69.9
CyPA > 13.0 ng/mL (ACS)	66.8	79.4	74.0	74.9

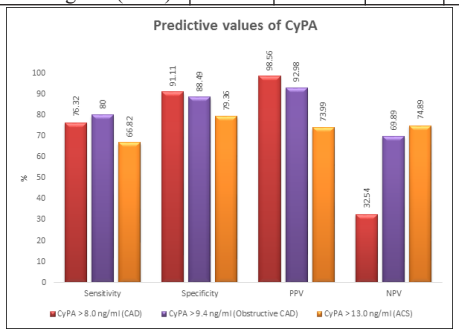

Figure 2: Predictive Values of CyPA levels

DISCUSSION:

The present study compared plasma CyPA levels with presence and severity of CAD and traditional coronary risk factors. Plasma CyPA levels were significantly higher in patients with obstructive CAD.

As CyPA is still a research molecule, its normal values and ranges for detection of presence and severity of CAD are not yet established. Therefore, ROC curves were used for estimation of cut off values of plasma CyPA for prediction of presence of CAD, obstructive CAD and ACS.

It was observed that plasma CyPA > 8.0 ng/mL predicted presence of CAD with 76.3% sensitivity, 91.1% specificity, 98.6% PPV and 32.5% NPV. High PPV suggests that plasma CyPA > 8.0 ng/mL is a good predictor for presence of CAD. Lower NPV suggests that plasma CyPA ≤ 8.0 ng/mL does not reliably rule out presence of CAD. CyPA was found to be an independent predictor of CAD. Satoh et al[3] reported significant correlation of plasma CyPA levels with presence and severity of CAD in a study on 320 patients. Ramachandran et al[9] and Shah et al[10] also stated significant association of plasma CyPA with presence of CAD. Findings in the present study are consistent with these studies.

Plasma CyPA > 9.4 ng/mL had 80 % sensitivity, 88.5 % specificity, 93.0 % PPV, and 69.9 % NPV in obstructive CAD (>70%) patients. This suggests that plasma CyPA > 9.4 ng/mL is a good predictor of obstructive CAD. Satoh et al[6] in his study reported that plasma CyPA levels > 15 ng/ml were significantly associated with CAD and suggested need for coronary revascularization.

Plasma CyPA levels > 13.0 ng/mL had 66.8% sensitivity, 79.4% specificity, 74.0% PPV and 74.9% NPV in ACS patients. This suggests that plasma CyPA > 13.0 ng/mL identifies patients at risk of developing ACS in near future and need aggressive management for the same. Also, a good NPV indicated that plasma CyPA ≤ 13.0 ng/mL puts the patient at low risk of having ACS. Studies conducted by Satoh et al[6], Ramachandran et al[9] and Shah et al[10] did not include ACS patients in their research. Ohtsuki et al[11] in their study reported association of CyPA ≥ 12 ng/ml with increased risk of AMI.

All this data suggests that estimation of plasma CyPA may be helpful in

indicating presence and severity of CAD. It can also predict stable angina patients at high risk of developing ACS in near future.

In the present study, it was found that median CyPA levels were significantly higher in patients with male gender, diabetes mellitus, dyslipidemia, and smokers. Median CyPA levels did not correlate significantly with age > 60 years, hypertension and family history of CAD. Several studies have established association of traditional risk factors like diabetes mellitus, hypertension, dyslipidemia, etc. with atherosclerosis and CAD.[12,13] Ramachandran et al[9] and Shah et al[10] observed that patients with CAD, irrespective of their diabetic status had significantly higher CyPA levels than those without CAD. Satoh et al[3] reported significant association of plasma CyPA with diabetes mellitus, dyslipidemia and smoking. Present study substantiated these findings. Gender association was not seen in study by Satoh et al[3]. Ramachandran et al[9] reported significantly higher plasma CyPA levels in patients with advanced age. Satoh et al[14] had previously demonstrated that ROS inducers, such as angiotensin II promote the secretion of CyPA. They reported significantly higher plasma CyPA levels in patients with hypertension.[3]

This suggests that plasma CyPA is a better and independent predictor of presence of CAD, obstructive CAD and ACS as compared to number of risk factors in terms of both ruling in and ruling out the disease.

There are few limitations. Plasma CyPA levels were compared with CAG, but intravascular ultrasound or fractional flow reserve was not performed. Traditional risk factors for CAD were compared, but novel risk factors for atherosclerosis like hyperhomocysteinemia and elevated CRP levels were not considered in the present study. Further studies should be done on a large population to assess the role of CyPA as a predictor, prognostic marker and therapeutic target in CAD.

CONCLUSION:

Estimation of plasma CyPA in patients with angina can predict presence and severity of CAD. Plasma CyPA levels of >8.0 ng/mL, >9.4 ng/mL and >13.0 ng/mL reliably predict presence of CAD, obstructive CAD and risk of ACS respectively with excellent specificity and good sensitivity. Plasma CyPA levels were significantly higher in males, diabetic patients, patients with dyslipidemia, and smokers.

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