ORIGINAL RESEARCH PAPER

Biochemistry

"COMPARATIVE STUDY OF CYCLOPHILIN A AND HS-CRP AMONG THE DIABETES MELLITUS PATIENTS WITH CORONARY ARTERY DISEASE"

KEY WORDS: Diabetes mellitus, coronary artery disease, hs-CRP, Cyclophilin A, HbA1c.

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ABSTRACT

Background: The secretive nature of Cyclophilin A (CypA) and its plasma expression in patients with diabetes mellitus and coronary artery disease (CAD) are significant possible biomarkers of the disease. Aim of this study was was to compare the plasma levels of CypA in type 2 DM patients with or without CAD with those in healthy participants in order to ascertain the possible role of CypA in inducing vascular disease in diabetic patients and to study the relationship of hsCRP with CypA levels.

Material and Method: Study included all the patients admitted under cardiology department of PMSSY Bangalore medical college, conducted between November 2017 to May 2019. The patients with known history of diabetes mellitus for 5-10yrs of duration and willing to be part of study with age >40 were included in present study. The patients were grouped as Group A with diabetes mellitus patients having CAD as cases and group B patients were diabetes mellitus patients without CAD as controls. Blood samples were analysed for analyzed for Cyclophilin A level, Hs-CRP, HBA1c.

Result: In present study, total of 71 patients were included among them 36 were in group A and 35 were in group B. 39 % were female and 61% are male subjects, with male to female ratio of 2:1. Statistically significant high levels of the hs-CRP and Cyclophilin A was seen in diabetes patients with CAD compared to DM patients without CAD. significant good strength of association between the two biomarkers Plasma Cyclophilin A and hs-CRP is present. The area under the curve for the CypA was higher than the hs-CRP to detect the vascular inflammatory changes

Conclusion: Demonstrated that CypA has a potential role in promoting vascular disease in diabetic patients and showed that CypA is a better biomarker for CAD compared to the hs-CRP.

INTRODUCTION

Multisystem complications such as microvascular disorders such as retinopathy, nephropathy and neuropathy and the macrovascular risk such as ischemic heart failure, stroke and peripheral artery disease are associated with diabetes mellitus (DM). Cyclophilin A is part of a variety of intracellular activities, such as intracellular signalling, protein trafficking and the regulation of other protein production.[1] The secretive nature of Cyclophilin A (CypA) and its plasma expression in patients with diabetes mellitus and coronary artery disease (CAD) are significant possible biomarkers of the disease. It is secreted by monocytes in response to hyperglycemia, acting as a possible marker in type 2 DM.[2] Because vascular inflammatory changes can hardly be measured using cardiac imaging techniques, the function of inflammation biomarker testing in peripheral blood is growing, with the high-sensitivity C-reactive protein (hsCRP) being the most deeply researched biomarker in cardiovascular disease. It stays stable in samples over a long period of time and can be checked very easily, quickly and cheaply.

HsCRP monitoring is useful for both primary and secondary cardiovascular disease prophylaxis and for those still suffering from cardiovascular disease (CVD). This measure is useful for the assessment of disease incidence, treatment effectiveness and outcome prognosis.[3] Plasma CypA is correlated with the amount of C reactive protein (CRP). There is a link between CypA plasma and CRP serum. There is an association between CypA plasma and CRP serum, a therapeutic proxy for vascular inflammation.[4] The objective of this study was to compare the plasma levels of CypA in type 2 DM patients with or without CAD with those in healthy participants in order to ascertain the possible role of CypA in inducing vascular disease in diabetic patients and to study the relationship of hsCRP with CypA levels.

MATERIAL & METHOD

Study included all the patients admitted under cardiology department of PMSSY Bangalore medical college, conducted between November 2017 to May 2019. The patients with known history of diabetes mellitus for 5-10yrs of duration and willing to be part of study with age >40 were included in present study. The patients were grouped as Group A with diabetes mellitus patients having CAD as cases and group B patients were diabetes mellitus patients without CAD as controls. Diabetes was assessed by recording HbA1c and/or fasting blood sugar (FBS) levels in accordance to ADA criteria[5] Already diagnosed cases of CAD with Type2 Diabetes from Cardiology was taken as cases (positive treadmill test in accordance with American Heart Association). Type 2 Diabetes patients diagnosed with no CAD was taken as controls by similar criteria.

5mL of fasting blood samples was collected from the cases and controls in EDTA and plain vacutainers. Samples allowed to clot for 2 hours at room temperature before centrifugation for 15 min at 1000×g at 2~8° C but for HbA1c without centrifugation and analyzed for Cyclophilin A level by sandwich immunoassay kit, Hs-CRP was measured by latex enhanced immunoturbidimetry method, HBA1c by BioRad D-10 analyser utilizes principles of ion-exchange high-performance liquid chromatography (HPLC).

STATISTICAL ANALYSIS:

All data was entered in the excel sheet and analyzed using the window-operated SPSS v21. The data is expressed as a mean standard deviation for data not usually distributed; the median and inter-quartile ranges have been shown. The mean difference between the normally distributed data was analyzed using the student t-test and the non-normally distributed data used by the Mann-Whitney U test. A p-value of <0.05 was considered statistically significant.

RESULTS

Total of 71 patients were included in the present study, grouped into

Group A: 36 already diagnosed Type 2 diabetic patients with Coronary arterial disease (CAD) as cases. (TMT positive)
Group B: 35 age and sex matched patients of type 2 diabetes mellitus without Coronary arterial disease (CAD) as controls. (TMT negative)

In present study, 39 % were female and 61% are male subjects, with male to female ratio of 2:1. Among 71 patients included, 36 underwent Coronary angiography and other 35 did not required / indicated for angiography.

Table 1: Mean age of the patients in two groups.

	Group A Mean ± SD	Group B Mean ± SD	p-value
Age in Years	57.5 ± 9.47	58.2 ± 10.47	.69

p-value <.05 statistically significant. Group A: DM with CAD; Group B: DM without CAD.

The mean age between the groups was not significantly different. No history of weight gain by patient in last few months.

Table 2: Showing the median value of hs-CRP and Cyclophilin A in both groups using Mann-Whitney U Test.

Mann-Whitney U Test	Group A (DM with CAD) Median (Minimum – maximum)	Group B (DM without CAD) Median (Minimum – maximum)	p-value
P. Cyclophilin A (ng/mL)	13.3 (1.44 – 60.06)	8.71 (0.5 – 31.6)	.001**
Hs-CRP (mg/dL)	2.77 (1.01-17.31)	0.92 (0.2-7.87)	.001**

P<.05 statistically significant; <.001 statistically highly significant.

Statistically significant high levels of the hs-CRP and Cyclophilin A was seen in diabetes patients with CAD compared to DM patients without CAD. (Table 2)

Table 3: Correlation of hs-CRP, Cyclophilin A and HbA1c levels.

		Plasma Cyclophilin A ng/mL	Serum hs-CRP in mg/dL
HbA1c %	r	.504**	.692**
Serum hs-CRP in mg/dL	r	.591*	

r- Pearson's correlation. *. Correlation is significant at the 0.05 level (2-tailed).
**. Correlation is significant at the 0.001 level (2-tailed).

There was a significant strength of association between the Hs-CRP, Cyclophilin A and HbA1c in present study. Strong positive correlation is present between HbA1c and Plasma Cyclophilin A concentration the patient with DM. similarly; a significant positive strength of association between the HbA1c and hs-CRP was seen in study. Also, significant good strength of association between the two biomarkers Plasma Cyclophilin A and hs-CRP is present. (Table 3)

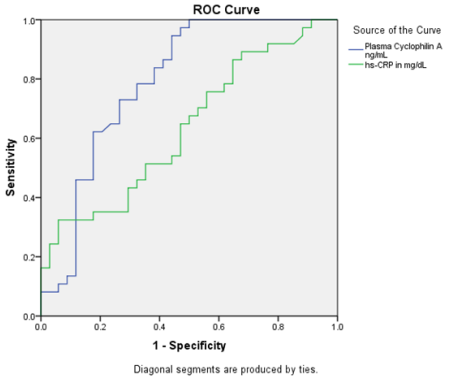

Table 4: Area Under the Curve for ROC analysis.

Test Result Variable(s)	Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval	
				Lower Bound	Upper Bound
Plasma Cyclophilin A ng/mL	.791	.056	.000	.680	.901
hs-CRP in mg/dL	.636	.066	.050	.507	.764

b. Null hypothesis: true area = 0.5

Table 5: Cutoff levels of the hs-CRP and CypA in patients with Diabetes mellitus and CAD.

	Cut-off	Sensitivity	1-specificity
Plasma Cyclophilin A ng/mL	10.08	91.3%	82.6%
hs-CRP in mg/dL	1.04	97%	50%

The area under the curve for the CypA was higher than the hs-CRP to detect the vascular inflammatory changes.

DISCUSSION

Plasma CypA is secreted by monocytes and vascular wall cells in response to inflammation and oxidative stress, but can also be secreted by or leaked from damaged cardiomyocytes and interstial fibroblasts. CypA expression and secretion increased by oxidative stress and vascular injury. They are the first to recognise CypA as a secreted redox-sensitive mediator, to establish CypA as a vascular smooth cell growth factor and to indicate a significant function in vascular disease pathogenesis..[6]

Complications involving the vulnerable atherosclerotic plaque are triggered by two major mechanisms, dyslipidemia and inflammation, although both are influenced by classic risk factors. Each mechanism provides additional information regarding the cardiovascular events and mortality.[6]

Plasma Cyclophilin A (CypA) was found to be significantly higher in Group-1 patients [13.3 (1.44 – 60.06)] compared to patients in Group-2 [8.71 (0.5 – 31.6)] (p-value <.001). Satoh et al., reported that plasma CypA levels were significantly higher in patients with significant coronary stenosis compared with those without (P<0.001). Patients with acute coronary syndrome have high plasma concentrations of CypA, and CypA is strongly expressed in the athermanous plaques of patients with acute myocardial infarction (AMI).[7]

A study performed by Yan et.al also showed that patients with ACD or ACS were considerably higher than those with regulated and stable angina. Increased levels of CypA can be a helpful indicator for predicting the risk of acute coronary disease.[8] Concordance, a study performed by Satoh et al. found that plasma CypA levels were substantially higher in DM patients with coronary artery disease than in DM patients without CAD.[7] Also glucose and HbA1c were positively associated with plasma CypA levels (r=0.504; p<.05) indicating a specific relation of plasma CypA levels with Hyperglycemia.

Hs-CRP levels among the Group-1 patients [2.73 (1.01 - 17.31)] was significantly higher than group-2 patients [0.98 (0.2 - 7.87)] (p-value <.001). similar findings was present in recent studies.[9–12]

The present study demonstrated, blood levels of serum hs-CRP and CypA showed significantly moderate positive strength of association (r=.591). Plasma CypA is associated with CRP levels, a clinical marker of vascular inflammation.[11] Similar study conducted by Satoh et al,[7] reported that hs-CRP correlate with plasma CypA levels in their patients with stenotic coronary arteries. Similar study by Ramachandran et.al., found that in patients with increased serum CRP level, plasma CypA was also elevated and reported that there was a positive association between both in patients with diabetes as well as in those with diabetes and CAD.[9]

In present study, ROC curve analysis showed that CypA (AUC= 0.791) is a better biomarker than hs-CRP (AUC= 0.636). One more study that analyzed ROC curve demonstrated that the plasma levels of CypA is useful for the diagnosis of coronary organic stenosis (c-statistic=0.80) and in predicting future cardiovascular intervention (c-statistic=0.79) and also found that plasma CypA level of more than 15 ng/ml remained highly related to CAD (P<0.001), and on comparing plasma levels of CypA and hsCRP, CypA were superior to hsCRP in terms of evaluation of the severity of CAD.[7]Also reported that plasma CypA level is a novel biomarker of CAD.[10]

CONCLUSION:

This study demonstrated that CypA has a potential role in

promoting vascular disease in diabetic patients and showed that CypA is a better biomarker for CAD compared to the hs-CRP. However combining CyPA levels with other biomarker such as hs-CRP would further improve the prognostic impacts for patients with CAD. Plasma CypA can be a potential novel marker for the risk of cardiovascular disease among the patient with diabetes mellitus.

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