

Assessment of plasma level of cyclophilin A in type 2 diabetic patients suffering from vascular diseases

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Received 4 July 2017

Accepted 7 August 2017

Benha Medical Journal

2018, 35:188–193

Background

Peptidyl-prolyl isomerase cyclophilin A (CypA) plays important roles in inflammation. However, little is known about the mechanisms by which CypA exerts its effects. It is secreted in human by monocytes activated by high glucose level. It has a role as an inflammatory mediator in vascular tissue damage.

Aim

This study aims to compare plasma levels of CypA in type 2 diabetic patients with or without coronary artery disease (CAD) with those in healthy participants to determine the potential role of CypA in promoting vascular disease in diabetic patient and to study the association of high-sensitivity C-reactive protein with CypA levels.

Patients and methods

The present study was conducted on 80 participants who were divided into four groups: group 1, which included apparently healthy individuals; group 2, which included patients with type 2 diabetes mellitus (DM) without CAD; group 3, which included patients with type 2 DM with CAD; and group 4, which included patients with CAD without DM. The plasma level of CypA was measured using a CypA enzyme-linked immunosorbent assay kit.

Results

The results showed an increase in the median CypA concentration in all patient groups in comparison with the controls ($P < 0.001$). Also, there was a statistically highly significant increase in the median CypA concentration in diabetic patients with CAD group when compared with only diabetic patients group ($P < 0.001$) and in patient with only CAD when compared with diabetic patients with or without CAD ($P < 0.001$).

Conclusion

This study demonstrated that CypA has a potential role in promoting vascular disease in diabetic patients and revealed that CypA is a good biomarker for CAD with or without DM better than high-sensitivity C-reactive protein.

Keywords:

coronary artery disease, cyclophilin A, high-sensitivity C-reactive protein, type 2 diabetes mellitus

Benha Med J 35:188–193

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2357-0016

Introduction

There are two types of diabetes mellitus (DM), type 1 and type 2, of which type 2 accounts for the majority (>85%). Both forms can lead to multisystem complications of microvascular endpoints, including retinopathy, nephropathy, and neuropathy, and macrovascular diseases including ischemic heart disease, stroke, and peripheral vascular disease [1]. The International Diabetes Federation showed the top 10 countries with the highest number of adults with diabetes in 2015, in which Egypt was at number 8 with 7.8 million diabetic patients [2]. The secretory nature of cyclophilin A (CypA) and its presence in plasma of patient with DM and coronary artery disease (CAD) underlines its potential as a marker for the disease [3]. CypA is a member of the peptidyl-prolyl isomerase family, a group of proteins that catalyze

cis-trans isomerization of peptidyl-prolyl bonds during protein folding and/or conformational change [4]. Plasma CypA is primarily secreted by monocytes and vascular wall cells in response to oxidative stress and inflammation, but can also be secreted by or leaked from damaged cardiomyocytes and interstitial fibroblasts [5]. CypA is a potential secretory marker of inflammation in type 2 DM. Expression of CypA reduces in circulating monocytes in patients with type 2 DM. CypA is secreted by monocyte in response to hyperglycemia [6]. As vascular inflammatory changes can hardly be evaluated using cardiac imaging methods,

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the role of inflammation biomarker testing in peripheral blood is increasing, with the high-sensitivity C-reactive protein (hsCRP) being the most profoundly studied biomarker in cardiovascular diseases. It remains stable in samples over long periods of time and can be tested quite simply, rapidly, and inexpensively. HsCRP testing is valuable in both primary and secondary cardiovascular diseases prophylaxis and for those who are already suffering from cardiovascular disease (CVD). This test is useful in evaluation of disease severity, treatment efficacy, and outcome prognosis [7]. Plasma CypA is associated with C reactive protein (CRP) levels. There is correlation between plasma CypA and serum CRP, a clinical marker of vascular inflammation [8]. This study aimed to compare plasma levels of CypA in type 2 DM patients with or without CAD with those in healthy participants to determine the potential role of CypA in promoting vascular disease in diabetic patient and to study the association of hsCRP with CypA levels.

Patients and methods

Patients

The present study was conducted on 80 patients, 42 male and 38 female, attending the Cardiology and Internal Medicine Departments at Benha University Hospital from October 2015 to October 2016. Informed written consent was obtained from all participants in the study, and this study was approved by the Research Ethics Committee. The study participants were divided into four groups: group 1, which included 20 apparently healthy individuals with matched age and sex; group 2, which included 20 patients with type 2 DM without CAD; group 3, which included 20 patients with type 2 DM with CAD diagnosed after detection of diabetes; and group 4, which included 20 patients with CAD without DM. Patients with cardiac disease other than CAD, nephropathy, retinopathy, inflammatory disease of any cause, liver or kidney disease, other systemic and metabolic disease, malignancy, and pregnant women were excluded from the study. All individuals in the study were subjected to full history taking and clinical examination. Blood samples were drawn from all participants to assess levels of fasting serum glucose, total cholesterol, triglycerides (TG), high-density lipoprotein-cholesterol, low-density lipoprotein (LDL)-cholesterol, urea, creatinine, alanine amino-transferase, creatine kinase-MB (CK-MB). These analyses were carried out using Biosystems A15 Auto-analyzer (Barcelona Spain NycoCard, California, USA). In addition, HbA1c was evaluated using NycoCard diagnostic medical device, and troponin I concentration was measured on the Mini

VIDAS instrument using VIDAS Troponin I Ultra (TNIU) kit (bioMerieux Inc., Durham, North Carolina, USA) for determination of human cardiac troponin I in human serum using the enzyme-linked fluorescence assay (ELFA) technique. The plasma level of CypA was measured by double-antibody sandwich enzyme-linked immunosorbent assay kit that was supplied by Cloud-Clone Corp. (Houston, Texas, USA). Blood for CypA were collected after overnight fasting under complete aseptic condition and were put into EDTA test tubes and centrifuged immediately at 3000 rpm for 15 min; the separated plasma was put into aliquots. Serum hsCRP was measured using solid phase enzyme-linked immunosorbent assay kit, which was supplied by BioCheck Inc. (Foster City, California, USA).

Statistical analysis

The collected data were tabulated and analyzed using SPSS version 16 (SPSS Inc., Chicago, Illinois, USA) software. Categorical data were presented as numbers and percentages, whereas quantitative data were expressed as mean $\pm$ SD, median, and range. The χ^2 -test or Fisher's exact test was used to analyze categorical variables. Quantitative data were tested for normality using Shapiro-Wilks test, assuming normality at P value more than 0.05; analysis of variance was used for normally distributed variables, and Mann-Whitney U -test, Kruskal Wallis test, and Spearman's correlation coefficient (σ) were used for non-normally distributed variables. The receiver operating characteristic (ROC) curve was used to determine cutoff value of CypA with optimum sensitivity and specificity in prediction of type 2 DM with or without CAD. The accepted level of significance in this work was stated at 0.05 ($P < 0.05$). A P value of more than 0.05 was considered nonsignificant, that of less than 0.05 was considered significant, and 0.001 or less was considered highly significant.

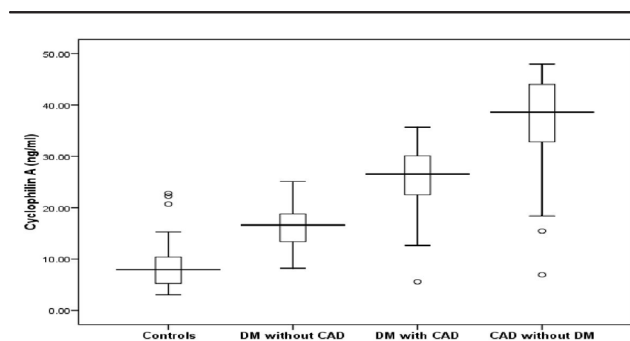
Results

There was a statistically highly significant increase in the median of CypA concentration in patient groups in comparison with the control group. Also, there was a statistically highly significant increase in the median of CypA concentration in diabetic patients with CAD group when compared with only diabetic patients group and in patient with only CAD when compared with diabetic patients with or without CAD group (Table 1 and Fig. 1). There was a statistically highly significant increase in the median hsCRP concentration in diabetic patients with CAD group in comparison with the control group, DM

Table 1 Comparing the studied groups regarding cyclophilin A (ng/ml)

Groups	n	Cyclophilin A (ng/ml)		KWT	P	Significant pairs
		Median	Range			
Group 1 (controls)	20	7.89	3.0–22.77	48.6	<0.001 (HS)	Group 1≠group 2Group 1≠group 3Group 1≠group 4Group 2≠group 3Group 2≠group 4Group 3≠group 4
Group 2 (DM without CAD)	20	16.63	8.2–25.1			
Group 3 (DM with CAD)	20	26.53	5.54–35.68			
Group 4 (CAD without DM)	20	38.61	6.91–48.0			

CAD, coronary artery disease; DM, diabetes mellitus; HS, highly significant, KWT, Kilowatt.

Figure 1

Box plot showing median and range of cyclophilin A (ng/ml). CAD, coronary artery disease; DM, diabetes mellitus.

group, and CAD group, and there was a statistically highly significant increase in the median hsCRP concentration in CAD without DM group in comparison with the control group (Table 2 and Fig. 2).

In diabetic patients without CAD group there was a highly significant positive correlation between the level of CypA and fasting blood sugar (FBS), HbA1c, and hsCRP. In diabetic patients with CAD group there was a highly significant positive correlation between the level of CypA and FBS, HbA1c, hsCRP, LDL. Also, there was a significant positive correlation between the level of CypA and CK-MB, troponin I, cholesterol, and TG. In CAD group there was a highly significant positive correlation between level of CypA and FBS, HbA1c, CK-MB, troponin I, and hsCRP and significant positive correlation between level of CypA and cholesterol, TG, and LDL (Table 3). The ROC curve analysis demonstrated that the level of CypA is better than hsCRP in predicting DM, CAD, and for exclusion of CAD in diabetic patient (Fig. 3). In patients with DM with CAD the best cutoff value for CypA was more than 22.4 ng/ml and at this point the sensitivity was 95% and specificity was 78.3%, with an area under the curve of 0.94. The best cutoff value for hsCRP was more than 3.42 mg/l and at this point the sensitivity was 80% and specificity was 66.7%, with an area under the

curve of 0.65. For exclusion of CAD in diabetic patient the best cutoff value for CypA was less than 20.4 ng/ml and at this point the sensitivity was 90% and specificity was 60%, with area an under the curve of 0.66. The best cutoff value for hsCRP was less than 3.42 mg/l and at this point the sensitivity was 60% and specificity was 41.7%, with an area under the curve of 0.51. Finally, in diagnosis of CAD the best cutoff value for CypA was more than 25.4 ng/ml and at this point the sensitivity was 85% and specificity was 81.7%, with an area under the curve of 0.896. The best cutoff value for hsCRP was more than 3.06 mg/l and at this point the sensitivity was 65% and specificity was 70%, with an area under the curve of 0.653.

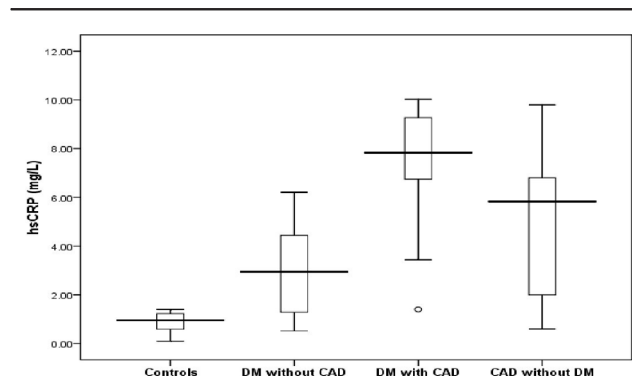
Discussion

Plasma CypA is secreted by monocytes and vascular wall cells in response to oxidative stress and inflammation, but can also be secreted by or leaked from damaged cardiomyocytes and interstitial fibroblasts [5]. CypA expression and secretion are increased by oxidative stress and vascular injury. These findings are the first to identify CypA as a secreted redox-sensitive mediator, establish CypA as a vascular smooth muscle cells (VSMC) growth factor, and suggest an important role for CypA in the pathogenesis of vascular diseases [9]. CAD occurs due to atherosclerosis of the coronary arteries of the heart [10]. 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 cardiovascular events and mortality [11]. The chronic DM is a major risk for cardiovascular disease. The incidence of CVD might be a foremost cause of morbidity and mortality in patient afflicted with DM [12]. In current study, there was a statistically highly significant increase in the median CypA concentration in all patient groups in comparison with the control group ($P < 0.001$); also, there was a statistically highly significant increase in the median CypA concentration in diabetic patients with CAD when compared with only

Table 2 Comparing the studied groups regarding high-sensitivity C-reactive protein (mg/l)

Groups	n	hsCRP(mg/l)		KWT	P	Significant pairs
		Median	Range			
Group 1 (controls)	20	0.95	0.1–1.4	47.4	<0.001 (HS)	Group 1≠group 3 Group 1≠group 4 Group 2≠group 3 Group 2≠group 4
Group 2 (DM without CAD)	20	2.95	0.52–6.2			
Group 3 (DM with CAD)	20	7.83	1.4–10.2			
Group 4 (CAD without DM)	20	5.83	0.6–9.8			

CAD, coronary artery disease; DM, diabetes mellitus; HS, highly significant; hsCRP, high-sensitivity C-reactive protein.

Figure 2

Box plot showing median and range of high-sensitivity C-reactive protein (hsCRP) (mg/l). CAD, coronary artery disease; DM, diabetes mellitus.

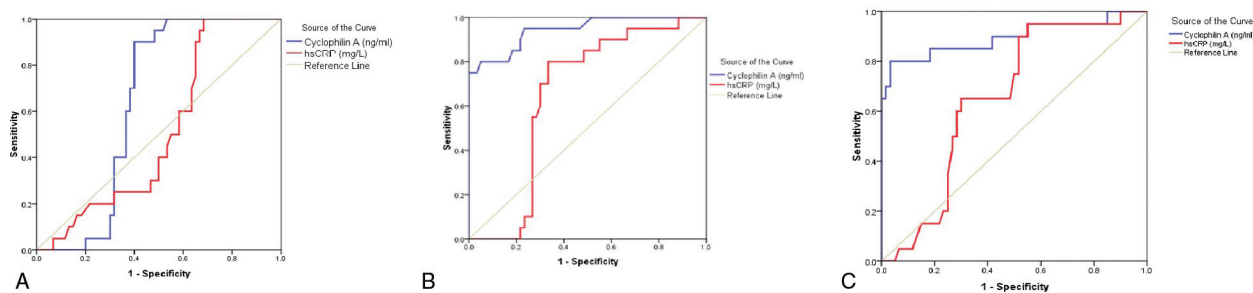
diabetic patients group ($P < 0.001$) and in patient with only CAD when compared with diabetic patients with or without CAD ($P < 0.001$). In concordance with these findings, Satoh *et al.* [13] reported that plasma CypA levels were significantly higher in patients with significant coronary stenosis 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) [13]. Yan *et al.* [14] also found that serum concentration of CypA in patients with acute coronary syndrome (ACS) (UA and AMI) was significantly higher than those with stable angina (SA) and controls ($P < 0.05$). Hence, increased concentrations of CypA may be a valuable marker for predicting the severity of acute coronary syndrome [14]. There was a study, which support these results, found that plasma CypA is secreted by monocytes and vascular wall cells in response to oxidative stress and inflammation, but can also be secreted by or leaked from damaged cardiomyocytes and interstitial fibroblasts [5], and another study reported that CypA has proinflammatory effects on endothelial cells and may play an important role in the pathogenesis of atherosclerosis [15]. Ramachandran *et al.* [3] found the same results and reported that patients with type 2 DM have higher circulating levels of CypA than the

normal population. Plasma CypA levels were increased in patients with DM and CAD suggesting a role of this protein in accelerating vascular disease in type 2 DM [3]. For the present study, correlation of CypA with the other studied variables is shown in Table 3. Ramachandran *et al.* [3] reported that age was positively associated with increased plasma CypA level, whereas, sex, serum levels of cholesterol, high-density lipoprotein, LDL, and TG were not associated with increase in CypA levels [3]. In contrast, Satoh *et al.* [13] found that age, diabetes, and dyslipidemia correlated with plasma CypA levels in their patients with stenotic coronary arteries. Also, Ramachandran *et al.* [3] found that fasting blood glucose and HbA1c were positively associated with plasma CypA levels indicating a specific relation of plasma CypA levels with hyperglycemia [3]. CRP belongs to the pentraxin protein family and is synthesized in hepatocytes and some extrahepatic tissues such as vascular smooth muscle, atherosclerotic plaques, and intracardial tissues [16]. Indeed, as vascular inflammatory changes can hardly be evaluated using cardiac imaging methods, the role of inflammation biomarkers testing in peripheral blood is increasing, with the hsCRP being the most profoundly studied biomarkers in CVD. It remains stable in samples over long periods of time and can be tested quite simply, rapidly and inexpensively [7]. The present study demonstrates highly significant positive correlation between level of CypA and hsCRP. Plasma CypA is associated with CRP levels, a clinical marker of vascular inflammation [8]. Also, Satoh *et al.* [13] reported that hsCRP correlate with plasma CypA levels in their patients with stenotic coronary arteries. Ramachandran *et al.* [3] found that in patients with increased serum CRP levels, plasma CypA was also elevated ($P = 0.016$) and reported that there was a positive association between higher hsCRP levels and elevated plasma CypA in patients with diabetes as well as in those with diabetes and CAD [3]. In the present study, ROC curve analysis showed that CypA (c -statistic=0.94) is a better biomarker than hsCRP (c -statistic=0.65), however for exclusion of CAD in diabetic patient c -statistic for CypA was 0.66, whereas it was 0.51 for hsCRP. Finally, in diagnosis of CAD

Table 3 Spearman's correlation coefficient between cyclophilin A and the studied variables

	Cyclophilin A							
	Controls		DM		DM+CAD		CAD	
	σ	P	σ	P	σ	P	σ	P
Age	-0.170	0.47	0.214	0.36	-0.141	0.55	-0.324	0.16
FBS	-0.093	0.69	0.852	<0.001 (HS)	0.735	<0.001 (HS)	0.776	<0.001 (HS)
HbA1c	0.189	0.42	0.860	<0.001 (HS)	0.745	<0.001 (HS)	0.752	<0.001 (HS)
CK-MB	0.052	0.82	-0.372	0.16	0.659	0.002 (S)	0.784	<0.001 (HS)
Troponin I	-0.264	0.26	0.150	0.53	0.605	0.005 (S)	0.779	<0.001 (HS)
Cholesterol	-0.355	0.13	0.124	0.61	0.611	0.004 (S)	0.529	0.017 (S)
Triglycerides	0.039	0.87	0.057	0.81	0.640	0.002 (S)	0.605	0.005 (S)
HDL	-0.284	0.22	-0.261	0.27	-0.790	<0.001 (HS)	-0.863	<0.001 (HS)
LDL	-0.179	0.45	0.056	0.81	0.696	<0.001 (HS)	0.465	0.039 (S)
ALT	0.05	0.83	0.272	0.25	0.004	0.98	0.290	0.21
hsCRP	0.405	0.08	0.989	<0.001 (HS)	0.750	<0.001 (HS)	0.907	<0.001 (HS)

ALT, alanine aminotransferase; CAD, coronary artery disease; CK-MB, creatine kinase-MB; DM, diabetes mellitus; HDL, high-density lipoprotein; HS, highly significant; hsCRP, high-sensitivity C-reactive protein; LDL, low-density lipoprotein; S, significant.

Figure 3

(a) Receiver operating characteristic (ROC) curve of the cyclophilin A (ng/ml) and high-sensitivity C-reactive protein (hsCRP) (mg/l) for prediction of diabetes mellitus (DM) with coronary artery disease (CAD). (b) ROC curve of the cyclophilin A (ng/ml) and hsCRP (mg/l) for exclusion of CAD in patients with DM. (c) ROC curve of the cyclophilin A (ng/ml) and hsCRP (mg/l) for prediction of CAD without DM.

c -statistic was 0.896 for CypA, while it was 0.653 for hsCRP. 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 [17]. Indeed, Satoh and Shimokawa [18] reported that plasma CypA level is a novel biomarker of CAD. Further studies are needed to establish the clinical significance of CypA in the pathogenesis of atherosclerotic CVD.

Conclusion

This study demonstrated that CypA has a potential role in promoting vascular disease in diabetic patients and revealed that CypA is a good biomarker for CAD with or without DM, better than hsCRP.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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