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Study of Cyclophilin- A & Hscrp as Predictors Biomarkers in Patients with Type 2 Diabetes Mellitus and Suggests Presence of Vascular Diseases

KEYWORDS: Hyperglycemia, Type 2 diabetes mellitus, Monocytes, Cyclophilin A, Vascular disease, Coronary artery disease (CAD), hsCRP (high sensitive C-reactive protein)

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ABSTRACT ***Aims/hypothesis:***

Cyclophilin A, an immunophilin is secreted from human monocytes activated by high glucose. its role as an inflammatory mediator of vascular tissue damage associated with inflammation and oxidative stress, we examined plasma levels of cyclophilin A in normal healthy volunteers and patients with type 2 diabetes(DM), with or without coronary artery disease (CAD).

***Methods**:*

Study subjects comprised of 30 normal healthy volunteers, 30 patients with DM and CAD, 30 patients Non Diabetic with CAD, 30 patients with diabetes. Diabetes was assessed by HbA1c levels while coronary artery disease was established coronary angiography. Plasma cyclophilin was measured using a cyclophilin A ELISA Kit. Relationship of plasma cyclophilin A levels with blood markers of type 2 diabetes. hsCRP is done by ELISA Kit(immuno-inhibition method).

Results: Plasma Cyclophilin levels were higher in diabetes patients with or without CAD compared to normal subjects ($P < 0.001$). Fasting blood sugar levels and HbA1C levels were positively associated with increased plasma cyclophilin. Increased level of hsCRP shows systemic inflammation in study group.

***Conclusions/interpretations**:*

Our study shows that patients with type 2 diabetes have higher circulating levels of cyclophilin A than the normal Healthy controls. Plasma cyclophilin levels were increased in patients with diabetes and coronary artery disease suggesting a role of this protein in accelerating vascular disease in type 2 diabetes. Considering the evidence that Cyclophilin A is an inflammatory mediator in atherogenesis. hsCRP levels are significantly higher in study groups as compared to healthy individuals.

Introduction

Cyclophilins are proteins belonging to the superfamily of immunophilins. They have been found in many types of cells in different organisms and all have peptidyl -prolyl cistrans isomerase (PPIase) activity. In diabetes, high glucose levels and ROS activate monocytes to secrete CypA via vesicles. Secreted CypA acts as a pro -inflammatory cytokine that activates endothelial cells and leukocytes, increasing inflammation in vessels and promoting atherogenesis. It is clear that diabetes and atherosclerosis can affect one another, with CypA being one of the factors connecting diabetes and atherosclerosis. [1] Cyclophilin A is also well recognized as a secreted growth factor that is induced by oxidative stress. The secretory nature of this protein and its presence in plasma of patients with DM and CAD underlines its potential as a marker of disease. [2] The progression of atherosclerosis is the development of an oxidizing environment because of the activation of macrophages that have become loaded with oxidized low density lipoprotein (LDL) and other lipids. These macrophages produce abundant reactive oxygen species (ROS) and secrete several growth factors that contribute to the progression of atherosclerosis.[3] Immunophilins are known to participate in Ca^{+2} homeostasis in different cells and are known to control Ca^{+2}channels in the ER-like IP3 receptors, whereas cyclophilins such as CyPA are probably involved in the regulation of sarcoplasmic/endoplasmic reticulum Ca^{+2} ATPase 2b (SERCA2b).[4]

C-reactive protein (CRP), a marker of systemic inflammation, & is emerging as an independent risk factor for cardiovascular disease. High CRP levels have been linked to an increased risk of thrombotic events including myocardial infarction. Elevated CRP levels have also been linked to an increased risk of later development of diabetes.[5]

More recently high sensitive CRP (hs-CRP) is used to detect the low level of inflammation. Type 2 diabetic patients have increased CRP values, and inflammation has been found to be related to insulin resistance syndrome, which may partly explain the high incidence of CVD in diabetic group. [6]

Materials and Methods

Place of study: Department of Biochemistry and Department of Medicine. MGM Medical College & hospital, Kamothe, Navi Mumbai

Study period **:** February 2015 to February 2016.

Study design : Prospective, case control study.

Age Group : 25 – 70 yrs. & Study included both genders.

The subjects selected for the study were categorized into the following four groups:

Group 1: 30 Healthy Controls.

Group 2: 30 patients with diabetes mellitus with CAD.

Group 3: 30 non-diabetic patients with CAD.

Group 4: 30 Patients with Diabetes mellitus without clinical Coronary artery disease.

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Written and verbal consent will be taken from patients and healthy individuals. Patients will be considered between February2015 to February 2016.

Informed consent was taken and the study was approved by the ethical committee of the

institution. Patients suffering from chronic renal disease, Chronic liver disease, Malnutrition HIV patient, Rheumatoid arthritis, Sepsis ,Asthma ,Malignancy Pregnant women.

5 ml of fasting blood sample was drawn from all subjects under the aseptic precaution and 2 ml

Of blood was drawn in postprandial period. Fasting samples were analyzed for routine blood parameters, fasting plasma glucose (FPG), HbA1c, & Lipid profile.

Postprandial sample was analyzed for postprandial plasma glucose (PPPG). Plasma Glucose was measured by HK G6P-DH, Determination of HbA1c in whole blood **(**Analysed on Bio-Rad D-10 analyzer) by Ion exchange HPLC methods. Similarly serum cyclophilin-A by estimated by ELISA method using commercially available Qayee-bio ELISA kit & hsCRP done by(immuno inhibition) ELISA kit.

Statistical analysis

Results are represented as mean ± standard deviation. Statistical analysis was done using

Student's t-test, and statistical significance was compared between the cases and the controls.

Pearson correlation between the study variables was performed to establish the relationship. Probability value (P) of <0.05 was considered as statistically significant. Statistical analysis was done using the Statistical Software: SPSS-16.

Results

Table 1: Comparison of FBS, PPBS and HbA1c in Control (Gr-I) & Study Group (Gr-II, III, IV)

Parameters	Group-I (Control) Mean± SD	Group-II (CAD with DM) Mean± SD	Group-III (CAD) Mean± SD	Group-IV **(T2DM) Mean± SD**
FBS ((mg/dl)	91.6±9.6	148.98±54.58**	96.7±9.42*	140.7±41.8**
PPBS (mm/dl)	129.4±10.6	257.1±56.3**	139.5±11.1**	234.9±62.4**
HbA1c (%)	5.5±0.4	8.1±1.0**	6.0±0.6**	7.8±0.8**

**p ≤ 0.05 significant, **p ≤ 0.001 highly significant & #p ≥ 0.05 non-significant.*

Table 2: Comparison of PON1, CyPA and hs-CRP in (Gr-I) & Study Group (Gr-II, III, IV)

Parameters	Group-I (Control) Mean± SD	Group-II (CAD with DM) Mean± SD	Group-III (CAD) Mean± SD	Group-IV (T2DM) Mean± SD
CyPA (Pg/ml)	186.0±26.5	273.84±33.4**	268.7±58.2**	259.1±39.0**
Hs-CRP (mg/l)	0.6±0.2	6.7±0.9**	3.7±0.6**	5.8±0.8**

p ≤ 0.05 significant,p ≤ 0.001 highly significant & #p ≥ 0.05 non-significant*

Fig 1: Shows serum CyPA levels in control & study groups

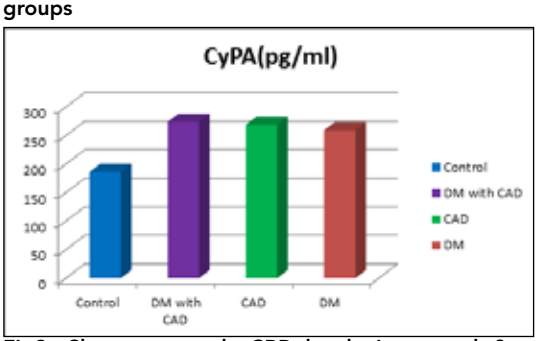

Fig2: Shows serum hs-CRP levels in control & study groups

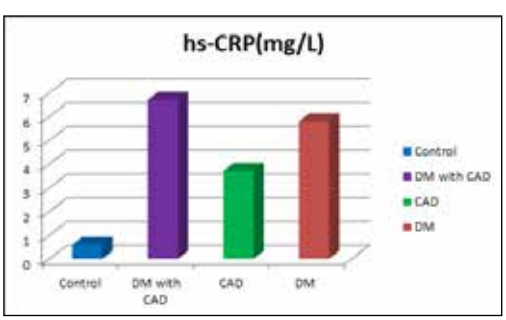

Fig 3: correlation of cyclophilin-A with hs CRP in CAD patients.

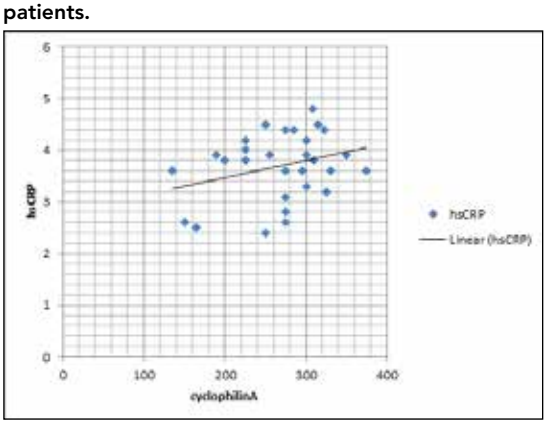

Fig 3: shows significant (r= 0.2991) Positive correlation between cyclophilin A & hs CRP in CAD patients

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DISCUSSION

CVD accounts for up to 80% of the deaths in persons with type 2 diabetes. The incidence of CVD mortality was 3-fold higher among individuals with diabetes who had suffered a myocardial infarction than among nondiabetic individuals.[7]

Table 1 shows mean levels of fasting plasma sugar; were significantly increase in group II & groups IV (P<0.001) as compared to the control group. There is significant rise FBS between group III & group I (P< 0.05).

Table 1 shows significantly increase Post-prandial plasma sugar level in group II & IV (P<0.001) as compared to the control group. There was slightly increased value in group III as compared to group I (P< 0.05).

Table 1 shows mean values of HbA1c, which are significantly high in group II & group IV as compared to the group I (p< 0.001); that suggesting poor glycemic control. However, in group III HbA1c values are slightly increased value as compared to group I (P<0.001) although it is in normal range.

Selvin et al.2012 evaluated 10 prospective studies and concluded that every 1% increase in HbA1c was associated with a 18% increase in hazard of CVD, 13% in CHD, 16% in fatal CHD, and 17% in stroke. Further they were done a meta-analysis of 5 RCTs (Randomized controlled trials) and showed, that during 5-year treatment, reduction of HbA1c by 0.9% resulted in a 17% significant reduction in non-fatal MI events, 15% in CHD events, but nonsignificant reduction in stroke events in type 2 diabetic patients. [8] Our study supports selvin et al if 1% increase in HbA1c can cause complication of CVD.

Table 2 shows mean level of serum cyclophilin A in the group I (control) & study groups (group II, group III, & group IV). There is highly significant elevation of Cyclophilin A in all study groups as compared to the control group (P<0.001). However the level of cyclophilin A is significantly increased in group II, as compared to group IV (P<0.001) (Fig 1).

A possible role for CyPA in atherosclerosis is becoming increasingly apparent. Consistently, plasma levels of CyPA were significantly increased in patients with CAD & DM as compared to control group. Several risk factors, such as hypertension, diabetes, smoking and aging, induce the generation of ROS and promote the secretion of CyPA. CyPA secretion is regulated by Rho-kinase activation, which is important for VSMC contraction and atherosclerosis. Consistently, plasma levels of CyPA were significantly increased in patients with CAD[9].

Ramachandran et al 2014 reported that Plasma cyclophilin levels were increased in patients with diabetes and coronary artery disease suggesting a role of this protein in accelerating vascular disease in type 2diabetes. Considering the evidence that Cyclophilin A is an inflammatory mediator in atherogenesis, the mechanistic role of cyclophilin A in diabetic vascular disease. [2]

Hyperglycemia in diabetes and related oxidative stress could contribute to secretion of cyclophilin A from circulating monocytes and a rise in plasma cyclophilin A levels as seen in our patients with diabetes.

Table 2 shows mean level of hsCRP in group I (control) & study groups (group II, group III, & group IV). In group II, group III, & group IV. We found highly significant increase hsCRP level as compared to group I (control) (P<0.001) (Fig 2).

Syed Shahid Habib et al.2013 results suggest that patients with angiographically evaluated CAD have significantly higher levels of hsCRP levels compared to healthy individuals and are correlated with the presence & severity of CAD.[10]

We observed that hsCRP levels are significantly higher in patients with CAD compared to healthy individuals (Fig2) our studies are supported by Habib SS & show systemic inflammation may show in study groups.

We observed a positive correlation between plasma cyclophilin A and serum CRP levels; that shows as inflammation increases hs-CRP & cyclophilin increases. CRP, an acute phase reactant has been developed as a surrogate marker of inflammatory mediators in coronary artery disease and diabetes.

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