Evaluation of Cystatin C and Intercellular adhesion molecule –1 as markers of preclinical coronary artery disease in normoalbuminuric early type 2 diabetes individuals

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Abstract

Introduction: The presence of sub-chronic inflammation characterized by elevated intercellular adhesion molecule 1 (ICAM-1) and other cytokines has been quite evident in diabetes-associated coronary artery diseases. Cystatin-c, primarily developed as an early renal dysfunction marker, is found to be associated with insulin resistance and inflammation independent of renal function. The present study was designed to evaluate Cystatin-c and ICAM-1 as markers of pre-clinicalcoronary artery disease in normoalbuminuric early type 2 diabetes mellitus individuals.

Materials and Methods: Post-screening, study participants of both genders aged 35-55 years were divided into group 1 (31 healthy controls) and group 2 (31 normoalbuminuric early diabetes cases with a diabetes duration of 2-7 years). Fasting plasma glucose (hexokinase method), HbA1c (high-performance liquid chromatography) and random urine microalbumin (immuno-turbidometry) were estimated. Serum ICAM-1 and Cystatin-c levels were quantified by ELISA. Data analysis wasperformed using SPSS version 20.

Results: The average age of diabetes and control groups was found to be 49.68±4.60 and 45.23±5.73 years respectively. The mean diabetes duration was found to be 5.58±1.50 years. Compared to controls, diabetes group showed elevated ICAM 1 (630.26±266.062 vs 856.58±233.36 ng/mL, p=0.001) and cystatin-c (1118.26±262.980 vs 1229.10±457.74 ng/mL, p=0.383) levels. Correlation of ICAM 1 with cystatin-c in both diabetes (r = 0.009, p = 0.96) and control (r = - 0.07, p = 0.68) groups was insignificant.

Conclusion: The significant increase in ICAM-1 levels over Cystatin-c in normoalbuminuric early diabetes individuals discriminates its superior role in assessing preclinical endothelial dysfunction and inflammation.

Keywords: Cystatin-c, ICAM-1, Normo-albuminuria, Preclinical coronary artery disease, Type 2 Diabetes mellitus

Introduction

Type 2 Diabetes Mellitus (T2DM) is now a global health priority.[1] Unlike the West, where the older individuals are often affected with diabetes, in countries like India, the prevalence is high among the young and middle-aged population. In addition, diabetes is often associated with significant morbidity in the form of chronic complications, adversely affecting the nation's health and economy.[2]

Endothelial dysfunction and atherosclerotic plaque formation are the primary processes contributing to the development of micro and macro-vascularcomplications in patients with diabetes. The processes are evident long before clinical manifestation (pre- clinical coronary artery disease). The stimulation of inflammatory cytokines such as intercellular adhesion molecule- 1 (ICAM-1) trigger endothelial dysfunction and play an important role in all stages of atherosclerosis.[3]

Diabetic nephropathy is a leading cause of end-stage renal disease globally. Renal disease is burdened witha huge risk of mortality due to cardiovascular disease.

Microalbuminuria is considered to be a reliable predictor of early-stage diabetic nephropathy and cardiovascular disease both among diabetes and non- diabetes subjects.[4] A novel surrogate marker cystatin-c initially proposed for early detection of diabetic nephropathy has been of recent interest as a marker for cardiac complications in diabetes and has shown to be strongly associated with the occurrence and severity of cardiovascular diseases (CVDs).[5,6]

Cystatin-c has been shown to be associated with insulin resistance and inflammation independent of the renal function.[7] This may explain the association between cystatin-c and cardiovascular disease in T2DM. Zhao et al. reported a significant relationship of elevated cystatin-c levels and the risk of prevalent heart disease in individuals without chronic kidney disease.[8] With an aim to assess promising biomarkers aiding in reducing morbidity, the present study was taken up to evaluate Cystatin-c and ICAM-1 as markers for pre-clinical coronary artery disease in normoalbuminuric early T2DM individuals with no known history of coronary artery disease (CAD) and nephropathy.

Materials and Methods

This case-control study was conducted over a period of one year in the department of Biochemistry of a tertiary care hospital in South India. The study participants were screened on the basis of their medical history and categorized in- to two groups namely; Group I: 31 apparently healthy subjects of both gender aged 35-55 years with FPG ≤ 100 mg/dl, HbA1c < 5.6% and random urine microalbumin < 20mg/L (< 30mg/24hr) as controls. Group II consisted of: 31 normoalbuminuric diabetes patients (defined as per American Diabetes Association criteria) [9] of both sex aged 35-55 years with 2-7 years duration of diabetes, no known history of diabetic nephropathy and CAD as cases. Individuals diagnosed with liver, renal, cardiovascular or other endocrine diseases, pregnant women, subjects on glucocorticoids or any herbal supplements interfering with glycaemic status, history of chronic inflammatory diseases, subjects with history of any infectious disease in the past two weeks, subjects with history of acute complications in the past one year, chronic smokers and alcoholics were excluded from the study.

The sample size was calculated by the formula $n = 2(Z\alpha + Z\beta)^2 \sigma^2/\delta^2$. Where $Z\alpha = 1.96$ at 95% Confidence Interval, $Z\beta = 1.28$ at 90% Power, $\sigma = 0.25$, $\delta = 0.18$

The study procedures were carried out after obtaining prior voluntary informed consent from all the participants.The protocol was approved by the institutional ethics committee (IEC KMC MLR 9-15/155). Blood samples were collected under aseptic conditions in a plain vacutainer and were centrifuged at 3000 rpm for 10 minutes to separate the serum which was then stored in pre-labelled Eppendorf tubes at -20 ^{o}C for further analysis. Fasting Plasma Glucose (FPG) was estimated by hexokinase enzymatic method on the autoanalyser COBAS 6000 (Roche, Switzerland), Glycated haemoglobin (HbA1c) was estimated by High-Performance Liquid Chromatography (HPLC) method on BioRad D10 autoanalyser and random urine microalbumin was estimated by the immunoturbidimetricmethod. The estimation of ICAM-1 and Cystatin-c was carried out by ELISA technique on ELx 800 ELISA platereader using Biovendor® Inc.(European Union) and Diaclone® Inc.(France) kits respectively.

Statistical analysis was done by SPSS version 20. Continuous variables are expressed as mean ± standard deviation (normally distributed data) or median interquartile range (skewed data). Groups were compared by student unpaired 't' test or Mann-Whitney U test. Correlation analysis was performed by Pearson's test where $p < 0.05$ was considered significant.

Results

Diabetes group (Group II) was comparatively older (49.68±4.60 years) than the controls- Group I (45.23±5.73 years) and the difference in age was statistically significant ($p = 0.001$). The male: female ratio was 22:9 and 15:16 respectively in the two groups. The mean diabetes duration was found to be 5.58±1.50 years. Values of fasting sugar, HbA1c, random urine microalbumin, ICAM-1 and cystatin-c levels were found to be 145.19±46.22 mg/dL, 7.87±1.52 %, 6.39±5.23 mg/L, 856.58±233.365 ng/mL and 1229.10±457.745 ng/mL respectively in the diabetes group. The same in the control group were found to be 93.23±4.19 mg/dL, 5.35±0.48 %, 4.81±4.15 mg/L, 630.26±266.062 ng/mL and 1118.26±262.980 ng/mL respectively. [Table 1] Except for cystatin-c, all other biochemical variables were significantly ($p \leq 0.02$) higher in the diabetes group. ICAM -1 did not correlate significantly with the glycemic parameters or with urine microalbumin in both the groups (Table 2). Similar was the finding of the correlation of Cystacin –C (Table 3) except with fasting glucose in control group ($r = 0.454$, $p<0.01$). ICAM 1 did not show any correlation with cystatin-c in either group (Figure 1)

Table 1. Comparison of clinical, demographic and biochemical variables among the two groups

Parameters	Controls (n=31)	Diabetes (n=31)	p-value
Age (years)	45.23±5.73	49.68±4.60	0.001*
Gender (male: female)	15:16	22:9	0.12
Duration of diabetes	-	5.58±1.50	-
FPG (mg/dL)	93.23±4.193	145.19±46.224	< 0.001 *
HbA1c (%)	5.35±0.486	7.87±1.522	< 0.001*
U. Micralb (mg/L)	4.81±4.151	6.39±5.232	0.02*
ICAM 1 (ng/ml)	630.26±266.062	856.58±233.365	0.001*
Cystatin-c (ng/ml)	1118.26±262.980	1229.10±457.745	0.383

FPG = Fasting plasma glucose, U.Micralb = Urine microalbumin, ICAM 1 = Intercellular cell adhesion molecule 1

Table 2. Correlation of **ICAM 1** with glycemic variables and urine microalbumin in both groups

Parameters	Correlations	Control (n=31)	Diabetes (n=31)
FPG	r-value	0.07	0.14
	p-value	0.70	0.42
HbA1c	r-value	0.18	0.16
	p-value	0.33	0.36
Urine microalbumin	r-value	0.11	0.21
	p-value	0.53	0.25

Table 3. Correlation of **Cystatin-c** with glycemic variables and urine microalbumin in groups

Parameters	Correlations	Control (n=31)	Diabetes (n=31)
FPG	r-value	0.45	-0.12
	p-value	0.01*	0.51
HbA1c	r-value	0.10	0.14
	p-value	0.56	0.42
Urine microalbumin	r-value	-0.03	0.02
	p-value	0.85	0.88

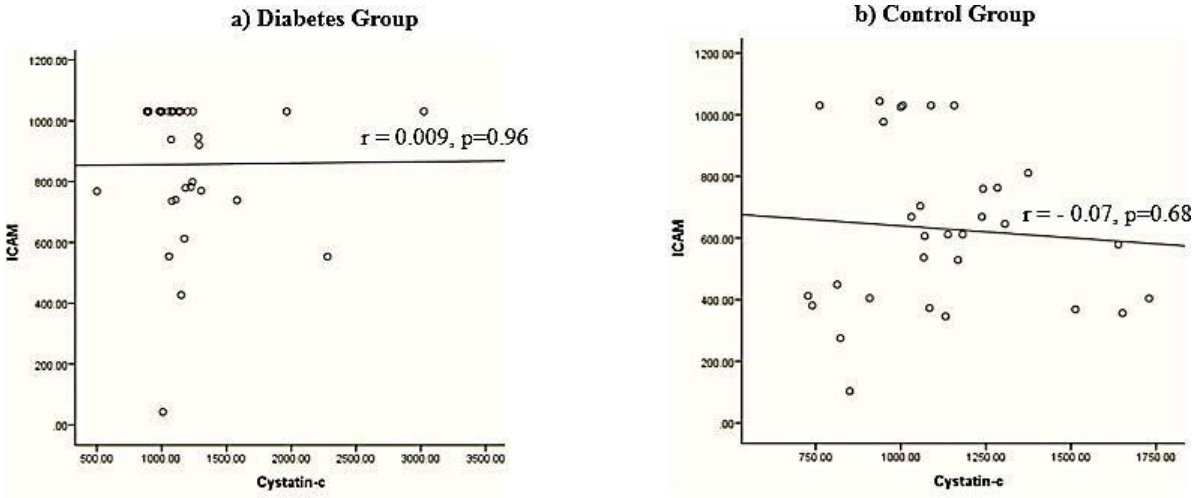

Figure 1 Correlation between Cystatin-c and ICAM in Diabetes and control groups

Discussion

There exists a strong association between Coronary Artery Disease (CAD) and diabetes mellitus and this has led tothe development of novel screening strategies of diabetes patients even before the patients are symptomatic.Even among non-diabetes individuals, a high blood glucose level is emerging as an independent risk factor for CAD.[18] Increased risk of CAD in patients with renal dysfunction is also well established[11]. The mechanism(s) of accelerated atherosclerosis in microalbuminuric patients could be multifactorial. Abnormal vasodilation, endothelial dysfunction, inflammation, insulin resistance, or abnormal coagulationmay be involved. Mortality to the extent of 40-50% is observed in stage 4 and 5 chronic kidney disease cases. In the present study, both groups were screened for microalbuminuria to rule out diabetic nephropathy and coronary artery disease in early T2DM patients. Still, the mean microalbumin levels were higher in the diabetes group, heralding the onset of structural changes in the early phase of the disorder itself, drawing attention to the need for cardiovascular and progressive renal disease risk stratification and robust management[12].

Several adhesion molecules like ICAM -1, VCAM -1 and E-selectin play a crucial role in the binding plasma leukocytes and their migration to the subendothelial space, which initiates the process of atherosclerosis. [15] High circulating levels of these molecules have been associated with adverse cardiac events (REF)[16] The significant elevation of ICAM 1 levels in the early diabetes group of this study further confounds the atherosclerotic risk and endothelial dysfunction in them

Cystatin-c, primarily developed and assessed as a marker of early renal dysfunction, has been studied for its role in predicting new-onset or deteriorating cardiovascular disease as it has been found to have a direct effect on atherosclerotic plaque formation and inflammation.[12] It has also been noted that T2DM patients with microangiopathy have significantly elevated cystatin-c levels than the apparently healthy individuals.[7] A study with similar hypothesis that cystatin-c may play a key role in the early detection of cardiovascular disease and peripheral neuropathy was done by Xubin et al. [13] who concluded that Cys –C is a biomarker of cardiovascular autonomic dysfunction. Bhat et al. found a statistically insignificant difference in Cys-C levels in diabetes patients with and without cardiac complications[5] There are several other studiesin favor of the clinical utility of Cys-C in atherosclerotic diseases. In our study however, we observed that there was no appreciable difference in the Cys-C levels between control group and the early diabetes group with no history of cardiac complications or nephropathy.

High concentration of cystatin-c are directly related to the process of inflammation and atherosclerosis.[14] Animal studies have revealed that cystatin C might interact with the inflammatory response, leading to activation of cathepsins and resulting in the degradation of collagen in the atheroma plaque, leading to an increased risk of rupture [22] BMC
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Clinical utility of serum cystatin C for prediction of multi-vessel disease by coronary angiography in type 2 diabetes mellitus patients with normal renal function

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The chronicity of diabetes mellitus often results in both micro and macro vessel complications. Although a good glycemic control largely results in better outcomes, a sizable number of patients still end up with complications. The challenge lies in the fact that it is very difficult to predict these in the early stages of diabetes. With the improved understanding of the pathogenesis involved, the attempt is to develop/identify biomarkers suggesting the impending complications. These would be beneficial as therapeutic targets too. We therefore included cystatin-c and tried to study its utility as a pre-clinical marker for CAD and compared it with ICAM-1 levels. In conclusion, the elevated ICAM-1 levels in diabetes patients is better marker of preclinical CAD as compared to cystatin-c, which was earlier hypothesized to have a predictive role. The study included normoalbuminuric individuals. However, it is observed that urinary microalbumin was significantly higher in the early diabetic group albeit within the normal range for microalbuminuria. The lack of correlation of both cystacin c and ICAM-1 with the study parameters calls for considering serial monitoring of microalbumin from the initial phase of diabetes to assess deterioration of renal functioning. ICAM 1 may be further explored as an ED marker.

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Conflict of Interest: The authors declare no conflicts of interest

Contributions: The study was conceptualized and planned by Dr. Poornima A Manjrekar (guide), project execution, data collection and manuscript preparation was done by Dr. Nisha Jha (post graduate student), review follow up of the study and proof reading was done by Dr. Anupama Hegde and Dr. Rukmini MS. Data analysis and manuscript editing was done by Yalla Durgarao.

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