ASSOCIATION OF CYSTATIN C, A MARKER OF PRE-CLINICAL RENAL DYSFUNCTION WITH ICAM-1 (INTERCELLULAR ADHESION MOLECULE-1) A MARKER OF PRECLINICAL CORONARY ARTERY DISEASE IN EARLY TYPE 2 DIABETES MELLITUS.

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AIM: To find the association of Cystatin-C, a marker of pre-clinical renal dysfunction with ICAM-1 (intercellular adhesion molecule-1) a marker of preclinical coronary artery disease in normoalbuminuric type 2 diabetes patients.

OBJECTIVES: To estimate Cystatin C and ICAM-1 in normoalbuminuric diabetic patients and compare them with normoalbuminuric healthy controls and to correlate cystatin-c with ICAM-1 and with markers of glycemic status.

METHODS: In this case control study, involving subjects of either sex, 31 normoalbuminuric diabetes patients aged 35-55 years with 2-7 years duration of diabetes and 31 age matched healthy controls on the basis of their Fasting Blood Glucose (FBG), Glycated haemoglobin (HbA1c) and spot urine microalbumin levels were selected. FBG was estimated by hexokinase method, HbA1c by HPLC method and spot urine microalbumin by immunoturbidimetric method on autoanalyzer. ICAM-1 and Cystatin-c were estimated by ELISA. Statistical analysis was done using SPSS version 20.

RESULT: FBG in cases and controls were 145±46mg/dl and 93±4mg/dl respectively with p value <0.001. HbA1c in cases and control were 8±1.44% and 5±0.02% respectively with p value <0.001. Difference in the values of Microalbuminuria (p-value-0.24) and Cystatin–c was not statistically significant between the two groups (p value 0.1) whereas ICAM-1 was found to be significant with p value <0.001.

CONCLUSION: Significant elevation of ICAM-I in cases of no known history of Coronary artery disease, indicates primordial endothelial dysfunction and inflammation. Cystatin-c does not prove to be a useful tool for assessment of coronary artery disease in early diabetic normoalbuminuric disease patients as compared to age matched normoalbuminuric healthy controls.

Keywords : Cystatin-c, ICAM-1, FBG, HbA1c, Microalbuminuria, Coronary Artery Disease, Diabetes Mellitus.

Title: Association Of Cystatin C, A Marker Of Pre-Clinical Renal Dysfunction With Icam-1 (Intercellular Adhesion Molecule-1) A Marker Of Preclinical Coronary Artery Disease In Early Type 2 Diabetes Mellitus.

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Introduction: Diabetes has long been known as a risk factor for coronary heart disease and is conservatively estimated to increase the risk of a fatal event by two fold[2.] Endothelial dysfunction and atherosclerotic plaque formation are the main processes which contribute to the development of micro- and macrovascular complications in patients with diabetes. Authors have advocated ICAM-1expression to be an early event in atherosclerotic lesions and its levels subsides after withdrawing the risk factor[4] like, rich cholesterol diet acts as an inducing factor in ICAM-1 expression[4]. Cystatin C has been shown to be associated with insulin resistance and inflammation independent of the renal function[7].

Aim: To find the association of Cystatin-C, a marker of pre-clinical renal dysfunction with ICAM-1 (intercellular adhesion molecule-1) a marker of preclinical coronary artery disease in normoalbuminuric type 2 diabetes patients.

Objectives: To estimate Cystatin C and ICAM-1 in normoalbuminuric patients with history of 2-7 years diabetes mellitus. Compare them with normoalbuminuric healthy controls to establish the utility of cystatin-c as a marker of endothelial dysfunction. To correlate cystatin-c with ICAM-1 and with markers of glycemic status (Fasting Plasma Glucose and HbA1c).

Materials and Methods: Case control study involving subjects of either sex were selected from KMC Hospital, Ambedkar Circle, Mangalore. Two such groups were taken up to form a total sample size of 62. Two such groups were taken up to form a total sample size of 62.

31 normoalbuminuric diabetes patients aged 35-55 years with 2-7 years of diabetes history with no known history of nephropathy and CAD were taken as diabetes group and 31 age matched healthy controls on the basis of their FBG, HbA1c and spot urine microalbumin levels were selected for the study. Blood glucose - estimation was done by automated analyzer using commercially available kits by the GOD –POD enzymatic method**.** Blood HbA1c was estimation was done by Bio-Rad D-10 automated analyser based on high performance liquid chromatography (HPLC). Spot urine microalbumin estimation was done by using automated analyser by immuno-turbidimetric method. Serum Cystatin C was analysed by Solid phase enzyme-linked immunosorbent assay (ELISA) in ELx 800 ELISA reader and kit from BIOVENDOR® Instruments, Inc. ICAM-1 was estimated by Solid phase enzyme-linked immunosorbent assay (ELISA) and the kit procured from DIACLONE® Instruments, Inc.

Statistical analysis done by SPSS version 20. Continuous variables are expressed as mean ± standard deviation (normal distributed data) and median interquartile range (skwed

data). Data analysis done using student independent 't' test and Mann-Whitney U test for group comparison, correlation analysis done by Pearson's correlation coefficient. Categorical P <0.05 were considered significant.

Results:

Table 1. COMPARISON BETWEEN CONTROL AND CASES

Parameters	Control Group (n = 31)	Diabetes Group (n = 31)	p-value
Age	45±6	50±5	0.001*
Gender M/F$	15/16	22:09	0.07
DM_duration	0	5±1.5	<0.001*
FBS	93±4	145±46	<0.001*
HbA1c	5±0.20	8±1.44	<0.001*
Urin_microalb	3 (3, 4)	5 (3, 7.5)	0.01*
ICAM-1	630±266	857±233	0.001*
Cystatin	1118±263	1229±458	0.24

Table. 1.
When comparison between control and cases were done, significant p value <0.001 was found for age, diabetes duration, fasting blood glucose and HbA1c and ICAM-1 respectively.
P value <0.01 was also found significant for urine microalbumin.

Table-2. COMPARISON BASED ON DURATION OF DIABETES

Parameters	DM_Duration < 5 years (n=9)	DM_Duration > 5 Years (n=22)	p-value
Age	50±5	49±4	0.5
FBS	133±41	150±48	0.34
HbA1c	7±1	8±1	0.2
Uri_microalb	4±0.8	7±6	0.01*
ICAM-1	722±126	912±247	0.03*
Cystatin	1212±462	1236±466	0.89

Table. 2.
When duration of diabetes was compared for <5 years duration and >5 years duration, urine microalbumin showed significant p value as 0.01

Table-3. CORRELATION ANALYSIS WITH ICAM

Parameters	correlation	Controls (n = 31)	Cases (n = 31)
Age	r	0.06	-0.341
	p	0.73	0.06
DM Duration	r	0	0.46
	p	0	0.01*
FBS	r	0.1	0.14
	p	0.57	0.42
HbA1c	r	0.4	0.18
	p	0.02*	0.31
Microalb	r	0.13	0.22
	p	0.47	0.23
Cystatin C	r	-0.39	0.01*
	p	0.83	0.96

Table.3.when comparison between ICAM-1 done with other factors like age, Diabetes Mellitus duration, FBS, HbA1c, urine microalbumin and cystatin-c. p value 0.01 was found to be significant for duration of diabetes.

Table-4. CORRELATION ANALYSIS WITH CYSTATIN C

Parameters	correlation	Controls (n = 31)	Cases (n = 31)
Age	r	0.39	0.33
	p	0.03*	0.06
DM Duration	r	0	-0.01
	p	0	0.98
HbA1c	r	0.22	0.2
	p	0.22	0.27
Microalb	r	-0.03	0.02
	p	0.85	0.91
FBS	r	0.45	-0.12
	p	0.01*	0.51

Table.4. When correlation analysis of cystatin c was done with other factors no significant p value was found.

DISCUSSION

Diabetic patients often are unaware of myocardial ischemic pain, and so silent myocardial infarction and ischemia are markedly increased in this population. There is a high chance of developing sudden cardiac death in those with diabetes. [10]

There is a high chance of developing sudden cardiac death in those with diabetes. Decreased kidney function is associated with adverse cardiovascular outcome. Cystatin C is a novel measure of kidney function that appears to have even stronger associations with mortality[10]

A case-control study done by Kim *et al*, was done to assess the relationship between serum cystatin C level and coronary artery disease in diabetic patients. Among 460 diabetic patients, 38 diabetic patients had CAD. The control group consisted of 38 diabetic patients who were matched to cases by age, sex, and presence/absence of diabetic nephropathy. Serum cystatin C level was found to be significantly higher in patients with diabetic nephropathy, both in CAD and non-CAD patients. However, serum cystatin C level did not differ between CAD and non-CAD patients, regardless of diabetic nephropathy. Hence we can conclude that, Serum cystatin C level is a marker of renal dysfunction, but not coronary artery disease, in diabetic patients.[18]

The study was conducted in Department of Biochemistry, RIMS in collaboration with Dept of Medicine, RIMS, Imphal, Manipur for a period of two years[10]. 60 patients were divided into two subgroups of 30 patients each. Dm-Group: DM-2 (controlled or without complications) and Dm+Cad -Group: DM-2 with confirmed cardiovascular complications. a significant difference of mean cystatin C as $P < 0.001$ was recorded among all the three groups, being indicating DM group p-value highest with 1.0 while DM+CAD group with 0.93, and the lowest in Control with 0.74[10]. As per the above finding, it was concluded that cystatin C differs significantly from Control group as well as from DM group to DM+CAD group.

In the current study main aim was to estimate Cystatin-c and ICAM-1 level in the serum of T2DM patients and to find their correlation with the FBG, HbA1c and random microalbumin levels. The present study found similar mean levels of serum cystatin c in diabetics cases without coronary artery disease in comparison to controls[14].

Cystatin C is less affected by age, sex and muscle mass, which may explain why cystatin C seems to better estimate true renal function[17]. Kim *et al* reported that they found no association between serum cystatin C level and CAD in diabetic patients and that serum cystatin C levels were significantly higher in patients with diabetic nephropathy than in patients without both in CAD patients and non-CAD patients[18].
A mendelian randomization study conducted by Sander W *et al,* Fall T, Soumaré A, Teumer A, Sedaghat S *et al* incorporated participant data from 16 prospective cohorts, Mendelian randomization analyses did not support a causal role of cystatin C in the etiology of CVD[16].

Bhat K *et al* conducted a study including 100 subjects, 50 healthy persons and 50 diabetic patients were analysed. The diabetic subjects were grouped into two those with cardiac complications (n=25) and those without cardiac complications (n=25). Statistically signifi cant (t= 9·5; p<0·001) difference was seen in Cystatin C levels between healthy and diabetic subjects. Although the Cystatin C level in diabetics with cardiac complications was marginally higher but the difference between two groups were statistically insignificant[5].

ICAM-1 correlates with the development and expansion of atherosclerotic lesions, the soluble molecule could be used to reflect the extent of the lesions. The expression of ICAM-1 increased significantly compared with that in the control group. Early intervention in Total Cholesterol levels can interrupt the development of atherosclerotic lesions through inhibiting the expression of ICAM-1. A study conducted by yiqing *et al*, suggests that ICAM-1 is not only elevated among diabetes patients but also among non diabetes individuals with insulin resistant, obesity, hypertension and dyslipidemia[28].
Microalbuminuria was measured on a spot of morning urine after an overnight fast that has been shown to be a reliable screening procedure for microalbuminuria. It provides semi-quantitative estimation of urinary albumin excretion adjusted to creatininuria, and determines participants with microalbuminuria (30–300mcg albumin/g creatinine) and those with normal albuminuria (<30 mcg/mg)[20] .

Rotondi N *et al*, found in their study that Microalbuminuria, but not cystatin C, is associated with carotid atherosclerosis beyond traditional cardiovascular risk factors among middle-aged adults. Cystatin C does not have a stronger relationship with carotid atherosclerosis in middle-aged adults than creatinine[20.] Microalbuminuria is considered to be a measure of generalized vascular leakiness for albumin secondary to endothelial dysfunction[21]

ICAM-1 play important roles in the firm attachment and transendothelial migration of leukocytes. These molecules have been observed consistently within the milieu of the atherosclerotic plaque[31]. Results of immunohistochemical studies show different levels of expression of these molecules that reflect their unique structural and functional characteristics.[31]

The present study is based on the hypothesis that circulating levels of ICAM-1 may be useful markers for increased expression of cellular adhesion molecules in atherosclerosis. The ICAM-1 was compared between patients and control subjects. The presence of ICAM-1 has been observed on normal arterial endothelium, consistent and strong expression of ICAM-1 showing its association with atherosclerotic lesions.[31]

CONCLUSION

We have found that on comparison between normoalbuminuric diabetes patients aged 35-55 years with a history of 2-7 years of diabetes duration with no known history of nephropathy and no history of CAD were compared with age matched apparently healthy controls on the basis of their age, sex, duration of diabetes, fasting blood glucose, HbA1c, spot urine microalbumin, ICAM-1 and Cystatin-C levels.

Significant difference was found for age, diabetes duration, fasting blood glucose and HbA1c and ICAM-1 respectively. When duration of diabetes was compared in cases for <5 years and >5 years duration of diabetes urine microalbumin was found to be affected by it.

ICAM-1 was increased in diabetes patients and showing its role in prediction of preclinical CAD as compared to cystatin-c, which was earlier thought of having its significant role in predicting pre-clinical CAD. Cystatin c was found to have no role in detection of preclinical CAD in young early diabetes patients with no known history of nephropathy and CAD.

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