

Correlation of Cystatin C and Cardiovascular Risk Markers in Uncontrolled Type 2 Dm

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ABSTRACT

Renal dysfunction is a major risk factor for cardiovascular disease. Cystatin C is identified as a promising marker of renal dysfunction and has emerged as a biomarker of cardiovascular risk. This study aims to estimate and correlate the levels of serum Cystatin C with cardiovascular risk markers in Type 2 Diabetic patients with and without glycemic control. The study population included Type 2 Diabetic patients aged between 35 and 60 years of either sex. Among 320 Diabetic patients, 120 patients were recruited and divided into group A (n= 54) with HbA1c \leq 6.5% Hb and group B (n=66) with HbA1c $>$ 6.5% Hb. Fasting blood samples were analyzed for FBG, Total cholesterol, TGL, HDLc, LDLc, Creatinine and HbA1c. Serum Cystatin C was estimated by immunoturbidimetric method and hs-CRP by particle enhanced immunoturbidimetric method. Non-HDLc and Cardiovascular risk ratios TC/HDLc and LDLc/HDLc was calculated. Diabetic patients with poor glycemic control (HbA1c $>$ 6.5%) had statistically significant higher value of serum cystatin C, hs-CRP, TC, TGL, LDLc and low HDLc as compared to diabetic patients with good glycemic control. Our data revealed high serum cystatin C concentration (1.30 ± 0.42 mg/L) which correlated well with the elevated endothelial inflammatory marker hs-CRP (14.02 ± 8.33 mg/L) and also with the mean of non-HDLc (177.3 ± 44.57 mg/dL). In the study, serum Cystatin C showed a positive correlation with HbA1c ($r = 0.9$) and with Cardiovascular risk markers hs-CRP ($r = 0.89$), non-HDLc ($r = 0.66$), TC: HDLc ($r = 0.48$) and LDLc: HDLc ($r = 0.72$). Serum Cystatin C, a preclinical marker of renal dysfunction can be used as a predictive marker of diabetic dyslipidemia and cardiovascular risk in poorly controlled Type 2 Diabetic patients.

Keywords: Cystatin C, HbA1c, TC/HDLc ratio, LDLc/HDLc ratio.

INTRODUCTION

Cardiovascular disease is the main cause of death in diabetic patients. Cystatin C, a marker of renal dysfunction⁽¹⁾. It is a naturally occurring cysteine protease inhibitor, encoded by the CST 3 genes. It is a low molecular weight (13.3 KDa) protein, produced by all nucleated cells at a constant rate⁽²⁾.

Unlike serum creatinine, cystatin C is considered as a reliable marker for evaluation of renal function, because it is freely filtered across the glomerular membrane, almost completely reabsorbed and not influenced by extra-renal factors like age, sex, muscle mass, exercise or diet^(3,4). Cysteine protease Cathepsins are a group of lysosomal proteolytic enzymes, which includes Cathepsins B, H, L, S and C that are involved in pathological mechanisms of inflammation, tumor invasion, breakdown of collagen and bone resorption⁽⁵⁾. It has been suggested that cystatin C can predict the risk of developing Chronic kidney disease, thereby signals the preclinical renal dysfunction. Studies have reported that renal dysfunction is a plausible link between increased cystatin C and impaired cardiovascular outcome. Also recently, it has been suggested that high cystatin C concentration are directly related to inflammation and atherosclerosis⁽⁶⁾.

A study had demonstrated the elastolytic activity of Cathepsins S, K and V which degrades the components of arterial extracellular matrix. The local imbalance between

elastolytic activity of Cathepsin and their inhibitor cystatin C promotes neovascularisation leading to microvessel formation and recruitment of inflammatory cells with accumulation of plasma lipids⁽⁷⁾. Thus studies have evidenced that cysteine proteases is a proatherogenic factor and increased concentration of cystatin C, a cathepsin inhibitor reflects a counterbalance of the damaging increased elastolytic activity, which are involved in the pathogenesis of atherosclerosis⁽⁸⁾. Few data have reported that serum cystatin C strongly predicts cardiovascular events and mortality than serum creatinine and estimated GFR⁽⁹⁾. Therefore, a better understanding is possible with serum cystatin C, endothelial inflammatory marker and cardiovascular risk marker to connect renal / heart dysfunction.

OBJECTIVE OF THE STUDY

The aim of the present study was to study the levels of serum cystatin C and correlate with hs-CRP, non-HDLc, TC / HDLc ratio and LDLc/HDLc ratio to assess the cardiovascular risk in type 2 Diabetic patients.

MATERIALS AND METHODS

The sample size and design of the study: Among 320 diabetic patients who visited the Diabetic outpatient clinic of our hospital, 120 patients were included in the study. Ethical clearance was obtained from the institutional

Table 1: Age, FBS and HbA1c values in male and female type 2 Diabetic patients.

Parameters	Male Type 2 DM n = 78 (Mean \pm S.D.)	Female Type 2 DM n = 42 (Mean \pm S.D.)	P value
Age (years)	52.82 \pm 9.35	53.22 \pm 9.2	0.9
FBS (mg/dL)	137.9 \pm 33.05	138.20 \pm 28.17	0.82
HbA1c % Hb	8.61 \pm 2.13	8.8 \pm 1.29	0.49

P value < 0.05 is considered significant

Values are expressed in Mean and Standard Deviation (S.D.)

Table 2: Biochemical parameters categorized by patients glycemic control (HbA1c)

Parameters	Group A Type 2 DM (n= 54) HbA1c \leq 6.5% Hb	Group B Type 2 DM (n=66) HbA1c > 6.5% Hb	P value
Total cholesterol mg/dL	169 \pm 33.02	227.3 \pm 47.76	0.005
Triglycerides mg/dL	66.7 \pm 17.02	129.1 \pm 76.39	0.004
HDLc mg/dL	45 \pm 6.08	32 \pm 5.79	0.17
LDLc mg/dL	85.72 \pm 16.07	143 \pm 29.5	0.002
Non-HDLc mg/dL	120 \pm 3.8	177.3 \pm 44.57	0.009
TC/HDLc ratio	3.83 \pm 0.48	6.2 \pm 1.24	0.019
LDLc/HDLc ratio	2.01 \pm 0.45	4.62 \pm 1.24	0.031
Cystatin C mg/L	0.6 \pm 0.22	1.34 \pm 0.42	0.017
hs-CRP mg/L	1.9 \pm 1.5	14.02 \pm 8.33	0.012

P value < 0.05 is considered significant

Values are expressed in Mean and Standard Deviation (S.D.)

Ethical Committee and informed consent was obtained from all the patients.

The study population: Inclusion criteria: Type 2 Diabetic patients aged between 35 to 60 years of either sex and duration of history of diabetes mellitus more than 1 year and less than 5 years were recruited. Divided into group A (n= 54) with HbA1c \leq 6.5% Hb and group B (n=66) with HbA1c > 6.5% Hb.

Exclusion criteria: Diabetic patients with infections, chronic kidney disease, hypertension, angina and acute coronary syndrome, coronary bypass surgery or percutaneous coronary interventions were excluded.

Fasting venous blood samples were collected from all the subjects. The serum sample was analyzed with Beckman Coulter reagents in Olympus AU 400 auto analyzer for fasting blood glucose (FBG), Total cholesterol (TC), Triglycerides, HDLc, creatinine by enzymatic method; LDLc by direct turbidimetric method. HbA1c was analyzed in whole blood sample by Immuno turbidimetric method. Serum cystatin C was estimated by Immuno turbidimetric method (Accurex) and hs-CRP by Particle enhanced Immuno turbidimetric method (Diasys) and the levels were expressed as mg/L.

Non-HDLc, Risk ratio (TC/HDLc) and LDLc/HDLc were calculated. For serum lipid reference level, National Cholesterol Education Program (NCEP) Adult Treatment Panel (ATP III) guideline was referred. Dyslipidemia was defined by presence of one or more abnormal serum lipid concentration. Diabetes was defined as per American Diabetes Association (ADA) criteria. Value of HbA1c was given as % of total Hb.

STATISTICAL ANALYSIS

Statistical analysis was performed with student's test and the results are expressed as mean \pm standard deviation

(S.D.). $P < 0.05$ was considered statistically significant. Pearson's correlation co-efficient was used to examine correlation between various parameters.

RESULTS

The data of the diabetic patients are expressed as mean \pm S.D. as on table (1). Among 320 type 2 DM patients 120 patients were included in this study, 78 were male and 42 were female subjects. The mean age \pm S.D. of male and female subjects were 52.82 \pm 9.35 years and 53.22 \pm 9.2 years respectively. The mean of HbA1c and FBG had no statistically significant difference between male and female patients.

In table (2), diabetic patients were classified into 2 groups as per their glycemic index. Group A (n= 54) consist of type 2 DM with HbA1c \leq 6.5% Hb Group B (n = 66) consist of type 2 DM with HbA1c > 6.5% Hb. Patients with HbA1c > 7% Hb had significantly higher value of serum cystatin C, hs-CRP, TC, triglycerides, LDLc, non-HDLc, TC/HDLc ratio and LDLc/HDLc ratio as compared to patients with HbA1c \leq 6.5% Hb figure (1). Correlation between serum cystatin C and cardiovascular risk markers are shown in table (3). Serum cystatin C showed a positive correlation with HbA1c, hs-CRP, non-HDLc, cardiovascular risk marker TC/HDLc and LDLc/HDLc ratio respectively.

DISCUSSION

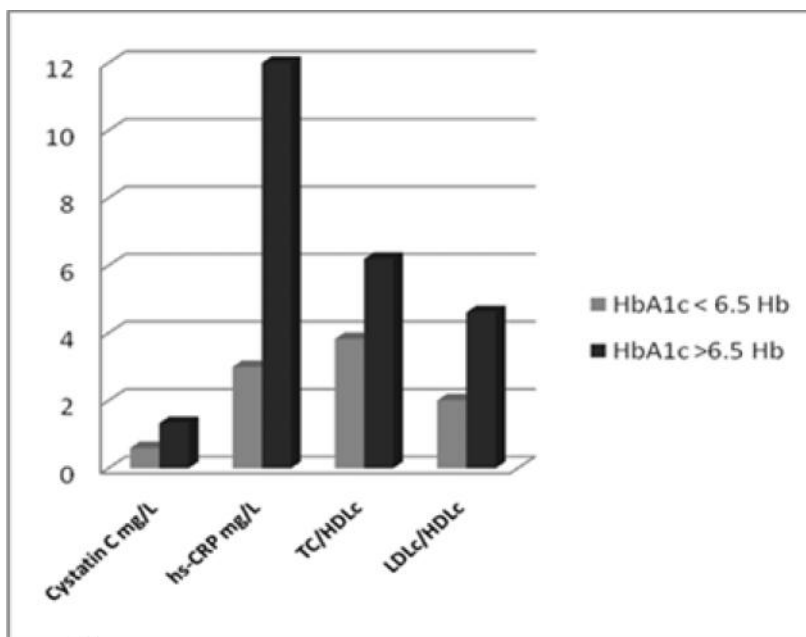
Cystatin C, an endogenous Cathepsin protease inhibitor is emerging as a marker of both chronic kidney disease and cardiovascular risk. It is considered to be a sensitive early marker of renal dysfunction. Though, Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation is a better means to estimate eGFR, the data of diabetic patients comparing the CKD-EPI and MDRD

Table 3: Correlation between cystatin C and cardiovascular risk ratios

Parameters	r - value	Correlation
HbA1c % Hb	0.9	'+'
hs-CRP	0.89	'+'
Non-HDLc	0.66	'+'
TC/HDLc	0.48	'+'
LDLc/HDLc	0.72	'+'

'+' : indicates positive correlation

Fig 1: Comparison of Cystatin C and Cardiovascular risk markers in DM patients with good and poor glycemic control (HbA1c)



equations are still limited⁽¹⁰⁾. Epidemiological studies indicate that serum cystatin C is superior to serum creatinine as an estimate for GFR and can be used to detect mild renal functional impairment and rapid changes in GFR.

In the present study, about 55% of diabetic patients had a poor glycemic control. Chronic hyperglycemia is associated with glycosylation of every protein in the body including lipoprotein, apolipoprotein, clotting factors etc. Overtime, a complex series of oxidation reaction results in formation of Advanced Glycation End (AGE) products. AGE induces excessive cross linking of collagen and other extracellular matrix proteins over the vascular wall, which leads to accumulation of LDL particles⁽¹¹⁾. Furthermore, AGE modified LDLc has prolonged half life and has direct effect on vascular endothelial cells which promotes atherothrombotic events.

In this present study, serum cystatin C is increased in DM with poor glycemic status. Like creatinine, the elimination of cystatin C is by glomerular filtration in the kidneys. Thus serum levels of cystatin C is elevated when kidneys function and glomerular function declines. Studies have reported that cystatin C can predict the risk of developing CKD, thereby signals the preclinical state of renal dysfunction^(12, 13).

Inflammation associated with atherogenic changes may be one of the mechanisms associated with cystatin C and cardiovascular risk. Our data revealed a correlation between cystatin C and hs-CRP ($r = 0.89$), suggesting that elevated cystatin C concentration are directly related to both elastolytic cysteine proteases and their inhibitor cystatin C are involved in the pathogenesis of atherosclerosis. This study reveals high prevalence of increased cholesterol, TGL, LDLc and low HDLc levels which are well known risk factors for cardiovascular disease. Interestingly, non-HDLc levels were elevated in DM with poor glycemic status and significantly correlated with cystatin C values ($r = 0.66$). TC/HDLc ratio and non-HDLc are considered to be better reflectors of apo-B containing particles⁽¹⁷⁾. According to National Cholesterol Education Program Adult Treatment Panel III, non-HDLc is recommended to assess the CVD risk in diabetic patients. These results are in accordance with the study done by Parick et al⁽¹⁴⁾. The authors showed that high cystatin C concentration is independently associated with cardiovascular risk factors and had a worse clinical risk profile.

Risk ratio TC/HDLc showed a strong correlation with serum cystatin C in our study. Some studies have found that non-HDLc and TC/HDLc are better predictors of apo

B containing particles, thereby predicts diabetic dyslipidemia⁽¹⁵⁾. Similarly, LDLc/HDLc ratio was significantly elevated in diabetics with poor glycemic status and had a good positive correlation with cystatin C levels. Metabolic reasons for increased LDLc/HDLc ratio could be i) decreased synthesis or clearance of HDLc ii) LDLc iii) LDL uptake by fibroblast is impaired. Epidemiological studies have found the LDLc/HDLc ratio as an excellent predictor of CHD risk. Khan HA et al showed the impact of glycemic control on various lipid parameters in which severity of dyslipidemia increases in patients with poor glycemic status⁽¹⁶⁾. Thus HbA1c and dyslipidemia are considered as independent risk factors of CVD. Hence based on the results derived from the present study, it could be inferred that an increase in serum cystatin C and cardiovascular risk ratios explains the state of pre-clinical kidney disease and the risk of progression to future cardiovascular events in type 2 diabetes mellitus.

CONCLUSION

Cystatin C is a potential and sensitive marker that signals the state of preclinical renal dysfunction. Our study showed an increase of serum cystatin C and correlated well with HbA1c, hs-CRP, non-HDLc and cardiovascular risk ratios: TC/HDLc and LDLc/HDLc, which can assess the dyslipidemic and proatherogenic status of the DM patients. Thereby, cystatin C could serve as a good predictive marker of preclinical renal and cardiovascular dysfunction in type 2 diabetes mellitus.

LIMITATIONS

However, our study is limited in that the study was conducted in a small sample size. Further it is not clear whether CVD has a direct effect of elevation of cystatin C. Thus, the relation need to be established further linking the molecular pathways and provides future directions for the study.

Conflict of Interest: none declared

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