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Research Article

Prognostic Significance of Cystatin C in Coronary Artery Disease

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Abstract: Cystatin C, an established marker of renal dysfunction, is gaining importance in dysfunction of other organs (systems) as well. Preliminary studies indicated a role for cystatin C as a prognostic marker in coronary artery disease (CAD). The aim of the study was to assess the of serum cystatin C levels in CAD cases and its comparison with controls. Comparison of serum cystatin C levels in CAD spectrum. Comparison of serum cystatin C levels in CAD cases based on risk factors,body mass index (BMI) and waist circumferance(WC).cystatin C levels in CAD cases based on risk factors,body mass index (BMI) and waist circumferance(WC). Study group comprised of 145 patients diagnosed as having CAD based on clinical and bio-chemical criteria. Control group included 66 age and sex matched subjects (non CAD cases) using the above mentioned criteria. In this study, significant increase of mean serum cystatin C levels was observed in CAD cases than controls. Highest mean cystatin C values were observed in Myocardial infarction (MI) than Unstable angina (UA) and Stable angina (SA).Highest mean cystatin C values were CAD cases with risk factors. Highest mean cystatin C values were CAD with increased BMI and WC. Cystatin C plays an important role in the development of CAD and serum cystatin C is a might have a role as a prognostic marker in patients with CAD.
Keywords: Coronary artery disease, Atherosclerosis, Cystatin C, Cathepsins, Extracellular matrix, Tissue remodeling,

INTRODUCTION

In recent years cystatin C has emerged as a potential marker for cardiovascular risk and predicts the cardiovascular events. Cystatin C is a naturally occurring protease inhibitor that protects the host tissue from cysteine proteases, which is a proatherogenic factor. Cystatin C is a reliable marker of renal functions and its plasma concentration is dependent completely on glomerular filtration rate (GFR) and emerged as a biomarker of cardiovascular risk.

Cystatin C is a non-glycated, low molecular basic protein that is a member of cystatin family of cysteine protease inhibitors. Cysteine protease comprises a group of lysosomal proteolytic enzymes, which includes cathepsins involved in pathological processes such as inflammation, tumor invasion, break down of collagen and bone resorption.The production of cystatin C is regulated by housekeeping genes expressed in all nucleated cells [1, 2].

Coronary artery disease (CAD) is the leading cause of death and is a major health burden worldwide [3]. One fifth of all deaths are due to CAD. By the year 2020, it will account for one third of all deaths. There are an estimated 45 million patients of CAD in India. Early and accurate diagnosis of coronary disease is very essential as it is associated with significant morbidity and mortality [4]. CAD is a condition in which there is an inadequate supply of blood and oxygen to a portion of the myocardium. The clinical spectrum of CAD is stable angina (SA), unstable angina (UA) and myocardial infarction (MI) [5].

Atherosclerosis is underlying cause of CAD. The pathophysiological origin of this disease depends on the formation and the 'stability' of atherosclerotic plaque [6]. Atherosclerosis is the result of a complex interaction between blood elements, disturbed flow, and vessel wall abnormality; involving several pathological processes: inflammation, with increased endothelial permeability, endothelial activation, monocyte

recruitment; growth and proliferation of the smooth muscle cells (SMCs) and lipid accumulation and necrosis. All these processes cumulatively lead to the formation of plaque [7, 8].

MATERIALS AND METHODS

The study was conducted in the department of biochemistry, Mamata Medical College and General Hospital, Khammam, India. The patients attending outpatient and wards of cardiology and general medicine departments of hospital and local cardiac centers were included in this study. All subjects were informed about the study and written informed consent was obtained from the patients admitted. The study was approved by the institutional ethical committee.

Study design

It was Cross-sectional comparative study.

Stastistical analysis

It was done using statistical analysis of software (SAS), version 9.3 and tests used were Analysis of variance[ANOVA] , Students' "t' test and Multiple comparison test.The results are expressed as mean ± standard deviation (SD). P< 0.05 was considered statistically significant.

Subjects

Study group comprised of 145 patients diagnosed as having CAD based on clinical and bio-chemical criteria using electrocardiogram(ECG), echocardiogram, cardiac biomarkers (myocardial enzymes and troponin) and tread mill test (TMT). Among these 145 CAD cases, 31 were diagnosed as SA , 32 were diagnosed as UA and 82 were diagnosed as MI .

Inclusion criteria

- Subjects in the age group of 30-50
- Subjects with risk factors diabetes mellitus(DM), hypertension(HTN) and smoking
- Subjects with DM, assessed based on history and WHO criteria
- Subjects with HTN, assessed based on history and JNC-7 criteria
- Subjects with normal kidney function

Exclusion criteria

- Alcoholics
- Subjects with of past history of CAD
- Subjects with altered kidney function (random urinary protein > 16 mg/dl where as serum creatinine > 0.9-1.3 mg/dl in males and 0.6 -1.1 mg/dl in females) [9].

Controls

66 sex and age matched subjects were recruited as control group (non CAD cases) using the same criteria.

Method: Immunoturbidimetric

Normal range of cystatin C: 0.55-1.2 mg/L

RESULTS:

CAD cases vs controls

Mean cystatin C value was significantly increased in CAD cases than controls (Table1a).

SA vs **UA** vs **MI**

In the spectrum of CAD (SA, UA and MI) mean cystatin C values have shown incremental increase. By anova p- value was significant (Table1a).

Multiple comparison test of spectrum

Cystatin C was significant among the groups except SA and UA(Table1b).

Distribution of cystatin C based on upper limit

When the upper limit of 1.2 mg/L was considered, 71 % had more than 1.2 mg/L in CAD cases **(**65 % CAD without risk and 77 % with risk**).**Only 15 % had more than 1.2 mg/L in controls **(**10 % in controls without risk and 19 % in controls with risk).

Controls without risk vs Controls with risk vs CAD without risk vs and CAD with risk

Mean cystatin C has shown significant incremental increase from controls without risk followed by controls with risk, CAD without risk and CAD with risk (Table 2).

Controls with normal BMI vs Controls with increased BMI vs CAD with normal BMI vs CAD with increased BMI

Cystatin C has shown incremental increase of mean values, from controls with normal BMI to controls with increased BMI, CAD with normal BMI and CAD with increased BMI. Highest mean value was observed in CAD with increased BMI (Table 2).

Controls with normal WC vs Controls with obese WC vs CAD with normal WC vs CAD with obese WC

Cystatin C has shown incremental increase of mean values, from controls with normal waist followed by controls with obese waist, CAD with normal waist and CAD with obese waist and increases were significant. Highest mean value was observed in CAD with obese waist (Table 2).

Multiple comparison test

p< 0.05 was considered statistically significant among the CAD cases , control groups and sub groups(Table 3).

Table 1a: Mean ± SD of Cystatin C of Controls (1) CAD cases (2) and SA (3), UA (4) and MI (5)

Parameter	Mean ± SD (1)	Mean ± SD (2)	P-value	Mean ± SD (3)	Mean ± SD (4)	Mean ± SD (5)	p-value
Cystatin C	0.9738 ± 0.2067	1.3883 ± 0.3822	< 0.0001	1.24 ± 0.43	1.33 ± 0.27	1.46 ± 0.38	0.01

Table 1b: Multiple comparison test: SA vs UA vs MI

Variable		SA	UA	MI
Cystatin C	SA		0.36	0.012
	UA	0.36		0.04
	MI	0.012	0.04	

Table 2: Mean ± SD of cystatin C

Controls without risk (1), Controls with risk (2), CAD without risk (3) and CAD with risk (4)					
Parameter	**Mean ± SD (1)**	**Mean ± SD (2)**	**Mean ± SD (3)**	**Mean ± SD (4)**	**p-value**
Cystatin C	0.8400 ± 0.1812	1.0853 ± 0.1549	1.3191 ± 0.3136	1.4462 ± 0.4247	< 0.0001
Controls with normal BMI (1), Controls with increased BMI (2), CAD with normal BMI (3) and CAD with increased BMI (4)					
Cystatin C	0.9500 ± 0.2089	0.9962 ± 0.2052	1.3552 ± 0.3568	1.4037 ± 0.3943	< 0.0001
Controls with normal WC (1), Controls with obese WC (2), CAD with normal WC (3) and CAD with obese WC (4)					
Cystatin C	0.9450 ± 0.2150	0.9882 ± 0.2034	1.2894 ± 0.3105	1.3966 ± 0.3854	<0.0001

Table 3: Multiple comparison test

Controls without risk (1) vs Controls with risk (2) vs CAD without risk (3) vs CAD with risk (4)					
Variable		**1**	**2**	**3**	**4**
Cystatin C	1		0.0028	< 0.0001	< 0.0001
	2	0.0028		< 0.0001	0.0007
	3	< 0.0001	< 0.0001		0.0211
	4	< 0.0001	0.0007	0.0211	
Controls with normal BMI (1), Controls with increased BMI (2), CAD with normal BMI (3) and CAD with increased BMI (4)					
Variable		**1**	**2**	**3**	**4**
Cystatin C	1		0.3	0.001	0.001
	2	0.3		0.001	0.001
	3	0.001	0.001		0.4
	4	0.001	0.001	0.4	
Controls with normal WC (1) vs Controls with Obese WC (2). CAD with normal WC (3) vs CAD with obese WC (4).					
Variable		**1**	**2**	**3**	**4**
Cystatin C	1		0.6121	0.0002	< 0.0001
	2	0.6121		< 0.0001	< 0.0001
	3	0.0002	< 0.0001		0. 1053
	4	< 0.0001	< 0.0001	0.1053	

DISCUSSION

Cystatin C: A marker of inflammation and atherogenesis

Cystatin C an endogenous inhibitor of cysteine proteases has emerged as biomarker of cardiovascular risk[2]. In the present study serum cystatin C was increased in CAD cases than controls. The results are in accordance with the study done by Azza Dandana *et al*. and Aditya Batra *et al*. [10, 11]. In the spectrum of CAD highest mean values were observed in MI group than UA and SA in the present study. Similar results were reported by Changjiang Ge *et al*. [12] and Gu Fei-fei *et al*. [13] Highest mean cystatin C values were observed in CAD with risk factors in the present study. The results are in accordance with the study done by Osama Tayeh *et al*. [14]. Higher cystatin C values were observed with increased BMI and WC in the present study. Similar results were reported by Deepa *et al*. [2] and Nadia Nour *et al*. [15].

Increased cystatin C levels are associated with high concentrations of C-reactive protein (CRP) which shows the link between inflammation and atherogenesis that contributes to cardiovascular risk [16,17]. There is an evidence that both elastolytic cysteine proteases and their inhibitors, an important one being cystatin C are involved in the pathogenesis of atherosclerosis. The elevation of cystatin C has been attributed to imbalance between proteases and inhibitors which determines the net effects on the cardiovascular system [18-21].

Remodeling of the extracellular matrix (ECM) is an important feature of many physiological and pathological processes. The ECM consists of elastins, collagens, and proteoglycans and is largely synthesized by smooth muscle cells (SMCs). Proteolytic enzymes, such as matrix metalloproteinases (MMPs) and cathepsin cysteine proteases, can degrade the ECM and contribute to pathophysiological processes, like atherosclerosis [22]. Cathepsins of the cysteine protease family are localized in lysosomes and endosomes, and degrade intracellular or endocytosed proteins [19, 23]. Cathepsins are secreted by macrophages, smooth muscle cells and endothelial cells . Human cathepsins have the capability to degrade low density lipoprotein (LDL), as well as lipid uptake and reduce cholesterol efflux from macrophages, aggravating foam cell formation [22].

Alternately inflamed cytokines associated with atherosclerosis stimulate the production of lysosomal cathepsins, and increased plasma cystatin C, a cathepsin inhibitor –which further counterbalances a potentially damaging increased elastolytic activity. The cathepsins are involved in the progression, the composition and rupture of atherosclerotic plaques [23-25]. Cystatin C is involved in the human immune defense via human poly mononuclear cell chemotaxis [26]. When there is a vascular injury, cytokine production is increased which in turn stimulate the production of cathepsins. This is counter balanced by their most abundant inhibitor cystatin C, which plays a prominent role in the tissue remodeling, especially in the post infarction period [27, 28]. During the process of atherosclerosis cystatin C is released into the circulation [29]. Increased cystatin C levels may reflect CAD associated with inflammation and atherosclerosis [30]. Increase of cystatin C in obese individuals could arise from enlarged adipocytes and macrophages [2, 15].

CONCLUSION

Serum cystatin C can be utilized as another simple non-invasive marker for identification of vulnerable plaques, severity of disease and stratification of future risk.

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