

Cystatin C as a marker of Cardio metabolic disorder in obese South Indian individuals

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Abstract: Background: Human obesity is strongly associated with cardio metabolic disease. Cystatin C is a naturally occurring protease inhibitor and marker of cardiovascular disease. The main objective of present study was to estimate the serum levels of Cystatin C in individuals with normal BMI, and obese, aged between 18-39 Yrs and to compare the levels of serum Cystatin C among these individuals and to correlate the levels of serum Cystatin C with cardio metabolic risk factors.

Material & Methods: The study population was taken from healthy volunteers of Mysore city, aged between 18-39 years of either sex. The study population was divided into 2 groups based on BMI. Each group contains sample size of 45. Fasting serum sample was analyzed for Blood glucose, Total cholesterol, Total Triglycerides, Direct HDL cholesterol, Direct LDL Cholesterol by enzymatic method and serum Cystatin-C by immunoturbidimetric method using auto analyser.

Results: The serum Cystatin C levels was significantly increased in obese groups, p value < 0.001 . The mean serum Cystatin C levels in normal BMI group was 0.78 ± 0.03 , and in Obese group is 1.15 ± 0.09 . In the study serum Cystatin C showed a positive correlation with serum glucose ($r=0.61$) serum triglycerides ($r=0.7$), Atherogenic index of plasma (AIP) ($r=0.80$), TCHOL: HDL ($r=0.71$), HDL: LDL ($r=0.70$) respectively and negative correlation with serum HDL ($r=-0.52$)

Conclusion: Serum Cystatin C could serve as a good predictive marker of preclinical cardio metabolic disorder in obese individuals.

I. Introduction

Obesity is an epidemic of the 21st century and is a major causative factor for many other metabolic disorders and premature deaths in developing countries. The risk of cardiovascular disease, hypertension, hyperlipidemia, diabetes mellitus and certain cancers increases many folds in association with obesity [1]. Recent evidence shows that the distribution of fat during early adulthood is associated with increased metabolic disease risk in later adulthood [2,]. The increase in cardiovascular events has necessitated the identification of possible predictors that can help in predicting atherogenicity.

Cystatin C is a naturally occurring protease inhibitor and marker of cardiovascular disease. Cysteine protease cathepsin, is a pro-atherogenic factor which is produced by adipose tissue and is increased in obese subjects [3]. Cysteine proteases comprises a group of lysosomal proteolytic enzymes which includes cathepsin B, H, L, S and C that are involved in pathological processes such as inflammation, tumor invasion, break down of collagen and bone resorption. [4] The activities of Cysteine proteases are controlled by naturally occurring inhibitory proteins such as Cystatins and $\alpha 2$ macroglobulin. These inhibitors functions to protect host tissues from destructive proteolysis. Cystatin C is a non glycosylated low molecular weight basic protein that is a member of Cystatin super family of Cysteine protease inhibitors. The production of Cystatin C is regulated by housekeeping genes expressed in all nucleated cells [5]. With the increasing prevalence of obesity worldwide there is an urgent need for better understanding of molecular mechanism linking obesity to metabolic and cardiovascular disease. Studies have shown that serum Cystatin C is consistently increased in obese individuals, whatever their renal status; strongly suggest a role for adipose tissue as a contributor to circulating concentration of Cystatin C. Various indices have been used for the diagnosis and prognosis of cardiovascular disease (CVD). Despite considerable advances the current approaches to evaluation for coronary heart disease (CHD) risk in asymptomatic individuals remain suboptimal. Atherogenic index of plasma (AIP) is a logarithmically transformed ratio of molar concentrations of triglycerides to HDL-cholesterol. It has been suggested that $AIP < 0.1$ is associated with low risk, $0.1-0.24$ with medium risk, and > 0.24 with high cardiovascular risk [6].

Hence the present study was undertaken to know the association of serum Cystatin C with the other cardio metabolic risk markers in obese South Indian individuals.

Objectives:

- 1) To estimate & compare the serum levels of Cystatin C in individuals with normal Body Mass Index and obese, aged between 18-39 years.
- 2) To correlate the levels of serum Cystatin C with cardio metabolic risk factors.

II. Material And Methods

The study population was taken from healthy volunteers of Mysore city, aged between 18-39 years of either sex. The study population was divided into 2 groups based on BMI, as per the Health Ministry of India guide lines [7]. Individuals with BMI of less than 23kg/m² were grouped into normal, and those with BMI more than 25kg/m² as obese. Each group contains sample size of 45. The sample size was estimated to be enough to detect a difference of 10% in serum cystatin C between 2 groups at 5% level of significance & 80% of power. Ethical clearance was taken from the Institutional Ethical Review Committee. A written informed consent was taken from the subjects.

Inclusion criteria: Healthy volunteers, aged between 18-39years of either sex.

Exclusion criteria: Those with history of infections, diabetes, hypertension, chronic kidney disease and cancers were excluded from the studies Data regarding age, sex, occupation, diet, physical activity, BMI, Blood pressure and others was collected in the form of questionnaires.

Four ml of fasting venous sample was collected from all the individuals in a plain vacutainers under aseptic precautions. Serum glucose by GOD-POD method [8], Serum Total Cholesterol by CHOD-PAP method [9], Direct HDL & Direct LDL Cholesterol by immune inhibition method [10, 11] & VLDL was calculated by Friedwald’s formula. Triglyceride by GPO-PAP methodology [12] and Cystatin C by immunoturbidimetric method. Cystatin C in the test sample binds to the specific polyclonal rabbit anti- Cystatin C antibody, which has been adsorbed to latex particles and agglutinates. The agglutination is detected as absorbance change at 546nm [13].

Statistical Analysis: The results were expressed as Mean ± Standard deviation. p<0.05 was considered statistically significant. Statistical analysis was performed using Epi info software and the test used was Student ‘t’ test. To correlate the serum Cystatin C with cardio metabolic risk markers Pearson’s correlation coefficient was worked out.

III. Results

The results of the present study is shown in table-1. Group1 represents individuals with BMI<22 (Normal) and Group2 with BMI>25(Obese). Data are expressed as Mean ± Standard deviation. Serum Cystatin C, Glucose, Triglycerides concentration were significantly increased in obese individuals when compared with non obese control group. The mean serum Cystatin C levels in normal BMI group was 0.78±0.03 mg/L, and in obese group 1.15±0.09 mg/L (p value<0.001).The mean Glucose levels in normal BMI was 80.5± 5.6 & in Obese group 93.2± 6.5 The mean AIP levels (log TG/HDL) are -0.14±0.06 in group 1 and 0.26±0.10 in group 2. AIP was significantly increased in obese group (p valve < 0.0001). Cardiac risk markers like total cholesterol/HDL and HDL/LDL ratio were significantly increase in obese group.

Table-1 -Comparison of serum valves between the two study groups.

	Group 1	Group-2	P-values
N	45	45	-----
Age (years)	26.1±5.25	30.65±6.47	-----
BMI(Kg/m ²)	20.7±1.6	29.6±3.63	0.0001*
Waist Circumference	81.6 ±11.3	95±12.6	0.0001*
Glucose(mg/dl)	80.5± 5.6	93.2± 6.5	0.0001*
Total Cholesterol(mg/dl)	132.2±9.44	139±19.3	0.0016
HDLCholesterol(mg/dl)	45.3±3.6	39.4±4.66	0.0002*
LDL Cholesterol(mg/dl)	99 ±8.8	102±14.30	0.0020
VLDL(mg/dl)	13.35±3.3	25.35±15.8	0.0023
Triglycerides(mg/dl)	115.6 ± 20.1	135.6 ± 40.6	0.0001*
AIP(log TG/HDL)	-0.14±0.06	0.26±0.10	0.0001*
T. Cholesterol/HDL(CRI-I)	2.83±0.3	4.16±0.67	0.0001*
HDL/LDL(CRI-II)	1.67±0.30	2.53±0.366	0.0001*
Non HDLc (TC-HDLc)	112.5±3.1	119.8±4.4	0.5
Cystatin C mg/L	0.70±0.033	1.15±0.09	0.0001*

N= number of subjects, p <0.0001 = highly significant.

Table 2- shows the correlation between the Serum Cystatin C and Atherogenic indices

Parameters	r-value	Correlation
Serum Glucose	0.61	Positive
AIP	0.8	Positive
HDL Cholesterol	-0.52	negative
Triglycerides	0.70	positive
TCHOL: HDL	0.71	Positive
HDL/LDL	0.70	Positive
NonHDLc/HDLc	0.60	Positive

In the study serum Cystatin C showed a positive correlation with serum glucose (0.61) Atherogenic index of plasma (AIP) (r=0.80), Triglycerides (r=0.70), TCHOL: HDL (Castelli’s Risk Index I) (r=0.71), HDL: LDL (Castelli’s Risk Index II) (r=0.70) Atherogenic coefficient (AC) {(NonHDLc)/HDLc}(r=0.60) respectively and negative correlation with serum HDL(r=-0.52).

Correlation between S. Cystatin and Log TG/HDL(AIP)

Figure 1: Scatter plot showing relationship between S. Cystatin C and AIP. Correlation coefficient value shows that there is strong positive correlation between S. Cystatin and AIP. p value for 2 tailed test is 0.032 which is less than 0.05 Which shows that the correlation between S.Cystatin C and Log TG/HDL is statistically significant.

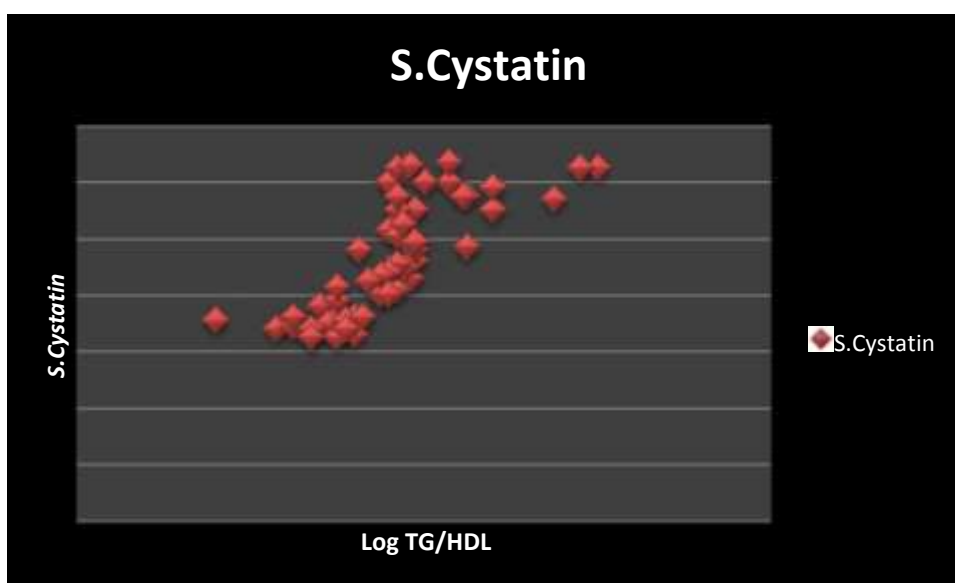
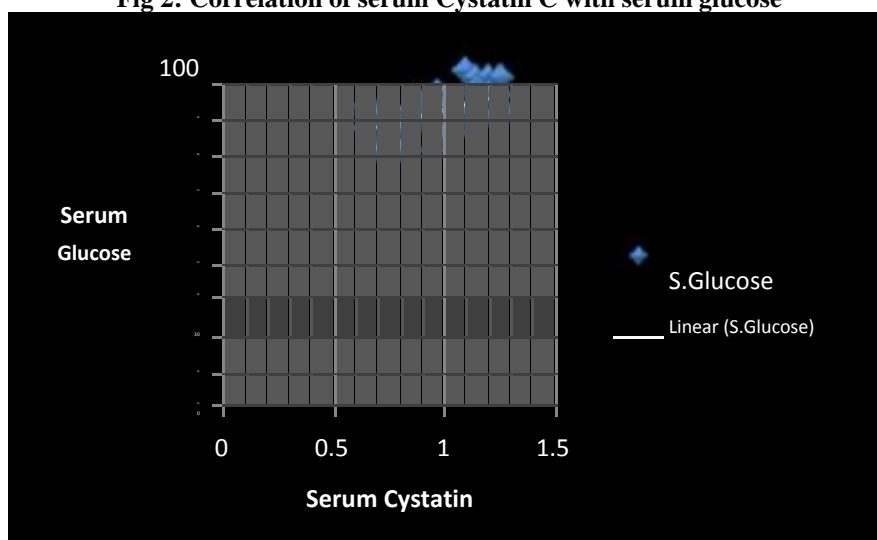


Fig 2: Correlation of serum Cystatin C with serum glucose



IV. Discussion

Cystatin C, an endogenous inhibitor of cathepsin proteases has emerged as a biomarker of cardiovascular risk and reduced renal function. Epidemiological studies indicate that serum Cystatin C is increased in obesity. In the present study serum Cystatin C is significantly increased in obese group when compared to normal weight individuals. The mean serum Cystatin C levels in normal BMI group was 0.70 ± 0.03 mg/L, and in obese group 1.15 ± 0.09 mg/L (p value < 0.001). These observations suggest that higher BMI is the main determinant of obesity – linked increase in serum cystatin C, and hence this study confirms the association between obesity and elevated cystatin C in humans. Serum cystatin C is consistently increased in obese individuals, whatever their renal status which strongly suggests a role for adipose tissue as a contributor to circulating concentrations of this protein in obesity. In support of this hypothesis, the study conducted by Nadia Naour et al [14] showed that cystatin C is highly expressed in human adipose tissue, equivalently in subcutaneous and omental fat depots, and that adipose tissue expression of cystatin C is increased in obesity. This increase could arise from enlarged adipocytes and macrophages, which express cystatin C mRNA and infiltrate the adipose tissue in obesity [15]. Based on its function as inhibitor of cysteine proteases, cystatin C has the potential to influence pathological process relying, at least in part, on deregulation of cathepsins. This includes atherosclerosis and other inflammatory-related disease [16]. Increased serum cystatin C might be part of regulatory mechanisms engaged to control the proatherogenic capacity of specific Cathepsins such as cathepsin S [17]. In this study, BMI was found to be significantly different between the two groups with most of the patients being obese. We found that the mean levels of serum TG were significantly higher in case group (135.6 ± 40.6) as compared to controls (115.6 ± 20.1). The mean serum HDLc levels were significantly lower in case group as compared to controls ($p < 0.05$). The deranged TG and HDLc levels may be attributed to obesity which is characterized by insulin resistance, so enhanced fatty acid esterification is observed due to elevated insulin levels. Moreover, the decrease in HDLc levels is due its enhanced catabolism. In this study, serum TC and LDLc did not show any significant difference between the two groups which is in agreement with Bhardwaetal [18].

In the present study serum cystatin C showed a positive association with cardio metabolic risk markers like Triglycerides ($r=0.70$), Atherogenic index of plasma (\log TG)/HDLc (AIP) ($r=0.80$), TCHOL: HDL (Castelli's Risk Index I) ($r=0.71$), HDL: LDL(Castelli's Risk Index II) ($r=0.70$) respectively and Atherogenic coefficient (AC) $\{(NonHDLc)/HDLc\}$ ($r=0.60$) and negative correlation with serum HDL($r=-0.52$). These results are in accordance with the study done by Parikh et al [19].

Interestingly in the present study serum glucose level increased as the cystatin C increased. Some researchers like Rishard P et al have also found such relation. The relation may need to be established further linking the molecular pathways and provides future directions for study [20] and hence this increased cystatin C could explain the risk of progression to pre-diabetes in these individuals.

In the recent years, several papers have confirmed the usefulness of cystatin C and its determination as a marker of early deterioration of GFR, being more sensitive than serum Creatinine [17]. Hence based on the results derived from the present study it could be inferred that an increase in serum cystatin C could explain a state of pre-clinical kidney disease in the study population.

V. Conclusion

With the increasing prevalence of obesity worldwide there is an urgent need for better understanding of molecular mechanism linking obesity to metabolic and cardiovascular disease. In the present study serum Cystatin C showed an increase with the increase in BMI of healthy individuals which strongly suggests a role for adipose tissue as a contributor to circulating concentration of Cystatin C. Higher Cystatin C concentration could possibly be associated with increased cardio metabolic risk and chronic kidney disease.

Hence serum Cystatin C could serve as a good predictive marker of preclinical cardio metabolic disease and early chronic kidney disease in obese individuals of aged 18-39 years, who are prone for future development of metabolic syndrome and its complications.

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