Correlation of Cystatin C with Atherogenic Indices in South Indian Obese Individuals

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ABSTRACT

Introduction: *Obesity is the leading cause for cardiovascular disease in the world. Cystatin C is a novel marker of cardiovascular disease and atherogenic indices which is used as an index for cardiac risk stratification. The objective of the study was to estimate the Cystatin C levels in non-obese and obese individuals aged between 18-39 years and to compare the levels of Cystatin C among these individuals. The study was also done with an objective to know the association of Cystatin C with atherogenic indices.*

Methodology: *The subjects were selected from healthy volunteers of Mysore aged between 18-39 years of either sex. They were grouped into two groups based on their BMI. Each group had sample size of 60. Sample were collected in fasting state and analyzed for Total Cholesterol, Triglyceride, LDL-Cholesterol & HDL cholesterol by enzymatic method and Cystatin-C by immune-turbidimetric method using Randox auto-analyzer.*

Results and Interpretation: *The mean serum Cystatin C level in non obese group was 0.7±0.03 mg/L, and in Obese group 1.15±0.09 mg/L (p value<0.001). Serum Cystatin C showed a positive correlation with serum triglycerides (r=0.7), Atherogenic index of plasma (AIP) (r=0.80), TCHOL: HDL (Castelli's Risk Index I) (r=0.71), HDL: LDL (Castelli's Risk Index II) (r=0.70) and Atherogenic coefficient (AC) {(Non HDLc)/HDLc} (r=0.60) respectively and negative correlation with serum HDL (r=-0.52).*

Conclusion: *Several indices have been derived from lipid profiles to establish an index for predicting the risk of having coronary event. Cystatin C, was strongly correlated with the Atherogenic index of plasma (AIP), hence AIP can be used as a better index for screening the preclinical Cardiovascular event in obese individuals as estimation of Cystatin C is cost effective.*

Key Words: *Atherogenic Index of Plasma (AIP), Atherogenic coefficient (AC), Castelli's Risk Index (CRI), Cystatin C, Obesity.*

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INTRODUCTION

Cardiovascular disease is one of the commonest cause of mortality and coronary artery disease (CAD) accounts annually for 12 million deaths. There is a steady increase in the prevalence of CAD in the Indian subcontinent, due to rapid change in demography and lifestyle of people consequent to economic development[1,2].

Obesity is the major cause for metabolic disorders and premature deaths in developing countries. It increases the risk of hypertension, dyslipidemia, diabetes mellitus and certain cancers[3]; however the underlying mechanism remains unclear. Recent evidences shows that body fat distribution during early adulthood is linked with increased metabolic disorder in later life[4,5]. Hence it necessitates the identification of possible biochemical predictors that can help in understanding 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[6]. Cysteine proteases are group of proteolytic enzymes which includes Cathepsin B, H, L, S and C that are involved in pathological processes such as inflammation, tumor invasion, breakdown of collagen and bone resorption[7]. The activities of Cysteine proteases are controlled by naturally occurring inhibitory proteins such as Cystatins and α2 macroglobulin. These inhibitors functions to protect host tissues from destructive proteolysis. Cystatin C is a non glycated low molecular weight basic protein expressed in all nucleated cells[8].

Various indices have been used for the diagnosis of cardiovascular disease (CVD). Despite considerable advances, the evaluation of coronary heart disease (CHD) risk in asymptomatic obese individuals remains unclear. In the absence of an abnormal lipid profile the possibility of CAD cannot be ruled out. Hence it becomes important to find out different combinations of these lipid profile parameters which can be used to identify such high risk individuals. In this direction Atherogenic index of plasma (AIP) is emerging strongly as an index for cardiac risk stratification.

Atherogenic index of plasma (AIP) is a logarithmically transformed ratio of molar concentrations of triglycerides to HDL-cholesterol. AIP<0.1 is associated with low risk, 0.1-0.24 with medium risk, and >0.24 with high cardiovascular risk[9]. Thus, the present study was conducted with the objective of assessing the

significance of Cystatin C with the lipid ratios like Atherogenic Index of Plasma (AIP), Castelli Risk Index (CRI) and Atherogenic Coefficient (AC) in identification of risk for CAD in obese individuals.

OBJECTIVES

1. To estimate the serum levels of Cystatin C in Obese individuals and compare it with non-obese.
2. To correlate the levels of Cystatin C with atherogenic indices.

MATERIAL AND METHODS

The study design was a cross sectional study and subjects were selected from healthy volunteers of Mysore district aged between 18-39 years of either sex. The subjects were grouped into two group based on Body Mass Index, as per the Health Ministry of India guide lines[10]. Individuals with BMI of less than 23 kg/m^2 were grouped as normal, and those with BMI more than 25 kg/m^2 as obese. Each group had sample size of 60. The study was undertaken after ethical clearance from the Institutional Ethical Review Committee and written informed consent was taken from the all subjects.

Exclusion Criteria: Individuals with diabetes, hypertension, infections, chronic kidney disease and cancers were excluded from the studies. Data regarding age, sex, occupation, diet, physical activity, BMI, Blood pressure were collected using pre tested semi structured questionnaire.

Four ml of fasting venous sample was collected from all the individuals in a plain red Vaccutainers under aseptic precautions. Serum glucose was estimated enzymatically by Glucose oxidase- Peroxidase method[11], Total Cholesterol by cholesterol esterase and oxidase (CHOD-POP) method[12], Direct HDL & Direct LDL Cholesterol by immune inhibition method[13,14] & VLDL was calculated by Friedwald's formula. Triglyceride by Glycerokinase and Glycerophosphate oxidase (GPO-PAP) methodology[15] and Cystatin C was estimated using immunoturbidimetric method[16].

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 to find the significance the test used was Student't' test. To correlate the serum Cystatin C with atherogenic indices Pearson's correlation co-efficient was worked out.

DISCUSSION

Epidemiological studies indicate that Cystatin C levels are elevated in renal dysfunction. The key finding in our study is that Cystatin C levels were significantly increased in obese group when compared to individuals with non obese. This observation suggests that higher BMI was associated with increase in Cystatin C levels in obese individuals which was independent of age & sex. In support to our results, the study done by Nevio Taglieri etal[17] showed that Cystatin C was highly expressed in human adipose tissue, equivalently in subcutaneous and omental fat depots and increased levels of Cystatin C could be a part of regulatory mechanism in controlling the activities of Cathepsin proteases which is involved in Inflammations.

Lipid profile refers to some routinely done biochemical tests. It includes serum Triglycerides (TG), serum total cholesterol (TC) and its sub fractions like HDLc and LDLc. The role of deranged lipid profile in the progression of CAD is a well known fact and deranged LDLc levels are the primary target for treatment. Various indices had been derived from lipid profiles to establish an index for predicting the risk of having coronary event[18,19].

In the present study serum Triglyceride (TG) was significantly increased in obese individuals with mean value of 135.6 ± 40.6 mg/dl as compared with non obese 115.6 ± 20.1mg/dl and serum High Density Lipoproteins (HDL) levels was significantly lower in Obese with $p<0.05$. These altered serum triglyceride (TG) and HDLc levels may be attributed to insulin resistance and increased HDL catabolism. Coronary Artery Disease (CAD) has been associated with alterations in lipid metabolism, which include hyper-triglyceridemia and significantly reduced HDLc, hence our study results was in agreement with Mudhaffar et al study[20.]

Interestingly the study showed higher AIP levels in obese individuals as compared with normal BMI with $p< 0.001$. AIP is a ratio calculated as Log TG/HDLc. In our observation AIP ratio was 0.26 in obese and -0.14 in non obese subjects. Hence AIP has diagnostic importance in predicting cardiovascular risk[21].

Castelli's Risk Ratio (CRI) is based on three important lipid parameters i.e. Total Cholesterol (TC) LDLc and HDLc. CRI-I calculated as the ratio of TC/HDLc and CRI-II as LDLc/HDLc, and interestingly we found significantly higher ratio levels in obese with mean value of 4.16±0.67 & 2.53±0.366 respectively in comparison with non-obese. The study did not show any significant difference in TC and LDLc levels between the two study groups whereas, the ratio based on these parameters showed a significant difference between the two groups. This clearly suggests the relevance of ratios over individual lipid parameters especially in situations where the drug management might be affected. The Canadian working group had chosen the TC/HDLc ratio as a secondary goal of therapy considering it to be a more sensitive and specific index of cardiovascular risk than total cholesterol, particularly in individuals with TG>300mg/dl[22,23]. Few studies have also found an association of TC/HDLc ratio with coronary plaques formation[24]. Atherogenic Coefficient (AC), calculated as Non-HDLc/HDLc or {(TC-HDLc)/HDLc} is a measure of cholesterol in LDLc, VLDLc, IDLc lipoprotein fractions with respect to HDLc. It reflects atherogenic potential of the entire spectrum of lipoprotein fractions.

Non HDLc is the second target of therapy after LDLc as per Adult treatment panel III guidelines especially in individuals with hyper-triglyceridemia[25]. In our study, the Atherogenic Coefficient (AC) was found to be significantly higher in obese individuals with p<0.001.
Moreover, it has been observed that obesity leads to rise in levels of small dense LDLc particles which are not measured routinely. Hence measurement of lipid ratios is equally important in indentifying the risk of Coronary artery disease in obese individuals which in turn helps in early diagnosis and effective treatment in order to postpone or control the disease process.
Since, atherogenic index of plasma was strongly correlated with the Cystatin C, hence AIP can be used as a better index for predicting the preclinical cardiovascular disease because of cost effectiveness in estimation of Cystatin C. Hence, AIP may be the diagnostic alternative for Cystatin C in predicting the early cardiovascular disease in obese individuals.

CONCLUSION

In summary, our study showed serum Cystatin C increases with increase in adiposity and it is highly correlated with that of atherogenic index of plasma (AIP). Hence, AIP can be used as better index for predicting the preclinical cardiovascular disease because of cost effectiveness in estimation of Cystatin C. Hence, AIP may be the diagnostic alternative for Cystatin C in predicting the early cardiovascular disease in obese individual there by helping the clinician in effective treatment management.

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RESULTS

Table 1: Comparison of serum valves between the two study groups

	Group 1	Group-2	P-values
N	55	55	-----
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.

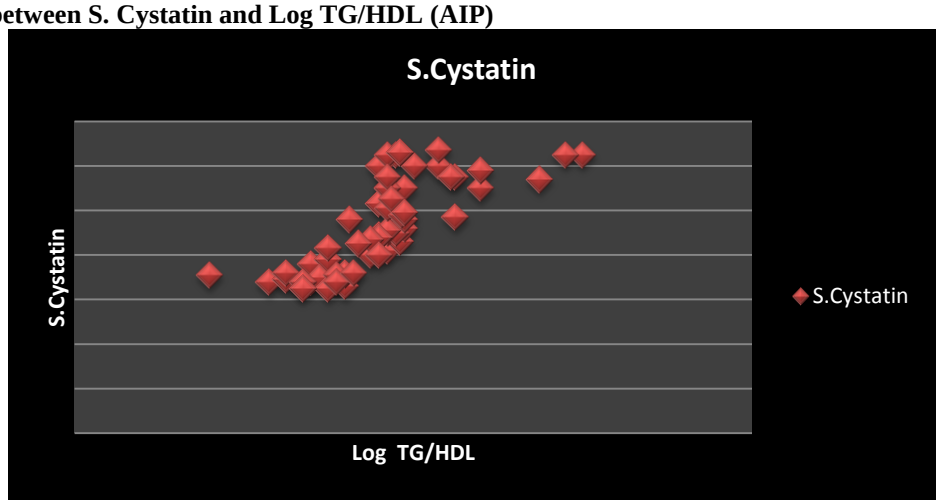
Serum Cystatin C, Triglycerides & Glucose concentration were significantly increased in obese individuals when compared with non-obese control group. Serum Cystatin C levels in non-obese subjects was 0.7±0.03 mg/L, and in obese group 1.15±0.09 mg/L with p<0.001. The mean AIP level (log TG/HDL) was -0.14±0.06 and 0.26±0.10, Castelli's Risk Index I was 2.83±0.366 & 4.16±0.67, Castelli's Risk Index II was 1.67±0.30 &2.53±0.36, Atherogenic Co-efficient was 2.93±0.16 &4.28±0.37 in non-obese and obese individuals respectively.

Table 2: Shows the correlation between the Serum Cystatin C and Atherogenic indices

Parameters	r-valve	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 Atherogenic index of plasma (AIP) (r=0.77), Triglycerides (r=0.70), 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) {(Non- HDLc)/HDLc} (r=0.60) and negative correlation with serum HDL (r=-0.52).

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



Graph 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 tests is 0.032 which is less than 0.05. This shows that the correlation between S.Cystatin C and Log TG/HDL was statistically significant.

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