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Correlation of Serum Cystatin C with Atherogenic indices in obese individuals

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ABSTRACT

Introduction: Human obesity is strongly associated with cardiovascular disease. Cystatin C is a naturally occurring protease inhibitor and marker of cardiovascular disease. The atherogenic indices are used as an index for cardiac risk stratification. Objectives: To estimate the serum levels of Cystatin C in individuals with normal BMI, and obese, aged between 20-39 Yrs and to compare the levels of Cystatin C among these individuals and to correlate the levels of serum Cystatin C with atherogenic index of plasma and other indices. Methodology: The study population was taken from healthy volunteers of Mysore city, aged between 20-39 years of either sex. The study population was divided into 2 groups based on BMI. Each group contains sample size of 60. Fasting serum sample was analyzed for Total Cholesterol, TG, LDL-Cholesterol & HDL cholesterol by enzymatic method and serum Cystatin-C by immune-turbidimetric method using auto-analysers. Statistical Analysis: Analysis of Variance [ANOVA] was used to compare the serum levels of Cystatin C in the two groups. To correlate the serum Cystatin C with atherogenic indices for predicting the cardiovascular risk factors, Pearson’s correlation co-efficient was worked out. Results: The mean serum cystatin C levels in normal BMI group are 0.7±0.03 mg/L, and in Obese group 1.15±0.09 mg/L (p value < 0.001). In the study 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), 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)

Conclusion: Several indices had been derived from lipid profiles to establish an index for predicting the risk of having coronary event. The atherogenic index of plasma was strongly correlated with the Cystatin C, hence AIP can be used as better index for predicting the preclinical cardiovascular disease because of cost effectiveness in estimation of Cystatin C.

Introduction

Cardiovascular disease is the commonest cause of mortality globally, accounting annually for nearly 12 million deaths, with coronary artery disease (CAD) being the major contributor. There is a steady increase in the prevalence of CAD in the Indian subcontinent, due to rapid changes in demography and lifestyle consequent to economic development [1, 2].

Human obesity is strongly associated with cardiovascular disease. Obesity is the 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 increase many folds in association with obesity [3]. The mechanism responsible for atherogenesis has been explored for ages. Recent evidence shows that the distribution of fat during early adulthood is associated with increased metabolic disease risk in later adulthood [4, 5]. 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 [6]. 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.[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 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 [8]. Various indices have been used for the diagnosis and prognosis of cardiovascular disease (CVD). Despite considerable advances that happened during the past decades, there is increasing awareness among scientists, epidemiologists, and clinicians that current approaches to evaluation of coronary heart disease (CHD) risk in asymptomatic individuals remain suboptimal. Hence in the absence of an abnormal lipid profile the possibility of CAD cannot be ruled out. It has been suggested that the different combinations of these lipid profile parameters can be used to identify such high risk individuals. Atherogenic index of plasma (AIP), a new marker of atherogenicity, and is strongly emerging index used as an index for cardiac risk stratification and to be significantly valuable for assessing atherogenic risk in the population. 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 [9]. Thus, the present study was conducted with the objective of assessing the significance of lipid ratios like Atherogenic Index of Plasma (AIP), Castelli Risk Index (CRI) and Atherogenic Coefficient (AC) in identification of at-risk for CAD beyond the routinely done lipid profile especially in insufficient resource situations [10, 11].

Objectives

1. To estimate the serum levels of Cystatin C in normal BMI, and obese individuals, aged between 20-39 Yrs.
2. To compare the levels of Cystatin C among these individuals and to correlate the levels of serum Cystatin C with atherogenic index of plasma & other indices.

Material and Methods

The study population was taken from healthy volunteers of Mysore city, aged between 20-39 years of either sex. The study population was divided into 2 groups based on BMI, as per the Health Ministry of India guide lines. 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 60. 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 20-39 years of either sex.

Exclusion criteria

Those with history of infections, diabetes, hypertension, chronic kidney disease and cancers were excluded from the studies.

Four ml of fasting venous sample was collected from all the individuals in a plain vactuainers under aseptic precautions. Serum Total Cholesterol by CHOD-PAP method, Direct HDL & Direct LDL Cholesterol by immune inhibition method, & VLDL was calculated by Friedwald’s formula. Triglyceride by GPO-PAP methodology and Cystatin C by immune turbidimetric 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 [12].

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’s t test. To correlate the serum Cystatin C with Atherogenic indices, Pearson’s correlation co-efficient was worked out.

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, Triglycerides concentration were significantly increased in obese individuals when compared with non obese control group. 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 increase in obese group ( p value < 0.0001). The mean serum Cystatin C levels in normal BMI group are 0.7±0.03 mg/L, and in obese group 1.15±0.09 mg/L (p value<0.001). The serum Cystatin C levels was significantly increased in obese groups. 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

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group 1</th>
<th>Group 2</th>
<th>P-values</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>60</td>
<td>60</td>
<td>----</td>
</tr>
<tr>
<td>Age (years)</td>
<td>26.1±5.25</td>
<td>30.65±6.47</td>
<td>--------</td>
</tr>
<tr>
<td>BMI(Kg/m²)</td>
<td>20.7±4.6</td>
<td>29.6±3.63</td>
<td>0.001*</td>
</tr>
<tr>
<td>Total Cholesterol(mg/dl)</td>
<td>132±9.44</td>
<td>139±19.3</td>
<td>0.016</td>
</tr>
<tr>
<td>HDL Cholesterol(mg/dl)</td>
<td>45.3±3.6</td>
<td>39.4±4.66</td>
<td>0.0002*</td>
</tr>
<tr>
<td>LDL Cholesterol(mg/dl)</td>
<td>99±8.8</td>
<td>102±14.30</td>
<td>0.0020</td>
</tr>
<tr>
<td>VLDL(mg/dl)</td>
<td>13.35±3.3</td>
<td>25.35±15.8</td>
<td>0.0023</td>
</tr>
<tr>
<td>Triglycerides(mg/dl)</td>
<td>115.6 ± 20.1</td>
<td>135.6 ± 40.6</td>
<td>0.0001*</td>
</tr>
<tr>
<td>AIP(log TG/HDL)</td>
<td>-0.14±0.06</td>
<td>0.26±0.10</td>
<td>0.0001*</td>
</tr>
<tr>
<td>T. Cholesterol/HDL(CRI-I)</td>
<td>2.83±0.3</td>
<td>4.16±0.67</td>
<td>0.0001*</td>
</tr>
<tr>
<td>HDL/LDL(CRI-II)</td>
<td>1.67±0.30</td>
<td>2.53±0.366</td>
<td>0.001*</td>
</tr>
<tr>
<td>Non HDLc (TC-HDLc)</td>
<td>112.5±3.1</td>
<td>119.8±4.4</td>
<td>0.5</td>
</tr>
<tr>
<td>Cystatin C mg/L</td>
<td>0.70±0.033</td>
<td>1.15±0.09</td>
<td>0.0001*</td>
</tr>
</tbody>
</table>

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

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

<table>
<thead>
<tr>
<th>Parameters</th>
<th>r-value</th>
<th>Correlation</th>
</tr>
</thead>
<tbody>
<tr>
<td>AIP</td>
<td>0.8</td>
<td>Positive</td>
</tr>
<tr>
<td>HDL Cholesterol</td>
<td>-0.52</td>
<td>negative</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>0.70</td>
<td>positive</td>
</tr>
<tr>
<td>TCHOL: HDL</td>
<td>0.71</td>
<td>Positive</td>
</tr>
<tr>
<td>HDL/LDL</td>
<td>0.70</td>
<td>Positive</td>
</tr>
<tr>
<td>NonHDLc/HDLc</td>
<td>0.60</td>
<td>Positive</td>
</tr>
</tbody>
</table>

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) {NonHDLc/HDLc}(r=0.60) and negative correlation with serum HDL(r=-0.52).

Correlation between S. Cystatin and Log TG/HDL

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 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.

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. 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. In support of this hypothesis, the study conducted by Nadia Naour et al. [11] 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.

Lipid profile refers to some routinely done biochemical tests to assess the atherogenic status of individuals at risk of coronary artery disease (CAD). It includes serum Triglycerides (TG), serum total cholesterol (TC) and its sub fractions like HDLc and LDLc. The Framingham heart study over years has established the role of deranged lipid profile in the progression of CAD and deranged LDLc levels are the primary target for treatment [13].

Several indices had been derived from lipid profiles to establish an index for predicting the risk of having coronary event. AIP, a new marker of atherogenicity, is directly related to the risk of atherosclerosis. People with high AIP have a higher risk of coronary heart disease than those with low AIP and vice versa. Identifying individuals at the highest risk of comorbidities of obesity is essential in order to identify those who might benefit most from management programs [14].

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.

CAD has been associated with alterations in lipid metabolism, which include hyper-triglyceridemia and significantly reduced HDLc. In this study, serum TC and LDLc did not show any significant difference between the two groups which is in agreement with Bhardwaj et al. [15].

In the current study, we observed that Atherogenic Index of Plasma (AIP) was significantly higher in cases as compared to controls (p < 0.001). AIP is a ratio calculated as (logTG)/HDLc. Studies have shown an inverse relationship that exist between TG and HDLc and that the ratio of TG to HDLc is a significant predictor of atherosclerosis. It has been suggested that AIP values of -0.3 to 0.1 are associated with low, 0.1 to 0.24 with medium and above 0.24 with high Cardiovascular risk. We observed AIP ratio of 0.26 in cases and -0.14 in controls which are in concordance with the suggested cut-offs. AIP may be the diagnostic alternative. Studies have shown its role in predicting cardiovascular risk and effectiveness of therapy [16].

Castelli’s Risk Ratio (CRI) is based on three important lipid profile parameters i.e. TC, LDLc and HDLc. CRI-I calculated as the ratio of [TC/HDLc] and CRI-II as [LDLc/HDLc] [17], was found to be significantly higher in cases compared to controls (p<0.001). In our study, we could not observe a 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 [18]. We observed CRI-I in our case group was > 4 in concordance with other studies [19]. Studies have shown the association of TC/HDLc ratio with coronary plaques formation [20]. In our study, CRI-II was also found to be above the upper limit for normal range i.e. >3 as observed in other studies [19]. 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 good cholesterol or HDLc. It reflects atherogenic potential of the entire spectrum of lipoprotein fractions. Non HDLc is the second target of therapy after LDLc as per ATPIII guidelines especially in individuals with hyper-triglyceridemia. In our study, this ratio based on Non-HDLc was found to be significantly higher in cases as compared to controls (p<0.001), even when there was no statistically significant difference observed between Non-HDLc among the two groups. Moreover, it has been observed that obesity leads to rise in levels of small dense LDLc particles which are not measured routinely. In such a scenario, it is essential to go beyond the routinely done lipid profile; especially, in centers with insufficient resources and owing to high cost of mostly available tests, these lipid ratios may prove a boon in the patient management. The atherogenic index of plasma was strongly correlated with the Cystatin C, hence AIP can be used as better index for predicting the preclinical cardiovascular disease because of cost effectiveness in estimation of Cystatin C. AIP may be the diagnostic alternative for Cystatin C in predicting the Cardiovascular disease.

Conclusion

This study concludes that atherogenic index of plasma could be used for identifying individuals at higher risk of cardiovascular disease in Indian population in the clinical setting especially when the absolute values of individual lipoproteins seem normal and in individuals with elevated TG concentrations. These can be easily calculated from the routinely done lipid profile parameters especially in centers where new tests are not possible due to cost factor. Thus, the use of these indexes should be encouraged to complement the existing profile of tests for identifying high risk individuals for CAD and effective drug management.

Acknowledgement

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References

