Comparison of lipid profile and new atherogenic indices among the coronary artery disease (CAD)-negative and -positive diabetic dyslipidemia subjects

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Received May 11, 2015. Accepted May 16, 2015.

Introduction

Diabetes mellitus (DM) is one of the most important diseases in modern society; according to the World Health Organization (WHO) study experts, an increase in the incidence of DM up to 300 million can occur, in 2025, among the persons aged older than 25 years. India alone would have 57 million diabetic patients, particularly of type 2 diabetes mellitus (T2DM), constituting 90% of the DM population.
Patients with T2DM are at a larger risk of developing vascular disorders: coronary artery disease (CAD)/coronary heart disease (CHD), stroke, peripheral arterial disease (PAD), cardiomyopathy, and congestive cardiac failure, for the reason that they are associated with a cluster of inter-related plasma lipid and lipoprotein abnormalities, including reduced high-density lipoprotein-cholesterol (HDL-c) and predominantly higher levels of small dense low-density lipoprotein-cholesterol (LDL-c) particles, very low-density lipoprotein-cholesterol (VLDL-c), and elevated triglycerides (TGs).\textsuperscript{[4,5]} Irrespective of the ethnic background, the risk of CAD among the diabetic patients is greater by a factor of 2 to 4 times when compared with nondiabetic subjects.\textsuperscript{[3,6]} Individuals with T2DM are at a higher risk of developing CAD than are non-T2DM patients. In addition, 75% of T2DM patients die as a consequence of cardiovascular diseases including CAD.\textsuperscript{[7,9]} The aim of this study was to compare the lipid profiles and new atherogenic indices among the CAD-negative and -positive diabetic dyslipidemia subjects and determine the use of atherogenic indices in the early prediction of CAD in diabetic subjects.

### Materials and Methods

#### Study Design
This cohort study was carried out at Dr. Ramesh Cardiac and Multispecialty Hospital, Ltd., Vijayawada, Andhra Pradesh, India. The study subjects were selected randomly, who were on a visit to the hospital for their general health checkup. The study protocol was approved by the Institutional Ethical Committee and was conducted during the period from 2012 to 2014. The selection of subjects was carried out by implementing certain inclusion criteria such as estimation of fasting blood glucose (FBG) and lipid profile, based upon selecting both, the cases and healthy subjects. Likewise, certain exclusion criteria followed were: subjects with hepatic, metabolic, and renal diseases and those who were on exogenous hormones supplements, on hormone replacement therapy, or on the use of lipid lowering drugs were excluded from the study. An informed written consent was obtained from all the study subjects who participated in our study.

#### Data Collection and Selection of Subjects
Systemic examination of each subject was carried out; it included their name, age, address, type of diet, occupation, physical exercise, present and past medical illness, and family history. Anthropometric assessments such as height in meter (m), weight in kilogram (kg), and body mass index (BMI) were done. The BMI was calculated by weight in kilograms divided by the square of the height in meter (kg/m\(^2\)). The selection of subjects was based upon the plasma lipid abnormalities and FBG cutoff values given by the expert panel of the National Cholesterol Education Program (NCEP).\textsuperscript{[9]} A total number of 194 subjects participated in our study, of which 65 people with diabetic dyslipidemia were considered as cases and 129 nondiabetic subjects with normal lipid profile selected as control subjects. Among the 65 subjects, 38 subjects were considered as CAD-negative subjects, enrolled individuals with normal clinical, cardiologic, and rest- ing and stress electrographic assessments. The rest of the 27 subjects were considered as CAD-positive subjects, enrolled individuals with abnormal clinical, cardiologic, and precordial pain and characteristic electrocardiographic changes.\textsuperscript{[10]}

#### Collection of a Blood sample and Estimation of Lipid Profile
Fasting blood samples were collected in the morning between 7 a.m. and 8 a.m. by venepuncture of antecubital vein with all aseptic precautions, using a dry disposable syringe under sterile conditions. Fresh plasma and serum were used for the estimation of FBG and total cholesterol (TC), TGs, and HDL-c, respectively. The tests were carried out in an automated clinical auto analyzer. Furthermore, LDL-c, VLDL-c, and non-HDL-c were calculated by using Friedewald's formula.\textsuperscript{[11]} In addition, atherogenic indices such as, Castelli’s Risk Index (CRI)-I = TC/HDL-c, CRI-II = LDL-c/ HDL-c, atherogenic coefficient (AC) = (TC – HDL-c)/HDL-c, TGs/HDL-c ratio, and atherogenic index of plasma (AIP) = log (TGs/HDL-c) were calculated from the individuals.

#### Statistical Analysis
The collected data were analyzed by using Graph Pad Prism, version 6. The differences in the groups were determined by performing the one-way analysis of variance (ANOVA) and the Tukey–Kramer multiple comparisons test; data were expressed either as mean ± standard error mean (SEM). The statistical significance was set at the p values of \( p < 0.05 \), \( p < 0.01 \), and \( p < 0.001 \), and \( p > 0.05 \) was considered as nonsignificant.

#### Result
Table 1 shows the mean ± SEM values of the age, weight, and BMI of the diabetic dyslipidemia and control subjects. Diabetic dyslipidemia subjects were further divided into CAD-negative and -positive subjects. Table 2 shows the mean ± SEM values of the FBG levels and lipid profiles of the diabetic and control subjects. The mean values of the FBG of CAD-negative and -positive group showed significantly higher values than the control nondiabetic subjects but did not show significantly different values when CAD-negative subjects were compared with CAD-positive subjects. Table 2 also shows the mean ± SEM values of the lipid profiles of both CAD-negative and -positive diabetic subjects, which were significantly different when compared with those of the control subjects, except the HDL-c value of CAD-negative subjects. The comparison between the CAD-negative and -positive diabetic dyslipidemia subjects revealed significantly higher values of TGs and VLDL-c and lower values of HDL-c in CAD-positive subjects.
shown that increasing BMI is associated with higher TC and LDL-c. However, these studies were limited by underrepresentation of obese subjects.\[13\] Higher levels of FBG and lipid profiles in both CAD-positive and -negative diabetic subjects were observed when compared with control subjects. Research studies have explained that the most common and serious effects of diabetes in adults is cardiovascular disorders (CVDs)\[14\] and the leading cause of death in patients with diabetes is CVDs than individuals without diabetes.\[15,16\] Research studies have also explained that, in diabetes, elevated levels of TGs are observed, owing to alterations in metabolism that include increased hepatic secretion of VLDL-c, impaired clearance of VLDL-c, and intestinally derived chylomicrons. These remnants include intermediate density lipoproteins (IDL-c) that are particularly involved in the development of atherogenesis in humans and in a number

Table 1: Comparison of age and BMI by Tukey–Kramer multiple comparisons test of controls with CAD-negative (CADN) and CAD-positive (CADP) diabetic dyslipidemia subjects

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control (n = 129), mean ± SEM</th>
<th>Subjects with dyslipidemia and diabetes (n = 65)</th>
<th>p, CADN vs. CADP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CADN, mean ± SEM (n = 38)</td>
<td>CADP, mean ± SEM (n = 27)</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>45.77 ± 1.36</td>
<td>54.71 ± 1.64**</td>
<td>51.37 ± 1.78ns</td>
</tr>
<tr>
<td>BMI</td>
<td>24.26 ± 0.39</td>
<td>26.48 ± 0.72ns</td>
<td>26.23 ± 0.87ns</td>
</tr>
</tbody>
</table>

**p < 0.01, significant; ns, nonsignificant.

Table 2: Comparison of FBG and lipid profile by Tukey–Kramer multiple comparisons test of controls with CAD-negative (CADN) and CAD-positive (CADP) diabetic dyslipidemia subjects

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control (n = 129)</th>
<th>Subjects with dyslipidemia and diabetes (n = 65)</th>
<th>p, CADN vs. CADP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mean ± SEM</td>
<td>mean ± SEM (n = 38)</td>
<td>mean ± SEM (n = 27)</td>
</tr>
<tr>
<td>FBG</td>
<td>87.96 ± 0.47</td>
<td>156.61 ± 7.48***</td>
<td>162.00 ± 8.63***</td>
</tr>
<tr>
<td>TC</td>
<td>153.71 ± 1.46</td>
<td>204.68 ± 8.56***</td>
<td>199.52 ± 10.25***</td>
</tr>
<tr>
<td>TGs</td>
<td>110.53 ± 2.32</td>
<td>166.61 ± 12.21*</td>
<td>339.59 ± 52.24***</td>
</tr>
<tr>
<td>LDL-c</td>
<td>87.99 ± 1.26</td>
<td>204.68 ± 8.56***</td>
<td>199.52 ± 10.25***</td>
</tr>
<tr>
<td>VLDL-c</td>
<td>22.10 ± 0.46</td>
<td>33.32 ± 2.44*</td>
<td>67.91 ± 10.44***</td>
</tr>
<tr>
<td>HDL-c</td>
<td>43.61 ± 0.46</td>
<td>41.89 ± 1.49ns</td>
<td>35.59 ± 1.40***</td>
</tr>
<tr>
<td>Non-HDL-c</td>
<td>110.10 ± 1.38</td>
<td>162.79 ± 7.33***</td>
<td>163.93 ± 9.28***</td>
</tr>
</tbody>
</table>

*p < 0.05, significant; **p < 0.01, significant; ***p < 0.001, significant; ns, nonsignificant.

Table 3: Comparison of atherogenic indices by Tukey–Kramer multiple comparisons test of controls with CAD-negative (CADN) and CAD-positive (CADP) diabetic dyslipidemia subjects

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control (n = 129)</th>
<th>Subjects with dyslipidemia and diabetes (n = 65)</th>
<th>p, CADN vs. CADP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mean ± SEM</td>
<td>mean ± SEM (n = 38)</td>
<td>mean ± SEM (n = 27)</td>
</tr>
<tr>
<td>CRI-I</td>
<td>3.55 ± 0.04</td>
<td>4.89 ± 0.10***</td>
<td>5.62 ± 0.19***</td>
</tr>
<tr>
<td>CRI-II</td>
<td>2.03 ± 0.03</td>
<td>3.07 ± 0.12***</td>
<td>3.21 ± 0.25***</td>
</tr>
<tr>
<td>TG/HDL-c</td>
<td>2.59 ± 0.06</td>
<td>4.05 ± 0.28**</td>
<td>9.50 ± 1.25***</td>
</tr>
<tr>
<td>AIP</td>
<td>0.39 ± 0.01</td>
<td>0.57 ± 0.02***</td>
<td>0.92 ± 0.04***</td>
</tr>
<tr>
<td>AC</td>
<td>2.55 ± 0.04</td>
<td>3.89 ± 0.10***</td>
<td>4.62 ± 0.19***</td>
</tr>
</tbody>
</table>

**p < 0.01, significant; ***p < 0.001, significant.

Table 3 shows the mean ± SEM values of atherogenic indices of diabetic dyslipidemia and control subjects. When compared with control subjects, both CAD-negative and -positive subjects showed higher values of indices that are significantly different. Among the CAD-negative and -positive subjects, CAD-positive subjects showed all indices that were significantly different, except CRI-II.

Discussion

In our study, higher BMI value was observed in diabetic patients than control subjects but was not significantly different. BMI has been widely used as an indicator of total adiposity; its limitations are clearly recognized by its dependence on race.\[15\] Epidemiologic studies done earlier have
of animal models[17,18]; these remnants are also responsible for the increased production of precursors of small-dense LDL-c particles.[19] Previous studies have also explained that plasma VLDL-c levels correlate with the increased density and decreased size of LDL-c particles.[20,21] In addition, LDL-c particles size and density are inversely related to the plasma levels of HDL-c.[22] Moreover, several studies have also suggested that development of insulin resistance was seen in patients with diabetic dyslipidemia[23–25]; furthermore, it was associated with hypertriglyceridemia, higher levels of VLDL-c, and low levels of HDL-c cholesterol.[26–28] These higher levels of triglycerides and VLDL-c particles may impair insulin action by inhibiting insulin binding to its receptor.[23,25] Garg et al.[29] proved and suggested that insulin resistance is the underlying mechanism in patients with hypertriglyceridemia and not vice versa. Lower levels of HDL-c in a diabetic person are explained by several mechanisms. First, diminished activity of lipoprotein lipase (LPL) may result in the excessive transfer of TGs from triglyceride-rich chylomicrons and VLDL-c particles in exchange for cholesterol esters from HDL-c particles, thus reducing the levels of HDL-c. Second, decreased activity of LPL resulting in reduced hydrolysis of TGs in chylomicrons particles may curtail the contribution of chylomicrons-derived nascent HDL-c particles. Further reduction of LPL activity and increased hepatic triglyceride lipase (HTGL) activity may explain markedly the lower levels of HDL-c when compared with nondiabetic subjects and patients with noninsulin-dependent diabetes mellitus (NIDDM).[30] In our study, results were concurrent with the earlier studies, such as elevated levels of TGs and VLDL-c and lower levels of HDL-c in CAD-positive subjects than in CAD-negative subjects, which may be owing to the early development of insulin resistance. Plasma lipids can be divided into the proatherogenic lipoproteins and antiatherogenic HDL-c. Assessment of the relative proportions of cholesterol in these two fractions can be valuable than the individual lipid measurements. One method is to compare the levels of HDL-c and non–HDL-c.[31] Another method is the use of atherogenic indices; these are powerful indicators of the risk assessment of CADs. The higher the values, the higher are the risks of developing CVDs and vice versa.[32] Atherogenic ratios such as, CRI-I, CRI-II, AC[33–36] TGs/HDL-c ratio,[37] and AIP[38] are calculated. We applied these indices for predicting the cardiovascular risks in diabetic dyslipidemia subjects. The average ratio of TC to HDL-c (CRI-I) of healthy individuals was about 3.5 or lower,[39,40] and in the case of LDL-c/HDL-c ratio (CRI-II), it was three or lower.[40,41] Another research study explained the association of TC/HDL-c with coronary plaques formation.[42] In the PROCAM study, it was observed that subjects with LDL-c/ HDL-c (CRI-II) > 5 showed six times higher rate of coronary events.[42] In our study, higher values of CRI-I and CRI-II in both CAD-negative and -positive subjects than the control subjects were observed, and higher value of CRI-I in CAD-positive subjects than CAD-negative subjects was also observed. CAD-positive group was confirmed by electrocardiographic changes and other clinical characteristics; so, the above-mentioned results indicate and support this index, which may be very useful in the prediction for CAD. In the case of CRI-II, both the subjects were near to normal values owing to the normal levels of TC in both CAD-negative and -positive subjects. da Luz et al.[37] explained that the ratio of TGs to HDL-c was found to be a powerful independent indicator of extensive coronary disease. The ratio TG/HDL-c, initially proposed by Gaziano et al.,[44] is an atherogenic index that has proven to be a highly significant independent predictor of myocardial infarction, even stronger than TC/ HDL-c and LDL-c/HDL-c.[44] Bampi et al.[39] reported that TGs/ HDL-c ratio is possible to approximately determine the presence and extent of CAD by noninvasive methods. The above-mentioned results indicate and support TGs/HDL-c ratio as a very useful predictor for the assessment of CVDs. AIP shows an inverse relationship that exists between TGs and HDL-c and that the ratio of TGs to HDL-c is a strong predictor of infarction, and it was used by some practitioners as a significant predictor of atherosclerosis.[45] Other researchers have suggested that, AIP is a highly sensitive marker of difference of lipoprotein in patients. 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 risks.[46] In our study, high values (p < 0.001) of AIP in diabetic subjects were observed. The CAD-positive subjects were already confirmed by electrocardiographic changes and other clinical characteristics; so the aforementioned result indicates and supports that this index may be very useful in the prediction for CAD. Atherogenic coefficient (AC) is a measure of cholesterol in LDL-c, VLDL-c lipoprotein fractions with respect to good cholesterol, or HDL-c. It reflects the atherogenic potential of the entire spectrum of lipoprotein fractions. The higher the values, the higher are the risks of developing CVDs and vice versa.[32] In our study, we observed high values of AC in both CAD-negative and -positive subjects than those in control subjects; we also observed higher values of AC in CAD-positive subjects than in CAD-negative subjects.

**Conclusion**

Our conclusion is that BMI is not an important predictor for the assessment of CVDs in diabetic subjects; we also observed the elevated levels of TGs, LDL-c, and VLDL-c and the reduced levels of HDL-c are important risk factors for the development of CVDs in diabetic subjects. We also observed the development of cardiovascular signs in CAD-positive group that may be owing to the reduced levels of HDL-c. On the basis of earlier studies and our results, we conclude, these atherogenic indices are powerful indicators to predict the risk of CADs based on the higher values of atherogenic indices observed in CAD-positive subjects than the CAD-negative subjects. These results indicate that atherogenic indices may be useful for identifying individuals at a higher risk of CVDs in the clinical practices, especially, and not markedly deranged or in centers with insufficient resources.
References


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How to cite this article: Ranjit PM, Guntuku GS, Pothineni RB. Comparison of lipid profile and new atherogenic indices among the coronary artery disease (CAD)-negative and -positive diabetic dyslipidemia subjects. Int J Med Sci Public Health 2015;4:1574-1579

Source of Support: Nil, Conflict of Interest: None declared.