Association of Lipid Abnormalities with High-Sensitivity C - reactive Protein in Patients Treated with Atorvastatin

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Abstract

Dyslipidemia and subclinical inflammation are the major determinants of cardiovascular disorders. For the management of dyslipidemia, statin is used and claimed to be effective in the reduction subclinical inflammation. In this study, lipidemic and subclinical inflammatory status in patients treated with stable statin (atorvastatin) therapy were evaluated in 111 Bangladeshi subjects. Lipid parameters and marker of subclinical inflammation (hsCRP) were measured by enzymatic endpoint techniques and by immunoturbidimetric method. Persistent single dyslipidemia is prominent for low-density lipoprotein (LDL) cholesterol (57.66%), followed by hypertriglyceridemia (55.86%), high-density lipoprotein (HDL) cholesterol dyslipidemia (54.06%), and hypercholesterolemia (20.72%). Regarding combined lipid abnormalities, elevated triglyceride (TG) combined with elevated LDL cholesterol was most frequent (35.13%). Persistent multiple combined lipid abnormalities for 3 lipid parameters (low HDL cholesterol, elevated LDL cholesterol and elevated TG) was observed for 19.82% subjects. Persistent subclinical inflammation (hsCRP ≥ 3.0 mg/l) was found to be high (36.94%), followed by moderate risk (33.33%) and low risk for CVDs (29.73%). hsCRP showed positive association total cholesterol (TC) and negative association with LDL cholesterol and HDL cholesterol. But after adjustment for age, sex, BMI, diabetes mellitus and duration of statin therapy, only HDL cholesterol showed significant inverse association with hsCRP (β= -0.314, p = 0.016). This study revealed that single or multiple combined dyslipidemia persists in subjects treated with statin. Large proportions of the subjects had subclinical inflammation which is inversely associated with HDL cholesterol on adjusting confounders.

Keywords: Dyslipidemia; Subclinical inflammation; Atorvastatin; High-density lipoprotein cholesterol; High sensitivity C-reactive protein

Introduction

Cardiovascular diseases (CVDs), the leading causes of death in the world are rising rapidly in low- and middle-income countries [1]. CVDs are the most prevalent cause of morbidity and mortality among patients with type1 or type2 diabetes [2]. In general, patients with diabetes aggregate other comorbidities such as obesity, hypertension, and dyslipidemia which also contribute to increase the risk for CVDs [3]. Disorder in lipid metabolism is one of the main determinants of cardiovascular risk. The primary target of lipid management is to achieve low-density lipoprotein (LDL) cholesterol at goal [4]. Statin therapy lowers the risk of cardiovascular events by reducing plasma cholesterol levels, and practice guidelines for patients with known cardiovascular disease emphasize the importance of reaching target goals for low-density lipoprotein (LDL) cholesterol [5]. For the management of dyslipidemia, statins or fibrates are commonly used. Statins (HMG-CoA reductase inhibitors) reduce CVD risk by approximately 23% per every 1 mmol/l (≈ 39 mg/dl) low-density lipoprotein (LDL) cholesterol lowering [6]. Atorvastatin, at doses ranging from 2.5 mg to 80 mg daily, can reduce LDL by 25% with the lowest dose and up to 60% with the maximal dose [7]. Beside lipid parameters, high sensitivity C-reactive protein (hsCRP) – an inflammatory cytokine and an independent predictor of CVD [8-13] is claimed to be reduced by statin treatment [14-19]. The US Food and Drug Administration approved a new use for statin therapy among those with elevated hsCRP and one additional risk factor, and the Canadian Cardiovascular Society recently issued new national guidelines indicating that statin therapy should be offered to those at “intermediate risk” who have elevated levels of hsCRP, even if LDL-C levels are low [20]. Though proven benefit of statin on CVD risk, patients with dyslipidemia remains at high risk for cardiovascular events even after LDL cholesterol, blood pressure and HbA₁c target have been achieved [21]. For example, in the DYSIS-Netherlands study, majority (71.77%) of patients receiving statin therapy fail to reach normal levels for lipid parameters [22]. However, no study has yet been carried out in Banglalee population regarding persistent dyslipidemia and subclinical inflammation in patients treated with statin.

On the basis of that finding and in the effort to address the clinical issues outlined above, we used a commercial assay to measure hsCRP and simultaneously measured plasma levels of serum total cholesterol, low-density lipoprotein (LDL) cholesterol, and high-density lipoprotein (HDL) cholesterol. Under this context, this study was undertaken to explore the status of lipid abnormalities and subclinical inflammation in patients on stable statin (atorvastatin) therapy in this population. We also sought to determine whether the measurement of markers of inflammation in addition to standard screening of lipid levels might provide a clinically useful method for improving overall prediction of the risk of cardiovascular events.

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Methods
Study designs and population

This cross-sectional study was conducted in the department of Clinical Biochemistry, Bangladesh Institute of Health Sciences (BIHS), Dhaka, Bangladesh during the period of January 2012 to June 2012. Subjects (111) were selected purposively from the outpatient department of BIHS according to inclusion-exclusion criteria. Subjects receiving statin monotherapy during last 3 months (apparently healthy adults) were included and patients with serious comorbid diseases (infection, stroke, myocardial infarction, major surgery, malabsorption, severe allergy, cancer, severe illness, liver dysfunction, chronic kidney disease (CKD), pregnancy, edema, oral contraceptive users, steroid or non-steroidal anti-inflammatory drugs users were excluded. Informed consent was taken before data collection and clinical examination. Before specimen collection history of diabetes, hypertension, blood pressure reading, height, weight were recorded. Data regarding habit of smoking, current medication or others were also recorded.

Anthropometric measurements

Anthropometric indices included height and weight. All the individuals were measured wearing light clothing without shoes and hats. Height was measured to the nearest 0.1 cm using a portable stadiometer and weight was measured to the nearest 0.1 kg using calibrated platform scales.

Blood pressure measurement

Blood pressure was measured to the nearest 1 mm Hg with mercury sphygmomanometers using standard recommended procedures [23]. Two readings each of systolic and diastolic blood pressures were recorded, and taken at 5 minutes intervals. The average of two readings was used in the data analysis. If two of the measurements differed by more than 5 mm Hg, an additional reading was taken.

Diabetes mellitus measurement

Diabetes mellitus is characterized by recurrent or persistent hyperglycemia, and is diagnosed by WHO diagnostic criteria for diabetes mellitus [24]. Blood glucose level is measured using the glucose oxidase method. The fasting blood glucose level, which is measured after a fast of 8 hours, is the most commonly used indication of overall glucose homeostasis. The metabolic response to a carbohydrate challenge is conveniently assessed by a postprandial glucose level drawn 2 hours after a meal or a glucose load. First-morning sample were collected fasting 10-12 hours prior to going in for blood collection.

Clinical measurements

Blood samples were obtained from the antecubital vein with the subject sitting comfortably in a chair in a quiet room and transfused into vacuum tubes containing EDTA in the morning after an overnight fasting period. After separation, blood samples were centrifuged for 10 minutes at 3000 rpm to obtain serum. Then serum was aliquoted into two microtubes, one preserved for lipid profile measurements and another was preserved at -20°C for hsCRP estimation until analysis.

Serum total cholesterol, triglyceride concentrations were measured by end point technique using Dimension® clinical chemistry system (Siemens Healthcare Diagnostics Inc. USA) using reagents (Cat. No. DF27, Siemens Healthcare Diagnostics Inc. USA). HDL cholesterol levels was measured by an fully automated reagent format Dimension® clinical chemistry system (Siemens Healthcare Diagnostics Inc. USA) and LDL cholesterol concentrations in serum were calculated by Friedewald’s formula [25]. hsCRP concentrations were determined immunotubidimetric by using BN ProSpec® system (Siemens Healthcare Diagnostics Products GmbH, Germany).

Statistical methodology

Statistical analysis was performed using MedCalc® version 11.4 for Windows, STATISTICA version 8.0 for windows. All data were expressed as mean ± SD (standard deviation) and/or percentage (%) as appropriate.

We used Pearson correlation to show the relationship of the difference between the two parameters to the mean of the two measurements [26]. To analyse that the factors affect the changes of hsCRP, a multivariate linear regression model was used to determine log (hsCRP) as a function of baseline log (hsCRP), gender, body mass index, systolic blood pressure, diastolic blood pressure, blood lipids, serum glucose (fasting blood glucose). Standardized β were used to compare the strength of the effect of each independent variable on the log (hsCRP), which with the largest standardized β (independent of the sign) has the strongest effect. Relationships between log (hsCRP) and these variables were also assessed by multiple regression analysis. The level for statistical significance was set at 0.05.

Results

Clinical characteristics

Total 111 dyslipidemic subjects were included in this study to explore residual lipid abnormalities and subclinical inflammation on statin therapy. All the subjects used statin (atorvastatin, 10 mg per day) with a mean duration of 2.35 ± 2.55 years. Characteristics of the study subjects are shown in Table 1 of the total subjects, 41 (37%) had BMI ≤ 25 Kg/m², 47 (42%) had BMI: 25-30 Kg/m² and 23 (21%) had BMI>30 Kg/m².

Correlation of hsCRP with the lipid measurements

Table 2 shows the correlation of lipid parameters with hsCRP in the study subjects. Twenty-three (20.72 %) of the study subjects had elevated serum total cholesterol (>200 mg/dl), 60 (54.06 %) had low-density lipoprotein cholesterol (>70 mg/dl) and 25 (22.72 %) had elevated triglyceride (>150 mg/dl). The correlation of hsCRP with the lipid measurements is shown in Table 2.

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**Table 1**: Baseline clinical characteristics of study subjects.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Mean±SD/Number</th>
<th>β value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>53 ± 11</td>
<td>0.114</td>
<td>0.2877</td>
</tr>
<tr>
<td>Gender (Male/Female, %)</td>
<td>55/56 (50/50)</td>
<td>0.138</td>
<td>0.5501</td>
</tr>
<tr>
<td>Body mass index (Kg/m²)</td>
<td>26.8 ± 4.5</td>
<td>0.094</td>
<td>0.3901</td>
</tr>
<tr>
<td>Hypertension</td>
<td>78 (71%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>124 ± 13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg)</td>
<td>81 ± 8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetes mellitus (%)</td>
<td>92 (83%)</td>
<td>0.099</td>
<td>0.4684</td>
</tr>
<tr>
<td>Smoker</td>
<td>15 (14%)</td>
<td>0.098</td>
<td>0.5784</td>
</tr>
<tr>
<td>Lipid status (mg/dl)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>23 (&gt;200mg/dl) / 20.72 %</td>
<td>0.733</td>
<td>0.1075</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>60 (&lt;35 mg/dl) / 54.06 %</td>
<td>-0.314</td>
<td>0.0157</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>62 (&gt;70 mg/dl) / 55.86 %</td>
<td>-0.602</td>
<td>0.1491</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>39 (&gt;150 mg/dl) / 35.13%</td>
<td>0.012</td>
<td>0.9469</td>
</tr>
</tbody>
</table>

| Medication | | | |
| Duration of atorvastatin therapy (years) | 2.35 ± 2.55 | -0.166 | 0.0912 |
Elevated CRP was higher (45%) in subjects with combined TG and hsCRP (Figure 2B) and 22 (19.82%) had lipid abnormalities with elevated hsCRP (elevated LDL cholesterol and elevated CRP) (Figure 2A); 22 (19.82%) had combined risk for HDL cholesterol and CRP (both were male and 27 (24.32%) were female. Of the total study subjects, 37 (33.33%) had moderate risk for CVD (hsCRP<1.0 mg/dl), 30.63% subjects had both low HDL cholesterol and elevated LDL cholesterol, and 21.62% had elevated TG and elevated LDL cholesterol, 35.13% subjects had both elevated TG, total cholesterol, and LDL cholesterol as well as decreased HDL cholesterol has been implicated with a variedly increased risk of CVDs both in cross-sectional and prospective studies [27-29]. The nature and extent of dyslipidemia, however, may vary depending on the ethnic, cultural and environmental background of a particular population. For the management of lipid abnormalities, statin and fibrates are commonly used in our population. Some studies have identified residual CVD risk on therapy in different population [30]; no study has yet been carried out to explore the prevalence of lipid abnormalities on stable statin therapy in this population. In the present hospital based study on Bangalee population, 111 statin treated subjects were included. Among the study subjects most of them (83%) were diabetic, 71% were hypertensive, 21 % were obese and 15% had a habit of smoking.

In the study subjects, when analyzed single lipid abnormalities, LDL dyslipidemia was found to be the most prominent one (present in 57.66% subjects) followed by hypertriglyceridemia (55.86%), HDL dyslipidemia (54.06%), and hypercholesterolemia (20.72%). When computing multiple combined lipid abnormalities, 19.82% subjects were found to have multiple combined dyslipidemia for HDL cholesterol, LDL cholesterol and TG. Moreover, 35.13% had both elevated TG and elevated LDL cholesterol, 30.63% subjects had both low HDL cholesterol and elevated LDL cholesterol, and 21.62% had low HDL cholesterol and elevated TG.

Another major focus of this study was to investigate the extent

### Variability of hsCRP and other risk factors by single lipid abnormality

Figure 2 shows distribution of mixed lipid and hsCRP risk factors in the study subjects. Thirty-three (29.73%) of the participants had low risk for CVD (hsCRP<1.0 mg/dl), 37 (33.33%) had moderate risk for CVD (hsCRP: 1.0-3.0 mg/dl) and 41 (36.94%) had high risk for CVD. Of the high risk subjects (hsCRP>3.0 mg/dl) 14 (12.61%) subjects were male and 27 (24.32%) were female. Of the total study subjects, 27 (24.32%) had combined risk for HDL cholesterol and hsCRP (both low HDL cholesterol and elevated hsCRP) (Figure 2A); 22 (19.82%) had combined risk for LDL cholesterol and hsCRP (elevated LDL cholesterol with elevated hsCRP) (Figure 2B) and 22 (19.82%) had combined TG and hsCRP risk factors (Elevated TG with elevated hsCRP) (Figure 2C). Elevated hsCRP was higher (45%) in subjects with low HDL cholesterol followed by 35.5% in subjects with elevated TG and 34.3% in subjects with elevated LDL cholesterol.

### Multivariable analysis

Multiple linear regression analysis considering hsCRP as dependent variable and lipid parameters as independent variables showed that hsCRP was positively associated with total cholesterol (β=1.136, p=0.010) and negatively associated with LDL cholesterol (β=-0.954, p=0.016) and HDL cholesterol (β=-0.299, p=0.020). But after adjustment for age, sex, BMI, diabetes mellitus and duration of statin (atrovastatin) therapy, only HDL cholesterol showed significant inverse association with hsCRP (β=-0.314, p=0.016) (Table 2).

### Discussion

Dyslipidemia is a prominent one among the traditional biochemical risk factors of CVDs. Elevated TG, total cholesterol, and LDL cholesterol as well as decreased HDL cholesterol has been implicated with a variedly increased risk of CVDs both in cross-sectional and prospective studies [27-29]. The nature and extent of dyslipidemia, however, may vary depending on the ethnic, cultural and environmental background of a particular population. For the management of lipid abnormalities, statin and fibrates are commonly used in our population. Some studies have identified residual CVD risk on therapy in different population [30]; no study has yet been carried out to explore the prevalence of lipid abnormalities on stable statin therapy in this population. In the present hospital based study on Bangalee population, 111 statin treated subjects were included. Among the study subjects most of them (83%) were diabetic, 71% were hypertensive, 21 % were obese and 15% had a habit of smoking.

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### Table 2: Correlation of hsCRP with the lipid measurements.

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<tr>
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<td>0.3901</td>
</tr>
<tr>
<td>DM</td>
<td>0.099</td>
<td>0.4664</td>
</tr>
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</tbody>
</table>

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#### Figure 1: Distribution of single and multiple combined lipid abnormalities among the study subjects.

#### Figure 2: Distribution and proportion of combined single lipid abnormality and elevated hsCRP among the study subjects.
of residual hsCRP, a non-traditional risk factor of CVDs and the coexistence of hsCRP risk with individual lipid parameters. Higher hsCRP has been found to be associated with CVDs by a number of cross-sectional studies [31] and it has been reasonably confirmed as a predictor of CVDs by substantial volume of longitudinal data [32]. Its independent role, additional to the traditional risk factors, has also been substantiated and particularly its additive role with individual lipids, have also been published [33]. The mean value of hsCRP was much higher than the upper cut-off limit (3.0 mg/l). Elevated hsCRP (>3.0 mg/l) was found to be high (36.94%), followed by moderate risk (33.33 mg/l) and low risk for CVDs (29.73%).

The coexistence of lipid abnormalities with elevated hsCRP has also been explored. Elevated hsCRP was observed for low HDL cholesterol and elevated TG (24.32%) but lower for elevated LDL cholesterol (19.82%). In this study, multiple linear regression analysis showed that subclinical inflammation is inversely associated with HDL cholesterol (β=-0.314, p=0.016) on adjusting confounding variables.

Conclusion

In conclusion, the elevated hsCRP levels may reduce in patients treated with atorvastatin. The screening of patients with elevated CRP levels may identify patients who have an increased risk for cardiovascular events although the use of CRP levels as a predictor of cardiovascular levels may identify patients who have an increased risk for cardiovascular heart disease, stroke, and mortality: an individual participant meta-analysis. Lancet 375: 132-140.
