Evaluation of Ischemia Modified Albumin in Hyperlipidemia Patients.

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Abstract:
Introduction: Lipid disorders are mainly related to abnormalities in metabolism of lipoproteins. Hypercholesterolemia is associated with oxidative stress, resulting into atheromatous plaque development and it reduces the capacity of albumin to bind cobalt owing to ischemia, resulting in an increased IMA (Ischemia Modified Albumin). IMA formation appears to be associated with oxidative stress and ischemia. The present study is undertaken to evaluate level of IMA in patients suffering from hyperlipidemia.
Method: Thirty patients of hyperlipidemia in the medical wards of S.S.G. Hospital, Vadodara and thirty age and sex matched healthy control were examined in the present study. IMA and lipid profile of all patient and controls were measured by colorimetric end point assay in fully automated biochemistry analyzer. mean values calculated and then compared with t test.
Result: Mean value of IMA in patients was 201.5±25.79 and in controls 110.4±19.04, which is highly significant(p= 0.001) and all Lipid Profile Parameter shows higher value in hyperlipidemic patients compared to controls, is found to be statistically significant (p<0.001) statistically significant.
Conclusion: Significant association is observed between IMA and lipids in patients with hyperlipidemia and IMA formation appears to be associated with oxidative stress, ischemic conditions and atheromatous plaque formation.
Keywords: Ischemia modified albumin, Oxidative Stress, Atheromatous plaque.

1. Introduction

Albumin is a multifunctional, most abundant protein in plasma. Among many functions performed by it, one of the main function is transport of metal ions, mainly copper, hence it has an inherent affinity for the metal ions, such as Co²⁺, Ni²⁺ etc. It involves coordination of metal ion to the N-terminal of albumin molecule, in particular to the first three amino acids; Aspartate, Alanine and Histidine. Any damage to the N-terminal of serum albumin causes reduction in binding of the metal ions. Albumin in which N-terminal is either damaged or occupied by copper is termed as Ischemia modified albumin (IMA).¹

Serum albumin of patients having cardiac ischemia, myocardial infarction (MI), atherosclerosis, coronary artery disease, angina etc. shows the presence of IMA. Other common condition prevailing in these patients is hypercholesterolemia. Hyperlipidemia comprises of various lipids like triglyceride (TG) and total cholesterol. Over and above, genetic pre-disposition, age, sex, hyperlipidemia is also caused due to various factors like smoking, obesity, physical inactivity, hypertension, atherogenic diet and diabetes mellitus.²

Hyperlipidemias are classified into three major types:
(I) Hypertriglycerideremia i.e. increased level of triglyceride in blood;
(II) Hypercholesterolemia I.e. increased level of cholesterol in blood;
(III) Combined hyperlipidemia in which there is rise in both cholesterol and triglyceride level in blood.

The guidelines of American Heart Association and National Cholesterol Education Program (NCEP) Adult Treatment Panel-III defined hyperlipidemia as a condition when serum cholesterol level is more than 240 mg/dl and serum triglyceride level is more than 150 mg/dl.³

Lipids being hydrophobic in nature, transported in blood in the form of lipoproteins. Lipoproteins are complexes of lipid and protein. The proteins present in lipoproteins are known as apolipoproteins. Fredrickson classified lipoproteins into five major classes based on their separation of electrophoretic mobility and density. They are termed as chylomicrons, very low-density lipoprotein (VLDL), low density lipoprotein (LDL), intermediate density lipoprotein (IDL) and high density lipoprotein (HDL).

Chylomicrons contain 88% triglyceride (TG), 5% cholesterol, 8% phospholipid (PL), 2% apoproteins i.e. apo-B-48, apo-A, apo-C-I, II, III, apo-E. VLDL consists of 56% TG, 22% cholesterol, 20% PL & 8% apoprotein i.e. apo-B-100, apo-C-I, II, III, apo-E. LDL contains 13% TG, 48% cholesterol, 28% PL, 21% apoprotein i.e. apo-B-100. HDL contains 4% TG, 34% cholesteryl ester, 53% PL, 57% apoprotein i.e. apo-A, apo-C-I, II, III & apo-E.
Lipid disorders are mainly related to abnormalities in metabolism of lipoproteins. Hypercholesterolemia is associated with increase in inflammatory and oxidative stress biomarkers resulting into atheromatous plaque development and it reduces the capacity of albumin to bind cobalt owing to ischemia, resulting in an increased IMA. IMA formation appears to be associated with oxidative stress and ischemia.

The present study is undertaken to evaluate level of IMA in patients suffering from hyperlipidemia.

II. Material And Method

Main aim of the study is to determine the level of IMA and Lipid Profile in Hyperlipidemic patients. The present study includes 30 patients of hyperlipidemia admitted medical wards of the S.S.G. Hospital, Vadodara. Total Cholesterol, Serum Triglyceride, Serum LDL, and Serum HDL were estimated. Serum VLDL was calculated using Friedewald’s equation as below:

\[ \text{Serum VLDL (mg/dl)} = \text{Total cholesterol} - (\text{LDL} + \text{HDL}) \]

Age and sex matched Healthy controls were patients relative & volunteers from the technical staff of the laboratory.

Inclusion criteria: Patients had normal albumin level and not undergone dietetic or therapeutic regime, which can modify serum lipid profile and had no renal disease. In controls also, lipid profile was estimated.

Method of IMA estimation: Albumin cobalt bind test (colorimetric end point assay), performed in Miura Fully automated biochemistry analyzer.

Sample and Specimen collection: Fasting Venous blood sample were collected in sterilized plain bulb. Samples were then centrifuged at 3000rpm for 5min and serum was separated.

Statistical analysis: The value of IMA and various lipid profile parameter are calculated, mean values found and then compared with student’s t test.

III. Results

In present study, the levels of IMA and lipid profile were estimated in hyperlipidemic patients. Thirty hyperlipidemic patients were selected and thirty healthy individuals served as control subjects. Out of these, 15 were males and other 15 were females in both. Table-1 shows mean value and SD of serum IMA levels and lipid profile parameters in controls and in patients.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Patients (Mean ± SD)</th>
<th>Controls (Mean ± SD)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IMA (units/ml)</td>
<td>201.5±25.79</td>
<td>110.4±19.04</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>197.4±56.19</td>
<td>81.37±25.35</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>266.6±3.09.05</td>
<td>147±14.04</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>VLDL-(mg/dl)</td>
<td>39.5±10.78</td>
<td>16.4±4.92</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LDL-C (mg/dl)</td>
<td>163.1±35.64</td>
<td>85.73±10.71</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HDL-C (mg/dl)</td>
<td>35.73±11.43</td>
<td>46.73±2.37</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

All Parameter shows higher value in patients compared to controls, is found to be statistically significant (p<0.001) statistically significance between patient and controls.

Figure 1- Correlation between Total Cholesterol and IMA in Patients

As shown in figure 1, the mean values of IMA when compared with total cholesterol of the patients it shows significant correlation (r=0.7).
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As shown in figure 2, the mean values of IMA when compared with Triglycerides, IMA level shows weak correlation with triglyceride levels ($r=0.4$).

IV. Discussion

Elevated levels of lipids are the major cause for various cardiovascular diseases such as atherosclerosis, ischemic heart disease, acute coronary syndrome, myocardial infarction, etc. Even though, elevated triglyceride do not accumulate directly in the vessel wall, its involvement in development of atherosclerosis is based on its association with the triglyceride rich lipoproteins i.e. VLDL and chylomicrons. Increased endogenous triglyceride levels cause rise in VLDL concentration, ultimately leading to elevation in smaller and denser LDL particles, which are more susceptible to modification by oxidation.

Elevated cholesterol has been shown to increase superoxide anion production that scavenges endothelial derived vasodilator, nitric oxide. Oxidative stress thus evoked in the body induces endothelial dysfunction by impairing normal vascular tone, increasing prevalence of intimal hyperplasia causing reduced blood flow to respective tissue leading to ischemic condition.

Prolonged ischemia, a pre-condition to infarction may lead to myocardial cell death. Thus identification of myocardial ischemia at earliest stage is pre-requisite to prevent devastating consequences of the disease. There are cardiac biomarkers such as CK-MB, LDH, Troponin, etc. identified significantly in recent past, but they are not sensitive to serve as an early marker of ischemia. This is because the above enzymes are released in the blood because of cell necrosis that is when the tissue is damaged. These enzymes are not released in the blood from intact cell. This fact aroused need to identify IMA as an early cardiac marker in ruling out ischemia, which is being studied, with great interest in our study.

In present study, the levels of IMA range from 167.5-255 units/ml with mean value 201.5±25.79 units/ml in hyperlipidemic patients. In controls, IMA ranges from 80-135 units/ml with mean value 110.4±19.04 units/ml which is significantly higher ($p>0.001$) in patients as compared to controls as shown in Table 1. The levels of triglyceride, total cholesterol, VLDL-C, LDL-C are also found to be raised ($p<0.001$) in the hyperlipidemic subjects as shown in Table 4. The levels of HDL-C is reduced ($p<0.001$) in these patients. Jardwiga Hartwich et al. noted increase in IMA level in postprandial lipemia. These patients also had raised triglyceride and cholesterol levels. Patients were on one of the four isocaloric fatty meals (oral fat tolerance test, OFTT). All types of OFTT transiently increased plasma triglyceride and LDL density.

Similar results were also observed by Marta M.M.F. Duarte et al., reported significant increase in IMA levels in hypercholesterolemic patients. They studied the association between IMA, lipid and inflammatory biomarkers in patients with hypercholesterolemia. They observed significant correlation between IMA and total cholesterol, LDL-C, ox-LDL, ox-LDL antibodies and hs-CRP levels. They further suggested that IMA formation appears to be associated with oxidative stress and atheromatous plaque development.

Hyperlipidemia shows elevation in LDL-C and VLDL-C which are well known to be involved in atheromatous plaque development occurring due to infiltration of ox-LDL through arterial wall and its engulfment by circulating macrophages leading to initialization of myocardial infarction.

HDL-C being widely accepted as atheroprotective, its decrease level in hyperlipidemia reflects proatherogenic disorders of triglyceride rich lipoproteins i.e. VLDL and chylomicrons. There seems to exist an inverse relationship between plasma levels of triglyceride and HDL-C. This holds true in practice because increase in VLDL and chylomicrons has been shown to stimulate the exchange of their triglyceride for cholesterol in HDL mediated by CETP in reverse cholesterol transport pathway. This exchange may result in return of cholesterol into peripheral cells, thus leading to its accumulation in peripheral tissues, including the arterial vessel wall.
Mastella A. K. et al. evaluated the levels of IMA in MI and prostate disease. They also studied the influence of HDL-C on IMA and CRP level. They observed that ability of IMA to discriminate IMA was higher than CRP. Significant correlation was observed between CRP and HDL, CRP and IMA, HDL and IMA. Similar correlation between HDL and IMA was observed in present study. IMA is increased in myocardial damage and decrease of HDL-C enhances the inflammatory response.

Debashis Roy et al. studied IMA increase under oxidative stress in patients undergoing radiofrequency (RF) ablation. They excluded patients with potential for myocardial ischemia in any vascular territory including peripheral vascular diseases. They observed that myocardial ischemia might not necessarily be the only trigger for IMA elevation in plasma, the thermal injury occurring to myocardial cell during RF ablation leads to rapid release of free radical causing oxidative stress and finally damage to N-terminal of albumin thus explaining role of oxidative stress as potential cause in IMA increase.

Long term hyperlipidemia results in atherosclerotic plaque formation in vascular endothelium. These results into ischemia, when the cardiac vessels are narrowed leading to myocardial ischemia terminating in MI. During this phase, albumin becomes modified at the N-terminal residues, thus decreasing its affinity to bind Co(ll) and increasing the IMA levels.

V. Conclusion

In hyperlipidemic patients, there is elevation in serum triglyceride, total cholesterol, LDL-C, VLDL-C levels and decrease in serum HDL-C level. These abnormalities in lipid content of hyperlipidemic patients leads to ischemia and resulting oxidative stress modifies N-terminal of albumin, reducing its cobalt binding capacity. This results in elevation of IMA levels. Thus, significant association is observed between IMA and lipids in patients with hyperlipidemia and IMA formation appears to be associated with oxidative stress, ischemic conditions and atheromatous plaque formation.

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