Diabetes Accelerates Age-Related Lipid Profile Disturbances in Cardiovascular Complications

Md. Ezaz Zafar1,2, Faizur Rahman3, K. R. Prasad3, M Nehal4

1,2Associate Professor, 3Professor, Dept. of Biochemistry, Katihar Medical College, Bihar, 4Professor, Dept. of Zoology, Ageing Research Laboratory, L.N. Mithila University, Bihar

*Corresponding Author:
Email: ezazzafar@yahoo.co.in

Abstract
Cardiovascular Complications (CVC) are predominant problems both in ageing and diabetes. The present study focuses on the effect of human ageing and diabetes, with special reference to serum lipid profile levels during cardiovascular complications in two age groups namely, 45±5 years and 65±5 years independent of obesity hypertension and nephropathy. The serum samples of study and control group includes estimation of serum total cholesterol, serum triglycerides, HDL-C, LDL-C and VLDL-C. Results shows, that the changes in the levels of lipid parameters follow a near similar trend in the two non-diabetic age groups with cardiovascular complications. Except the HDL-C which decline, total cholesterol, LDL-C, VLDL and triglycerides increases.

In diabetes, however, the said lipid parameters are not so similarly affected in the stated age subjects. One interesting observation is the near similar levels of lipid parameters found in diabetic 45±5 y and non-diabetic 65±5 y age groups with Cardiovascular complications. It is, therefore, suggested that diabetes accelerates age related disturbances in the lipid profile.

Key Words: Diabetes, Ageing, Lipid profile and CVC

Introduction
Cardiovascular complications are predominant problems both in ageing and diabetes. The risk factors continue to predict Cardiovascular disease in older individuals1-4 including lipoprotein abnormalities & diabetes along with other complications.

There is ample evidence that total Cholesterol, LDL, cholesteral and HDL – Cholesterol levels are all univariant predictors of risk of coronary disease in both men and women over 65 years of age.1-6 HDL in particular is an important predictor of risk7-8, because HDL & triglycerides levels are strongly and inversely, related it would not be surprising that triglycerides continue to be predictors of coronary risk in older individuals.

Patients over 65 years of age with established coronary diseases represent a group of particular interest. Elevated Cholesterol levels substantially increase the risk of recurrent myocardial infarction (MI) or death in such men and women.6 In the Framingham study, Cholesterol levels over 275 mg/dl (7.1 mmol/L) were associated with a fourfold increase in risk of recurrent infarction or in coronary death, and almost a threefold increase in risk from all-cause mortality compared with Cholesterol levels less than 200 mg/dl.

Lipoproteins are altered in diabetes. The quantitative changes most commonly seen are an increase in the TG-rich lipoproteins and a decrease in HDL.9-12 These changes can be seen at and even before the diagnosis of diabetes.

Despite the relative lack of attention to diabetes mellitus in the literature on geriatrics, this is the third most prevalent life threatening disease among older people after atherosclerosis and cancer. Approximately 20% of those over age 80 are diabetic18 and there is 17% prevalence of "mild and easy to treat" diabetes among Finns over age 85.20 According to the International Diabetes Federation bulletin (2000), India had nearly 33 million diabetic constituting around 1-2% of the total population. According to WHO estimates, India would have 75 million diabetics by 2020.

Due to the ageing of the global population, the prevalence of diabetes and the combination of diabetes and advanced age is expected to increase considerably. Diabetes appears to be an important risk factor for significant cognitive decline and dementia in the elderly. The challenge for the next decades will be to unravel the complex interaction between the mechanisms of ageing and diabetes.19

The present study focuses on the effects of human ageing and diabetes, with special reference to serum level lipid profile during CVC in two age groups namely, 45±5 years and 65±5 years, independent of
obesity hypertension and nephropathy. The parameters include estimation of serum total cholesterol; HDL – C, LDL – C, VLDL and triglycerides.

**Research Design and Methods**

The studies were carried out in human subjects at Katihar Medical College & Hospital, Katihar. Individuals consent and management permission were duly obtained.

**No. of Subjects:** Minimum 15 in each categories.

<table>
<thead>
<tr>
<th>S. No</th>
<th>Age group &amp; Patho-physiological state.</th>
<th>Fasting Plasma glucose level</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>25±5 y – Non-diabetic, without CVC</td>
<td>60 - 100 mg/dl</td>
</tr>
<tr>
<td>2</td>
<td>45±5 y – Non-diabetic without CVC</td>
<td>70 - 100 mg/dl</td>
</tr>
<tr>
<td>3</td>
<td>45±5 y – Non – diabetic with CVC</td>
<td>70 - 100 mg / dl</td>
</tr>
<tr>
<td>4</td>
<td>45±5 y – Diabetic with CVC</td>
<td>150 - 220 mg / dl</td>
</tr>
<tr>
<td>5</td>
<td>65±5 – Non-diabetic without CVC</td>
<td>70 - 110 mg/dl</td>
</tr>
<tr>
<td>6</td>
<td>65±5 – Non – diabetic with CVC</td>
<td>70 - 110 mg / dl</td>
</tr>
<tr>
<td>7</td>
<td>65±5 – Diabetic with CVC</td>
<td>140 - 217 mg / dl</td>
</tr>
</tbody>
</table>

**Methods**

Serum total Cholesterol, HDL, Cholesterol & Triglycerides were estimated by enzymatic method. The VLDL was determined simply from the Triglycerides divided by five & LDL was determined by using Friedewald equation. \( LDL = C = S - total Cholesterol - HDL + VLDL \)

**Estimation of Total Cholesterol:** The free Cholesterol produced by hydrolysis of Cholesteryl ester & preexisting free Cholesterol are oxidized by Cholesterol oxidase to liberate \( \text{H}_2\text{O}_2 \), which reacts with 4- amino antipyrine (4AAP) to form a quinoneimine, a red color compound which is read at 510 nm.

**HDL – Cholesterol – Estimation:** In the presence of Phosphotungstate and divalent Cation i.e. magnesium LDL, VLDL, and Chylomicrons are precipitated. After centrifugation the HDL Cholesterol remains present in the supernatant and it is estimated by using Cholesterol reagent.

**Triglycerides Estimation:** Triglycerides is hydrolyzed by lipase into glycerol & free fatty acid. Glycerol reacts with ATP in presence of Glycerokinase to form Glycerol 3 phosphate which further reacts with \( \text{O}_2 \) catalyzed by Glycerol Oxidase to form dihydroxy acetone phosphate & \( \text{H}_2\text{O}_2 \). This \( \text{H}_2\text{O}_2 \) again reacts with 4- aminoantipyrine in the presence of 3, 5 – dichloro – 2 – hydroxybenzene Sulfonate Catalyzed by peroxidase to form a pink Coloured quinoneimine dye. The absorbance is recorded in green filter.

**Results**

Results show that the change in the levels of lipid parameters follow a near similar trend in the two non-diabetic age groups with Cardiovascular complications as shown in the Table 1. The data shown in the table are the mean value of all 15 subjects with ±S.E.M. Except the HDL-C decline, total Cholesterol, LDL – C, VLDL and triglycerides increase with age.

Among non-diabetic middle aged subjects the level of cholesterol was found 203.5±3.37 which was further increased to 237±5.85 in same age group with CVC. In old age non-diabetic people it was 224±3.72, which further increased to 260.73±4.79. Although the value of cholesterol among non-diabetic, middle aged and old aged people was within normal limit, but when it was compared with their younger group the increased was significant. Similarly the LDL-C, VLDL-C and triglycerides also increased significantly with age when compared to there younger counterpart. The value were further increased among the subjects with CVC in comparison to the subjects without CVC of same age group. Level of HDL-C was found in decreasing order in similar fashion.

Among the diabetic subjects with CVC of both age group the level of cholesterol, LDL-C, VLDL-C and triglycerides was very much higher than non-diabetic subjects of same age group without CVC. Although these levels was higher among diabetic subjects of 65±5 y age groups to the diabetic subjects of 45±5 y group, but difference was not very high. But the level of HDL-C was in decreasing order.

However, among diabetic the said lipid parameters are not so similarly affected in the stated age subjects. One interesting observation is the near similar levels of lipid parameters found in diabetic 45±5 y and non-diabetic 65±5 y age groups with Cardiovascular complications.

**Types of Subjects**

The subjects selected for this study were non-obese, non-hypertensive & free from nephropathy. The entire subjects were divided into seven groups according to their age and patho-physiological state as shown in the Table below.
**Table 1:** Lipid profile in different age group with and without cardiovascular complication in diabetic and non-diabetic subjects

<table>
<thead>
<tr>
<th>Age (in years)</th>
<th>25±5</th>
<th>45±5</th>
<th>65±5</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Physiological State</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-diabetic without CVC</td>
<td>Non-diabetic without CVC</td>
<td>Non-diabetic with CVC</td>
<td>Diabetic with CVC</td>
</tr>
<tr>
<td>Serum total cholesterol (mg/dl)</td>
<td>174.46±6.90</td>
<td>203.5±3.37</td>
<td>237.8±5.85</td>
</tr>
<tr>
<td>S. HDL-C (mg/dl)</td>
<td>60.53±1.34</td>
<td>56.7±0.79</td>
<td>48.26±1.02</td>
</tr>
<tr>
<td>S. LDL-C (mg/dl)</td>
<td>89.86±5.43</td>
<td>113.4±1.85</td>
<td>143.13±5.98</td>
</tr>
<tr>
<td>S. VLDL-C (mg/dl)</td>
<td>24.73±1.09</td>
<td>33.4±1.22</td>
<td>45.73±0.95</td>
</tr>
<tr>
<td>S. Triglycerides (mg/dl)</td>
<td>122.33±5.36</td>
<td>162.0±6.13</td>
<td>228.7±4.79</td>
</tr>
</tbody>
</table>

All the values in the table are mean±S.E.M.

CVC – Cardiovascular complication.

**Discussion**

It is well known that the disorders of lipoprotein metabolism result from abnormal synthesis, processing or Catabolism of Plasma lipoprotein particles. More than half of patients with angiographically confirmed coronary heart disease before age 60 years have a familial lipoprotein disorders. This association is most striking among younger patients and declines with increasing age at first myocardial infarction. This suggests the presence of genetic factors that accelerate age-associated Cardiovascular changes seen in the general population. Four types of lipoprotein abnormalities are observed: elevated LDL – Cholesterol; reduced HDL Cholesterol, increased triglycerides and VLDL – Cholesterol.

The metabolic syndrome i.e. the clustering of high serum triglyceride, small dense LDL-particles, low serum HDL-cholesterol levels, hypertension, insulin resistance and a prothrombotic state, is an important risk factor for the development of CVD in older persons including very old people.

Cholesterol levels over 240 mg/dl are associated with a three-fold increased risk of death from ischaemic heart disease in men relative to cholesterol levels below 200 mg/dl, and there is a continuous risk gradient as the...
cholesterol rises. Elevated total cholesterol primarily reflects elevated LDL cholesterol which constitutes 70 percent of serum cholesterol. Low HDL levels, usually accompanied by elevated plasma triglyceride levels represent the most common dyslipidemia associated with CHD.

There is an overall inverse relationship between serum triglycerides & HDL cholesterol levels in the general population. This relationship primarily arises from the fact that high serum triglycerides stimulate cholesterol ester transfer from HDL to triglyceride rich lipoproteins an effect mediated by plasma C E transfer protein.

Effect of Diabetes on lipoproteins: The commonest abnormality in diabetes is hypertriglyceridemia due to an excess of very low density lipoprotein(VLDL). Lipoprotein lipase depends for its full activity on insulin, and VLDL clearance is reduced in poorly controlled patients with IDDM. In NIDDM patients, there is also overproduction of VLDL & apoprotein (apoB). Insulin deficiency or resistance increases production of non-esterified fatty acids from adipose tissue by the action of hormone sensitive lipase and these provide a substrate for hepatic triglyceride synthesis Hypertriglyceridermia in diabetics, therefore, usually responds to intensified insulin treatment.

LDL levels are also raised in association with poor glycemic control, but a substantial improvement in blood glucose is required to lower LDL. Insulin stimulates LDL receptor activity, increasing LDL clearance, while non enzymatic glycosylation of apo B reduces its affinity for the receptor, thereby, slowing down LDL removal. HDL levels vary inversely with VLDL. Since reduced lipoprotein lipase activity impairs Catabolism of VLDL and hence transfer of lipids and apoproteins to HDL. In NIDDM, HDL levels are low especially in association with hyper triglyceridermia, whereas in IDDM the levels are normal. Glycosylation of HDL occurs in vivo and in animal studies glycosylated HDL is removed faster than normal. Glycosylation of HDL occurs in vivo and in clinical studies glycosylated HDL is removed faster than normal.

Shared mechanism of Pathogenesis of CVC during diabetes and ageing: Both ageing and diabetes are associated with a loss in function of cardiovascular system and increased CVC risk factor. Epidemiological data from the Multiple Risk Factor indicate that the risk for the cardiovascular death is increased two to three fold in type-2 diabetic individuals. Moreover, after a first myocardial infarction, cardiovascular morbidity and mortality are increased in patients with diabetes compared with non-diabetic patients. The reason why the diabetic patients have such a high risk for CVC are probably multifactorial and include dyslipidemia, hypertension, inflammation, oxidative stress and accumulation of AGEs. Typically, patients with type-2 diabetes are characterized by hyper triglyceridemia and low HDL-cholesterol levels. Diabetic patients also exhibit alteration in post prandial lipid transport.

One commonly reported problems of CVC in both ageing and diabetes is a reduction in the ability of the peripheral vasculature to vasodilate. A similar mechanism might be involved in reducing vascular reactivity with age and diabetes.

The mechanism for reduced resting and post ischaemic blood flow with both ageing and diabetes has been linked to a reduction of the vascular endothelial cells ability to produce nitric oxide, a potent vasodilator substance. This may be due to a defect in nitric oxide synthesis, decreased nitric oxide sensitivity or reduced availability of L-arginine, the precursor of nitric oxide. Ageing and diabetes effect endothelial function by a similar mechanism, so that it might be inferred that diabetes potentiated the loss in endothelial function with age.

Non-enzymatic protein glycation is also a common feature in both ageing and diabetes. Glucose irreversibly modifies long-lived macromolecules by forming advanced glycation end products (AGEs) as a function of glucose concentration and time. The formation of AGEs is also associated with the increased production of ROS, thus, linking the pathophysiological model of non-enzymatic glycation to oxidative stress and in addition, increased circulating levels of AGEs and glycation of basement membranes of vessel walls may affect vascular function, as endothelial oxidative damage and endothelial dysfunction have been observed in the presence of AGEs. Furthermore, AGEs may be responsible for quenching of the vasodilating compound and nitric oxide. In tissues affected by diabetic complications the amounts of AGEs are generally increased, leading to structural changes in the extracellular matrix, as well as to modifications to cell membranes and intracellular components.

The important correlation observed in this study, which lies in the serum lipid and lipoprotein components among elderly and diabetics is, the changes which observed with non-diabetic elderly at the age of 60-65 years with CVC, match to those found among diabetics at the age of 40-45 years with CVC. Serum total cholesterol increases with age, CVC and diabetes. Interestingly, S-total cholesterol among diabetic middle age patients with CVC appears to be similar to those found in non-diabetic and diabetic elders with CVC, suggesting that
diabetes coupled with cardiovascular disorders accelerate the cholesterol elevation much earlier. Similarly LDL-C and VLDL-C are also found to go up with age, CVC and diabetes. However, HDL-C decreases with age and diabetes in CVC pointing to the fact that the good cholesterol starts depleting as a function of age. Diabetes and cardiovascular disorders only add fuel to the fire in this depletion. Likewise, the triglycerides also increase with age, which is further enhanced by diabetes.

On the basis of the present study and evidences available from the various other investigation regarding the changes in lipoprotein during ageing and diabetes, it may be suggested that diabetes accelerates age related disturbances in the lipid profile.

References