IS CARDIOVASCULAR RISK MORE IN DIABETICS BECAUSE OF LOWER APOLIPOPROTEIN A1 LEVELS RATHER THAN HIGHER APO B /APOA1 RATIO?

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ABSTRACT

Diabetics are known to have increased risk of cardiovascular diseases. Apolipoprotein B/ A1 ratio is considered to be a good biochemical marker for Coronary Artery Disease (CAD) risk assessment. The study was designed to estimate serum levels of apolipoprotein A1 , B and calculate Apo B/Apo A1 ratio in thirty diagnosed patients of type II diabetes attending the diabetic clinic in Lady Hardinge Medical College and Associated SSK Hospital, comparing them with age and sex matched controls and assessing their significance levels. Fasting Plasma Glucose ,Apolipoprotein A1 and apolipoprotein B in serum were measured by on fully automated analyser SYNCHRON CX9 using Kit from Randox . Statistical analysis was done by using SPSS version 17.0.It was found that Fasting plasma glucose was significantly higher in the study group compared to control group ($p<0.0001$). Apolipoprotein A1 was lower in patients of diabetes mellitus as compared to controls and the difference was very highly significant $p<0.0001$. Apolipoprotein B levels as well as Apo B/Apo A1 were significantly higher in diabetics compared to controls with $p<0.01$ for both. It emerged from the study that apolipoprotein A1 levels are more significant compared to apolipoprotein B and ratio of Apo B/Apo A1 and therefore is a better assessor of CAD risk in diabetics.

KEY WORDS: Apolipoprotein A1, Apolipoprotein B, CAD, Diabetes Mellitus

INTRODUCTION

The prevalence of diabetes mellitus has been increasing worldwide with an expected doubling of diabetic population from 171 million to 366 million between 2000-2030. The greatest relative increase will occur in Middle Eastern Crescent, Sub Saharan Africa and India[1]. This diabetic population is predisposed to an increase risk of both micro and macrovascular complications and some

50% of people with diabetes die of cardiovascular disease[2]. Atherosclerosis which may began early in presence of diabetes, and lipid and lipoprotein abnormalities can be a cause of increased cardiovascular complications in such patients[3,4,5,6].

Diabetic patients are also known to be at increased risk of dylipidemias[7]. Dyslipidemia in diabetes is characterized by elevated triglycerides, low high-density lipoprotein (HDL) cholesterol levels, and increased small dense low-density lipoprotein (LDL) [8,9,10,11]. The risk of developing a coronary heart disease(CHD) in diabetic patients is so well established that ATP III guidelines recognizes diabetes as an CHD- risk equivalent and categorises it in a high risk category[12] .

There are four major groups of lipoproteins that are known, namely chylomicrons, very low density lipoprotein (VLDL), low density lipoprotein(LDL) and high density lipoprotein (HDL).The protein moiety of a lipoprotein is known as apolipoprotein which can be of different types with different functions . Apo B is the major protein component of VLDL and LDL in serum and forms an important part of their structure, while apo A1 is a major protein component of HDL[13]. Population studies have suggested that levels of serum apolipoproteins like Apolipoprotein B, Apolipoprotein A1 or ApoB/Apo A1 ratio are more strongly related to the risk of cardiovascular disease than cholesterol fractions of lipoproteins[14,15,16].

Our aim in this study was to see the variations of serum level of these apolipoproteins in diabetic (type 2) patients, compare with age and sex matched controls and to thereby analyse which of these is more significantly different in the two groups and best for use as a biochemical marker of CAD risk in diabetics.

MATERIAL AND METHODS

Study sample comprised of 30 diagnosed type II diabetes mellitus patients attending the diabetic clinic in Lady Hardinge Medical College and Associated SSK Hospital. All the patients were on oral hypoglycaemic agents. Thirty age and sex matched controls with no history suggestive of diabetes mellitus were also enrolled. Detailed history and clinical examination was done after taking informed onset. The procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional or regional) and with the Helsinki Declaration of 1975, as revised in 1983.

Blood sample was drawn after an overnight fast and collected in two vials. 3ml of venous blood sample was collected in a vial containing sodium fluoride and potassium oxalate (in ratio 1:3) for fasting plasma glucose estimation and another 3ml venous blood sample was collected in plain vial for serum apolipoprotein-B and apolipoprotein A1 estimation which was allowed to clot at room temperature. It was then centrifuged at 3500rpm for 5 min. Serum sample was stored in aliquots at -20°C till batch analysed for serum Apo A1 and Apo B. Analysis was done within 8 weeks as per their stability suggested in the protocol provided with kit. Fasting Plasma Glucose was measured by Glucose Oxidase method (Randox). Glucose present in the plasma is oxidized

by the enzyme glucose oxidase (GOD) to gluconic acid with the liberation of hydrogen peroxide, which is converted to water and oxygen by the enzyme peroxidase (POD).4 aminophenazone, an oxygen acceptor, takes up the oxygen and together with phenol forms red - violet quinoneimine dye as indicator and absorbance is measured at 540 nm.

Determination of Apolipoprotein A1 and Apolipoprotein B in serum was performed by immunoturbidometric immunoassay on SYNCHRON CX9 using Kit from Randox (U.K).Sample containing human Apo A1 and apo B were made to react with the respective specific antiserum to form an insoluble complex which was measured turbidometrically at 340 nm. Lot specific information was used for reconstitution of calibrator (Lot No.499LP). After successful calibration, Lipid Controls (Lot no. LE2662) were run in dilution of 1:20(For Apo A1) and Undiluted for Apolipoprotein B with normal saline before samples were analysed. Results obtained were acceptable in the range provided with the controls. Apolipoprotein A1 and B concentrations were thus determined for the samples.

RESULTS

Table 1 show the baseline characteristics and fasting plasma glucose levels in the two groups. The study group and control group were comparable to each other with respect to age and sex. Fasting plasma glucose was significantly higher in the study group compared to control group as represented by $p<0.0001$. Table 2 is showing the levels of apolipoprotein A1, B and apoB/apoA1 ratio in the two groups. Apolipoprotein A1 was lower in patients of Diabetes Mellitus as compared to controls and the difference was very highly significant $p<0.0001$. Apolipoprotein B levels as well as Apo B/Apo A1 were higher in cases of Diabetes Mellitus as compared to controls and this difference is significant statistically with $p<0.01$ for both.

DISCUSSION

In our hospital based case control study we have found that apolipoprotein B levels are significantly higher in cases as compared to controls ($p<0.01$). Apolipoprotein B is the major apolipoprotein in chylomicrons, VLDL, intermediate-density lipoprotein, and LDL. It is an essential structural part in these lipoprotein particles. The genetic inability to secrete apolipoprotein B causes the absence of these lipoproteins in plasma [17]. Furthermore, mutations in the ApoB gene can cause low levels of Apo B and LDL cholesterol and may be associated with protection from premature coronary artery disease [18]. In addition, ApoB acts as a ligand for the LDL receptor, mediating the cellular uptake and degradation of LDL [19]. One molecule of ApoB exists per lipoprotein particle, and thus the quantity of ApoB in fasting plasma is a measure of the number of LDL and VLDL particles. In fact, the plasma levels of “non-HDL cholesterol”, which includes both LDL and VLDL, are correlated with plasma apolipoprotein B levels[20,21]. However, in contrast to the constant 1: 1 molar ratio of ApoB per LDL and VLDL particle, the amount of cholesterol in these lipoproteins varies widely. Therefore, plasma ApoB levels may be a better assay of the concentration of atherogenic lipoprotein particles than are LDL cholesterol or non-HDL

cholesterol levels[22,23]. Association of high Apo B concentration with increased CHD incidence has been demonstrated in Asian population[24]. Snehalatha and Ramachandran et al have shown that the apolipoprotein B and A1 provide better information regarding the risk of CHD[25].

Also, in our study apolipoprotein A1 levels are reduced in diabetic subjects compared to controls with the resultant apoB/Apo A1 ratio significantly higher in cases compared to controls. This reduction of apolipoprotein A1 was more significant ($p<0.0001$) compared to elevation of apolipoprotein B ($p<0.01$). ApoA-I is the major apolipoprotein in HDL, and it is probably important in protecting against premature atherosclerosis. Genetic defects that cause the inability to synthesize ApoA-I cause very low plasma concentrations of HDL cholesterol and premature coronary artery disease in the fourth and fifth decades [26,27,28,29]. The potential mechanisms of the cardio protective effects of apo A-I include enhancement of reverse cholesterol transport, attenuation of oxidative stress, increased peroxonase activity, and enhanced anticoagulant activity[30].

Expression of the apo A-I gene is regulated primarily at the transcriptional level. The apo A-I gene promoter contains a TATA-like motif close to the transcriptional start site, while further 5', several *cis* elements regulate expression of the gene in either a positive or negative manner in response to changes in the hormonal or metabolic status [31]. Thyroid hormones, retinoids, estrogens, and glucocorticoids have been shown to induce apo A-I promoter activity and gene expression through proximal promoter elements located between nucleotides -235 and -144 (relative to the transcriptional start site [32]. Further 5'from the transcriptional start site, an insulin response core element (IRCE) is located between nucleotides -404 and -411[33]. This element binds to the ubiquitous transcription factor Sp1 and is responsible for the induction of the apo A-I gene by insulin. Thus, expression of the apo A-I gene is subject to regulation by level of insulin, which is reduced in diabetics and this explains the finding of reduced apoA1 levels in our study group compared to controls.

More and more concern has been attracted by ApoB/ApoA1 ratio, a novel predictor for high risk of atherosclerosis. Many studies showed high ApoB and a high ApoB/ApoA-I ratio were strongly related to increased coronary risk[16,22,34]. Walldius and Jungner reviewed articles recently published on ApoB/ApoA-I ratio and risk of atherosclerosis and concluded that the cholesterol balance determined as the ApoB/ApoA-I ratio has repeatedly been shown to be a better marker than lipids, lipoproteins and lipid ratios [35].Results indicated that the ApoB/ ApoA-I ratio was a simple, accurate and new risk factor for cardiovascular disease.

However in our study apolipoprotein A1 levels were more significantly different in diabetics as compared to apolipoprotein B ($p<0.01$) or ApoB/Apo A1 ratio ($p<0.01$). As explained above, it can be because of the decreased insulin level affecting the transcription of apolipoprotein A1 in diabetics.

CONCLUSION

We have found significantly elevated levels of atherogenic apolipoprotein B

levels, apoB/apo A1 ratio and reduced levels of antiatherogenic apolipoprotein A1 levels in Diabetic subjects compared to healthy controls. Out of all these apolipoprotein A1 emerged as the most significant biochemical marker. So, we hereby advocate the use of apolipoprotein A1 levels over and above apolipoprotein B and ratio of Apo B/Apo A1 for assessing risk of cardiovascular disease in diabetics. However ours being a pilot study, a prospective study with a larger sample size and follow up should be done to confirm the findings of the present study.

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Table 1. Baseline characteristics of the two groups

Baseline Characteristics	Study Group (n=30) (Mean±S.D)	Control Group (n=30) (Mean±S.D.)	P Value
Age (in years)	50.57±8.98	47.13± 4.96	0.071
Sex Distribution	Males= 15(50%) Females=15(50%)	Males=12(40%) Females= 18(60%)	0.601
Fasting Plasma Glucose	152.05±69.21	86.76± 15.40	**<0.0001***

*highly significant

Table 2. Apolipoprotein A1, B levels and Apo B/Apo A1 ratio in study and control group

Apolipoprotein levels	Study Group (n=30) (Mean±S.D)	Control Group (n=30) (Mean±S.D.)	P Value
Apolipoprotein B	98.861±18.61	73.84±12.63	**<0.01***
Apolipoprotein A1	69.81±18.61	100.26±6.13	**<0.0001****
ApoB/Apo A1 ratio	0.986±0.22	0.737±0.12	**<0.01***

*highly significant;**very highly significant