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ASSOCIATION OF HbA1c WITH SERUM LIPID PROFILE AND LIPOPROTEIN (a) IN TYPE 2 DIABETES MELLITUS

Mahantesh Patil[1], Nirmal Kumar[2], Aliya Nusrath[2], Shubha Jayaram[1], Rajeshwari A.[2]

[1]Department of Biochemistry, MMC & RI, Mysore, KA, India
[2]Department of Biochemistry, AIMS & RI, B.G.Nagara, Mandya, KA, India

E-mail of Corresponding Author: dr_mgpatil@yahoo.co.in

ABSTRACT

Background: Diabetes mellitus (DM) is a group of metabolic disorders of carbohydrate metabolism. Also it is proposed thatunderutilization of glucose is associated with changes in lipid profile. Lipoprotein (a) [Lp (a)] is regarded as an independent indicator of risk development of vascular disease which is also a diabetic complication. Changes in lipid profile are also well related with severity of DM as adjudged by glycated Hb (HbA1c).

Objectives: The study intends to find the association between Lipid profile, Lp (a) and HbA1c levels in type 2 diabetic patients.

Methods: A case-control study was conducted in Adichunchanagiri Institute of Medical Sciences, B.G.Nagara, Karnataka from 1st Jan to 15th June 2012. Study involved 80 participants of which 40 were patients admitted with diagnosis of DM and other 40 were healthy controls. Blood samples were collected in fasting state and analyzed for FBS, PPBS, HbA1c, TAG, VLDL, HDL, LDL and Lp(a) and values were tabulated for statistical evaluation.

Results: In DM patientssignificant changes in the following parameters were observedcompared to controls. FBS, PPBS, HbA1c &Lp (a) levels increased significantly (P<0.001), HbA1c/HDL, HbA1c/LDL & HbA1c/Chol ratios also increased significantly (P<0.001). Also the levels of TAG, VLDL &Chol/HDL were significantly increased with P<0.008, P<0.011 & P<0.003 respectively. The levels of HDL were significantly reduced in patients with DM compared to controls with P<0.002. There is no significant change observed in Cholesterol, LDL & HbA1c/Lp (a) levels.

Conclusions: Along with lipid profile and Chol/HDL ratio, study of HbA1c/HDL, HbA1c/LDL & HbA1c/Chol ratios may be helpful in risk assessment of coronary heart disease in DM and association between Lp (a) and HbA1c needs to be further evaluated.

Keywords: Diabetes Mellitus, Glycated hemoglobin (HbA1c), Lp (a)

INTRODUCTION

Diabetes mellitus (DM) is an iceberg disease. The metabolic dysregulation associated with DM causes secondary pathological changes in multiple organ systems that impose a tremendous burden on the individual with diabetes and on the health care system. In the United States, DM is the leading cause of end-stage renal disease (ESRD), no traumaticlower extremity amputations, and adult blindness. It also predisposes to cardiovascular diseases.

With an increasing incidence worldwide, DM will be a leading cause of morbidity and mortality for the foreseeable future.[1]The number of adults with diabetes in the world will rise from 135 million in 1995 to 300 million in the year 2025. Presently, India, China, and the United States are the countries with the largest number of people suffering from diabetes and this trend is expected

to continue till the year 2025,.[2, 3]In India alone, diabetes is expected to increase from 40.6 million in 2006 to 79.4 million by 2030.[4]

Most of the longstanding macro and micro vascular complications are also more common among Indian diabetics as compared to other races and ethnic groups. Recent studies have shown that the prevalence of coronary heart disease (CHD) in Indian diabetics may be as high as in the migrant population. [4]

Changes in lipid-profile are a consequential event in DM. Due to these changes distribution and function of various fractions of lipids are affected. Many Studies have evaluated the risk factors for CHD in DM patients and observed high fasting blood sugar (FBS) and post prandial blood sugar (PPBS), total cholesterol (Chol), low density lipoproteins (LDL), triglycerides (TAG) levels and low high density lipoproteins (HDL) levels when compared to controls. [5]

Glycated heamoglobin (HbA1c) is considered a gold-standard measure of chronic glycemia in diabetic patients. In the Rancho Bernardo study, HbA1c was a better CHD predictor than fasting or 2-h glucose.[6]HbA1c was strongly associated with atherosclerosis as measured by carotid IMT (intima-media thickness). [7, 8]The ADA recommends measurement of HbA1c in patients with both type 1 and 2 diabetes, first to document the degree of glycemic control, then as part of continuing care.[9] Changes in lipid profile is also well related with severity of DM as adjudged by HbA1c.

Lipoprotein (a) [Lp (a)] is a distinct class of lipoprotein that is structurally related to LDL, because both lipoprotein classes possess one molecule of Apo B-100 per particle and have similar lipid compositions. However, unlike LDL, Lp (a) contains a carbohydrate rich protein [Apo (a)]. Apo (a) is a unique component and has significant homology with plasminogen. [10]

The serum level of Lp (a) is an independent indicator of risk development of vascular disease.[11] A clear correlation was found between the serum level of Lp(a) and its accumulation in the vessel wall.[12] Recently much interest has been focused on Lp(a) as an important marker of CHD.The level of Lp (a) is genetically determined, and when elevated, cannot be lowered by alterations in food intake or by most of the cholesterol lowering agents. Diabetic patients are reported to have higher Lp(a) values than non-diabetic persons and levels are still more significantly elevated in patients with diabetic complications.[13] The data on relationship between Lp(a) and diabetes is scarce and the data on Lp(a) in Asian Indian diabetics is still meagre.[14] Therefore the present study has been undertaken to assess the serum concentration of Lp(a) in patients with diabetes mellitus and to study any association of HbA1C with lipid profile and Lp(a) levels.

METHODOLOGY

This case control study was conducted at Adichunchanagiri Institute of Medical Sciences, Karnataka, India. A total number of 80 subjects participated in the present study. Forty controls and 40 clinically diagnosed cases of diabetes mellitus patients attending out- patient and inpatient departments of ShriAdichunchanagiri Hospital and Research Center (SAH&RC) were included in the study. Age and sex matched healthy individuals are taken as control group. The study was approved by ethical and research committee of SAH&RC. Patients with signs and symptoms of obstructive jaundice, hypothyroidism, hypopituitarism, epileptic patients, psychiatric disorders & nephrotic syndrome were excluded from study.

Non-probability convenient sampling method was adopted for sample selection. After obtaining informed consent from patients and controls the data was collected using semi structured questionnaire. The questionnaire included the following information like, socio demographic data, detailed medical historyand relevant clinical examination data.

Collection of blood sample

Under aseptic precautions,7ml of Blood sample in fasting state was drawn from controls and clinically diagnosed cases of DM.Then the blood sample was divided into 3 test tubes, marked as 1, 2 and 3 and analyzed respectively for blood glucose (FBS & PPBS), Lp(a) & other lipid parameters (TAG, Chol, HDL, VLDL & LDL) and HbA1c.

1. Test tube 1 containing 2ml of blood with anticoagulant was used for estimation of blood glucose by Glucose Oxidase method. [15]

2. Test tube 2 containing 3ml of blood with no anticoagulant was allowed to clot and serum was separated. Serum was used for measurement of-TAG by GPO-Trindermethod[15,16], Cholesterol by CHOD-POD method[17], HDL by Phosphotungstic acid method[18], VLDL was calculated by formula (TG/5)[8], LDL was derived by Fredrickson-Friedwald formula [(TC-HDL) – TG / 5] [8] & [Lp(a)] was estimated by Immunoturbidometric method.[19]

3. Test tube 3 containing 2ml of whole blood was used for estimation of HbA1c by Affinity Chromatography. [20, 21]

The chemicals and reagents used for the procedures were of analytical grade.

Descriptive statistical analysis has been carried out in the present study. Results on continuous measurements are presented as Mean ± SD and results on categorical measurements are presented in Number (%). Significance is assessed at 5 % level of significance.Statistical significance between two groups (Inter group analysis) was studied using Student t-test (two tailed, independent). Chi-square/Fisher Exact test has been used to find the significance of study parameters on categorical scale between two groups.

RESULTS

The results of the study parameters FBS, PPBS, HbA1c, TAG, Chol, HDL, LDL, VLDL, Lp (a), are depicted in tabular form.

Table 1 shows the demographic distribution between control group and patient group. Samples are age and gender matched with P=0.113.

Table 2 gives the results of FBS, PPBS & HbA1c levels of patients and control group presented as Mean±SD. There is increase in all 3 parameters in patients compared to controls which is statistically strongly significant ($p<0.001$).

Table 3 gives the results of lipid profile and Lp (a) levels of patients and control group expressed as Mean±SD. There is strongly significant increase in TAG, HDL, VLDL & Lp (a) levels in patients compared to controls, whereas cholesterol & LDL showed no statistical significance.

Table 4 gives the results of Association between HDL, LDL, Chol and Lp (a) with HbA1c (HbA1c / HDL, HbA1c / LDL, HbA1c / Chol & HbA1c / Lp (a)) & Chol / HDL levels of patients and control group. HbA1c / HDL, HbA1c / LDL & HbA1c / Chol ratios shows statistically strongly significant results in patients when compared to controls, whereas HbA1c/Lp(a) ratio shows no significance.

DISCUSSION

The present study shows FBS & PPBS values of patients higher than upper limit which correlated well with the clinical diagnosis. HbA1c is done to monitor the control of blood glucose in DM. Several studies have shown the positive correlation of HbA1c with duration of DM and as a strong predictor of risk (cardiovascular diseases) for diabetes complications. [22]

HDL, LDL, TAG and Chol are well known risk factors for complications of DM like CHD. In a study by H. Surekha Rani Et.al., it is observed that FBS and PPBS, Chol, VLDL, LDLs, TAGs were high and the levels of HDLs were low compared to controls. [5] In diabetic dyslipidemia, high TAG tend to coexist with low HDL and small, dense, undesirable (more atherogenic) type of LDL in their blood (even though their LDL level may be normal). In the present study we found significantly increased levels of TAG and VLDL

and decreased HDL levels. But no significant change was observed with serum levels of LDL and total cholesterol.

Studies have shown that high Lp(a) in blood is a risk factor for CHD, cerebrovascular disease(CVD), atherosclerosis,thrombosis, and stroke.[23] Present study also shows statistically significant increase ($p<0.001$) in the levels of Lp(a) in cases compared to the controls predicting the possibilities of CHD in diabetic complications.

The mean values of Chol/HDL ratio are significantly higher in diabetics than non-diabetics. Increased Chol/HDL ratio increases the risk of coronary artery disease.

Study by Elizabeth et.al, observed that LDL and HDL cholesterol were significantly associated with HbA1c. HDL cholesterol was inversely associated with HbA1c where as LDL cholesterol was positively associated with HbA1c in diagnosed diabetics. [24] In present study there is no significant change in the LDL and total cholesterol levels between cases and controls. However there is a significant difference in the HbA1c/LDL & HbA1c/Chol ratios between cases and controls. Similarly we also observed a significant difference in HbA1c/HDL ratio ($p<0.001$) indicating the inverse relation between HDL and the HbA1c levels.

An important risk factor evaluated in the study, Lp (a), showed a significant rise in the serum levels in DM patients compared to controls but its levels were not significantly associated with HbA1c levels which is a standard risk factor for the CHD.

CONCLUSION

Lp (a) may not be an independent risk factor for CHD in patients with DM and Chol/HDL, HbA1c/LDL & HbA1c/Chol may be better indicators in determining the risk factors of CHD in DM than evaluating the parameters individually.

However further studies with large sample size are required to evaluate the correlation between the Lp (a) and other lipoprotein factors with HbA1c to assess the risk for development of CHD in DM.

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Table 1: Comparison of gender distribution in DM

	Controls		Cases	
	No	**%**	**No**	**%**
Age	50.10±12.86		49.50±9.66	
Male	26	65.0	16	40.0
Female	14	35.0	24	60.0
Total	40	100.0	40	100.0
h/o of DM (yrs)	5 ± 0.7	---	---	---
BMI	24.38 ± 3.19		25.1 ± 2.83	

Samples are age and gender matched with P=0.11

Table 2: Levels of FBS, PPBS, and HbA1c in controls and cases

Sugar parameters	Controls	Cases	Significance
FBS (mg/dl)	86.20±8.28	167.70±59.80	t=6.037;p<0.001**
PPBS (mg/dl)	107.20±19.11	238.30±81.31	t=7.019;p<0.001**
HbA1c	5.41±0.30	9.44±2.31	t=7.742;p<0.001**

Results are presented in Mean ± SD

+ Suggestive significance (P value: 0.05<P<0.10);* Moderately significant (P value: 0.01<P ≤ 0.05);** Strongly significant (P value: P≤0.01)

Table 3: Levels of Lipid parameters in controls and cases

Lipid profile	Controls	Cases	Significance
Triglycerides (mg/dl)	128.60±28.25	195.25±102.65	t=2.800;p=0.008**
Total cholesterol (mg/dl)	169.20±24.57	174.25±42.86	t=0.457;p=0.650
HDL (mg/dl)	45.05±6.64	37.25±8.03	t=3.348;p=0.002**
LDL (mg/dl)	100.95±21.22	100.40±34.83	t=0.060;p=0.952
VLDL (mg/dl)	24.95±5.98	36.60±18.59	t=2.667;p=0.011*
Lipoprotein(a) (mg/dl)	20.16±6.26	46.20±22.92	t=4.783;p<0.001**

Results are presented in Mean ± SD

Table 4: Association between HDL, LDL, Chol and Lp (a) with HbA1c &Chol/HDL levels in controls and cases

Lipid profile	Controls	Cases	Significance
HbA1c/ Lp(a)	0.31±0.14	0.36±0.43	t=0.47083;p=0.641
Chol/HDL	3.81±0.68	4.79±1.21	t=3.160;p=0.003**
HbA1c/HDL	0.12±0.02	0.26±0.07	t=9.026;p<0.001**
HbA1c/LDL	0.06±0.01	0.10±0.04	t=5.466;p<0.001**
HbA1c/Chol	0.03±0.01	0.06±0.01	t=7.640;p<0.001**

Results are presented in Mean ± SD