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ESTIMATION OF SERUM LIPOPROTEIN (A), LIPID PROFILE AND HBA1C IN PATIENTS WITH TYPE 2 DIABETES MELLITUS - A CASE CONTROL STUDY

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ABSTRACT

Objective: Diabetes is a major worldwide health problem, leading to markedly increased mortality and serious morbidity. It is characterised by chronic hyperglycemia resulting from a diversity of aetiologies and environmental and genetic factors acting together. Type 2 diabetes mellitus itself leads to dyslipidemia like elevated triglyceride levels and low HDL levels, which are known risk factors for coronary artery disease (CAD). Increased lipoprotein (a)[Lp(a)] concentrations are predictive of CAD. The study was designed to estimate serum lipoprotein (a) and glycosylated hemoglobin (HbA1c) in south Indian patients with type 2 diabetes mellitus and compare them with healthy controls.

Material and Methods: The study included 50 patients of type 2 diabete mellitus and 50 age and sex matched controls. Fasting venous blood sample was analysed for fasting blood glucose(FBS), glycated hemoglobin (HbA1c), lipid profile and serum Lp(a). Statistical analysis was done using student t test.

Results: The serum Lp(a) and HbA1c levels were significantly elevated in type 2 diabetics compared to healthy controls ($p<0.001$). There was significant elevation in the levels of FBG ,total cholesterol, total triglyceride, LDL cholesterol and TC/HDL-cholesterol ratio in type2 diabetics compared to the control group($p<0.05$).

Conclusion: Diabetic patients have higher levels of serum Lp(a) and HbA1c compared to healthy controls. Serum Lp(a) associated with lipid abnormalities - high total cholesterol, high triglyceride ,high LDL and low HDL makes diabetics prone for CAD.

Key Words: Type 2 diabetes mellitus, Glycated hemoglobin, Lipid profile, Lipoprotein a

INTRODUCTION

Diabetes mellitus (DM) is world-wide in distribution. Diabetes mellitus is a chronic metabolic disorder that is often associated with unacceptably high disease burden especially in developing countries and the cardiovascular complications of DM are highly contributory to this scenario[1]. In India, diabetes is not an epidemic anymore but has turned into a pandemic, according to the International Journal of Diabetes in developing countries which labelled India the diabetes capital of the world. Mainly because India now has the highest number of diabetes patients in the world. The International Diabetes Federation estimates that the number of diabetic patients in India more than doubled from 19 million in 1995 to 40.90 million in 2007. It is projected to increase to 69.9 million by 2025.Currently upto 11 percent of India's urban population and 3 percent of rural population above the age of 15 has diabetes. The most prevalent is type 2 diabetes[2]. Well studied and documented CVS risk factors in DM include components of metabolic syndrome namely dyslipidemia, central obesity and hypertension[3]. Other CVS risk factors that have not been widely studied in Indian population include C-reactive protein, hyeuricemia, HbA1c and lipoprotein (a). Lipoprotein (a) [Lp(a)] consists of an LDL like particle and the specific apolipoprotein (a) [apo(a)],which is covalently bound to the apo B of the LDL like particle. The structure of Lp(a)

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resembles LDL and its atherogenic properties can be explained by its binding to glycosaminoglycans and inhibition of fibrinolysis. The atherogenic properties of Lp(a) are expressed over 30 mg/dl[4].

Persistent hyperglycaemia causes glycosylation of the proteins especially hemoglobin[5]. Haemoglobin glycation, estimated by percentage of glycated haemoglobin (HbA1c) ,was first used clinically 30 years ago to asses the degree of chronic hyperglycaemia among diabetic patients in whom values reflect weighted mean glucose levels over the preceding 3 months period; it is useful for characterizing dysglycemia in population studies because it is simpler to perform than the oral glucose tolerance test(GTT) [6]. In Diabetics an increase in HbA1c of 1 percent was associated with a 20 % to 30% increase in mortality associated with cardiovascular events[7].

MATERIALS AND METHODS

Study Participants: The present study comprises 50 patients with Non-Insulin dependent diabetes mellitus (NIDDM) reporting to Hanagal Shree Kumareshwar Hospital and Research Centre. The criteria for the diagnosis of DM was according to the criteria of the American Diabetes Association (ADA) 2007 guidelines. [8]

50 subjects of similar age, sex and socioeconomic status served as controls. The controls were free from any major aliment which could affect the parameters under study (the clinical history or investigative results showed no involvement of any organ).The exclusion criteria included type 1 DM, Gestational diabetes , other specific causes for diabetes, micro- and mcro-vascular complications frank proteinuria detected by albustix, cigarette smoking and on lipid altering drugs like oral contraceptive, diuretics, beta blockers and lipid lowering drugs. Subjects with lipid altering diseases: hepatobiliary disease, hypothyroidism, chronic kidney disease and nephrotic syndrome were also excluded.

Informed written consent was obtained from all the subjects enrolled for the study. Institutional ethical committee clearance was obtained for the study (Ref No:SNMC/09-10/612).The study was conducted from June 2009 to February 2010. A detailed history was taken to know duration of the disease, treatment history and any complication of the disease.

All the subjects underwent clinical examination including anthropometric measurements. The anthropometric measurements comprised of waist circumference, height, body weight and body mass index (BMI) was calculated as weight in Kg/height in m^2.The waist circumference was determined by applying a tape measure to the midpoint between the inferior margin of the last rib and the crest of the ileum.

Biochemical analysis-The fasting blood sample, 2 ml in fluoride bulb for sugar estimation and 5ml in plain bulb for lipid profile estimation was collected from the cubital vein with aseptic precaution. Serum was separated by centrifugation at 3000 rpm for 10 minutes.

The following parameters were studied.

1. Fasting blood glucose –Enzymatic, GOD-POD, end point colorimetric, single reagent chemistry.(Trinder P and Teitz N W by autospan kit method).
2. Serum total cholesterol – cholesterol oxidase / peroxidase method. CPT diagnostics kit.
3. Serum HDL cholesterol – phosphotungstate / magnesium precipitate method. Ident i kit.
4. Serum Triglycerides – Glycerol phosphate oxidase / peroxidase method, Ident i kit.
5. Serum VLDL cholesterol – calculated by formula Triglyceride / 5
6. Serum LDL cholesterol – calculated by formula LDL cholesterol = TC – (HDL-C+VLDL-C)
7. TC/HDL-C Ratio
   All the parameters read using semi auto analyser (Erba, Transasia).
8. Glycosylated Haemoglobin (HbA1c) was determined using Biorad equipment.
9. Lp (a) levels were determined using immunoturbidimetric methods.

The statistical analysis was done using student't' test and p value < 0.005 was considered statistically significant.

RESULTS

The descriptive characteristics and the glycemic status of the control and diabetics subjects are shown in table 1. There was no statistical significant difference seen in age and height of control and diabetic patients ($p<0.05$). The weight, WHR, BMI, levels of fasting blood glucose and HbA1c in diabetes mellitus patients were significantly elevated in comparison to healthy controls ($P<0.001$) (table 1).

The levels of serum total cholesterol, serum triglycerides, serum LDL-C , serum VLDL-C, TC/HDL-C ratio and serum Lp(a) showed statistically significant elevation in DM subjects compared to control subjects ($P<0.001$). The level of serum HDL-C was statistically significantly decreased in diabetes mellitus patients compared to healthy controls ($P<0.001$) .The results are shown in table no 2.

DISCUSSION

The number of people affected with type 2 DM has reached epidemic proportions worldwide. It is estimated

that by 2020, there will be approximately 250 million people who will be affected worldwide [9]. Diabetes is associated with a variety of metabolic abnormalities, principal among which is hyperglycaemia and disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action or both. The long term relatively specific effects of diabetes include development of retinopathy, nephropathy and neuropathy. The patients with diabetes are also at increased risk of cardiac, peripheral arterial and cerebrovascular disease [10].

HbA1c reflects average plasma glucose over the previous 8 to 12 weeks. It can be performed at any time of the day and does not require any special preparations such as fasting .These properties have made it the preferred test for assesing the glycemic control in people with diabetes . More recently there has been substantial interest in using it as a diagnostic test for diabetes and as a screening test for persons at high risk of diabetes[11].

The main cause of the major feature of diabetic dyslipidemia is the increased free fatty acid release from insulin resistant fat cells.[11] The increased flux of free fatty acids into the liver in the presence of adequate glycogen stores promotes triglyceride production which in turn stimulates the secretion of apolipoprotien B (apo B) and VLDL-C .This leads to enhanced hepatic VLDL-C production[12].The abnormally increased TGs enrich high -density lipoproteins and low density lipoproteins leading to high levels of potentially atherogenic particles and low levels of HDL-C .[13]

The study showed significantly decreased level of the HDL-C in diabetic as compared to the healthy controls. The reduced HDL-C may be due to decrease in the activity of hepatic lipase resulting in decrease VLDL clearance leading to reduced HDL-C synthesis is the primary abnormality[14] .The TC/HDL-C ratio is a sensitive and specific index of cardiovascular risk. The TC/HDL-C ratio is regarded as a predictor of CHD risk, especially with value >6.0 [15]

Atherosclerosis is one of the common complications of DM and abnormal lipoprotein metabolism may account for the increased frequency of atherosclerotic lesions in diabetics. Elevated Lp(a) and low HDL were independent risk factors for premature coronary artery disease [16]. Lp(a) interferes with the fibrinolytic function of plasminogen, thereby promoting the thrombotic events. It inhibits tissue plasminogen activator (t-PA) binding to fibrin and also suppresses the fibrin and fibrinogen fragment dependent enhancement of plasminogen activation by t-PA in some assay system [17]. Lp(a) also inhibits plasminogen activation by streptokinase. It has been shown to compete for the binding of plasminogen to monocytoid cell and epithelial cells[18]. Tetraectin, a plasma protein ,binds reversibly to plasminogen and enhances plasminogen activation by t-PA. Lp(a) was found to bind to tetrenectin with higher affinity than Glu or Lys plasminogen [19]. Systemic atherosclerosis measured as the peripheral occlusive arterial disease is strongly associated with serum Lp(a) in type 2 DM [20].

The Lp(a) levels have also been suggested to play a pathogenic role in the development of complications like gangrenous foot lesions i patients of DM [21].

Type 2 DM has a strong genetic component [22]. The genetic basis of dyslipidemia has been well established [23]. The genetic predisposition of both type 2 DM and the deranged Lp(a) levels may be a common basis of the two events occuring together. Lp(a) concentration in serum is also affected by apo(a) phenotypes. According to their electrophoretic mobilities Lp(a) has six phenotypes-F,B,S1.S2,S3 and S4 as compared to apo B-100. Family studies are compatible with the fact that Lp(a) glycoprotein phenotypes are controlled bya number of autosomal alleles at a single lous. Comparison of Lp(a) levels in different phenotypes revealed a highly significant association of phenotype with concentration .Phenotype B,S1 and S2 are associated with high and phenotype S3 and S4 with low plasma concentration [24]. It may be possible that increased Lp(a) concentrations in diabetics is due to the presence of apo(a) phenotypes and added to this are increased levels of insulin in patients with type 2 DM[25].

CONCLUSION

In the present study the concentration of serum Lp(a), total cholesterol, serum triglyceride, LDL cholesterol and HbA1c is increased in the circulation of diabetic subjects than in controls, it may be the effect of hyperglycaemia on the the rate of synthesis , transcription and translation of apo (a). Glycosylation prolongs the half-life of proteins, this may add to higher levels of Lp(a) and HbA1c in diabetics compared to controls.

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Table 1: Comparison of descriptive characteristics and glycemic status between control and diabetic subject

Parameters	Control		NIDDM			
	Mean	SD	Mean	SD	p-value	Statistical significance
Age(years)	50.45	17.85	52.18	16.93	0.1745	N.S
Height(cms)	168.17	9.23	166.21	12.94	0.2684	N.S
Weight (Kg)	76.98	14.29	86.28	19.77	0.034	S.S
WHR	0.93	0.23	1.02	0.11	0.0231	S.S
BMI (kg/m^2)	27.92	5.13	28.89	5.75	0.0232	S.S
FBS (mg/dl)	90.3	5.78	122.8	9.13	<0.001	H.S
HbA1c (%)	5.19	0.71	7.58	1.69	<0.001	H.S

BMI-body mass index, WHR-waist hip ratio, FBS-fasting blood sugar,
HbA1c-glycosylated haemoglobin

Table 2: Comparison of lipid profile and Lp(a) between control and diabetic subjects

Parameters	Control		DM			
	Mean	SD	Mean	SD	p-value	Statistical significance
TC(mg/dl)	178.8	30.91	237.5	28.77	< 0.01	S.S
TG(mg/dl)	135.0	41.64	193.8	16.16	< 0.01	S.S
HDL(mg/dl)	54.9	23.84	39.98	46.50	< 0.001	H.S
LDL(mg/dl)	145.81	7.13	169.85	82.42	< 0.001	H.S
VLDL(mg/dl)	27.58	2.33	38.69	13.63	< 0.01	S.S
TC/HDL-C Ratio	3.25	1.29	5.94	0.61	<0.01	S.S
Lp(a) (mg/dl)	20.67	18.86	29.88	24.97	< 0.01	S.S

TC-total cholesterol; TG;total triglyceride; LDL-low density lipoprotein; HDL-high density lipoprotein; VLDL-very low density lipoprotein; Lp(a)-lioprotein a