PHYSIOLOGY OF GLYCOSYLATED HAEMOGLOBIN, AND THE IMPACT OF GLYCAEMIC CONTROL ON LIPID PROFILE IN ADULT MEN WITH TYPE-2 DIABETES MELLITUS

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ABSTRACT

Looking at the effects of insulin deficiency on carbohydrate and lipid metabolism, diabetes mellitus is now being called more a disease of lipid metabolism than carbohydrate metabolism. A cross-sectional study was conducted during March 2005 to March 2006 to study the glycosylated haemoglobin (HbA1c) and lipid levels in normal adults and with type -2 diabetes mellitus and its relation to glycaemic control. Comparison of lipid levels was made between group of diabetic patients and the normals. The lipid fractions i.e. Total cholesterol, triglycerides, and low-density lipoprotein levels of poorly controlled diabetic (HbA1c > 8 %), were respectively higher ($p<0.001$), ($p<0.01$) and ($p<0.001$) than those of the control group. Later the lipid levels decreased drastically with glycaemic control ($p>0.05$, $p>0.05$ and $p<0.05$ respectively). Increased levels of low-density lipoprotein may be a contributory factor to the high risk of atherosclerosis induced coronary artery disease observed in diabetes mellitus patients. Reduction of blood glucose levels is likely to reduce low density lipoprotein levels and the risk of complication, with the lowest risk being in those with glycosylated hemoglobin values in the normal range i.e. less than 8.0%

Keywords: *Glycosylated hemoglobin, Glycaemic control, Lipid profile, Type-2 Diabetes Mellitus*

INTRODUCTION

Diabetes mellitus (D.M) is the commonest endocrine disease affecting mankind. The incidence of disease is on a rise not only in developed countries but also in developing countries. Diabetes mellitus is a metabolic disorder characterized by elevated fasting and postprandial blood glucose level due to insufficiency of either secretion or action of endogenous insulin which in turn leads to nonenzymatic glycosylation of proteins like haemoglobin forming glycosylated haemoglobin .This leads to variety of multisystem complications, mainly in the blood vessels of eye, kidney, heart, nervous system and integument. The heavy toll of death each year is due to its complications. The complications are more prevalent among the people of lower socioeconomic status because of negligence, illiteracy, poverty etc. The normal glycosylated haemoglobin levels ranges from 4.0 to 7.0.

Coronary artery disease (CAD) especially Myocardial Infarction (MI) has reached enormous proportions striking more and more young subjects especially in patients with diabetes mellitus. Among the very many risk factors identified for acceleration of the atherosclerotic process and thus predisposition to ischemic heart disease are hyperlipidemia and hyper lipoproteinemias. Cholesterol was incriminated as an etiologic factor for atherosclerosis long back when cholesterol was found in the atheromatus plaques. Various studies around the world have well established that low density lipoprotein (LDL), and very low density lipoprotein (VLDL) are atherogenic whereas high density lipoprotein (HDL) is a protective factor against coronary

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atherosclerosis. The basis of this finding is whenever the glycaemic level increases as in the diabetes mellitus patients, the low density lipoproteins will get glycated. This chemically modified glycated LDL causes inhibition of the ability of LDL to interact with the LDL receptors. This in turn inhibits the ability of the LDL to be metabolized by the LDL receptor pathway. Thus plasma LDL levels increases and atherosclerosis occurs early in life[1]. Another mechanism shows chronic hyperglycemia leads to nonenzymatic glycation of LDL and collagen fibers leading to formation of Schiff base which in turn forms amadori products. This causes tissue damage by reactivity and protein cross linking. The glycated LDL also binds with glycated collagen leading to heavy cholesterol deposition which is the first step in the pathogenesis of atherosclerosis leading to coronary artery disease[2]. Free radicals may react on a variety of biomolecules, including lipids, carbohydrates, proteins, nucleic acids, and macromolecules of connective tissue, generating reactive oxygen species and resulting in oxidative stress. Increased lipid peroxidation caused crosslink formation between single molecules of proteins and oxidation of LDL particles and led to early occurrence of atherosclerotic changes[3]. Increased LDL-cholesterol and triglyceride levels provide evidence for increased lipid peroxidation and possible tissue damage by free radicals. This increase the risk of vascular complications of diabetes mellitus and atherosclerosis[4].

In the present study, glycosylated haemoglobin levels were estimated in normal adult men, then the effect of glycaemia control on lipid profile of adult men having type -2 diabetes mellitus (DM) was investigated.

MATERIAL AND METHODS

The study group comprise of 50 adult men above 30 years of age having Type-2 diabetes mellitus. The control group comprise of 50 healthy adult men above 30 years of age.

Cases and controls are collected from outpatients and inpatients visiting R.L.Jalappa hospital and S.N.R. hospital attached to Sri Devraj Urs Medical College, Kolar, Karnataka, India. History was taken in detail with informed consent and general physical examination was done after the clearance from ethical committee. Subjects with history of hypertension, lung or cardiac disease, smoking and alcoholism, on any drug affecting lipid levels of plasma are excluded from the study. Blood Samples are collected between 7 AM to 8 AM after overnight fasting. Glycosylated hemoglobin (HbA1c levels) is estimated by using Glycohemoglobin Reagent set, provided by POINTE SCIENTIFIC INC. Lipid levels (Total serum cholesterol, HDL cholesterol, LDL cholesterol, and triglycerides) were estimated by standard method (Dr.Lange LP 700 equipment). Low density lipoproteins are calculated by FRIEDEWALD'S FORMULA[5] i.e.

Low Density Lipoprotein= Total Cholesterol- High Density Lipoprotein -Triglycerides /5

RESULTS

100 male subjects were selected as per the criteria laid down in the methods and materials section for the present study. They were grouped as study group (diabetic patients) and control group. Lipid levels and glycosylated hemoglobin levels were estimated in both groups. The data collected have been statistically analyzed and discussed. Lipid levels and glycosylated hemoglobin levels were compared between the two groups and the results discussed. The data was arranged into suitable tables from the master chart for discussion under different headings. Analysis was performed using SPSS 8.0 statistical package for windows. Continuous variables are expressed as

the mean + standard deviation and qualitative data as percentages. Comparison of patient's features was performed using Student's t test for paired and unpaired data. Pearson correlation coefficient test (r- value) was carried out to know the correlation of HbA1C and LDL.Chi-square test was carried out to evaluate the significance of coronary artery disease in different groups. The mean difference is significant at P<0.05 level. Conclusion was drawn based on outcome of this statistical treatment.

Table -1 : Distribution of subjects depending on age

AGE GROUP IN YEARS	DIABETICS	CONTROLS
30-39	12	10
40-49	16	20
50-59	11	10
>60	11	10
TOTAL	50	50

Table-1 shows the distribution of the subjects according to the age. The youngest subject in the study group is aged 32 years and the oldest aged 68 years. The youngest subject in the control group is aged 34 years and the oldest aged 64 years. The mean age of the study group was 48.56 years and control group was 48.96 years. The maximum incidence of the coronary artery disease was found in the age group above 40 years.

Table-2 shows the mean values and standard deviations of the various lipid fractions in diabetics in comparison to that of controls. It can be seen that mean value of all lipid fractions TC, TG, LDL, TC/HDL, LDL/HDL are higher in the diabetics (214.68 + 28.72 mg/dl, 170.40 + 61.20 mg/dl, 146.0+24.12 mg/dl, 6.20+1.4, 4.22+0.84) when compared to control (152.20+32.62 mg/dl, 138.42+50.20mg/dl, 82.06 + 27.6 mg/dl, 3.58 +1.02, 1.93+0.82) and is statistically significant. The HDL-Ch was higher in controls than diabetics and is a safety factor.

Table-3: HbA1C levels and LDL levels in diabetics and controls

	DIABETICS	CONTROLS
HbA_{1c} LEVELS	9.8 $\pm$ 2.42	5.6 + 1.26
LDL LEVELS(mg/dl)	146 $\pm$ 24.12	82.06 + 27.6
SIGNIFICANCE	r =+0.9999, P<0.001	r =+0.9709, P<0.001

Table 3 shows HbA1C levels and LDL levels in study and control groups. LDL levels are significantly higher in the diabetes patients whose glycosylated hemoglobin levels are elevated. There is a moderate positive correlation between the HbA1C levels and LDL levels in the diabetic group (Pearson correlation coefficient r = + 0.999, P<0.001).

Table-2: Comparison of lipid levels in diabetics and controls

TEST	DIABETICS HbA1C >8 n=50	CONTROLS n=50	t- value	p-value	SIGNIFICANCE
TC(mg/dl)	214.68 + 28.72	152.20 + 32.62	10.16	<0.001	VHSS
TG(mg/dl)	170.40 + 61.20	138.42 + 50.20	02.82	<0.01	HSS
HDL-C (mg/dl)	34.60 + 8.14	42.46 + 9.2	04.51	<0.001	VHSS
LDL-C (mg/dl)	146.0 + 24.12	82.06 + 27.6	12.34	<0.001	VHSS
TC/HDL	6.20 + 1.4	3.58 + 1.02	10.69	<0.001	VHSS
LDL/HDL	4.22 + 0.84	1.93 + 0.82	13.40	<0.001	VHSS

NOTE: VHSS: very highly statistically significant HSS: highly statistically significant
SS: statistically significant NSS: not statistically significant

Table-4: Comparison of lipid levels in diabetics and control after diabetes control with treatment

TEST	DIABETICS CONTROLLED HbA_{1c} < 8 n=50	CONTROLS n=50	t-value	p-value	SIGNIFICANCE
TC(mg/dl)	168.14 + 18.56	160.28+32.62	1.48	> 0.05	NSS
TG(mg/dl)	152.30 + 42.12	142.32+40.14	1.21	> 0.05	NSS
HDL-C(mg/dl)	38.62 + 4.86	44.42 + 6.92	4.83	< 0.001	VHSS
LDL-C(mg/dl)	99.06 + 19.2	87.4 + 26.7	2.51	< 0.05	SS
TC/HDL	4.35 + 1.06	3.60 + 1.02	3.57	< 0.001	VHSS
LDL/HDL	2.56 + 0.81	1.96 + 0.80	3.75	< 0.001	VHSS

Table-4 shows the mean values and standard deviations of the various lipid fractions in diabetics after they were treated by diet, oral hypoglycemic drugs and insulin for 5-6 months. It can be seen that mean value of all lipid fractions TC, TG, LDL, TC/HDL, and LDL/HDL decreased with glycaemic control (168.14$\pm$18.56 mg/dl, 152.30 $\pm$ 42.12 mg/dl, 99.06 $\pm$19.2 mg/dl, 4.35$\pm$ 1.06, 2.56 $\pm$ 0.81 respectively).

DISCUSSION

Diabetes mellitus acts as a major risk factor for atherosclerosis either alone or in combination with other major risk factors such as diet, smoking, body weight etc.It has been found that patients with type-2 diabetes mellitus suffer from dyslipidemia which in turn leads to various vascular complications. In this study, glycosylated haemoglobin levels was estimated and found to be 5.6+1.26 in normal adults and 9.8+2.42 in diabetics. Lipid levels were compared between type-2 diabetes mellitus patients and the normal subjects. The results are analyzed and discussed below.

The present study has shown that the lipid fractions i.e. Total cholesterol (TC), triglycerides (TG), and low-density lipoprotein (LDL) levels of poorly controlled diabetic (HbA1c > 8 %), were respectively higher ($p<0.001$), ($p<0.01$) and ($p<0.001$) than those of the control group which are statistically significant. Later lipid levels decreased drastically with glycaemic control by diet, oral hypoglycemic drugs and insulin injections ($p>0.05$, $p>0.05$ and $p<0.05$ respectively). This goes in favor of many other studies.

The finding of the present study is in conformity with the earlier studies

Edward P Feener et al. showed both metabolic and hormonal imbalances contribute to the pathogenesis of diabetic vascular diseases and vasculopathies. One hallmark of diabetic vascular disease is thickening of the basement membrane, which develops in relation to the duration of diabetes and the degree of glycaemic control. An increase in extracellular matrix proteins, mainly from type IV collagen, laminin, and fibronectin, reduces the elasticity and alters the filtration properties of the basement membrane [2].

Ercyias F et al. in their study showed, increased levels of Malondialdehyde (MDA), MDA/LDL index, and dyslipoproteinemia showed that especially metabolically poorly controlled DM children are at high risk of atherosclerosis and vascular complications of DM and that there is a significant relationship between the lipid profile and oxidative stress. Thus, it may be appropriate to evaluate MDA in addition to routine laboratory assessments in evaluation of type 1 DM paediatric patients[4].

Formation of glycated hemoglobin is essentially reversible, and the blood levels depend on both the life span of the red blood cell (average 120 days) and the blood glucose concentration. Because the rate of formation of glycated hemoglobin is directly proportional to the concentration of glucose in the blood. The glycated hemoglobin concentration represents the integrated values for glucose over the preceding 6 to 8 weeks. This provides an additional criterion for assessing glucose

controls because glycated hemoglobin values are free from day to day glucose fluctuations and are unaffected by exercise or recent food ingestion[6]. The glycated hemoglobin (HbA1C) test measures the percentage of that glycated hemoglobin, offering a snapshot of the average blood sugar control for the past few months.

Abraha et al. determined whether abnormal lipid levels in children with Type 1 diabetes mellitus are the result of poor metabolic control or may in part be determined by genetic factors in 141 children with Type 1 diabetes. They concluded that both glycaemic control and familial factors may be important determinants of lipid levels with diabetes. Both may contribute to the subsequent risk of cardiovascular disease[7].

Alagozlu H et al. in their study investigated the lipid levels in non-obese, type 2 diabetes mellitus patients and a control group and concluded that gaining metabolic control in diabetes is crucial in pulling back lipid, lipoprotein and apolipoprotein levels to a desired level[8].

Lp (a) concentration was positively correlated with body mass index ($P < 0.05$) and HbA1c ($P < 0.05$). No association was found between Lp (a) and sex, age, other lipidic parameters, microalbuminuria, type of treatment and presence of cardiovascular disease. These findings may suggest that glycaemic control could have a modulatory role on Lp (a) concentration in NIDDM patients[9].

Habib S et al. analyzed serum lipoprotein (a) [Lp (a)] levels in Pakistani patients with type 2 diabetes mellitus (DM).The study concluded that serum Lp(a) levels are significantly raised in type 2 DM and have a positive correlation with serum total and LDL-c levels[10].

Hicham Mohammadi et al. in their study showed, increased levels of LDL-cholesterol and triglycerides levels showed that especially poorly controlled diabetes mellitus children present a high risk of atherosclerosis and vascular complications of diabetes mellitus. They showed a significant relationship between the lipid profile and the poor glycaemic control in diabetic children[11].

The accumulation of ceroid in macrophages is shown to be related to LDL oxidation rather than LDL glycation, per se, as it too occurs at a maximum of approximate 25 mM. Oxidative sequelae of protein glycation appear to be a major factor in LDL-macrophage interactions, at least with respect to ceroid accumulation[12].

Recent experimental findings suggest that overproduction of reactive oxygen and nitrogen species, lowered antioxidant defense and alterations of enzymatic pathways in humans with poorly controlled diabetes mellitus. This can contribute to endothelial, vascular and neurovascular dysfunction. Consequences of oxidative stress are damage to DNA, lipids, proteins, disruption in cellular homeostasis and accumulation of damaged molecules[13].

Seema Singla et al. suggest that Lp (a) levels are increased in type 2 diabetic patients. The elevated Lp (a) levels do not reflect the glycaemic status and are also independent of increase in LDL: HDL ratio suggesting different metabolic pathways and genetic connection[14]. High levels of serum lipoprotein (a) have been associated with increased risk of coronary artery disease[15].

Wagner AM et al. studied the effect of improving glycemic control on low-density lipoprotein particle size in 33 type-2 diabetes

mellitus patients in a longitudinal intervention study. It was found that with glycaemia control a significant reduction in the LDL cholesterol, ApoB and an increase in HDL cholesterol and Apo AI were seen[16].

Although the level of hyperglycemia is clearly a risk factor for microvascular complications in diabetic patients, its role in macrovascular complications remains controversial. They concluded that both hyperglycemia and common cardiovascular risk factors are important predictors of all-cause and cardiovascular mortality in diabetic subjects[17].

The most commonly recognized lipid abnormality in non-insulin-dependent diabetics is hypertriglyceridemia, which is known to be an independent risk factor for coronary heart disease in diabetics. Hypertriglyceridemia can be produced by two mechanisms, increased synthesis of very-low-density lipoprotein triglyceride and removal defect of plasma triglyceride. It has been a matter of debate whether insulin always stimulates hepatic VLDL secretion but it is generally accepted that insulin deficiency results in an impairment of plasma triglyceride clearance[18]. Therefore, we propose here that plasma lipid levels of diabetic subjects must be more strictly controlled in order to avoid an increased risk for coronary heart disease.

CONCLUSION

Lipid levels were elevated in poorly controlled diabetic patients. Among the different lipids, the elevation of Low-density lipoprotein was contributing more for the development of coronary artery disease. Glycaemic optimization and lifestyle intervention is a good tool to improve the components of diabetic dyslipidaemias. Reduction of glycosylated hemoglobin is likely to reduce the risk of complication with the lowest risk being in those with glycosylated hemoglobin values in the normal range i.e. less than 8.0%

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