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Correlation between glycosylated hemoglobin level and lipid profile in patients with Diabetes mellitus

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Abstract
The present study was conducted in the Department of Biochemistry, M.N.R. Medical College, Sangareddy District, Telangana, State, India. A total of n=75 subjects were selected, comprising n=50 subjects (n=25 male and n=25 female) with known Diabetes mellitus and n=25 non-diabetic healthy controls. The study was aimed to investigate the pattern of dyslipidemia among the subjects and understand its association with glycosylated hemoglobin (HbA1c) level in diabetic and healthy controls. Biochemical parameters were estimated by following standard methods. The mean and SD values of fasting blood glucose (164.56 ± 10.41) and post prandial blood glucose (228.60 ± 12.66) were significantly ($p<0.05$) increased in diabetic cases compared to non-diabetic controls (84.44 ± 5.04 and 123.64 ± 6.20 respectively). The mean and SD of HbA1c (8.88 ± 0.49) in diabetic cases were significantly higher compared to controls (5.08 ± 0.45). Poor glycemic control indicated by HbA1c level >8 was seen in n=34 (68%) of diabetic patients. Poor glycemic control was associated with dyslipidemia in n=24 (48%) diabetic patients. Whereas, 6 cases (12%) showed worse glycemic control with HbA1c level > 9%, and 8 cases showed good glycemic control with HbA1C level ≤ 6%. The mean±SD of serum total cholesterol (216.48 ± 10.87), triglycerides (214.84 ± 8.98), LDL-cholesterol (149.52 ± 5.06) were significantly ($p<0.05$) high in diabetic cases over controls (167.68 ± 6.83, 154.84 ± 5.38 and 92.12 ± 5.36 respectively). HDL-cholesterol (31.83 ± 4.42) was significantly reduced in diabetic patients compared to controls (46.94 ± 6.84). The lipid profile values in female diabetic and non-diabetic subjects were slightly increased compared to male subjects. However, the values were not statistically significant. This study showed a significant correlation between levels of glycosylated hemoglobin (HbA1c) and lipid profile. This may help in predicting the lipid profile levels from the degree of glycemic control and therefore, identifying the patients with increased risk of diabetic complications.

Keywords: Diabetes mellitus, glycosylated hemoglobin HbA1c, lipid profile, serum cholesterol, triglycerides

1. Introduction
Diabetes mellitus (DM) is a chronic metabolic disorder characterized by increase in blood glucose levels due to absolute or relative deficiencies in insulin secretion, insulin action or both. This increase in blood sugar level increases the risk of both micro and macrovascular and chronic renal complications in diabetic patients.

Diabetes is a global disease with rapid increase in both developed and developing countries [1]. Prevalence of diabetes is reported to be higher in Asians.

The lipid abnormalities such as increased cholesterol, elevated LDL, high triglycerides (TG) and low high-density lipoprotein (HDL) are considered risk factors contributing to the mortality and morbidity in diabetics.

Glycated hemoglobin (GHB), reported as hemoglobin A1c (HbA1c), is routinely measured to check the glycemic control over a preceding 8-12 weeks of time. It is used as an indicator for the state of glycemic control, progression of the disease and the development of diabetic complications. Elevated HbA1c has been regarded as an independent risk factor for coronary heart disease (CHD) in patients with or without diabetes [2].

HbA1c level ≥ 6.5% can be used for diagnosis of DM. Ravipati *et al* [3]. also observed a direct correlation between HbA1c concentration and the severity of coronary artery disease (CAD) in diabetic patients. Diabetes Complications and Control Trial has established HbA1c as the gold standard for glycemic control, and proposed HbA1c at ≤7% as critical value for reducing the risk of vascular complications [4].

In the present study, serum lipid profile parameters, blood glucose levels and glycosylated hemoglobin levels were estimated in patients with diabetes mellitus and compare with non-diabetic healthy controls.

The lipid abnormalities in diabetic dyslipidaemia are prevalent in diabetes mellitus because insulin resistance or deficiency affects key enzymes and pathways in lipid metabolism. Diabetic dyslipidemia using World Health Organization (WHO) criteria [5, 6] is characterized by serum triglyceride levels 150-400 mg / dl (1.7 to 4.5 mmol / L), total cholesterol (TC) > 200 mg / dl (> 5.2 mmol / L), low density lipoprotein cholesterol (LDL-C) > 135 mg / dl (> 3.5 mmol / L), high density lipoprotein cholesterol (HDL-C) <35 mg / dl (< 0.9 mmol / L) in men or < 40 mg / dl (< 1.0 mmol / L) in women.

Taking these findings into account, the present study was aimed to investigate the pattern of dyslipidemia among the subjects with diabetes and non-diabetic healthy controls and also understand its association with glycosylated hemoglobin (HbA1c) level.

Material and Methods

The present study was conducted in the Department of Biochemistry, M.N.R. Medical College, Sangareddy District, Telangana, State, India. A total of n=75 subjects were selected in this study with age ranging from 40 to 55 years. The study group comprised of n=50 subjects (n=25 male and n=25 female) with known Diabetes mellitus, age range from 40 to 55 years. The normal healthy adult volunteers (n=25) working in MNR Medical College participated in the study served as controls and their age range was 40 - 52 yrs. Controls were healthy adults who were excluded from diabetes.

Patients presenting to the Medicine outpatient Department with past history of Diabetes mellitus for the last 3 years, were taken as study group cases in this study. Subjects with normal renal function tests and normal random blood glucose were treated as controls. The patients excluded in this study were smokers, hypertensives, hyperlipidemics, pregnant women and some other chronic disorders. Patients having proteinuria due to other reasons like hypertension, urinary tract infection, heart failure and some other chronic disorder were excluded from the study.

An informed consent was taken from all the subjects to participate after explaining the objective of the study. Approval for the study protocol was obtained from the institutional research ethical committee. The variables collected were age, gender, fasting and postprandial blood glucose, glycosylated hemoglobin (HbA1c), total cholesterol (TC) and triglycerides (TG), high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) of all the subjects.

Sample collection

Blood samples of all these subjects were collected in anticoagulant tubes. For biochemical investigations serum was separated by centrifugation at 4000 rpm for 10 minutes and the plasma was separated and used for further analysis. The samples were estimated for the variables such as blood glucose level (BSL), total cholesterol, triglycerides, high-density lipoprotein cholesterol and low-density lipoprotein cholesterol and glycosylated hemoglobin.

After overnight fasting, under aseptic conditions 8 ml of venous blood samples were collected in clean glass tubes and 1 ml of sample was taken in an EDTA coated tube for the estimation of HbA1c.

Blood sugar levels were estimated by glucose oxidase and peroxidase (GOD-POD) end point assay method [7]. Glycosylated hemoglobin (HbA1c) was determined by high-performance liquid chromatographic (HPLC) method described by Little *et al* [8]. Photometric determination of total cholesterol (TC) was done according to CHOD-PAP method [9], serum triglycerides (TG) were estimated by enzymatic colorimetric technique using glycerol kinase according to GPO-POD [10]. HDL-Cholesterol (HDL-C) was estimated by phosphotungstic acid precipitation followed by enzymatic analysis in supernatant fraction [11] and LDL-Cholesterol (LDL-C) was determined by using Friedewald's equation [12].

All these laboratory parameters for fasting blood glucose (FBG), post prandial blood glucose (PPBG), lipid profile parameters were analyzed using Human reagent kits and with the help of semi auto analyser (Humalyser 3500, Germany) kit. Total cholesterol and Triglycerides were analyzed by enzymatic kit (Auto pure manufactured kits) method by using Olympus auto-analyzer.

Reference ranges of various parameters according to the kits manufacturer are as follows; FBG (70-100 mg/dl), PPBG (<140 mg/dl), HbA1c (5.5%), TC (150-200 mg/dl), TG (50-200 and 40-150 mg/dl in male and female respectively), HDL-C (30-60 and 35-75 mg/dl in male and female respectively) and LDL-C (90-200mg/dl).

Statistical analysis

The data was expressed as mean and standard deviation (mean ± SD). The relation of HbA1c and various blood lipid parameters evaluated by student's t-test were used to compare the significance between study group diabetic patients and non-diabetic healthy control. $p \leq 0.05$ was considered as statistically significant.

Results and discussion

In the present study, a total of n=75 diabetic subjects were included. Study group subjects comprised n=50 diabetic patients which included 25 males and 25 were females. The age of the study group subjects ranged from 40 - 55 years and the mean age 52.92 years. 25 subjects (n=25) were normal healthy individuals treated as controls. The age of the healthy controls ranged from 40 – 52 years and the mean age was 48.72 years.

In our study, we assessed the biochemical profile in study group diabetic subjects and healthy controls. For this, we studied the plasma glucose levels, glycosylated hemoglobin, serum triglycerides, serum total cholesterol, serum HDL cholesterol and serum LDL cholesterol to compare all these parameters of diabetes cases with those of healthy control subjects. The study also was to see the correlation of HbA1c levels with lipid profile parameters in the diabetic patients with those of non-diabetic control subjects.

Table 1: Biochemical parameters of diabetic patients and non-diabetic healthy controls.

Parameters (mg/dl)	Patients (n=50)	Controls (n=25)
	Mean ± SD	Mean ± SD

FBS	164.56 ± 10.41*	84.44 ± 5.04
PPBG	228.60 ± 12.66*	123.64 ± 12.91
TC	216.48 ± 10.87*	167.68 ± 6.83
TG	214.84 ± 8.98 *	154.84 ± 5.38
HDL-C	37.83 ± 3.04*	46.94 ± 6.84
LDL-C	149.78 ± 15.06*	92.12 ± 5.36
HbA1c	8.88 ± 0.49*	5.08 ± 0.45

FBS-fasting blood sugar, PPBG-postprandial blood glucose, TC- serum total cholesterol, TG-serum triglycerides, HDL-C- high density lipoprotein cholesterol, LDL-C- low density lipoprotein cholesterol, HbA1c- glycosylated hemoglobin

The mean and standard deviation values of biochemical parameters of study group diabetic patients and non-diabetic healthy controls are presented in Table 1 and Fig. 1. This data shows that the mean ± SD values of HbA1c level and lipid profile parameters TC, TG and LDL-C were significantly increased in the diabetic study group compared to normal healthy control subjects. However, serum HDL-C values were significantly reduced in diabetic cases compared to non-diabetic controls.

The mean and SD values of fasting blood glucose (164.56 ± 10.41) and PPBG (228.60 ± 12.66) were significantly ($p<0.05$) increased in diabetic cases compared to non-diabetic healthy controls (84.44 ± 5.04 and 123.64 ± 6.20 respectively). The mean and SD values of HbA1c was observed to be 8.88 ± 0.49 in diabetic patients which is significantly higher compared to healthy controls 5.08 ± 0.45 (Table 1). Levels of HbA1c is referred to be an important marker for the assessment of glycemic control 8 – 12 weeks prior to its measurement, which moves in parallel with diabetic induced complications [13].

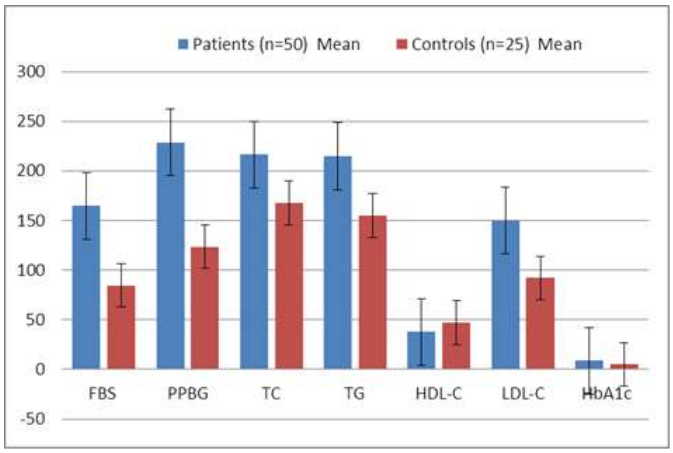

Fig 1: Biochemical parameters (with error bars) of diabetic patients and non-diabetic healthy controls; FBS-fasting blood sugar, PPBG-postprandial blood glucose, TC- serum total cholesterol, TG-serum triglycerides, HDL-C- high density lipoprotein cholesterol, LDL-C- low density lipoprotein cholesterol, HbA1c- glycosylated hemoglobin.

In this study, poor glycemic control indicated by HbA1c level >8 was seen in n=34 cases (68%) of diabetic patients. Poor glycemic control was associated with dyslipidemia in n=24 (48%) cases of study group patients. Whereas, 6 cases (12%) showed worse glycemic control with HbA1c level > 9%, and 8 cases showed good glycemic control with HbA1C level ≤ 6%. The age group with maximum patients with both dyslipidemia and higher HbA1c levels was seen maximum in the individual of the age group 50-55 years (data not presented). Poor and worst glycemic control is considered as risk factor for complications in diabetes. Strict glycemic control lowers the risk of micro- and macrovascular complications of diabetes mellitus.

Lipid profile parameters (Table 1) were significantly different in diabetic patients and non-diabetic controls. The mean and SD values of serum total cholesterol were 216.48 ± 10.87, triglycerides (214.84 ± 8.98), LDL-cholesterol (149.52 ± 5.06) were significantly ($p<0.05$) higher in study group diabetic cases compared to their values in healthy controls with 167.68 ± 6.83, 154.84 ± 5.38 and 92.12 ± 5.36 respectively. HDL-cholesterol were significantly reduced in diabetic patients (31.83 ± 4.42) compared to healthy control individuals (46.94 ± 6.84). These findings are in accordance with previous studies which suggest that lipid abnormalities are higher in diabetic patients than in non-diabetic controls [14, 15]. Diabetes is a metabolic disorder having a wide range of lipid abnormalities.

The gender wise lipid profile parameters of diabetic and non-diabetic subjects are presented in Table 2 and Figure 2. The lipid profile values (serum total cholesterol, serum triglycerides, serum LDL-C) in male and female diabetic cases were found to significantly increase over their values in male and female non-diabetic controls. Serum HDL-C was significantly reduced in diabetic cases over healthy controls. The lipid profile values in female diabetic and non-diabetic subjects were slightly increased compared to male subjects. However, the values were not statistically significant. Several factors are likely to be responsible for diabetic dyslipidaemia. These include differences in coagulation, the pattern of obesity between men and women, and possible role for hyperinsulinemia.

Table 2: Gender-wise comparison of biochemical parameters studied in diabetic patients and non-diabetic healthy controls.

Parameters (mg/dl)	Patients (n=50)		Controls (n=25)	
	Male	Female	Male	Female
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
FBS	163.8 ± 10.41*	165.32 ± 10.56 *	86.62 ± 6.62	82.16 ± 12.46
PPBG	225.72 ± 12.47*	227.48 ± 13.04	123.04 ± 5.86	127.88 ± 6.65
TC	215.92 ± 8.74 *	219.04 ± 8.27*	165.08 ± 7.28	169.28 ± 12.43
TG	214.64 ± 7.07*	217.04 ± 8.27*	152.39 ± 8.98	159.56 ± 4.96
HDL-C	37.09 ± 1.61*	38.12 ± 3.82*	46.96 ± 3.79	46.68 ± 3.96
LDL-C	144.08 ± 3.96*	149.96 ± 6.02*	91.16 ± 5.39	92.12 ± 5.36
HbA1c	9.02 ± 0.47*	8.52 ± 0.50*	4.32 ± 0.48	4.91 ± 0.72

n = number of subjects; SD - standard deviation; *- statistically significant ($P < 0.05$); FBS - fasting blood sugar; TC - total cholesterol; TG - total triglycerides; HDL-C - high density lipoprotein cholesterol, LDL-C - low density lipoprotein cholesterol

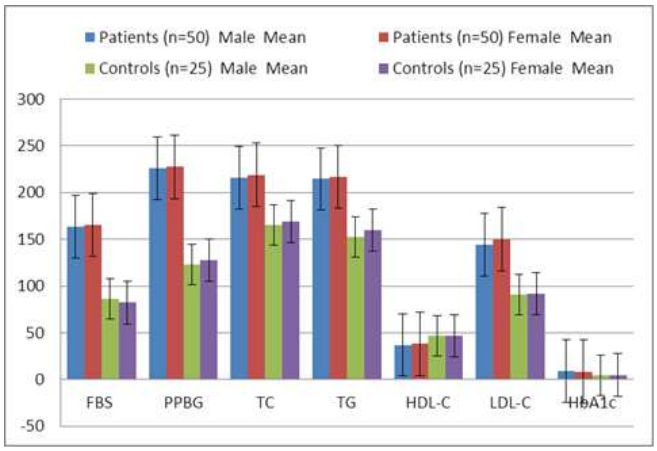

Fig 2: Gender-wise comparison of biochemical parameters (with error bars) studied in diabetic patients and non-diabetic healthy controls; FBS- fasting blood sugar, PPBG- postprandial blood glucose, TC- serum total cholesterol, TG- serum triglycerides, HDL-C- high density lipoprotein cholesterol, LDL-C- low density lipoprotein cholesterol, HbA1c- glycosylated hemoglobin.

Abnormality of cholesterol metabolism may lead to cardiovascular diseases and heart attacks. Our study indicates the high prevalence of hypercholesterolemia, hypertriglyceridemia, high LDL, and low HDL levels which are risk factors for cardiovascular diseases which is due to poor glycemic control in diabetic patients.

In the present study, results showed the increase of serum lipid profile parameters which are positively correlated with HbA1C levels. Maximum number n=34 (68%) of diabetic cases had serum TG level more than 176 mg/dl. HbA1C was significantly ($p<0.05$) increased among diabetic cases compared to healthy controls. The value of serum TG was increased with increase in the percentage of HbA1C. A positive correlation was observed between serum TG and HbA1C among the study group subjects. Our results showed a significant correlation between HbA1c with fasting blood glucose level and are in agreement with earlier reports [16, 17].

Glycemic control has been shown to prevent nephropathy and other systemic complications. The fasting and post prandial blood sugar levels were found to be significantly higher in diabetic cases compared to normal healthy individuals indicating poor glycemic control which is an indicator of diabetic nephropathy. Strict glycemic control lowers the risk of nephropathy and of other diabetic complications.

Significant correlation between HbA1c and cholesterol, triglycerides, HDL and LDL was observed in the study group diabetic patients. Our results are in agreement with previous reports [18] and suggested the importance of glycemic control and normalizing the dislipidemia.

In the present study, the levels of HbA1c and lipid profile levels did not significantly differ between the two genders. This indicates diabetes confers a markedly increase risk of events in both males as well as in females [19].

Bellomo *et al.* [20] reported high triglycerides and low HDL, but not hypercholesterolemia as the main features of dislipidemia observed in patients with metabolic syndrome. Significantly high serum triglyceride levels have been found in diabetic patients with coronary heart disease (CHD) as compared to non-diabetic patients [21].

The above discussion clearly indicated the clinical significance of various lipid parameters including total cholesterol, triglycerides, HDL and LDL in predisposing diabetic patients to cardiovascular complications.

In this study, a significant correlation was observed between levels of glycosylated hemoglobin (HbA1c) and lipid profile. This may in turn help in predicting the lipid profile levels from the degree of glycemic control and therefore, identifying the patients with increased risk of diabetic complications.

Earlier studies evidenced correlation between glycemic control and dyslipidemia [18]. Hussain *et al.* [22] found a positive correlation between HbA1c and high triglycerides suggested that HbA1c can be used as a potent marker for dyslipidemia and mitigate the macro- and micro-vascular complications.

Conclusion

This study showed a strong association between lipid profile and HbA1c levels. There is a need to propagate awareness about the importance of monitoring biochemical and biophysical parameters amongst diabetic individuals. We feel that, the individuals suffering from diabetes should be educated about regular monitoring of the lipid profiles and their glycemic status, to avoid complications associated with diabetes mellitus.

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