ORIGINAL RESEARCH PAPER
Volume-8 | Issue-11 | November - 2019 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr
INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

EVALUATION OF GLYCATED HEMOGLOBIN AS A PROGNOSTIC MARKER OF DYSLIPIDEMIA IN DIABETES MELLITUS TYPE 2 IN NORTH INDIAN POPULATION

Biochemistry

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ABSTRACT

Hyperglycemia is the main feature of diabetes mellitus type 2 which cause the non enzymatic glycation of haemoglobin and influence the lipid metabolism. In this comparative study, plasma of 150 Subjects (50-poor control (HbA1c >7%), 50-good control (HbA1c <7%) and 50-Controls) were analyzed for blood glucose and HbA1c and serum for lipid profile. A significant increase in TC, TG, VLDL-C and LDL-C concentrations were found in poor control than good control and Controls (*'p'*= 0.000) except HDL-C level which is not significantly increase in poor control (*'p'*= 0.132). In poor control HbA1c show the positive correlation with TC, TG, VLDL-C (*'p'*= <0.05) except HDL-C (*'p'*= 0.133). In good control HbA1c show significant correlation with lipid profile but less significant than poor control. This dual biomarker- HbA1c, glycemic control as well as lipid profile can be used for screening and early diagnosis of dyslipidemia.

KEYWORDS

Glycated hemoglobin, Dyslipidemia, Diabetes mellitus

INTRODUCTION

Diabetes mellitus is a heterogeneous metabolic disorder characterized by the presence of hyperglycemia due to impairment of insulin secretion, defective insulin action or both. The chronic hyperglycemia of diabetes is associated with relatively specific long term microvascular complications affecting the eyes, kidneys and nerves, as well as an increased risk for cardiovascular disease (CVD).[1] Patient with diabetes mellitus type 2 (T2DM) have a combination of insulin resistance and dysfunctional β-cells but do not require insulin to sustain life although insulin eventually will be required to control hyperglycemia and keep HbA1c below 7% in 90% of patients.[2]

HbA1c are post-translational modifications formed by the slow non-enzymatic attachment of glucose to haemoglobin over the lifetime of the red cell, the degree of haemoglobin glycation can be used as an index of average glycaemia over the preceding weeks and months.[3]

Disorders of lipid metabolism are common and prominent in diabetes, and are important risk factors for the high frequency of atheromatous complications in the disease.[4] Hyperlipidemia is one of the most risk factors for coronary artery disease (CAD) which is more prevalent among adults with T2DM` than in the general population with a four to six fold greater cardiovascular mortality.[5]

The aim of this study was to assess the relationship between HbA1c and serum lipid profile as well as to evaluate the importance of HbA1c as an indicator of dyslipidemia in patients with T2DM in North Indian population.

MATERIAL AND METHODS

Upon ethical clearance, this hospital based Retrospective study was conducted in biochemistry department on **150 subjects** (50 patients were diagnosed with good controlled type 2 diabetes mellitus with HbA1c <7%, 50 patients were diagnosed with poor control type 2 diabetes mellitus with HbA1c >7%, 50 healthy **controls** in the similar age group) attending OPD of general medicine of Rama Medical College, Hospital & Research Centre, Kanpur, Uttar Pradesh, India. Detailed information of the patients was collected with the help of pre-test proforma that included age, sex and family or personal history of chronic diseases.

Inclusion criteria

- Subjects between 31–75 years age group were considered.
- Both males and females were included.

Exclusion criteria

- Type 1 diabetes mellitus
- Pregnant & Lactating mothers
- Smoking and alcoholic individuals
- Patients having coronary heart disease, familial dyslipidemia, Thyroid disease, Chronic renal failure, Pancreatitis, Hepatic diseases etc.
- Patients with history of using drugs that significantly affect lipid metabolism.
- Patients with low hemoglobin level (female < 12 mg/dl , male <13 mg/dl)

Method of Analysis:

Under all aseptic precautions, 5ml each fasting and post meal samples were obtained from each study subject with the help of disposable syringe and needle. Serums were separated and used for various biochemical assays.

Blood glucose assays:

Plasma glucose were estimated on Erba CHEM-5 *Plus* semi auto analyzer by GOD-POD method.[6]

Glycated haemoglobin :

HbA1c was estimated on Erba CHEM-5 *Plus* semi auto analyzer by Ion Exchange Resin method.[7]

Lipid profile:

The lipids were analyzed on Erba Chem 5-*plus* semi-auto analyzer according to the protocol mentioned in the test kits from Erba Mannheim.

a) Serum Total cholesterol (TC) was estimated by CHOD-PAP end point method.[8]
b) Serum Triglycerides (TG) was estimated by Trinder's method.[9]
c) High Density Lipoprotein- cholesterol (HDL-C) was estimated by direct method.[10]
d) Very Low Density Lipoprotein- cholesterol (VLDL-C) and Low Density Lipoprotein- cholesterol (LDL-C) were calculated by Friedewald's formulae.[11]

- VLDL = TG/5 mg/dl
- LDL-C = TC – (HDL+TG/5) mg/dl

Statistical analysis:

Statistical analysis was done by using statistical software **SPSS VERSION 21.0** and the results were expressed as Mean ± SD. The comparisons of serum levels of these parameters between poor control, good control and controls have been done using *Independent't' test*. Pearson's correlation coefficient was also used to find the correlation between HbA1c and serum concentration of lipids.

RESULTS

TABLE 1- T-TEST FOR EQUALITY OF MEANS BETWEEN POOR CONTROL AND CONTROL

Parameters	Control (Mean ± S.D.)	Poor control (Mean ± S.D.)	*'t' value*	*'p' value"*
FBG (mg/dl)	88.78 ± 11.09	173.82 ± 51.61	11.389	0.0001**

PPBG (mg/dl)	114.84 ± 13.64	265.48 ± 50.73	20.273	0.0001**
HbA1c (%)	5.67 ± 0.52	8.73 ± 1.44	14.036	0.0001**
TC (mg/dl)	174.96 ± 43.72	209.82 ± 69.55	3.00	0.003*
TG (mg/dl)	118.14 ± 27.34	194.84 ± 100.14	5.224	0.0001**
HDL-C (mg/dl)	51.68 ± 9.74	49.08 ± 7.16	-1.520	0.132
LDL-C (mg/dl)	92.98 ± 31.47	115.40 ± 56.60	2.448	0.016*
VLDL-C (mg/dl)	26.50 ± 8.08	38.88 ± 21.51	3.808	0.0001**

A 'p' value < 0.05 was considered significant.
A 'p' value < 0.001 was considered highly significant.

FBG, PPBG, HbA1c, TG, and VLDL-C in poor control are highly significant with control ('*p*'= 0.0001). TC and LDL-C are also found statistical significant increased in poor control than control (*'p'*= 0.003 and *'p'*= 0.016 respectively) but the difference in HDL-C level between poor control and control is not significant ('*p*'=0.132).

TABLE 2- T-TEST FOR EQUALITY OF MEANS BETWEEN GOOD CONTROLAND CONTROL

Parameters	Control (Mean ± S.D.)	Good control (Mean ± S.D.)	*'t' value*	*'p' value*
FBG (mg/dl)	88.78 ± 11.09	126.00 ± 37.39	6.746	0.0001**
PPBG (mg/dl)	114.84 ± 13.64	243.52 ± 53.73	16.360	0.0001**
HbA1c (%)	5.67 ± 0.52	6.05 ± 0.594	3.370	0.001*
TC (mg/dl)	174.96 ± 43.72	197.94 ± 49.55	2.459	0.016*
TG (mg/dl)	118.14 ± 27.34	149.08 ± 58.57	3.384	0.001*
HDL-C (mg/dl)	51.68 ± 9.74	50.1 ± 9.71	- 0. 812	0.419
LDL-C (mg/dl)	92.98 ± 31.47	107.08 ± 37.93	2.023	0.046*
VLDL-C (mg/dl)	26.50 ± 8.08	34.46 ± 14.92	3.316	0.001*

A 'p' value < 0.05 was considered significant.
A 'p' value < 0.001 was considered highly significant.

FBG, PPBG in good control were highly significant with control ('*p*'= 0.0001). HbA1c, TC, TG, LDL-C and VLDL-C were statistical significant increased in good control than control but the HDL-C between good control and control was not significant ('*p*'= 0.419).

TABLE 3- PEARSON CORRELATION BETWEEN HbA1c AND LIPID PROFILE LEVELS IN POOR CONTROL AND GOOD CONTROL.

Lab variables	HbA1c Group	N	Pearson Correlation	*'p' value*
TC (mg/dl)	Poor control	50	0.221	**0.027**
	Good control	50	0.135	**0.181**
TG (mg/dl)	Poor control	50	0.048	**0.0001**
	Good control	50	0.164	**0.102**
HDL-C (mg/dl)	Poor control	50	0.151	**0.133**
	Good control	50	0.006	**0.954**
LDL-C (mg/dl)	Poor control	50	0.159	**0.015**
	Good control	50	0.197	**0.050**
VLDL-C (mg/dl)	Poor control	50	0.406	**0.0001**
	Good control	50	0.129	**0.200**

N = Number of observations
A 'p' value < 0.05 was considered significant.
A 'p' value < 0.001 was considered highly significant.

In poor control highly significant Positive correlation was seen between HbA1c and serum lipids concentrations except HDL-C (*'p'*= 0.133). In good control, lipid profile was not significantly increase with HbA1c as compare with poor control.

DISCUSSION

The present study was conducted in the Department of Biochemistry, Rama Medical College, Hospital & Research Centre, Kanpur, Uttar Pradesh, India. The result of the present comparative study showed that diabetes mellitus disease is more prevalent in male in North Indian population. The higher prevalence of T2DM disease in men suggests that less sensitive to insulin might be involved. My study indicates that there was a trend toward a higher prevalence of poor control and good control in the age group 45 - 60 years.

According to WHO diabetes diagnosis criteria FBG should be >126 mg /dl and PPBG should be >200 mg /dl. In my study, diabetic patients of poor control and good control followed the WHO criteria and their FBG and PPBG were more than 126 mg/dl and 200 mg/dl respectively. Good control included newly diagnosed diabetic patients and poor control included prolonged diabetic patients.

HbA1c is formed by non enzymatic glycation of hemoglobin. HbA1c of poor control was find > 7% and good control was find < 7%. Similar finding were reported by *Rosmee and Shyamal Koley*[12], *Alekandra Klisic et al*.[13]

In normoglycemic subjects, a carbohydrate moiety is attached to a small proportion of hemoglobin A, thus creating what is called as glycosylated or glycated hemoglobin. It has three distinct fractions: A1a, A1b and A1c. The A1c fraction accounts for 60% of bound glucose. Non-diabetic individuals have HbA1c values in the range of 3 - 6%.

In conditions of sustained hyperglycemia, the proportion of hemoglobin that is glycated increases substantially. This glycation is the result of postprandial modification of haemoglobin A molecules, the binding of glucose is a non-enzymatic process that occurs continuously during the life of the red blood cell. Thus the amount of glycated hemoglobin reflects the glycemic control of a patient during the 6 – 8 week period before the blood sample was obtained.[14]

In my clinical data analysis 70% diabetic patients were found to have dyslipidemia in poor control than good control (32% dyslipidemia) and control. The findings were in agreement with the previous studies*;* *Santosh Gosavi* [15] , *A.Valarmathi et al.*[16] , *Sherwani et al.*[17], *Rosmee and Shyamal Koley*[12], who reported higher TC, TG, VLDL-C and LDLC values in diabetic patients except HDL-C.

The abnormal lipid profile observed in type 2 diabetes mellitus is said to be related to insulin resistance as reported in previous studies, which leads to increased release of free fatty acids from fatty tissue, impaired insulin dependent muscle uptake of free fatty acids and increase fatty acid release to the hepatic tissue [18] which has been closely associated with diabetic dyslipidemia, hypertension[19] and enormous risk to cardiovascular diseases. Chronic hyperglycemia causes glycation of apolipoproteins and interferes with the normal pathways of lipoprotein metabolism.[20]

The possible reason for high serum cholesterol in diabetes may be due to decrease muscular exercise or inhibition of cholesterol catabolism. High serum cholesterol in T2DM enhanced lipolysis leads to high free fatty acid levels in plasma and consequent accumulation of fat in liver. Due to this, more Acetyl-COA is now available which cannot be efficiently oxidized by TCA cycle because the availability of oxaloacetate is limited. The stimulation of gluconeogenesis is responsible for the depletion of oxaloacetate. The excess of Acetyl-COA therefore is diverted to cholesterol leading to hyperchole sterolemia[21,22]

Hypertriglyceridemia is the most common alteration of lipoproteins in T2DM. It is caused by hyperglycemia and insulin resistance that together lead to: (1) Overproduction of VLDL triglyceride (2) Defective clearance of VLDL triglyceride (3) Decreased activity of lipoprotein lipase and (4) Decreased production of apolipoprotein B. Also the composition of VLDL is altered such that the proportion of cholesterol increases and this increases the propensity for atherosclerosis.

Hyperglycemia causes increased activity of hepatic lipase that leads to increased clearance of HDL while impaired catabolism of VLDL causes decreased formation of HDL. Thus the HDL levels decrease in T2DM.[23]

However, to some extent, we have succeeded in correlating diabetes mellitus with the altered lipid profile but the results inferred may not be considered as the reflection of larger population because our study involved a small sample size due to limited period. So, there is an absolute need for large study designs to answer the questions to whether diabetes mellitus is associated with increased risk for CVD and one of the most important and frequent complications with a high premature mortality and morbidity rate, dyslipidemia should be better assessed, prevented, and treated as early as possible to avoid or reduce vascular damage.

CONCLUSIONS

From my study there is a significant correlation between HbA1c and various circulating lipid parameters. In this present study a significant difference in lipid parameters in two groups (<7.0% and >7.0%) of HbA1c. This may indicate that HbA1c can be used as a potential biomarker for predicting dyslipidemia in patients with T2DM in addition to glycemic control. The DCCT also established HbA1C as the gold standard of glycemic control. Hence, early diagnosis can be accomplished through relatively inexpensive blood testing and may be utilized for screening high-risk patients with DM for timely intervention with lipid-lowering drugs.

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