Research Article (Open access)

Glycated Hemoglobin as a Dual Biomarker in Type 2 Diabetes Mellitus Predicting Glycemic Control and Dyslipidemia Risk

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ABSTRACT- Diabetes mellitus is associated with hyperglycemia and patients are at an increased risk of cardiovascular disease. The present study was carried out to evaluate the diagnostic value of Glycated hemoglobin (HbA1c) in predicting risk of development of diabetic dyslipidemia. 70 clinically diagnosed cases of type 2 diabetes mellitus with the age range 30-75 years were included in the study group. Out of which 35 diabetic patients with good glycemic control were included under Group A and 35 diabetic patients with poor glycemic control were included under Group B. 70 age and sex matched healthy individuals served as controls. HbA1c demonstrated positive and significant correlation with total cholesterol (TC), low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C) and LDL/HDL-C, non-HDL-C and TC/HDL-C ratio. Patients with HbA1c value > 7.0% had significantly higher value of TC, Triacylglycerol (TAG), LDL-C, LDL-C/HDL-C ratio, non-HDL-C and TC/HDL-C ratio as compared to the patients with HbA1c ≤ 7.0%. However, there was no significant difference in value of HDL-C between two groups. Thus HbA1c can be used as a potential dual marker of glycemic control and dyslipidemia in type 2 diabetes mellitus.

Keywords: - Type2 Diabetes Mellitus, Glycated hemoglobin, Dyslipidemia, Cardiovascular disease, Lipid Profile panel

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INTRODUCTION

Diabetes mellitus (DM) is a hereditary, chronic and endocrine-metabolic disorder. [1] DM is a group of metabolic disease characterized by hyperglycemia resulting from defects in insulin secretion, insulin action or both.[2] Epidemiological studies have demonstrated that type 2 diabetes mellitus (DM) is a well-known risk factor for the development of cardiovascular disease, cerebrovascular disease, and peripheral vascular diseases. Alterations in lipid and lipoprotein profile contribute to atherosclerosis in type 2 diabetes. [3] Diabetic dyslipidemia is generally characterized by increased plasma triglyceride (TG) and decreased high-density Lipoprotein cholesterol (HDL-C) concentrations, a preponderance of small, dense low-density lipoprotein (LDL), and an increased apolipoprotein B concentration. Although the major focus on the connection between lipids and CHD is on LDL-cholesterol (LDL-C), the Adult Treatment PanelIII has recognized the important roles of HDL-C & TGs, calling this combination an atherogenic dyslipidemia.[4] Dyslipidemia is elevation of plasma cholesterol, triglycerides (TGs), or both, or a low high-density lipoprotein–Cholesterol (HDL-C) level that contributes to the development of atherosclerosis, which may be primary (genetic) or secondary and diagnosed by measuring plasma levels of total cholesterol (TC), TGs, and individual lipoproteins. It is traditionally classified by patterns of elevation in lipids and lipoproteins.[5] Dyslipidemia is a well-recognized and modifiable risk factor that should be identified early to institute aggressive cardiovascular preventive management.[6] Patients with type 2 DM are at greater risk of developing vascular diseases because of lipid changes. Lipid abnormalities and insulin use is critically discussed in diabetics.[7] The most typical lipoprotein pattern reported in diabetes, also known as diabetic dyslipidemia or atherogenic dyslipidemia consists of moderate elevation in TG levels, low HDL-C cholesterol values, and low density lipoproteins cholesterol (LDL-C) (especially small dense LDL particles.[8] The atherogenic index of plasma (AIP), defined as logarithm [log] of the ratio of plasma concentration of triglycerides to high-density lipoprotein (HDL) cholesterol, has recently been proposed as a predictive marker for plasma atherogenicity and is positively correlated with cardiovascular disease risk.15 AIP's significance as a marker is based on the following facts: it is found increased in cohorts at high risk for CAD, it is positively correlated with the fractional esteri-

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Received: 19 September 2015/Revised: 30 September 2015/Accepted: 16 October 2015

fication rate of HDL-C (FERHDL), which is perhaps the most dependable marker for the atherogenic capacity of the lipid-lipoprotein profile and it is inversely correlated to LDL-C particle size (an indirect indicator of LDL particle).[9]

The amount of glycated hemoglobin (HbA1c) reflects the glycemic control of a patient during the 6 – 8 week period before the blood sample was obtained. The amount of HbA1c correlates well with fasting and postprandial blood glucose levels. At present HbA1c is the best surrogate marker we have for setting goals of treatment. 5 The Diabetes complications and control trial (DCCT) established HbA1c as the gold standard of glycemic control. The level of HbA1c value 7.0% was said to be appropriate for reducing the risk of cardiovascular complications.[10]

MATERIALS AND METHODS

The study was conducted at Department of Biochemistry, King George Medical University Lucknow, India.

Inclusion criteria

70 clinically diagnosed cases of type 2 diabetes mellitus with the age range 30-75 years were included in the study group. Out of which 35 diabetic patients with good glycemic control were included under Group A and 35 diabetic patients with poor glycemic control were included under Group B. 70 age and sex matched healthy indiviguals served as control Venous blood was collected from the subjects after an overnight or 12 hours of fasting samples were analyzed for fasting plasma glucose is a better term because these days we estimate glucose levels in plasma, lipid profile and glycated haemoglobin. Diabetes was diagnosed as per American Diabetes Association (ADA) criteria.[11] for serum lipid reference level, National Cholesterol Education Programme (NCEP) Adult Treatment Panel III (ATP III) guideline was referred.[12]

Exclusion criteria

The patients with type 1 Diabetes Mellitus, Chronic kidney disease stage 3 and above, recent major surgery, infection, retinopathy, nephropathy, diabetic foot were excluded.

Biochemical Assessments

1. HbA1c –Estimated using Direct Enzymatic Assay method.[13]
2. FBSL -Glucose oxidase method commonly known as the GOD-PAP (End-Point) method.[14]
3. TG – Enzymatic colorimetric (End point) method.[15]
4. TCH -Enzymatic colorimetric (End point) method.[16, 17]
5. LDL & HDL - by precipitation method using a reagent that consists of modified polyvinyl sulfonic acid (PVS) and polyethylene-glycol methyl ether (PEGME).[18]

Very Low density lipoprotein cholesterol was calculated using the Friedewald's formula [32]VLDL-C (mg/dl): Triglyceride/5

The statistical test used in present study mean±SD and percentage.Unpaired t-test was used to compare the study parameters between cases and controls. The Pearson correlation coefficient was calculated among the study parameters. The p-value<0.05 was considered significant.

RESULTS

This studied show result that HbA1c demonstrate the positive and significant correlation with total cholesterol, triglycerides, LDLc, Non HDLc and a negative correlation with HDLc. There is also a good correlation between HbA1c and lipid profile. The patients were classified into two groups depending on their glycated hemoglobin (HbA1c); Good Glycemic Control (GGC) group having HbA1c < 7.0% (n= 35) and Poor Glycemic Control (PGC) group having HbA1c>7.0% (n= 35).

Table 1: Correlation of HbA1c levels with other parameter

Parameter	Mean ± SEM	Correlation with HbA1c r- value	Inference
HbA1c (%)	7.53 ± 0.27	-	-
FBG (mg/dl)	143.84 ± 4.89	0.81	Direct
TG (mg/dl)	152.66 ± 6.96	0.40	Direct
TCH (mg/dl)	176.55 ± 4.54	0.26	Direct
HDL (mg/dl)	37.96 ± 1.06	- 0.19	Inverse
LDL (mg/dl)	107.02 ± 4.49	0.31	Direct

**Statistically highly significant

Thus, direct correlation of HbA1c was observed with FBG, TG, TCH, and LDL. While inverse correlation was observed between HbA1c and HDL.

Table: 2 Comparison between 2 groups of cases

Parameter	PGC group (n=35)	GGC group (n=35)	P-value	t-value
FBG	172.52 ± 5.48	113.22 ± 3.29	<0.0001**	54.8
TG	170.35 ± 11.25	132.28 ± 4.37	<0.0001**	18.6
TC	189.14 ± 8.07	164.89 ± 3.49	<0.0001**	16.3
LDL	119.4 ± 7.59	96.51 ± 3.36	<0.0001**	16.3
HDL	37.32 ± 1.42	41.16 ± 1.34	<0.0001**	11.6

Thus, statistically highly significant 'p' values were obtained for FBS and statistically significant 'p' values TG, LDL in PGC group.

Tablet 3: Correlation between HbA1c with TG, Cardiovascular risk ratios, Non-HDL-C

Parameters	r - value	Correlation
HbA1c & Fasting blood glucose	0.75	'+'
HbA1c &HDLc	-0.25	'+'
HbA1C &LDLc	0.61	'+'
HbA1c & TC/HDLc	0.54	'+'
HbA1c &LDLc/HDLc	0.66	'+'
HbA1c & Non-HDLc	0.47	'+'
Correlation	**Negative**	**Positive**
Small	0.03 to 0.1	0.1 to 0.3
Medium	0.5 to 0.3	0.3 to 0.5
Strong	1.0 to 0.5	0.5 to 1.0

HbA1c showed a highly significant positive correlation with Non-HDLc (r = 0.47) than the other cardiovascular risk ratios.

DISCUSSION

Diabetes is a common non-communicable disease. Diabetes mellitus is a chronic illness that requires continuing medical care, patient education, and support to prevent acute complications and to reduce the risk of long-term complications. Control of blood glucose in patients with diabetes can be assessed by several methods. These include assessment of glycated hemoglobin (HBA1c), fasting blood sugar (FBS), and Lipid profile. The gold standard for assessment of glycaemic control at follow up is the glycated hemoglobin level.[19] High concentrations of glucose can increase the glycation of common proteins such as HbA1c, formed through the non-enzymatic attachment of glucose to hemoglobin, which is commonly considered to reflect the integrated mean glucose level over the previous 8–12 weeks, the time period being dictated by the 120-day lifespan of the erythrocyte.[20] The concentration of HbA1c predicts diabetes complications because it reflects more harmful glycation sequelae of diabetes, such as retinopathy and nephropathy, which are understood to be due to harmful advanced glycation end products. [21-23] Moreover, HbA1c is undoubtedly a user friendly and stable test with very minimal biological variability and which is not affected by factors which otherwise has considerable impact on glucose measurement.[24-26] So the compliance of diabetic subjects is increased which is an important and welcome feature in diabetic management for patient as well as the treating physicians. But despite its good compliance, a large number of medical conditions are associated with alterations in the HbA1c values. Hematological conditions such as the presence of hemoglobin variants, iron deficiency, and hemolytic anemia, the presence of carbamylated hemoglobin in uremia, a variety of systemic conditions, including certain forms of dyslipidemia, malignancies, and liver cirrhosis, various medications, and finally, pregnancy are among the factors that influence the HbA1c measurement. [27,28] So, the present study was performed to relate FBS with HbA1c to search a better alternative of HbA1C in developing country like India where relatively costly but more sensitive test HbA1c is the beyond the reach of most patients attending clinics at state hospitals.

Numerous factors like stress, acute illness, medication, venous stasis, posture, sample handling, food ingestion, prolonged fasting and exercise can alter fasting plasma glucose. [29] Recent studies have also shown that HbA1c predicts cardiovascular complications in diabetic subjects.

Glycated hemoglobin (HbA1c) is the marker for long term glycemic control. Elevated HbA1c indicates poor glycaemic control and predicts the risk for the development of cardiac complications in diabetes. Thus elevated HbA1c in diabetes mellitus patients predicts increased risk for development of CVD. [30] studied 278 subjects to evaluate the diagnostic value of HbA1c in predicting cardiovascular risk. The findings indicate that HbA1c can provide auxiliary information about the extent of circulating lipids and Atherogenic Index of Plasma (AIP), and can thus be used as a predictor of cardiovascular risk in diabetics.[30]

From the data, sensitivity, specificity and positive predictive value was also calculated, to predict good control of diabetes (HbA1c<7%) was considered as per American Diabetic association (ADA) guidelines. PPBS showed better performance than Fasting glucose, in detection of better glycemic control status. Result of the study indicate that PPBS level increased in all three groups and has a strong relationship with the rising of HbA1C. The gold standard for assessment of glycaemic control at follow up is the glycated haemoglobin level.

Our study also showed a significant correlation between HbA1c and Non-HDLc. Non-HDLc was shown to be the stronger predictor of CVD in diabetic population. [2, 10]

CONCLUSION

The finding of the study suggests significant correlation between HbA1c and lipid profile. It provides valuable supplementary information about the extent of circulating lipids besides its primary role in monitoring long-term glycemic control. Thus, dual biomarker capacity of HbA1c (glycemic control as well as lipid profile indicator) may be utilized for screening high-risk diabetic patients As the relative risk of cardiovascular disease is higher in diabetic patients, so good glycemic control and timely intervention with statins may prevent or delay the occurrence of adverse cardiovascular events.The diabetes complications and control trial (DCCT) established HbA1c as the gold standard to assess glycemic control. As elevated HbA1c and dyslipidemia are independent risk factors of CVD, diabetic patients with elevated HbA1c and dyslipidemia can be considered as a very high risk group for CVD. Improving glycemic control can substantially reduce the risk of cardiovascular events in diabetics.

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