

Research Article

Role of glycated hemoglobin (HbA1c) as a dual marker to predict glycemic status and dyslipidemia in type II diabetes mellitus

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ABSTRACT

Background: Type 2 diabetes mellitus (DM) is an endocrinological disease associated with hyperglycemia characterized by both insulin resistance and defective insulin secretion. Glycated hemoglobin (HbA1c) is a routinely used marker for long-term glycemic control. This study is aimed at investigating the relationship between glycemic control and serum lipid profile and to evaluate the role of glycated haemoglobin as an independent risk factor for cardiovascular diseases in patients with type-2 diabetes and to evaluate the diagnostic value of glycated hemoglobin (HbA1c) in predicting diabetic dyslipidemia as a marker of circulating lipids. The aim of the present study was to estimate glycated hemoglobin and lipid profile in patients with type 2 DM and compare it with controls (healthy subjects). The association of glycated hemoglobin with lipid profile evaluated.

Methods: This study was conducted in 150 subjects, out of whom 75 were type 2 diabetes mellitus patients (cases) and 75 were non diabetic healthy subjects (controls). The sera were analyzed for HbA1c, fasting blood glucose (FBG), total cholesterol, triglycerides, high-density lipoprotein cholesterol (HDL) and low-density lipoprotein cholesterol (LDL).

Results: A significantly increased level of glycated hemoglobin (HbA1c) is observed in cases compared to control. HbA1c showed direct and significant correlations with cholesterol, triglycerides and LDL and inverse correlation with HDL in cases when compared to controls. Our study also revealed a significant positive correlation between lipid profile and glycated hemoglobin.

Conclusions: These findings clearly suggest that HbA1c can provide valuable supplementary information about the extent of circulating lipids besides its primary role in monitoring long-term glycemic control. Further studies are warranted to reinforce the potential of HbA1c as a biomarker for screening of high-risk diabetic patients.

Keywords: Type 2 diabetes mellitus, Glycated hemoglobin HbA1c, Circulating lipids, Diabetic dyslipidemia

INTRODUCTION

Diabetes is a global endemic with rapidly increasing prevalence in both developed and developing countries.¹ DM is a group of metabolic disease characterized by hyperglycemia resulting from defects in insulin secretion, insulin action or both. Uncontrolled diabetic patients are

characterized by hyperglycemia, hyper insulinemia, protein glycation and oxidative stress which cause early appearance of diabetic complications.

The chronic hyperglycemia is associated with long-term damage dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart and blood

vessels.² There is a high risk of cardiovascular disease (CVD) in People with type 2 diabetes; Diabetic patients with accompanied dyslipidemia are silent targets for cardiovascular deaths which represent the top killer in this population.³

Diabetes mellitus is a common secondary cause of hyperlipidemia, particularly, if glycaemic control is poor, which in-turn is an important risk factor for atherosclerosis and coronary heart disease. Diabetes mellitus increases the risk of atherosclerotic vascular disease because of associated dyslipidemia, exhibit an atherogenic lipid profile, which greatly increases their CVD risk compared with people without diabetes.

Glycosylated haemoglobin (HbA1c) is commonly used as a marker of glycaemic status. Glycated haemoglobin (HbA1c) was called as unusual haemoglobin in patients with diabetes when it was first discovered.

After that discovery, it was established that HbA1c could be used as an objective measure of glycaemic control and a validated relationship between A1C and average glucose across a range of diabetes types and patient populations by an International Expert Committee recommendation which was later adopted by WHO (International Expert Committee, 2009; World Health Organisation, 2011). HbA1c has been proposed as a dual marker for glycaemic control and coronary artery disease (CAD) risk factor.⁴

The American diabetes association (ADA) estimates that the risk of diabetes-related mortality increases 25% for each 1% increase in HbA1c. It has also been estimated that each percentage point increase in HbA1c correspond to a 35% increase in the risk of microvascular complications and an 18% increase in the risk of myocardial infarction (fatal and non-fatal). The reduction or control of blood glucose level may lower the lipid risk factor for cardiovascular diseases.⁵

There is evidence of close relationship between poor glycaemic control and progression of dyslipidemia.⁶ Most frequent complication of DM is atherosclerosis; it affects major vascular beds leading to various metabolic abnormalities. Intensive glycaemic control means the glycohaemoglobin (HbA1c) or blood glucose values are normal or near normal range, no matter how simple or complex the treatment regimen. A strong correlation has been shown between lipid profile and CAD.

The Framingham study has demonstrated a linear increase in CAD risk with increment of TC level from 180 mg upward. The study established that individuals with HDL cholesterol less than 35 mg/dl have 8 times increase in CAD incidence than those with HDL cholesterol more than 65 mg/dl.⁷

The Lipid Research clinics Coronary Primary Prevention Trial concluded that a 1% fall in the TC reduced the CAD

risk by 2%.⁸ Helsinki heart study concluded that a mean 12% rise in HDL cholesterol and an 11% fall in LDL cholesterol were both correlated with a 34% decline in CAD. This study was carried out to; compare lipid profile between uncontrolled and controlled diabetics, and find out age and gender distribution of uncontrolled diabetics in a group of diabetic patients. The aim of the study is to evaluate the HbA1c as a marker of glycaemic control and lipid profile.

METHODS

The study was approved by the Ethics Committee; a written informed consent was obtained from all participants for participation in this study. A total of 150 patients (aged 30-70 years) with type-2 DM recruited from Institute's Medicine and Endocrinology departments. The diagnosis of type-2 DM was confirmed by biochemical investigations as per WHO criteria.

Patients were excluded when diagnosed with type 1 DM, acute complications such as severe infection, major operations, trauma, GI disorders, severe cardiovascular/respiratory diseases, pregnant and breast feeding women. Patients taking supplements such as antioxidants, vitamins, minerals were also excluded. Age and sex matched 150 controls were recruited after clinical and biochemical evaluation.

The baseline demographic data and family history were obtained. 3 mL of venous blood sample was collected. The serum was used for analysing fasting blood glucose (FBG), Total cholesterol (TC), HDL-cholesterol (HDL-C), Triglycerides (TG). HbA1c concentrations were measured using NGSP method. All the above mentioned parameters were measured using the autoanalyzer Beckman Coulter DXC 600.

HbA1c was estimated by high performance liquid chromatography using D10. Serum lipid levels were referred by National Cholesterol Education Program (NCEP) Adult Treatment Panel III (ATP III) guidelines. According to NCEP-ATPIII guideline, hypercholesterolemia is defined as TC>200 mg/dl, high LDL-C when value >100 mg/dl, hypertriglyceridemia as TG >150 mg/dl and low HDL-C when value 30 mg/dl.⁹

Statistical analysis: Statistical analysis was done by SPSS version 17.0. Pearson's correlation test was performed to examine correlations between various parameters. Independent samples t-test (2-tailed) was used to compare means of different parameters. All Values are expressed as mean±standard error of mean. The results were considered statistically significant when p<0.001.

RESULTS

A total of 150 subjects were included in our study, 75 Type 2 DM patients and 75 gender matched non-diabetic apparently healthy control subjects (52 males and 23

females). The age group of cases and controls were between 30-70 years with a mean age 54.36±11.25 in cases and 51.81±10.25 for controls. Table 1 also shows

significantly increased HbA1c, fasting and post prandial blood sugars in cases than controls with p value <0.001.

Table 1: Comparison of biochemical parameters between case and control groups.

Parameters	Cases (n=75)	Controls (n=75)	p value
HbA1c	9.4±1.80	4.71±0.67	<0.001**
FBS mg/dl	218.98±63.11	81.96±19.04	<0.001**
PPBS mg/dl	226.84±89.64	113.56±42.39	<0.001**

Table 2: Comparison of lipid parameters between case and control groups.

Lipid parameters	Cases	Controls	p value
Cholesterol mg/dl	257.30±56.39	188.58±39.59	<0.001**
Triglycerides mg/dl	253.18±58.98	112.56±55.44	<0.001**
HDL mg/dl	30.98±5.21	42.65±6.55	<0.001**
LDL mg/dl	191.17±86.17	98.74±28.30	<0.001**
VLDL mg/dl	51.31±11.79	31.59±11.56	<0.001**
L/H Ratio	6.59±1.52	3.98±1.84	<0.001**

The mean value of HbA1c and FBS were slightly higher in males in comparison to female patients and the differences were statistically significant. Among the circulating lipids, TC TG, LDL-C and VLDL were significantly higher, hypercholesterolemia was found in 68 (90%) individuals (Table 2). Similarly, hypertriglyceridemia was found in 69 (92%) individuals, increased LDL-C was found in 55 (73%) individuals and decreased HDL-C was found in 65 (86%) individuals and

highly significant positive correlation was observed between FBG and HbA1c. The value of r was 0.7245, P<0.01. There was a strong positive correlation HbA1c and PPBS with r value 0.7356., p<0.01.) HbA1c also demonstrated direct and significant correlations with cholesterol with r value 0.6445, TG with r value 0.5426, LDL-C with r value of 0.3584, VLDL r value 0.2245, L/H R value of 0.3416 with a strong positive correlation. Whereas HDL-C showed negative correlation with r value -0.4965 (Table 3).

Table 3: Pearson correlation of HbA1c with FBS,PPBS and Lipid profile in cases.

Lipid parameters	Cases r value	Correlations	p value	Significance
HbA1c /FBS	0.725	Positive	0.001	<0.001**
HbA1c /PPBS	0.7356	Positive	0.001	<0.001**
HbA1c /Cholesterol mg/dl	0.6445	Positive	0.001	<0.001**
HbA1c /Triglycerides mg/dl	0.5426	Positive	0.001	<0.001**
HbA1c /HDL mg/dl	-0.4965	Negative	0.003	<0.001**
HbA1c /LDL mg/dl	0.3584	Positive	0.002	<0.001**
HbA1c /VLDL mg/dl	0.2245	positive	0.05	<0.10
L/H RATIO	0.3416	positive	0.002	<0.001**

DISCUSSION

Diabetes mellitus (DM) is a hereditary, chronic and endocrine-metabolic disorder. 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. 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. In the present study we have estimated the diagnostic value of HbA1c as a dual

marker. Diagnosis of diabetes rests on the measurement of plasma glucose levels. According to American Diabetic Association FBS >126mg/dl is diagnostic value of diabetes. The mean value of HbA1c were higher in cases (9.05 ± 2.45) in comparison to control (4.71 ± 0.67) patients but the differences were statistically significant $< 0.001^{**}$. The mean value of FBS were higher in cases (218.62 ± 97.86) than in comparison with control (81.96 ± 19.04) but the differences were statistically significant $< 0.001^{**}$.

Among the circulating lipids, TC and total cholesterol were significantly higher in patients than control. Diabetic patients were classified into 2 groups as per their glycemic index; first group consists of patients with HbA1c value $\leq 7.0\%$ 13 patients found in this group (26%), and second group consists of patients with HbA1c value $> 7.0\%$, 62 patients found in this group (74%). Patients with HbA1c value $> 7.0\%$ had significantly higher value of TC ($p=0.001$), TG ($p=0.001$), LDL-C ($p=0.001$), Non-HDL-C, LDLC/HDL-C ratio as compared to patients with HbA1c value $\leq 7.0\%$.

A highly significant correlation between HbA1c and FBG is observed in our study which is similar to various studies.¹⁰ We also observed a significant correlation between HbA1c and TC/ HDLc and LDLc/ HDLc. Several investigations have reported significant correlation between HbA1c and lipid profile and suggested the importance of glycemic control in normalizing dyslipidemia.¹¹ Present 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.¹²

The Diabetes Complications and Control Trial (DCCT) established HbA1c levels $< 7\%$ appropriate for reducing the risk of vascular complications and also as the gold standard of glycemic control.¹³ HbA1c is directly related to the severity of coronary artery disease (CAD) in diabetic patients.¹⁴ Whereas, improving the glycemic control can substantially reduce the risk of cardiovascular events in diabetics.¹⁵ Each 1% reduction in HbA_{1c} was associated with reduction in risk of 21% for any end point related to diabetes, 21% for deaths related to diabetes, 14% for myocardial infarction, and 37% for micro vascular complications.

Thus reduction in HbA_{1c} is associated with reduction in diabetes related risk complication.¹⁶ Type 2 diabetic patients are at a much higher risk of cardiovascular diseases than the non-diabetic. Thus the risk of cardiovascular events in diabetics can be reduced by improving the glycemic control.¹⁷ Type 2 diabetic patients have markedly increased risk of coronary heart disease than similarly dyslipidaemic non diabetic subjects. Low HDL and HDL2 cholesterol, high VLDL cholesterol, and high total and VLDL triglycerides are powerful risk indicators for coronary heart disease events in patients with type 2 diabetes mellitus.

The Lipid Research clinics Coronary Primary Prevention Trial concluded that a 1% fall in the TC reduced the CAD risk by 2%.⁸ Helsinki heart study concluded that a mean 12% rise in HDL cholesterol and an 11% fall in LDL cholesterol were both correlated with a 34% decline in CAD. Significant dyslipidemia has been encountered among type 2 diabetic subjects. Very few investigators have reported significant correlations between HbA_{1c} and lipid profiles and suggested the importance of glycemic control in normalizing dyslipidemia.¹⁸⁻²⁰ Also many studies have shown the beneficial effects of reducing lipids on cardiovascular system.²¹ However these patients had a lower level of HDL cholesterol when compared to patients with good glycemic control. It has been reported that HDL cholesterol is inversely, and non-HDL cholesterol directly, associated with CAD risk in diabetes patients. The cause of dyslipidaemia in type 2 diabetes mellitus may be due to impaired liver apolipoprotein production which in turn regulates the enzymatic activity of lipoprotein lipase and cholesterol ester transport protein.²²

Control subjects showed low levels of FBS, TC, TG and LDL levels where as young type 2 diabetic patients with poor glycemic control showed significantly higher TC, TG and LDL similar to other studies.^{23,24} Ahmad khan in 2007 suggests that HbA1c can predict serum lipid levels in both male and female diabetic patients and HbA1c was regarded as an independent risk factor for coronary heart disease.²⁵ They observed a direct correlation between HbA1c and the severity of coronary artery disease (CAD) in diabetic patients. Whereas, improving the glycemic control could reduce the risk of cardiovascular events in diabetic patients. Moreover, reducing cardiovascular risks resulted in the improvement of HbA1c even in the absence of any specific intervention targeted at improving glycemic control. Another study in 2011 showed that cardiovascular disease (CVD) is significantly higher in people with high levels of HbA1c.²⁶

The purpose of this study was to evaluate correlation between glycated hemoglobin (HbA1c), fasting (FBS) and 2 hours postprandial (2hpp) blood sugars with serum lipid levels in type 2 diabetic patients. Giansanti et al also observed significantly higher levels of hypercholesterolemia and hyperlipidemia in type 2 diabetic patients with CVD as compared to diabetic patients without CVD.²¹ Early therapeutic interventions, aiming to reduce triglycerides and LDL and to increase HDL, significantly reduce cardiovascular events and mortality in patients with type 2 diabetes. Onat et al suggested that fasting triglycerides are predictive for future CVD independent of age, diabetes, total cholesterol and HDL.²⁸ The above discussion clearly indicates the clinical significance of various lipid parameters including total cholesterol, triglycerides, HDL and LDL in predisposing diabetic patients to cardiovascular complications. Significant correlations between HbA1c and all these lipid parameters and a linear relationship between HbA1c and dyslipidemia point towards the usefulness of HbA1c

for screening high-risk diabetic patients. Hyperglycaemia increases complications in diabetes mellitus by generating reactive oxygen species, resulting on oxidative stress. Increased lipid peroxidation causes crosslink formation between single molecules of amino acids and LDL particles.

In metabolically poorly controlled diabetic patients, glycation of LDL increases with hyperglycemia. This elevated level of LDL is explained by decreased catabolism of LDL, decreased activity of cholesterol ester transfer protein and lipoprotein lipase activity.²⁹ It has been suggested that non enzymatic glycosylation of the LDL particle itself result in its increased incorporation in the arterial wall. Further glycosylation of lysine groups on apolipoprotein B causes inhibition of the ability of LDL to interact with the LDL receptor.

This in turn inhibits the ability of LDL to be metabolized by the LDL receptor pathway. Thus plasma LDL levels are high and atherosclerosis occurs very early in life. Because of its critical importance in atherogenesis, LDL cholesterol is a focus of current guidelines for determination of the risk of cardiovascular diseases. The above discussion clearly indicates the clinical significance of various lipid parameters in predisposing diabetic patients to cardiovascular complications.

Significant correlations between HbA_{1c} and all lipid parameters and a linear relationship between HbA_{1c} and dyslipidemia point towards the usefulness of HbA_{1c} for screening diabetic patients at high risk of developing CAD.³⁰ Type 2 diabetes mellitus is commonly associated with an abnormal lipoprotein phenotype which is characterised by increased TAG, decreased HDLc and an accumulation of small dense LDLc particles (The so called atherogenic dyslipidemic phenotype).³¹

Our study also showed a significant increase in TC/HDLc and LDLc/HDLc ratios between control and patient groups and also in between controlled and uncontrolled diabetic group. An increased CAD risk was suggested due to the increase in total cholesterol/ HDLc ratio. Schmitt et.al; suggested that LDLc uptake by fibroblasts may be impaired in diabetic patients. This leads to increase in the LDLc/ HDLc ratio in type 2 diabetes.³² LDLc/ HDLc is actually a purer ratio than TC/ HDLc, because LDLc is a measure of good cholesterol, whereas total cholesterol is the sum of HDL, LDL and VLDL. Non- HDL seems to be a better choice, as it includes triglyceride rich lipoproteins, which plays an important role in atherogenesis in type 2 diabetes.³³

CONCLUSION

The findings of the study ensure HbA_{1c} predicts serum 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 HbA_{1c}

(glycemic control as well as lipid profile indicator) may be utilized for screening high-risk diabetic patients for timely intervention with lipid lowering drugs and thus preventing adverse cardiovascular events. It has been estimated that reducing HbA_{1c} levels by 0.2% could lower the mortality by 10%.

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