ORIGINAL ARTICLE

Investigation of HbA1c as a potential biomarker for assessing dyslipidemia and atherogenicity in individuals with type 2 diabetes mellitus

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ABSTRACT:
Aim: Investigation of HbA1c as a potential biomarker for assessing dyslipidemia and atherogenicity in individuals with type 2 diabetes mellitus. **Methods:** This study conducted in the department of medicine. Total 140 diagnosed cases of DM type 2 in the age group of 20-70 years were include in this study. The patients were divided into 2 groups depending on the levels of glycated hemoglobin. Venous blood samples from all the subjects were collected after at least 6 hours fasting and analyzed for fasting plasma glucose (FPG), 2 hours post prandial glucose levels(2hPG), serum total cholesterol(TC), triglycerides(TG),HDL-C, Very low density lipoprotein cholesterol(VLDL-C) & LDL-C analysis was done by standard methods. The atherogenic index of plasma (AIP) was calculated by the formula base 10 logarithm of the ratio of TG to HDL-C. **Results:** Out of the 140 patients studied, there were 60 males and 80 females. The maximum number of patients was in the age group of 50-60 years. The levels of FBG, TC, TG,VLDL-C LDL-C, HbA1c, AIP, ratios of TC/HDL-C and LDL-C/HDL-C are increased, while the levels of HDL-C are decreased in patients with HbA1c >7% as compared to patients with HbA1c ≤ 7% and these values are highly significant. The patients with HbA1c >7% had dyslipidemia as the most prominent feature in the lipid profile. Direct and significant correlation of HbA1c with FBG, 2Hpg were found. It also shows direct and highly significant correlation of HbA1c with TC, TG, LDL-C, TC/HDL-C, LDL-C/HDL-C and AIP, and inverse correlation between HbA1c and HDL. the patients with HbA1c >7% were more prone to cardiovascular risk as their levels of AIP fall in the high risk group i.e AIP > 0.22 and the association was highly significant proving that glycemic control contributes significantly to the future risk of cardiovascular problems. The lipid parameters of patients with HbA1c <7% were in the normal range but the AIP levels still fell in the risk group. **Conclusion:** The findings of this research suggest that individuals with inadequate glycemic control have an atherogenic lipid profile, and it is possible to predict dyslipidemia and atherogenicity using the measurement of Glycated haemoglobin.
Keywords: Lipid profile, type 2 diabetes mellitus, Glycosylated haemoglobin, glycemic control, atherogenic index of plasma

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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, hyperinsulinemia, 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

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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 glycemic control and progression of dyslipidemia.[6] Most frequent complication of DM is atherosclerosis; it affects major vascular beds leading to various metabolic abnormalities. Intensive glycemic 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.

MATERIAL AND METHODS
This study conducted in the department of medicine, after taking the approval of the protocol review committee and institutional ethics committee. After taking informed consent detailed history was taken from the patient or the relatives. The technique, risks, benefits, results and associated complications of the procedure were discussed with all patients. Total 140 diagnosed cases of DM type 2 in the age group of 20-70 years, attending the diabetic OPD and admitted to the medicine wards. Patients with complications like retinopathy, nephropathy, history of heart disease, hepatic disorders or any other chronic illness were excluded from the study. The patients were divided into 2 groups depending on the levels of glycated hemoglobin (HbA1c); good glycemic control was defined as HbA1c < 7.0%(< 53mmol/mol) and poor glycemic control as HbA1c>7.0% (> 53mmol/mol).[9] Venous blood samples from all the subjects were collected after at least 6 hours fasting and analyzed for fasting plasma glucose (FPG), 2 hours post prandial glucose levels(2hPG), serum total

cholesterol(TC), triglycerides(TG) using kit based method on autoanalyser XL-640 Erba. HDL-C was estimated by kit based on precipitation method[10] & Very low density lipoprotein cholesterol(VLDL-C) & LDL-C analysis was done by applying Frieldwald's formula[11] i.e. VLDL-C = TG/5 where TG is less than 400 mg/dl, LDL-C was calculated as :TC - (HDL-C+ VLDL-C). HbA1c was calculated by Ion Exchange Resin Method. The atherogenic index of plasma (AIP) was calculated by the formula base 10 logarithm of the ratio of TG to HDL-C.[12] For serum lipid reference level, National Cholesterol Education Programme (NCEP) Adult Treatment Panel III (ATP III) guideline was referred. According to NCEP-ATPIII guidelines, hypercholesterolemia is defined as TC >200 mg/dl, high LDL- C with a value >100 mg/dl, hypertriglyceridemia as TG >150 mg/dl and low HDL-C with a value <40 mg/dl. Dyslipidemia was defined by presence of one or more than one abnormal serum lipid concentration.[13]

STATISTICAL ANALYSIS
Demographic and lipid parameters were presented as Mean ± SD. Categorical variables were expressed in actual numbers and percentage. Demographic and lipid parameters were compared by performing unpaired t-test. Categorical variables were compared by performing chi-square test. P value <0.05 was considered as statistically significant. SPSS version 25.0 was used for data analysis.

RESULTS
Out of the 140 patients studied, there were 60 males and 80 females. The maximum number of patients were in the age group of 50-60 years. Table 1 shows the comparison of studied parameters between the good and poor glycemic control group. The levels of FBG, TC, TG,VLDL-C LDL-C, HbA1c, AIP, ratios of TC/HDL-C and LDL-C/HDL-C are increased, while the levels of HDL-C are decreased in patients with HbA1c >7% as compared to patients with HbA1c ≤ 7% and these values are highly significant. The patients with HbA1c >7% had dyslipidemia as the most prominent feature in the lipid profile. This shows that glycemic control is extremely necessary to curb the future risk of cardiovascular disease, which can be contributed to the atherogenic lipid profile.

Table 1: Demographic and Lipid parameters categorized by patient's glycemic control

Parameters	Glycated haemoglobin		p-value
	≤ 7.0%	>7.0%	
Age in years	50.38± 5.47	58.74± 7.98	<0.0001
FPG	159.88 ±20.34	190.24 ±17.23	<0.0001
2hPG	213.88 ±22.38	250.10± 26.32	0.0002
TC	175.88± 14.97	224.97± 13.58	<0.0001
TG	151.20± 13.34	224.39± 22.31	<0.0001
VLDL-C	32.12± 5.97	44.12± 10.22	<0.0001
HDL-C	55.76± 10.32	48.25± 4.78	<0.0001
LDL-C	88.95± 12.35	130.34± 19.98	<0.0001
AIP	0.45± 0.21	0.66± 0.19	<0.0001

TC/HDL-C	3.47 ±0.63	4.96± 1.58	<0.0001
LDL-C/HDL-C	1.76± 0.53	2.87 ±126	<0.0001

Table 2 shows direct and significant correlation of HbA1c with FBG, 2hPG. It also shows direct and highly significant correlation of HbA1c with TC, TG, LDL-C, TC/HDL-C, LDL-C/HDL-C and AIP, and inverse correlation between HbA1c and HDL.

Table 2: Correlation of HbA1c with FBG, 2hPG and lipid parameters

Parameters	r-value	p-value
FPG	0.51	<0.0001
2hPG	0.43	<0.0001
TC	0.65	<0.0001
TG	0.66	<0.0001
VLDL-C	0.68	<0.0001
HDL-C	-0.34	<0.0001
LDL-C	0.53	<0.0001
AIP	0.75	<0.0001
TC/HDL-C	0.63	<0.0001
LDL/HDL-C	0.54	<0.0001

Table 3: Association of AIP and HbA1c

AIP	≤7	>7	p-value
<0.11 (low risk)	0	2	
0.11 -0.22(Intermediate risk)	4	0	0.033
>0.22 (High risk)	30	110	

Table 3 shows that patients with HbA1c >7% were more prone to cardiovascular risk as their levels of AIP fall in the high risk group i.e AIP > 0.22 and the association was highly significant proving that glycemic control contributes significantly to the future risk of cardiovascular problems. The lipid parameters of patients with HbA1c <7% were in the normal range but the AIP levels still fell in the risk group.

DISCUSSION

Insulin impacts the liver apolipoprotein production which regulates the enzymatic activity of lipoprotein lipase and Cholesterol ester transport protein. These could be the likely causes of dyslipidemia in Diabetes mellitus as reported by Goldberg.[14] Over and above this, insulin deficiency also reduces the activity of hepatic lipase and several other steps in the production of biologically active lipoprotein lipase may also be altered in DM.[15] A number of studies using tracer kinetics in humans have demonstrated that liver production of apolipoprotein B (apoB), the major protein component of VLDL and LDL, is increased in type 2 diabetes. Increased lipolysis in adipocytes due to poor insulinization results in increased fatty acid release from fat cells. The ensuing increase in fatty acid transport to the liver, which is a common abnormality seen in insulin-resistant diabetes, may cause an increase in VLDL secretion.[16]. A second regulatory process may be a direct effect of insulin on liver production of apoB and other proteins involved in degradation of circulating lipoproteins. In some studies insulin directly increased degradation of newly synthesized apoB.[17] Therefore, insulin deficiency or hepatic insulin resistance may increase the secretion of apoB, in turn increasing LDL-C and VLDL-C levels. Our study shows direct and significant correlation of HbA1c with FBG, 2hPG, as also shown by previous studies.[18,19] Khan HA et al. also found similar correlations of HbA1c with TC, TG, LDL-C and HDL-C .[20] We also found a direct and significant correlation of HbA1c with AIP, TC/HDL-C and LDL-C/HDL-C ratio. Thus the severity of dyslipidemia increases in patients with increased HbA1c value. Elevated levels of HbA1c and dyslipidemia are independent risk factors of cardiovascular diseases and hence, diabetic patients with elevated HbA1c and dyslipidemia are considered as high risk group for cardiovascular disease. Improving glycemic control can reduce the risk of cardiovascular events in diabetes. in our study patients with HbA1c >7% were more prone to cardiovascular risk as their levels of AIP fall in the high risk group i.e AIP > 0.22 and the association was highly significant proving that glycemic control contributes significantly to the future risk of cardiovascular problems. Dobiasova et al.[21] was observed the patients with type 2 diabetes with poor glycemic control have the highest AIP. Dobiasova et al. also observed a higher FERHDL compared with nondiabetic individuals.[21] We hypothesize that even if the lipid profile appears to be in normal range, the AIP levels when calculated can be in the risk group emphasising the calculation of this simple ratio every time a lipid profile is asked for. AIP, therefore, reflects the delicate metabolic interactions within the whole lipoprotein complex.[21]

In addition, they are more likely to have a predominance of small, dense LDL particles compared with nondiabetic controls.[22] All of these

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factors suggest that AIP is a suitable marker for plasma atherogenicity and cardiovascular risk in patients with type 2 diabetes. Earlier studies targeted total cholesterol and HDL-C levels to reduce the risk for CVD, but the levels of triglyceride were ignored as a contributor towards this risk. Since Gaziano et al. reported that "the ratio of triglycerides to HDL was a strong predictor of myocardial infarction[23], additional findings have been made regarding relationship between HDL-C and TGs. Tan et al.[12] compared the results of AIP analysis with those of a TG/HDL-C ratio analysis used in another study.[24] The P values for AIP were consistently lower than those for TG/HDLC. Although an independent, inverse relationship between HDL-C and cardiovascular risk has been demonstrated beyond any doubt, the contribution of TGs to cardiovascular risk has been underestimated. This may have been attributable to the high variability of plasma TG concentrations (which decreases the statistical significance of assessments), the lack of information on the role of TGs in biochemical mechanisms, or the incessant efforts to find an atherogenic marker independent of other lipids. In reality, any therapeutic hypolipidemic intervention leads to bigger or smaller changes in the spectrum of plasma lipids and apoproteins, including changes in lipoprotein particle sizes and changes in cholesterol esterification and lipolytic rates.[25] Thus, TGs play the role of a regulator of lipoprotein interactions and not the role of an independent risk marker. This claim is supported by evidence that an increased plasma concentration of TGs is associated with (a) an increased incidence of coronary artery disease, (CAD)[26] (b) an increased population of small, dense LDLs and (c) enhanced cholesteryl ester (CE) mass transfer from HDL to apolipoprotein B (apoB)-containing lipoproteins.[27]

CONCLUSION

The findings of the current research lead to the conclusion that individuals who exhibit inadequate glycemic control possess an atherogenic lipid profile. Furthermore, it was shown that levels of Glycated haemoglobin might serve as a predictor for the presence of dyslipidemia and atherogenicity. The atherogenic index of plasma (AIP) is a distinct statistic that has a strong correlation with cardiovascular risk. The calculation of this metric may be easily performed whenever a lipid profile is requested, enabling the assessment of the patient's cardiovascular risk.

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