



STUDY OF HbA1c AS A BIOMARKER IN DYSLIPIDEMIA AND ATHEROGENICITY IN TYPE 2 DIABETES MELLITUS

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ABSTRACT

HbA1c is being used to assess the glycemic control for many years. This study was done to evaluate the importance of HbA1c in predicting dyslipidemia and atherogenicity in type 2 Diabetes. **Methods:** 200 type 2 diabetic patients were taken as subjects. Fasting and post meal blood sugar, Glycated haemoglobin (HbA1c), lipid profile, lipid ratios and atherogenic index of plasma (AIP) was analysed in these patients. The patients were divided into 2 groups depending on their HbA1c; Good Glycemic Control was defined as having HbA1c \leq 7.0% and Poor Glycemic Control as HbA1c $>$ 7.0%. **Results & Discussion:** We found a significant increase in the levels of blood glucose, total serum cholesterol (TC), triglyceride, LDL cholesterol (LDL-C) and VLDL cholesterol (VLDL-C), TC/HDL-C, LDL-C/HDL-C, atherogenic index of plasma (AIP) and a significant decrease in the levels of HDL cholesterol (HDL-C) in patients with HbA1c $>$ 7% as compared to patients with HbA1c \leq 7%. HbA1c had a direct and significant correlation with TC, TG, VLDL-C, LDL-C, TC /HDL-C, LDL-C/HDL-C, and an inverse correlation with HDL-C. AIP correlates with cardiovascular risk very well, and the association between HbA1c with various lipid parameters and atherogenic ratios suggests the importance of glycemic control in order to control dyslipidemia and future risk of cardiovascular disease in type 2 diabetics.

KEYWORDS: Lipid profile, type 2 diabetes mellitus, Glycosylated haemoglobin, glycemic control, atherogenic index of plasma.

INTRODUCTION

Diabetes is a global endemic with rapidly increasing prevalence in both developed and developing countries and there is a high risk of cardiovascular disease (CVD) in people with type 2 diabetes.^[1] Although there is an increase in the prevalence of type 1 diabetes also, the major driver of the epidemic is the more common form of diabetes, namely type 2 diabetes, which accounts for more than 90 per cent of all diabetes cases. The International Diabetes Federation (IDF) estimates the total number of diabetic subjects to be around 40.9 million in India and this is further set to rise to 69.9 million by the year 2025.^[2] Glycated Hemoglobin (HbA1c) is a routinely used marker for long term glycemic control. HbA1c predicts the risk for development of diabetic complications in diabetic patients. Now-a-days, elevated levels of HbA1c have been regarded as an

independent risk factor for cardiovascular disease in subjects with or without diabetes.^[3] The Diabetes complications and control trial (DCCT)^[4] carried out by National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), USA, established that, HbA1c is the gold standard of glycemic control. The level of HbA1c value \leq 7.0% was said to be appropriate for reducing the risk of cardiovascular complications. HbA1c is directly related to the severity of coronary artery disease (CAD) in diabetic patients. Estimated risk of CAD has shown to be increased by 18% for each 1% increase in absolute HbA1c value in diabetic population.^[5] Dyslipidemia is one of the major established risk factors for cardiovascular disease in diabetes mellitus.^[6] The characteristic features of diabetic dyslipidemia are increased levels of

triglyceride(TG) concentration and low density lipoprotein cholesterol (LDL-C) particles decreased high density lipoprotein cholesterol (HDL-C). Although the major focus is on the connection between lipids and CVD is on LDL-C, the National Cholesterol Education Program under Adult Treatment Panel III has recognized the important roles of HDL-C and TGs, calling this combination an atherogenic dyslipidemia.^[7]

Individuals with coexisting diabetes and metabolic syndrome have the highest prevalence of CVD. Early therapeutic interventions, aiming to reduce triglycerides and LDL and to increase HDL-C, significantly reduce mortality in type 2 diabetic patients.^[8,9]

Several lipoprotein-related indices [plasma concentrations of lipids (LDL-C, HDL-C, and TGs), molar ratios (TG/HDL-C and LDL-C/HDL-C), and particle size (LDL-C and HDL-C) have been used to predict CVD risk. The total cholesterol/HDL-C and LDL-C/HDL-C molar ratios have good predictive value for future cardiovascular events.

Recently, another molar ratio, log TG/HDL-C popularly known as Atherogenic Index of Plasma (AIP), has been used as a significant and independent predictor of CHD.^[10] AIP has been proposed as a predictive marker for plasma atherogenicity and is positively correlated with cardiovascular disease risk. AIP's significance as a marker is based on the following facts:

1. It is found increased in cohorts at high risk for CAD;
2. It is positively correlated with the fractional esterification rate of HDL-C (FERHDL), which is perhaps the most dependable marker for the atherogenic capacity of the lipid-lipoprotein profile; and
3. It is inversely correlated to LDL-C particle size^[11]

Tan et al.^[10] compared the results of AIP analysis with those of a TG/HDL-C ratio analysis used in another study.^[12] The P values for AIP were consistently lower than those for TG/HDL-C. An AIP of < 0.11 is considered as low risk, 0.11- 0.21 intermediate risk and >0.21 as high risk^[13,14].

With this preview, this study was undertaken with the aim of evaluating the importance of HbA1c in predicting the diabetic dyslipidemia and atherogenicity.

MATERIALS AND METHODS

This study was done at Indira Gandhi Govt. Medical College & Hospital, Nagpur between January 2014 and September 2014 with 200 diagnosed cases of DM type 2 in the age group of 25-75 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 clearance was taken from the institutional ethical committee. Informed and written consent was taken from the patients, with the explanation of the procedure of 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).^[15] Venous blood samples from all the subjects were collected after at least 8 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^[16] & Very low density lipoprotein cholesterol(VLDL-C) & LDL-C analysis was done by applying Friedwald's formula^[17] 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, (Accurex Manufacturers). The atherogenic index of plasma (AIP) was calculated by the formula base 10 logarithm of the ratio of TG to HDL-C.^[10]

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.^[18]

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. Statistical software STATA version 12.0 was used for data analysis.

RESULTS

Out of the 200 patients studied, there were 74 males and 126 females. The maximum number of patients were in the age group of 51-60 years.

Table 1 and Figure 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 patients glycemic control

Parameters	Glycated haemoglobin		p-value
	≤ 7.0%	>7.0%	
Age in years	51.18± 9.32	57.35± 8.40	<0.0001, HS
FPG	160.38 ±39.51	189.95 ±46.02	<0.0001, HS
2hPG	215.58 ±63.55	250.21± 55.94	0.0002
TC	177.76± 25.10	226.82± 42.71	<0.0001, HS
TG	148.05± 32.96	224.49± 53.29	<0.0001, HS
VLDL-C	31.86± 6.59	44.96± 10.76	<0.0001, HS
HDL-C	56.03± 11.98	48.32± 8.78	<0.0001, HS
LDL-C	89.88± 16.78	131.78± 40.11	<0.0001, HS
AIP	0.46± 0.11	0.66± 0.15	<0.0001, HS
TC/HDL-C	3.26 ±0.59	4.87± 1.40	<0.0001, HS
LDL-C/HDL-C	1.68± 0.49	2.85 ±1.14	<0.0001, HS

P <0.001- HS (highly significant)

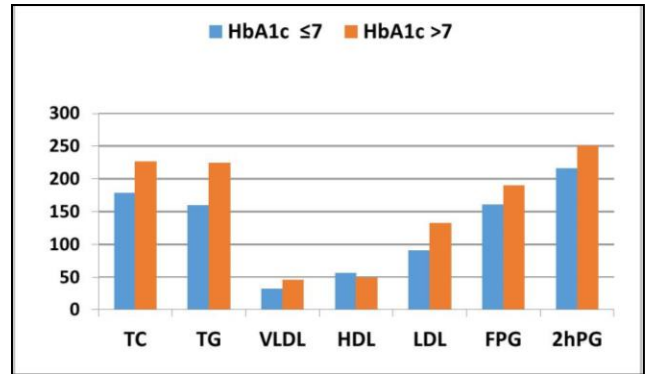


Figure 1. Comparison of lipid and glucose profile in good (HbA1c ≤ 7) & poor (HbA1c > 7) glycemic control group.

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^[19]. 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.^[20] 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.^[21] 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.^[22] Therefore, insulin deficiency or hepatic insulin resistance may increase the secretion of apoB, in turn increasing LDL-C and VLDL-C levels.

Table 2 shows direct and significant correlation of HbA1c with FBG, 2hPG, as also shown by previous studies.^[23,24] It also shows direct and highly significant correlation of HbA1c with TC, TG (figure2), LDL-C (figure 3), TC/HDL-C, LDL-C/HDL-C and AIP (figure 4), while Figure 5 shows inverse correlation between HbA1c and HDL. Khan HA et al. also found similar correlations of HbA1c with TC, TG, LDL-C and HDL-C^[25]. 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.

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

Parameters	r-value	p-value
FPG	0.4936	<0.0001, HS
2hPG	0.4084	<0.0001, HS
TC	0.6607	<0.0001, HS
TG	0.7273	<0.0001, HS
VLDL-C	0.7219	<0.0001, HS
HDL-C	-0.3941	<0.0001, HS
LDL-C	0.5842	<0.0001, HS
AIP	0.7147	<0.0001, HS
TC/HDL-C	0.6854	<0.0001, HS
LDL/HDL-C	0.6040	<0.0001,HS

P < 0.001- HS (highly significant)

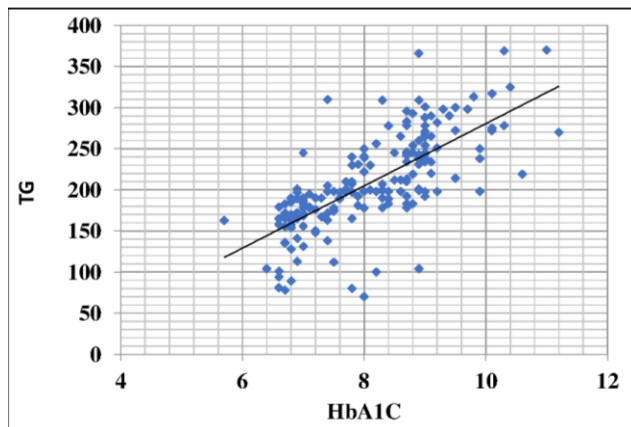


Figure 2. Direct correlation between HbA1c & TG levels, r=0.73

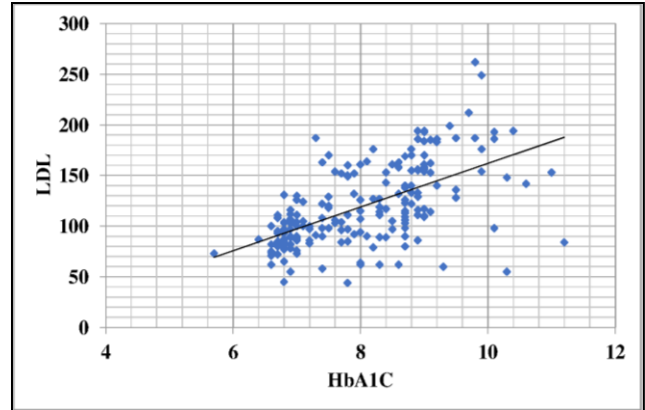


Figure 3. Direct correlation between HbA1c & LDL-C levels, r= 0.58

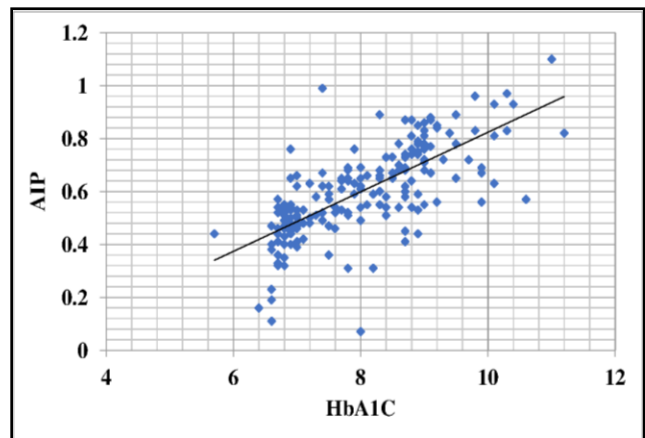


Figure 4. Direct correlation between HbA1c & AIP levels, r= 0.71

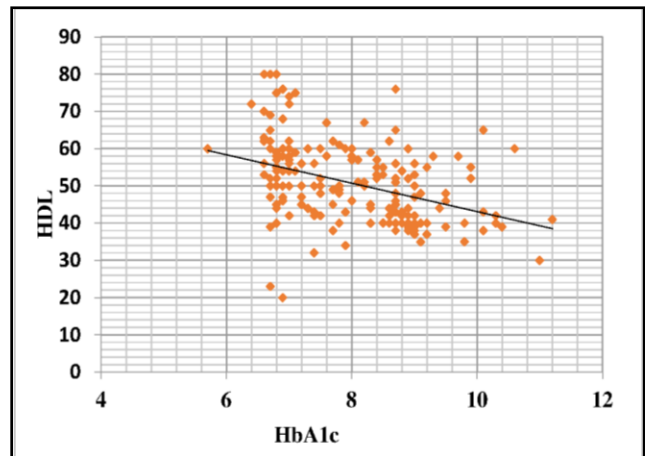


Figure 5. Inverse correlation between HbA1c & HDL-C levels, r = - 0.39

Table 3. Association of AIP and HbA1c

AIP	≤7	>7	p-value
<0.11 (low risk)	0	1	0.026, S
0.11 -0.22(Intermediate risk)	3	0	
>0.22 (High risk)	57	139	

p< 0.05 - S (significant)

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. We also found that the lipid parameters of patients with HbA1c <7% were in the normal range but the AIP levels still fell in the risk group, Table 1. 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.^[13]

DISCUSSION

In accordance with Dobiasova et al^[13] it is observed from table 3, 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.^[26] In addition, they are more likely to have a predominance of small, dense LDL particles compared with nondiabetic controls.^[27] All of these 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^[28], additional findings have been made regarding relationship between HDL-C and TGs. Tan et al.^[10] compared the results of AIP analysis with those of a TG/HDL-C ratio analysis used in another study.^[12] The P

values for AIP were consistently lower than those for TG/HDL-C.

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.^[29] 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)^[30] (b) an increased population of small, dense LDLs and (c) enhanced cholesteryl ester (CE) mass transfer from HDL to apolipoprotein B (apoB)-containing lipoproteins.^[31]

CONCLUSION

It can be concluded from the present study that patients with poor glycemic control have a atherogenic lipid profile and Glycated haemoglobin predicts dyslipidemia and atherogenicity. Atherogenic index of plasma, is an independent parameter which correlates very significantly with the cardiovascular risk. This parameter is easy to calculate every time a lipid profile is asked for, so that the cardiovascular risk of the patient can be assessed.

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