HbA₁C AS A PREDICTOR OF LIPID PROFILE IN TYPE 2 DIABETIC PATIENTSJosephine Latha Pushparaj¹, S. Selvapandian Kirubakaran²**HOW TO CITE THIS ARTICLE:**

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ABSTRACT: BACKGROUND: Diabetes is a chronic metabolic disorder and a well-known risk factor for atherosclerotic diseases. Atherosclerosis is mainly due to alterations in lipoprotein profile. HbA₁C is a marker of long term exposure to chronic hyperglycemia. HbA₁C and dyslipidemia are independent risk factors for atherosclerotic diseases. **AIM:** To evaluate the correlation between HbA₁C and dyslipidemia in type 2 diabetic patients and to find out whether HbA₁C can predict dyslipidemia. **MATERIALS AND METHODS:** We conducted a cross-sectional study of 103 type 2 diabetic patients attending diabetic outpatient clinic at Mahatma Gandhi Memorial Govt. Hospital, Tiruchirappalli. Venous blood samples were collected from all the subjects after at least 8-10 hours of fasting. Fasting and post-prandial Blood Glucose, Lipid Profile and HbA₁c were estimated by standard methods. NCEP-ATP III guideline was referred to define dyslipidemia. The data were analyzed using SPSS version 21 software. **RESULTS:** In our study, the mean values of HbA₁C, TGL, VLDL and TGL/HDL were found to be significantly higher ($p < 0.05$) in women. 98% of the subjects under study were dyslipidemic. 45% of the subjects had four abnormal lipid parameters, 29%-three, 7%-two and 14%-one abnormal parameter. The prevalence of inadequate glycemic control ($\text{HbA}_{1\text{C}} \geq 7$) in the study population was 66%. The mean values of all the lipid parameters and atherogenic risk ratios were found to be higher in the $\text{HbA}_{1\text{C}} \geq 7$ group and were statistically highly significant. Pearson's correlation test, showed that HbA₁C was positively correlated with TC($p < 0.05$), TGL($p < 0.001$), LDL($p < 0.05$), VLDL($p < 0.001$), TC/HDL($p < 0.001$), TGL/HDL($p < 0.001$), LDL/HDL($p < 0.001$) and negatively correlated with HDL($p < 0.001$). By linear regression analysis, it was also found that HbA₁C could predict hypercholesterolemia ($p=0.024$; $R^2 = 0.049$), hypertriglyceridemia ($p=0.000$; $R^2 = 0.254$), high LDL ($p=0.045$; $R^2 = 0.039$), high VLDL ($p=0.000$; $R^2 = 0.116$), low HDL ($p=0.000$; $R^2 = 0.316$) and the atherogenic ratios TC/HDL ($p=0.000$; $R^2 = 0.196$), TGL/HDL ($p=0.000$; $R^2 = 0.399$) and LDL/HDL ($p=0.000$; $R^2 = 0.141$). **CONCLUSION:** We therefore conclude that HbA₁C is not only a marker of chronic exposure of hyperglycemia but can also predict dyslipidemia. Lifestyle modifications and earlier intervention by lipid lowering therapy can reduce the cardiovascular mortality of this risk group. However for intervention by lipid lowering therapy, more prospective studies with large sample sizes are essential.

KEYWORDS: Atherosclerosis, diabetic complications, dyslipidemia, HbA₁C, Glycated hemoglobin.

INTRODUCTION: Diabetes is a metabolic disorder resulting either from insulin deficiency or insulin resistance. According to the International Diabetes Federation (IDF), 382 million people have diabetes in 2013; by 2035 this will rise to 592 million. 80% of people with diabetes live in low- and middle-income countries.¹ The Indian Council of Medical Research-Indian Diabetes study (ICMR-INDIAB), a national diabetes study, has projected that India currently has 62.4 million people with diabetes and 77.2 million people with prediabetes.² IDF data reveal that the prevalence of diabetes in

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India exceeds that of the United States and is ranked second in the world in diabetes prevalence, just behind China.

Many epidemiological studies have established that type 2 diabetes mellitus (DM) is a well-known risk factor for the atherosclerotic cardiovascular, cerebrovascular, and peripheral vascular diseases. The pathogenesis of atherosclerosis in type 2 diabetes is mainly because of alterations in lipid and lipoprotein profile.³ Diabetic dyslipidemia is generally characterized by increased plasma triglyceride (TG) and decreased high-density lipoprotein cholesterol (HDL-C) concentrations, a predominant small, dense low-density lipoprotein (LDL), and an increased apolipoprotein B concentration. The Adult Treatment Panel III has recognized the important roles of HDL-C and TGs, calling this combination an atherogenic dyslipidemia.⁴

HbA_{1c} (Glycated hemoglobin) is a marker for long term glycemic control. The chronic exposure to increased glycemic level was associated with increased risks of all-cause mortality and cardiovascular outcomes in type 2 diabetes, according to the meta-analysis of 26 prospective studies⁵. In a meta-analysis, Selvin et al. ⁶ evaluated 10 prospective studies and concluded that every 1% increase in glycated hemoglobin (HbA_{1c}) was associated with an 18% increase in hazard of CVD, 13% in CHD, 16% in fatal CHD, and 17% in stroke incidence after controlling potential confounders.

Elevated levels of HbA_{1c} are associated with increased risks for cardiovascular outcomes among patients with type 2 diabetes and independent from other conventional risk factors. From all these studies we understand that diabetic patients with higher HbA_{1c} levels and dyslipidemia should be closely followed due to their higher risks of cardiovascular diseases and all-cause mortality. With this background we wanted to evaluate the correlation between HbA_{1c} and dyslipidemia in type 2 diabetic patients.

MATERIALS AND METHODS: We conducted a cross-sectional study of 103 type 2 diabetic patients attending diabetic outpatient clinic at Mahatma Gandhi Memorial Govt. Hospital, Tiruchirappalli. Informed consent was obtained from all the subjects. Patients with microvascular and macrovascular complications were excluded from the study.

Venous blood samples were collected from all the subjects after at least 8-10 hours of fasting. Fasting Blood Glucose (FBG) and post-prandial Blood Glucose (PPBS) were estimated by Glucose oxidase-Peroxidase method. Serum Total cholesterol (TC) was estimated by cholesterol esterase method and High Density Lipoprotein (HDL) by cholesterol esterase method after precipitation using phosphotungstate method. Lipase method was used to estimate Triglycerides (TGL). All these estimations were done in an auto-analyzer.

Indirect Low Density Lipoprotein (LDL), was calculated by Fried Wald's formula [$LDL-C = TC - (HDL-C + triglyceride/5)$], where the triglyceride level was less than 400 mg/dL. The risk ratios (TC/HDL-C, TGL/HDL-C and LDL/HDL-C) were calculated from the estimated lipid profile values. HbA_{1c} was estimated by using Ion exchange chromatography (Crest A Coral clinical system, USA). National Cholesterol Education Programme (NCEP) Adult Treatment Panel III (ATP III) guideline was referred to define dyslipidemia.

According to NCEP-ATP III guideline, hypercholesterolemia is defined as TC > 200 mg/dl, high LDL when value > 100 mg/dl, hypertriglyceridemia as TG > 150 mg/dl and low HDL when value < 40 mg/dl in men and 50 mg/dl in case of women. Dyslipidemia was defined by presence of one or more than one abnormal serum lipid concentration.⁷

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STATISTICAL ANALYSIS: The resulting data were analyzed using SPSS software version 21. The means of different parameters were compared by the Independent samples test (2 tailed). The correlations were examined using Pearson's correlation test. The predictability of HbA1C was tested by linear regression analysis.

RESULTS: We studied 103 diabetic subjects among which 51 were male and 52 were female. The mean values of HbA₁C, TGL, VLDL and TGL/HDL ratio were found to be significantly higher ($p < 0.05$) in women in our study which is shown in Figure-1.

Fig. 1: Mean values of HbA₁C, TGL, VLDL and TGL/HDL in male and female

98% of the subjects under study were dyslipidemic according to NCEP-ATP III guidelines. The results of means of all the biochemical parameters are given in Table-1 as their mean $\pm$ SD (standard deviation). 47(45%) of the study population were found to have four abnormal lipid parameters, 30(29%) of them 3 abnormal parameters, 8(7%) have two abnormal parameters and 15(14%) one abnormal parameter. Only 3 people (2%) have normal lipid parameters.

	Minimum	Maximum	Mean $\pm$ SD (n = 103)
FBS (mg/dl)	72.00	294.00	148.88 $\pm$ 41.09
PPBS(mg/dl)	162.00	392.00	244.16 $\pm$ 52.51
TC(mg/dl)	127.00	363.00	213.16 $\pm$ 46.99
TGL(mg/dl)	74.00	360.00	183.79 $\pm$ 40.79
HDL(mg/dl)	26.00	56.00	35.36 $\pm$ 6.03
LDL(mg/dl)	63.20	285.80	138.40 $\pm$ 43.27
VLDL(mg/dl)	14.80	72.00	37.71 $\pm$ 7.86
HbA1C (%)	5.10	13.30	7.49 $\pm$ 1.39
TC/ HDL ratio	3.14	12.96	6.21 $\pm$ 1.80
TGL/ HDL ratio	2.18	11.61	5.39 $\pm$ 1.62
LDL/ HDL ratio	1.51	10.21	4.04 $\pm$ 1.53

Table 1: Means of the Biochemical parameters of the diabetic patients

(FBS-fasting blood sugar, PPBS-postprandial blood sugar, TC-total cholesterol, TGL-triglycerides, LDL-low density lipoprotein, VLDL-very low density lipoprotein, HDL-high density lipoprotein)

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Further the subjects were grouped under two categories according to their HbA_{1c} level: HbA_{1c} $\geq$ 7 % (inadequate glycemic control) and HbA_{1c} $<$ 7 % (adequate glycemic control). The results are shown in table-2 as their mean $\pm$ standard deviation. The prevalence of inadequate glycemic control in the study population was 66%. The mean values of all the lipid parameters and atherogenic risk ratios were found to be higher in the HbA_{1c} $\geq$ 7% group and were statistically highly significant.

The difference in fasting and postprandial blood sugar levels was not statistically significant between the groups.

Variable	HbA _{1c} $\geq$ 7% (n=68) (Mean $\pm$ SD)	HbA _{1c} $<$ 7 % (n=35) (Mean $\pm$ SD)	p value Sig 2 tailed	95% Confidence Interval of the Difference	
				Lower	Upper
FBS(mg/dl)	150.49 $\pm$ 37.74	145.77 $\pm$ 47.36	0.584	-12.301	21.728
PPBS(mg/dl)	244.84 $\pm$ 53.55	242.83 $\pm$ 51.18	0.855	-19.765	23.784
TC(mg/dl)	221.78 $\pm$ 49.44	196.40 $\pm$ 37.03	0.009*	6.545	44.214
TGL(mg/dl)	197.25 $\pm$ 39.03	157.63 $\pm$ 30.39	0.000**	24.619	54.624
HDL(mg/dl)	32.87 $\pm$ 2.98	40.20 $\pm$ 7.42	0.000**	-9.371	-5.293
LDL(mg/dl)	145.30 $\pm$ 45.80	124.98 $\pm$ 34.69	0.023*	2.825	37.810
VLDL(mg/dl)	39.16 $\pm$ 7.81	34.90 $\pm$ 7.27	0.009*	1.103	7.403
TC/HDL ratio	6.79 $\pm$ 1.65	5.09 $\pm$ 1.56	0.000**	1.036	2.370
TGL/HDL ratio	6.05 $\pm$ 1.37	4.10 $\pm$ 1.27	0.000**	1.407	2.509
LDL/HDL ratio	4.45 $\pm$ 1.51	3.24 $\pm$ 1.23	0.000**	0.630	1.801

Table 2: Biochemical parameters categorized by patient's HbA_{1c}

*statistically significant at $<$ 0.05 level, **statistically significant at $<$ 0.001 level

From Pearson's correlation test, it was found that HbA_{1c} was positively correlated with TC ($r = 0.222$, $p < 0.05$), TGL ($r = 0.504$, $p < 0.001$), LDL ($r = 0.198$, $p < 0.05$), VLDL ($r = 0.340$, $p < 0.001$), and negatively correlated with HDL ($r = -0.562$, $p < 0.001$). HbA_{1c} was also positively correlated with the risk ratios: TC/HDL ($r = 0.443$, $p < 0.001$), TGL/HDL ($r = 0.632$, $p < 0.001$), LDL/HDL ($r = 0.375$, $p < 0.001$). All the correlations were statistically significant and the correlations are shown in figures 2(a-h). By linear regression analysis, it was also found that HbA_{1c} can predict hypercholesterolemia ($p=0.024$; $R^2 = 0.049$), hypertriglyceridemia ($p=0.000$; $R^2 = 0.254$), high LDL ($p=0.045$; $R^2 = 0.039$), high VLDL ($p=0.000$; $R^2 = 0.116$), low HDL ($p=0.000$; $R^2 = 0.316$) and the atherogenic ratios TC/HDL ($p=0.000$; $R^2 = 0.196$), TGL/HDL ($p=0.000$; $R^2 = 0.399$) and LDL/HDL ($p=0.000$; $R^2 = 0.141$).

Figure 2: Correlation of HbA1C with lipid parameters

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DISCUSSION: The present study was done so as to evaluate the correlation of glycated hemoglobin with the lipid profile parameters and to find out whether HbA_{1c} can predict dyslipidemia. The results of our study clearly showed that the lipid parameters TGL, VLDL and TGL/HDL ratio were higher in women. Also in glycemic parameters only HbA_{1c} was higher in women compared to men and not FBS and PPBS. Similar results of high HbA_{1c} and TGL in type 2 diabetic women compared to men were found in some previous studies.⁸⁻¹⁰ This might be due to the alteration in the protective effect of estrogen in diabetes¹¹. Moreover the protective effect of estrogen on body fat distribution and insulin action may be decreased by the interaction of inflammatory factors with female sex hormones and low grade inflammation may have a greater role in altering insulin action in women.¹²

Diabetes Mellitus leads to various patterns of lipid abnormalities, the most common being hypertriglyceridemia and decreased HDL level. 98% of the diabetic patients in the study were found to be dyslipidemic. 45% of the subjects showed all the four lipid parameters abnormal, (i.e.) hypercholesterolemia, hypertriglyceridemia, high LDL levels and low HDL levels. Only 2% had normal lipid parameters. Similar results of high prevalence of hypercholesterolemia, hypertriglyceridemia, high LDL-C and low HDL-C levels which are well known risk factors for cardiovascular diseases were shown in the study of Sehran et al in Pakistan.¹³ Increased prevalence of hyper triglyceridemia and low HDL cholesterol levels were reported in the Framingham Heart Study¹⁴ and the UK Prospective Diabetes Study (UKPDS).¹⁵ The abnormalities of lipoproteins in diabetic patients are being continuously proved by many studies done in various regions.¹⁶⁻¹⁸

Insulin deficiency results in increased lipolysis in adipocytes releasing increased amount of free fatty acids. Poor insulinization also leads to decrease in the enzymatic activity of lipoprotein lipase (LpL) and hepatic lipase resulting in elevated VLDL and remnant lipoproteins. Cholesteryl Ester Transfer Protein (CETP) leads to the exchange of triglycerides from increased amount of VLDL to Cholesteryl Esters in HDL and LDL resulting in reduced level of HDL and increased amount of small dense LDL particles.¹⁹

Further we analyzed the parameters by grouping the subjects under two categories according to their HbA_{1c} level, HbA_{1c} $\geq 7\%$ (inadequate glycemic control) and HbA_{1c} $< 7\%$ (adequate glycemic control). 66% of the patients had inadequate glycemic control. The association of HbA_{1c} levels and diabetic complications was established by The Diabetes complications and control trial (DCCT) published in 1993²⁰ in type 1 diabetes, and the U.K. Prospective Diabetes Study, published in 1998 in type 2 diabetes²¹. In 2009, HbA_{1c} test with a threshold of 6.5% was recommended to diagnose diabetes, by an International Expert Committee that included representatives of the ADA, the International Diabetes Federation (IDF), and the European Association for the Study of Diabetes (EASD)²², and the same criteria was adopted by ADA since 2010²³. The level of HbA_{1c} value $\leq 7.0\%$ was said to be appropriate for reducing the risk of micro vascular complications.²⁴

The diabetic patients with HbA_{1c} value $> 7.0\%$ exhibited a significant increase in total cholesterol, triglycerides, LDL, VLDL and significantly lower levels of HDL in the present study. The atherogenic ratios TC/HDL, LDL/HDL and TGL/HDL also showed significant increase in patients with HbA_{1c} value $> 7.0\%$. Further HbA_{1c} was found to be positively correlated with total cholesterol, LDL cholesterol, triglycerides, the atherogenic ratios and negatively correlated with HDL, which is in accordance with many previous studies.²⁵⁻²⁹

In the study done by Khan HA et al²⁶ to show the effect of glycemic control on various lipid parameters, the diabetic patients were divided into 3 groups according to their HbA_{1c} levels: group 1,

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HbA_{1c}<6%; group 2, HbA_{1c} >6%–9% and group 3, HbA_{1c}>9%. Alterations in the lipid parameters were statistically significant in the three different groups except for LDL-C. Dyslipidemia was found to be severe in patients with elevated HbA_{1c}. Zhe Yan et al ²⁸ in their study found that TC/HDL-C, LDL-C/HDL-C ratios were gradually increased, with increased HbA_{1c} level and the difference was significant among groups (P<0.05). Our study also showed that HbA_{1c} can predict lipid parameters and the atherogenic ratios from linear regression analysis.

CONCLUSION: Atherogenic dyslipidemia is the most important feature exhibited by Diabetic patients which increases their cardiovascular risk when compared to non-diabetics. Diabetes has now become a global endemic in both developing and developed countries. Hence it is the need of the hour for early detection and prevention of this non-communicable disease.

We conclude from our study that HbA_{1c} is not only a marker of long term glycemic control but can also predict lipid parameters and the atherogenic ratios so that lifestyle modifications and earlier intervention by lipid lowering drug therapy can reduce the cardiovascular mortality of this risk group. However for intervention by lipid lowering therapy, more prospective studies with large sample sizes are essential.

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