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Glycated HbA1c: A Potential Biomarker for Diagnosis of Type 2 Diabetes Mellitus and its Correlation with Dyslipidemia.

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ABSTRACT

Glycated Haemoglobin (HbA1c) is currently one of the best, widely used and accepted test for monitoring the glycaemic control in diabetic patients for about 3 decades i.e. it estimates average blood glucose levels of previous 8-12 weeks. Despite the progress made in the development of anti-diabetic agents, the ability to maintain tight glycaemic control still remains a challenge. The main objective of the present study was to evaluate the diagnostic value of HbA1c in predicting dyslipidemia in type 2- diabetic patients. Fifty type-2 diabetic patients were included in the present study. Estimation of HbA1c, fasting blood glucose and lipid profile was done. Further diabetic patients were classified in 2 groups based on their glycaemic index: group 1 consisted of patients with HbA1c $\geq 7.0\%$ and group 2 consisted of patients with HbA1c $\leq 7.0\%$. A positive correlation was found between HbA1c and lipid parameters but a negative correlation was found between HbA1c and HDL. The patients with HbA1c $\geq 7.0\%$ (diabetic dyslipidemia) had significantly increased level of triglycerides (TG), total cholesterol, increased fasting blood glucose but significantly decreased HDL-cholesterol as compared to the patients with good glycaemic control (diabetic group1). The mean HbA1c value in diabetic dyslipidemia was 9.54 ± 1.85 which was extremely higher than diabetic group having mean HbA1c 5.49 ± 0.68 . The mean triglycerides of diabetic dyslipidemia patients was 191.41 ± 72.73 and group 2 had mean TG of 123.86 ± 35.08 . The mean total cholesterol of group 1 was 201.88 ± 48.25 which was again higher than group 2 with a mean total cholesterol of 169.5 ± 37.62 . But the mean value of HDL-cholesterol in diabetic dyslipidemic group 1 was lower i.e. 38.65 ± 7.05 than group 2 diabetic patients with a mean HbA1c of 44.53 ± 4.22 . Hence in diabetic dyslipidemia HDL showed a negative correlation with HbA1c. The mean FBG in group 1 diabetic dyslipidemic was higher i.e. 200.25 ± 72.34 and group 2 diabetic patients had mean FBG of 114 ± 18.53 . The findings suggest that type 2- diabetic patients with dyslipidemia are at increased risk of cardiovascular diseases. The association between HbA1c with various lipid parameters suggested the importance of glycaemic control in order to prevent the future risk of dyslipidemia in type-2 diabetic patients. Thus in a nut shell, the diabetes associated dyslipidemia lead to conclusion that good glycaemic control would be useful to prevent the possibilities of the development of diabetic dyslipidemia and various other complications related to diabetes.

Keywords: HbA1c, biomarker, diabetes, dyslipidemia

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INTRODUCTION

Diagnosis of diabetes has been dependent upon the various glucose tests over a number of years. A World Health Organization (WHO) consultation has concluded HbA1c as the most widely used clinical test that can be used to diagnose diabetes mellitus [1]. HbA1c also serves as a marker that measures the average blood glucose levels over the period of 8-12 weeks [2]. American Diabetes Association (ADA) has recently recommended HbA1c as a diagnostic test for DM after achieving HbA1c goal $\leq 7\%$ and this has revolutionized the significance or importance of this test as a diagnostic test for assessing the adequacy of glycemic control [3, 4]. A number of attempts have been made in order to reduce diabetic dyslipidemia by making improvements in HbA1c test results. The Diabetes Control and Complications Trial (DCCT) carried out by National Institute of Diabetes Digestive and kidney diseases in USA have established HbA1c as a gold standard method of glycemic control [5]. HbA1c levels $<7.0\%$ was said appropriate in order to reduce the risks of cardiovascular complications [6]. HbA1c do have a positive correlation with Triglycerides (TG's), Low density Lipoproteins (LDL), Total Cholesterol and a negative correlation with high density lipoproteins (HDL)[7]. Poorly controlled diabetic patients with HbA1c levels $>7\%$ have higher values of Total cholesterol, LDL cholesterol, Total cholesterol/HDL cholesterol ratios as compared to the good glycemic controlled diabetic patients having HbA1c values $<7\%$. Thus HbA1c is used as a dual potential biomarker of glycemic control and of circulating lipids in type 2 DM patients [8].

MATERIAL AND METHODS

The study was conducted in the department of biochemistry of Govt. Super Speciality Hospital , Jammu, over a period of 3 months from January 23 to April 24,2015. A total of 50 subjects participated in the study (23 male and 27 female). Participants with a minimum ≤ 5 year history of diabetes and maximum ≥ 5 year history of diabetes were included in the study. The study was completed covering. Patients were selected from indoor and outdoor patient department. The age group selected was from 14 – 75 years. The mean age of males 49.79 ± 15.25 and mean age of females was 48.70 ± 10.0 . Majority of the patients were stabilized on drugs and few were insulin dependent. All the three parameters i.e. HbA1c, blood glucose and lipid profile estimation was performed on Architect c System and AEROSET System by using special kits supplied by Abbot Laboratories.

- HbA1c levels were determined by using (REF 2k96-20) MULTIGENT HbA1c which is a 4 reagent kit supplied by Abbot Laboratories, Inc. Abbot. 1L 60064 USA Abbot.
 - Blood glucose in the venous blood was determined by using (REF 3L82-21) R1 which is a single glucose reagent kit, ready-to-use supplied by Abbot Laboratories, Inc. Abbot. 1L 60064 USA Abbot.
 - Dyslipidemia- The assessment of dyslipidemia was based on estimation of lipid parameters in the fasting blood samples viz.; serum triglyceride, total cholesterol. HDL- cholesterol. All these parameters were done on Architect c System and AEROSET System by using special kits supplied by Abbot Laboratories.
1. Serum Triglycerides was estimated by (REF 7D74 -21) R1 Triglyceride which is supplied as a liquid, ready-to-use, single reagent kit, supplied by Abbot Laboratories, Inc. Abbot. 1L 60064 USA Abbot.
 2. Serum Total cholesterol was estimated by (REF 7D62) R1 cholesterol, which is supplied as a liquid, ready-to-use, single reagent kit, supplied by Abbot Laboratories, Inc. Abbot. 1L 60064 USA Abbot.
 3. Serum HDL- cholesterol was estimated by using (REF 7D64) R1 HDL-cholesterol, which is supplied as a liquid, ready-to-use, single reagent kit, supplied by Abbot Laboratories, Inc. Abbot. 1L 60064 USA Abbot.

Selection of patients:

Type- 2 Diabetic Patients were selected on the following criteria

- Duration of diabetes. Diabetic Patients having either maximum ≥ 5 history of diabetes or minimum ≤ 5 year history of diabetes.
- Drug dependent or insulin dependent
- Smoker or non smoker
- Alcoholic or non alcoholic

- Vegetarian or non vegetarian

Specimen collection and preparation for analysis:

Blood Collection- Fasting blood samples were collected from patients in the indoor and outdoor patient department of the concerned hospital from 10.00 am to 1.00 pm. Approx.5ml blood was taken from the patients for estimation of three biochemical parameters: HbA1c, blood glucose and Lipid profile. Therefore separate test tubes were required for each test at the time of collection. One for HbA1c test and another for Lipid and blood glucose estimation.

Serum separation- Serum was separated from blood by centrifugation and was later used for analysis of blood glucose and lipid profile estimation. Whole blood sample was used for estimation of HbA1c.

METHODOLOGY

Name: The MULTIGEN HbA1c Immunoassay

The MULTIGENT HbA1c assay was used for the quantitative in vitro measurement of percent HbA1c in human whole blood on the ARCHITECT c System and on the AEROSTAT SYSTEM. The assay measures the concentration of the HbA1c relative to the concentration of the total Haemoglobin.

PRINCIPLES OF PROCEDURE:

The assay consisted of two separate concentration measurements. The glycated haemoglobin (HbA1c) and the total Haemoglobin (THb) . The two concentrations were used to determine the percent HbA1c or haemoglobin fraction. The individual concentration values of HbA1c and THb generated by the assay were used only for calculating the percent HbA1c or (haemoglobin fraction) and must not be used individually for diagnostic purposes. The whole blood specimens were first pre-treated with the MULTIGENT Haemoglobin Denaturant. The erythrocytes were lysed and the haemoglobin was degraded by the proteolytic enzyme pepsin in order to form hemolysate. Both the total Hb and HbA1c concentrations were determined from the same hemolysate.

RESULTS

Fifty individuals participated in the study. Out of which 27 were females and 23 were males. Participant's age ranges from 18-75 years. In the first part of the present study based on various HbA1c cutoff values among 50 subjects, 35 were Diabetic dyslipidemic, 5 were diabetic, 5 were Pre- diabetic and 5 were normal. In second part of the present study all 50 subjects were categorized into 2 groups based on their HbA1c cutoff value $\geq 7.0\%$ and $\leq 7.0\%$. Group 1 consisted of patients with HbA1c $\geq 7.0\%$ had extremely poor control on diabetes, while on the other hand patients who had HbA1c $\leq 7.0\%$ were kept under group 2. Results of this study depicted that HbA1c had a positive correlation with lipid parameters and FBG but a negative correlation was found between HbA1c and HDL. Patients having poor control on diabetes were found to have decreased HDL cholesterol and those having good glycemic control had high HDL levels. Among 35 diabetic dyslipidemic individuals, 14 were males and 21 were females. The mean age \pm SD of total male and female subjects was calculated. The mean value of HbA1c in females was slightly higher than males. Similarly the mean level FBG in females was higher than males. Among circulating lipids Total Cholesterol, Triglycerides were found higher in females than in males. The mean value of TC in females was higher than males. The mean TG in overall female population was found to be extremely higher than males. But the mean values of HDL in females were slightly higher than males

(Table no.1)

The patients with high HbA1c $\geq 7.0\%$ values had high FBG,TC, TG from rest of the groups but extremely low HDL cholesterol as compared to diabetic with a high mean value of HDL, pre-diabetic had higher mean value of HDL than diabetics and normal group had the extremely high mean values of HDL cholesterol (Table no.2). With higher HbA1c values, the severity of dyslipidemia increases in patients. Diabetic patients with elevated HbA1c and dyslipidemia are considered as a very high risk for cardiovascular diseases.

Table 1: Evaluation and Comparison of Lipid profile parameters and HbA1c results of Total Males and Females subjects. (n=50)

Total no. of Patients (n=50)	Males (n=23)	Females (n=27)
	Mean ±SD	Mean ± SD
Age (yrs)	49.79±15.25	48.70±10.0
HbA1c (%)	8.21 ± 2.12	8.67±2.46
T.Cholesterol (mg/dl)	188.17± 43.11	195.5±47.38
T.G (mg/dl)	156.43± 62.36	184.34±76.09
HDL(mg/dl)	40.08±2.96	40.70±8.99
FBG (mg/dl)	166.73±72.4	180.88±74.11

Table 2: Evaluation and Comparison of lipid Parameters based on various HbA1c cutoff values in Dyslipidemic, Diabetic, Pre- Diabetic and in Normal Subjects.

Total no. of Patients (n=50)	n=35	n=5	n=5	n=5
	HbA1c≥7.0(%)	HbA1c ≥6.5 (%)	HbA1c 5.4-6.4(%)	HbA1c ≤5.4(%)
	Mean ±SD	Mean ± SD	Mean ±SD	Mean ±SD
HbA1c (%)	9.54 ± 1.85	6.6±0.14	6.14±0.18	5.1±0.33
T.Cholesterol (mg/dl)	201.88± 48.25	152.8±49.84	185.8±39.38	170±14.26
T.G (mg/dl)	192±72.23	99.8±17.29	137.4±53.83	134.4±4.5
HDL(mg/dl)	38.65±7.05	41.8±1.09	43.2±0.83	48.6±5.31
FBG (mg/dl)	200.25±72.34	111±13.58	118.8±26.36	109.2± 16.05

Table 3: Evaluations and Comparison of Lipid Profile results based on glycemic control (HbA1c ≥7.0 & ≤ 7.0%) in Type -2 diabetic Patients.

Parameters	Glycated Hemoglobin (HbA1c)	
	Group 1 (Poor glycemic control)	Group 2 (Good glycemic control)
	Diabetic dyslipidemia	Diabetic
	≥ 7.0 (%)	≤ 7.0 (%)
	Mean ± SD	Mean ± SD
Total no. of Patients = (50)	n=35	n= 15
TG (mg/dl)	191.41 ±72.73	123.86 ± 35.08
T. Cholesterol (mg/dl)	201.88 ±48.25	169.5 ± 37.62
HDL (mg/dl)	38.65 ±7.05	44.53 ± 4.22
FBG (mg/dl)	200.25 ±72.34	114 ± 18.53

Out of 35 dyslipidemic individuals, 14 were males and 21 were females. The mean value of HbA1c in dyslipidemic individuals was extremely higher than those having HbA1c ≤7.0 %.The mean values of TC in dyslipidemia was higher than those having HbA1c ≤ 7.0%. Similarly mean TG was found to be higher in dyslipidemic patients and lower in case of those having good control on diabetes. But mean values of HDL in dyslipidemia was less than those having good control on diabetes. (**Table no. 3**)

DISCUSSION

Glycated hemoglobin is considered as a dual biomarker for long term glycemic control and a predictor of diabetic dyslipidemia in type -2 diabetic patients. Lipid abnormalities are common in type -2 diabetic patients. Dyslipidemia makes diabetes prone to cardiovascular diseases and other complications of atherosclerosis. Persistent increase in blood glucose levels i.e. hyperglycemia causes glycosylation of all proteins especially collagen cross linking and matrix proteins of arterial wall. This process slowly and gradually causes dysfunctioning of the endothelial cells, which is a contributing factor of the atherosclerosis [9, 10, 11]. The actual pathogenesis of diabetic dyslipidemia is not known so it still remains a challenge to find out the exact crux of the process; many evidences from the research studies suggest that insulin resistance has a central role in the development of diabetic dyslipidemia. The main cause of diabetic dyslipidemia is the increased free fatty acids that are released from insulin resistant fat cells [12, 13]. The increased influx of free fatty acids into the liver in presence of adequate amounts of glycogen that is stored into the liver promotes

triglyceride production, which in turn promotes the secretion of VLDL and apolipoprotein B and cholesterol. Thus due to impaired ability of insulin to inhibit the free fatty acid release leads to increased production of hepatic VLDL cholesterol which in turn increases the degree of hepatic fat accumulation. The features of Diabetic dyslipidemia are highly varied; however, the most common ones are high triglyceride concentration, low HDL cholesterol, high total cholesterol, and high LDL cholesterol [2]. The diabetes itself, particularly hyperglycemia causes extreme risk of cardiovascular diseases in type-2 diabetes patients. Therefore tight glycemic control could improve the lipid profile of diabetic patients and can reduce the associated risk of cardiovascular disease.

The classification of subjects according to the gender and HbA1c cutoff values portray that most of the type 2 diabetic patients experience a poor glycemic control. An observation from this study demonstrated a positive correlation between HbA1c and triglycerides, total cholesterol, FBG and HDL in both the genders. Although the mean values of HbA1c and total cholesterol do not have much differences in common but females were found to exhibit the higher levels of both (**Table 1**). The mean HbA1c fasting blood glucose was found higher in females than males. Among circulating lipids triglycerides were found extremely higher in females than males but mean HDL cholesterol was slightly lower in females than in males. Diabetes confirms a marked increase in lipid profile in both the genders but females were found to have increased susceptibility of cardiovascular diseases. Diabetic women's are subjected to adverse changes to vascular function and other cardiovascular risk factors than diabetic men [14]. The results of lipid profile showed that female diabetic patient had significantly higher levels of cholesterol, triglycerides, fasting blood glucose and HbA1c levels than males. Results of this study are in accordance with the studies performed by different scientists in the different areas [15,16].

In the present study diabetic patients were classified into 2 groups as per their glycemic index: group 1 consists of 35 patients with HbA1c value $\geq 7.0\%$ and second group consists of 15 patients with HbA1c value $\leq 7.0\%$. It was found that diabetic patients with HbA1c value $\geq 7.0\%$ exhibited a major increase in TC, TG, FBG and significantly lower HDL cholesterol as compare to the patients with HbA1c value $\leq 7.0\%$ (**Table 3**). Patients having HbA1c $\geq 7.0\%$ were considered as diabetic dyslipidemic and those who had HbA1c was $\leq 7.0\%$ were diabetic but maintaining a good glycemic control. The present study also revealed high prevalence of hypercholesterolemia, hyper triglyceridemia, hyperglycemia and low HDL cholesterol in diabetic dyslipidemic patients. All these diabetic dyslipidemic patients were found to have high risk factors for cardiovascular diseases. This study showed that a positive correlation between HbA1c and TG, HbA1c and TC, HbA1c and FBG and a negative correlation was seen between HbA1c and HDL-cholesterol in both the groups. In this study diabetes confirms a markedly increased risk of cardiovascular events in both the groups however group 1 with HbA1c $\geq 7.0\%$ showed an extremely high risk of cardiovascular diseases [12]. Ikhlas *et al* have shown a high prevalence of dyslipidemia in type 2 diabetic subjects and also have confirmed that hypertriglyceridemia, hypercholesterolemia are more common in diabetic subjects than in control ones [10,11]. A highly positive correlation between HbA1c and FBG was seen in the present study which was in conformity with various studies [1]. Results regarding dyslipidemia and HbA1c were also in accordance with the study conducted by Khan *et al* [8].

The present study illustrated that most of the diabetic patients maintained a poor glycemic control that adversely affected their lipid profile and made them prone to cardiovascular diseases. This study also confirmed higher prevalence of dyslipidemia in diabetic patients than in non-diabetic patients. The severity of dyslipidemia increased in diabetic patients with the higher HbA1c. So diabetic patients with elevated HbA1c and dyslipidemia can be considered as a very high risk group for cardiovascular diseases. The DCCT performed by National Institute of Diabetes Digestive and kidney diseases in USA have considered HbA1c as a gold standard method of glycemic control [5]. HbA1c levels $\leq 7.0\%$ was said appropriate in order to reduce the risks of cardiovascular complications [6]. Improving glycemic control can considerably reduce the risk of cardiovascular diseases up to a great extent. Significant correlations between HbA1c and various lipid parameters and significant differences between lipid parameters of two groups with (HbA1c $\geq 7.0\%$ and $\leq 7.0\%$) signified that HbA1c can be used a potential biomarker in predicting dyslipidemia in type 2- diabetes mellitus in addition to glycemic control.

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