GLYCATED HbA1c: A POTENTIAL BIOMARKER FOR DIAGNOSIS OF TYPE 2 DIABETES MELLITUS AND ITS CORRELATION WITH DYSLIPIDEMIA

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CERTIFICATE

This is to certify that the present thesis entitled **"Glycated HbA1c: A Potential Biomarker for diagnosis of Type 2 Diabetes Mellitus and its Correlation with dyslipidemia",** is the outcome of the original piece of work carried out by Miss. Soofi Nusrat Ara (Registration No: 113000075) herself under my guidance and the contents of her thesis did not form a basis of the award of any previous degree to her and to the best of my knowledge to anybody also. The thesis has not been submitted by the candidate for any research degree in any other University.

The dissertation is fit for submission to the partial fulfillment of the conditions for the award of M.Sc. in Clinical Biochemistry. Further certified that the candidate in habit and character is a fit and proper person for the award.

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LIST OF CONTENTS

ABBREVIATIONS

DM	Diabetes Mellitus
T1DM	Type-1 Diabetes Mellitus
T2DM	Type-2 Diabetes Mellitus
CVD	Cardiovascular Disease
DCCT	Diabetes Control and Complication Trials
NGPS	National Glycohemoglobin Standardization
ADA	American Diabetes Association
WHO	World Health Organization
NIDDK	National Institute of Diabetes and Digestive and Kidney Diseases
USA	United States of America
UKPDS	United Kingdom Prospective Diabetes Study
IEC	International Expert Committee
IDFACE	International Diabetes federation and American College of Endocrinology
IFCC	International Federation of Clinical Chemistry
IFCCLM	International Federation of Clinical Chemistry and Laboratory Medicine
AIC	Hemoglobin A1c

ABSTRACT

Glycated Hemoglobin (HbA1c) is currently one of the best, widely used and accepted test for monitoring the glycemic 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 glycemic 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. In the present study 50 type-2 diabetic patients had participated. Estimation of HbA1c, fasting blood glucose and lipid profile were done. The overall mean value HbA1c of males was 8.21±2.12 and in females overall mean HbA1c was 8.67± 2.46. Further diabetic patients were classified in 2 groups based on their glycemic index: group 1 consists of patients with HbA1c ≥7.0% and group 2 consists of patients with HbA1c ≤ 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 ≥ 7.0% (diabetic dyslipidemia) had significantly increased level of triglycerides, total cholesterol, LDL cholesterol and increased fasting blood glucose but significantly decreased HDL-cholesterol as compared to the patients with good glycemic control (diabetic group1). The mean HbA1c value in diabetic dyslipidemia was 9.54± 1.85 which was extremely higher than diabetic group having decreased mean HbA1c 5.49 ± 0.68. The mean TG of diabetic dyslipidemia patients was 191.41± 72.73 and group 2 had mean TG of 123.86 ± 35.08. The mean LDL in diabetic dyslipidemia was 198.36±63.3, while as in good glycemic group mean value of LDL was 156.22± 38.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 with a mean HbA1c of 44.53± 4.22. 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. These findings imply that type 2- diabetic patients with dyslipidemia are at increased risk of cardiovascular diseases. The association between HbA1c with various lipid parameters suggests the importance of glycemic control in order to prevent the future risk of dyslipidemia in type-2 diabetic patients. Thus in a nut shell, the diabetes associated dyslipidemia leads to conclusion that good glycemic control would be useful to prevent the probability of the development of diabetic dyslipidemia and various other complication related to diabetes. Monitoring HbA1c levels is an easy, cost effective and less time confusing tool to access diabetes as well as diabetes associated dyslipidemia.

CHAPTER 1

Introduction

INTRODUCTION

Background

Diabetes mellitus, a globally epidemic and rapidly increasing metabolic disorder, characterized by the rise in blood glucose level called "hyperglycemia", either due to insulin deficiency or insulin resistance is one of the major causes of premature illness and death worldwide [1]. The world wide prevalence of diabetes mellitus estimated in the year 2010 was approximately 285 million(6.4%) and the number is projected to increase upto 1.552 million (7.7%) by the year 2050 [2,3]. DM is of two types, T1DM which is insulin independent accounts for 5% of prevalence while as type 2 DM i.e. insulin dependent the major one accounts for 95 % prevalence in diabetic patients [4]. Diabetic patients with uncontrolled blood glucose are mostly hyperglycemic, which consequently causes various diabetic complications [5]. The chronic hyperglycemia or persistent blood glucose elevations increases the risk for various long term vascular complications thus leading to failure of most of the vital organs of the body [6]. These diabetic complications comprises of coronary artery diseases, heart attack, stroke, heart failure, Diabetic nephropathy(kidney failure), Diabetic neuropathy(loss of sensation of nerves especially in feet), Diabetic retinopathy(eye failure), gas gangrene and gastroporesis (slow emptying of the stomach), while in contrast to this short term diabetic complications in case of surgery is poor wound healing [7]. Type 2 diabetes mellitus patients have a high risk of cardio vascular diseases (CVD). Diabetic patients are often accompanied by artherogenic lipid profile or dyslipidemia and are the soft targets of cardio vascular deaths [8]. In contrast to this most of the individuals may also carry unnoticed dyslipidemia, hence they have elevated levels of Triglycerides (TG's) total cholesterol(TC) low density lipoproteins (LDL)and decreased high density lipoproteins(HDL) [9].

Glycated Hemoglobin: (HbA1c)

Diagnosis of diabetes has been dependent upon the various glucose tests over a number of years. The Diabetes Complication and Clinical Trials (DCCT) has established Glycated Hemoglobin (HbA1c) as a gold standard method in the analysis of patients glycemic control status with levels,<7% deemed appropriate for reducing the risk of various vascular complication [10]. HbA1c is a routinely used marker for long term glycemic control [11]. A World Health Organization (WHO) consultation has concluded HbA1c as the most widely used clinical test that can be used to diagnose diabetes mellitus [12]. HbA1c also serves as a

marker that measures the average blood glucose levels over the period of 8-12 weeks. i.e. levels of previous 2-3 months prior to the measurement [13]. The cut of value of 48mmol/L (6.5%) should be coded as diabetes and the levels above this 6.5 % significantly correlates with the increased risk of various diabetic complications [14]. Glycated hemoglobin A1c is a form of hemoglobin that is primarily used to identify the average Plasma glucose over a prolonged period of time; approximately for the period of 4-12 weeks. It is formed in a non enzymatic pathway by hemoglobin's normal exposure to elevated plasma levels of glucose [15]. HbA1c testing has been found attractive because it measures chronic hyperglycemia rather than instantaneous blood glucose [16]. HbA1c levels of 7.0% or greater is strongly associated with the development of micro vascular and macro vascular complications[17]. Micro vascular complication of diabetes mellitus consists of diabetic retinopathy, diabetic neuropathy, and diabetic nephropathy while as macro vascular complications consists of various forms of CVD's like coronary artery diseases, heart failure and stroke. The reference range found in a healthy individual is about 4%-5.9% [18]. American Diabetes Association(ADA) has recently recommended HbA1c as a diagnostic test for DM after achieving HbA1c goal less than $\leq$ 7% and this has revolutionized the significance or importance of this test as a diagnostic test for accessing the adequacy of glycemic control [19].

HbA1c Historical Background:

The significance of HbA1c in monitoring the degree of blood glucose control in diabetic patients was first proposed in 1976 by 2 researchers namely *Ceramic and Koening*[20]. HbA1c testing is being considered as one of the best and finest achievements in the history of DM and is still on its way to celebrate 50 years of existence. Being the most important and accepted diagnostic procedure for monitoring average blood glucose control. Furthermore HbA1c has emerged as a marker of glycemic control and the predictor of cardio vascular complications and is also used as a screening tool for diagnosis of diabetes mellitus [21]. In the year 1955, for the first time researchers from different parts of the glob described that adult hemoglobin contains a heterogeneous molecule but the significance of this finding was not explained till the complication of the year 1969. B y that time Rahbar*et a.*, described that unusual hemoglobin found in diabetic patients was actually HbA1c [22].

By the end of 1970's, the nature of HbA1c Reaction was explained i.e. glycation of HbA1c is a spontaneous reaction in which glucose molecules are covalently bound with hemoglobin

molecules at amino terminals of the beta-globin chains [23]. In the year 1980, HBA1c testing was considered a widely accepted biomarker in the clinical practice [24]. Glycated HbA1c which is a form of hemoglobin is irreversibly glycated at one of the N-terminal valines of the beta Hb chains, but this definition did not exclude the hemoglobin that is being additionally glycated at the other sites of alpha or beta Hemoglobin chains[25]. *Huisman and Dozy* in 1962 [26], observed slight increase in HbA1c fractions of in 4 of their diabetic patients, who were already on tolbutamide, a hypoglycemic drug, but both of them failed to characterise this phenomenon in vitro. Thus Rahbar,*et. al*. after 5 years of documenting this unusual clinical finding in the year 1969 described HbA1c an abnormal hemoglobin in diabetes mellitus [27]. Rahbar, while screening the process of abnormal hemoglobin in diabetic patients demonstrated abnormal fraction of HbA1c was found in slight higher amounts in the blood of diabetic patients. In the year 1969, further investigation in diabetic patient with poorly controlled blood glucose leads to the finding of a new component called diabetic hemoglobin component in RBC's [28]. Later on many structural studies were carried on such diabetic patients and this so called abnormal hemoglobin was found to be identical to the HbA1c fraction. Further more studies on this abnormal hemoglobin increased in direct proportion to the degree of hyperglycemia [29 30]. The use of HbA1c to diagnose DM with a reference value of ≥6.5% was recommended by the International Expert Committee (IEC). However there is an important need for HBA1c standardization with a reference assay given by Diabetes Control and Complication Trials (DCCT), also Standardization of HbA1c can also be done by another method that is certified by National Glycohemoglobin standardization Programme (NGSP) [31].

Formation of Glycated HbA1c:

As we know the normal life span of erythrocytes is 120 days. During the hyperglycemic conditions, the erythrocytes circulate into the blood, at this moment one or both N-terminals valines residues of beta chain of Hb slowly and gradually under goes irreversible non enzymatic glycation process [32]. HbA1c formed in this way constitutes about 80% of the glycated hemoglobin. The time period for required for glycation of hemoglobin gets covered over the entire life span of RBC's [33]. A peculiar pattern is being followed for the formation or glycation of hemoglobin. At the initial step, about 25%of glycation occurs in first two months of the life span of erythrocytes, next 25% of glycation process occurs in the third month of erythrocyte formation , while as remaining HbA1c is formed during the senescence of the RBC,s thereby covering the period of 2 months prior to the HbA1c measurement. With

the result, senescent or older RBC's have more HbA1c concentration as compared to the reticulocytes. More over behind this process, the entire process leads to the formation of rationale i.e. HbA1c represents an average blood glucose concentration over the period of 4-8 weeks [34,35],.During abnormal glucose metabolism, hyperglycemia induces excessive or bulk production of early glycation products, which is an acute reversible change. These glycation products are formed both inside and outside the cells.

As the glucose molecules rapidly attaches itself to the amino group of proteins (N-terminals) through the nucleophilic addition reaction (a non enzymatic process)to form an unstable aldamine -shiffs base adducts, these adducts them reach to the equilibrium level that is directly proportional to the blood glucose concentration. And the reaction subsequently goes to the Amadori rearrangement reaction to form one more stable Keto-amine linkage called as AGES- Advanced glycation end products [36]. This in turn reaches to the equilibrium over the period of 4-12 weeks. This reaction is critically irreversible, meaning once the protein hemoglobin molecule gets glycated, it remain so until the end of its life span. In this way glycated hemoglobin is formed [37].

Once the Hemoglobin molecule is glycated within the erythrocytes, this build up of glycated hemoglobin within the RBC's reflects the average level of blood glucose to which red blood cells have been exposed during its entire life span. In contrast to this older or senescent RBC's loosed their ability to metabolize glucose and hence they remain permeable to glucose. Due to this phenomenon the concentration of intracellular and extra cellular glucose becomes equal. The HbA1c assay measures total glycation of hemoglobin in both glycated younger and more glycated older RBC's. From this observation it follows that HbA1c level is directional proportional to the average blood concentration over the previous 4-12 weeks.Thus by measuring glycated hemoglobin, we can access the effectiveness of the therapy by monitoring long term blood glucose therapy [38].

HbA1c Clinical significance: A Useful tool to assess or to predict the risk of Diabetic Complications:

Macro vascular Complications:(Correlation with HbA1c): Today, the world is facing an escalating global epidemic of diabetes. About 220 million people worldwide have been diagnosed with diabetes. Patients with type 2 diabetes often exhibit an elevated artherogenic lipid profile which consequently increases the risk of cardiovascular diseases as compare to the people with diabetes. Moreover 60% of people with type 2 DM die of CVD [39]. Also

people with co-existing diabetes and metabolic syndrome i.e. dyslipidemia, hyperglycemia and hypertension have been found with the highest prevalence of CVD [40, 41]. Despite some progress made in the anti-diabetic agents, the ability to maintain tight glycemic control in order to prevent diabetic complications still remains a challenge. HbA1c is strongly associated with various lipid parameters and artherogenic ratios and therefore this association suggests the importance to maintain tight glycemic control in order to control diabetic dyslipidemia and future risk of cardiovascular diseases [42].

A number of attempts have been made in order to reduce diabetic dyslipidemia by making improvements in HbA1c test results. The DCCT [43] carried out by National Institute of Diabetes Digestive and kidney diseases in USA have established HbA1c as a gold standard method of glycemic control. HbA1c levels ≤7.0% was said appropriate in order to reduce the risks of cardiovascular complications [44]. 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) [45]. 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≤7.0 %. Thus HbA1c is used as a dual potential biomarker of glycemic control and of circulating lipids in type 2 DM patients [46].

Microvascular Complications:

The Clinical utility of HbA1cas an important diagnostic tool to assess the risks of diabetic complications was proposed in DCCT publications 47 and United Kingdom Perspective of Diabetic Study (UKPDS) [48].The microvascularcomplication of DM includes diabetic retinopathy, diabetic nephropathyand diabetic neuropathy. UKPDS in 1988 confirmed a relationship between HbA1c levels and microvascular complications in 1988. An HbA1c level > 6.8% is a strong predictor of diabetic retinopathy [49, 50].

HbA1c Vs Blood Glucose Testing:

Major practical problems have found to be associated with Fasting Blood Glucose (FBG) and Oral Glucose Tolerance Tests (OGTT's) as both the tests are time consuming and needs special patient preparation. In OGTT's patient should be kept of strict diet for at least 3 days and an overnight fasting. The test is time consuming taking at least 3 hours and involves 3 blood samples which is labor insensitive. More over Blood Glucose Test is being poorly

tolerated by the diabetic patients who have vomiting, nausea, delayed gastric emptying and issues of venous access. Thus it often gives invalid results; hence there comes a need of repetition of this blood glucose testing. Therefore HbA1c is considered a better and efficient diagnostic tool, there by negating the need of OGTT's and is less time consuming and requires no special patient preparation [51]. Australia has recommended an HbA1c value of 48mmol/l(6.5%) or above for diagnosis of DM [52]. This recommendation is association with National Health and Medical Research Council guidelines for the treatment and management of DM.A proposal has also submitted to Medicare by ADS in order to accept the measurement of HbA1c for diagnosis but unfortunately this proposal remains still under consideration [53].

Diagnosis of Diabetes:

Diabetes is confirmed with HbA1c value ≥6.5% on two occasions in an asymptomatic individual, means if the person is asymptomatic, a second increased HbA1c value is necessary to confirm the diagnosis i.e. both HbA1c values should be greater than or equal to 6.5%(>48mmol/L).While in contrast to this, if a person is symptomatic, a single HbA1c test is sufficient if HbA1c value comes above 48mmol/L[54]. If HbA1c levels increases between 42-47mmol/mol (5.7-6.4%) then the individual is coded as pre-diabetic, for such patients yearly HbA1c is recommended.C11ys is a red codeused for pre-diabetes, whereas the mean temporary code was EMISNQPR215was given by EMIS [54].

Table 1: Diagnostic Criteria for Diabetes Mellitus with normal an abnormal Glucose Tolerance [54]

	Fasting Plasma Glucose mmol/L	2 hours Glucose mmol/L	Random Glucose mmol/L	HbA1c Mmol/L
Normal.	<6.0	<7.8	<11.1	<42mmol/L <6.0%.
Impaired Fasting Glucose.	6.1-6.9	<7.8	–	–
Impaired glucose Tolerance (IGT).	<7.0	7.8 - 11.0	–	–
Pre-Diabetes.	–	–	–	42-47 mmol/L 5.7-6.4%.
Diabetes mellitus.	>7.0	>11.1	>11.1	>48 mmol/L >6.5%.

Correlation between HbA1c and Mean Plasma Glucose:

The relationship between HbA1c and plasma glucose is quiet complex. Mostly elevated HbA1c levels are found in those diabetic patients whose blood glucose levels remain persistently high. On the other hand, diabetic patients with tight glycemic control sometimes do also have HbA1c levels that are nearly close or within the reference range. According to IDFACE, recommended HbA1c values is below 6.5%, while ADA has recommends HbA1c value below 7.0% for most of the diabetic patients [55]. An HbA1c of 6% on average corresponds to the mean plasma glucose of 135mg/dl.

For every 1 % increase of HbA1c, mean plasma glucose increases by 35mg/dl. For a non-diabetic individual, the recommended HbA1c value is 3.5%-5.5%. In case of DM, a value of 6.5% is considered as good glycemic control. Although many clinical research laboratory findings have considered HbA1c an index of mean plasma glucose over the preceding weeks to months but, HbA1c values does not truly reflect glycemic control for the period of last 2-3 months as it is claimed to be, rather it is being measured to the more recent weeks. Mean plasma glucose during the month, Preceding HbA1c measurement covers 50% of the result, during the next 30-60 days, prior or before HbA1c measurements mean plasma glucose contributes another 25%, similarly during the period of 60-120 days which is the entire life span of the RBC'S prior the measurements contributes the final 25% [56]. Various HbA1c levels comparable to the different blood glucose levels have been given in the table. The following Master equation given below represents the approximate mapping between HbA1c and EAG (Estimated Average Glucose) measurements [57].

EAG (mg/dl) = 28.7× hemoglobinAIC – 46.7

EAG (mg/dl) = 1,59× hemoglobin AIC – 2.59

If HbA1c level goes beyond the percentage i.e. ≥ 6.5% has been level of HbA1c has been found drug treatment for DM [58].

Table 2: Correlation between HbAIc level and Mean Plasma Glucose levels.

HbA1C (%)	6	7	8	9	10	11	12
MPG	135	170	205	240	275	310	354

MPG=Mean Plasma Glucose

Standardization of Glycated Hemoglobin Measurement:

Glycated HbA1c level must be reliable and consistent across the whole globe. Standardization calibrations are extremely important parameters so far as diagnosis is considered. Un till the year 1999, a vast variety of HbA1c assays were being used by different laboratories for the measurement of HbA1c, these include many forms of chromatographic techniques, like cation or ion-exchange High Performance Liquid Chromatography (HPLC) used by 50 % laboratories, Chromatography (more than 30% laboratories), Immunoassays (15%) [59]and Electrophoretic methods(<5%) and Boronate Affinity Chromatography [60]. There is no doubt that different variety of methods used by different laboratories yields different HbA1c results. Near about 30 Different Laboratory Methods are available to measure HbA1c levels. Such A lack of standardization have consequently resulted in great variation in the results of the same sample (a variation of4.0%-8%) [61].

This makes it extremely difficult to correlate the patient results among different laboratories. So this has caused the major comparability problems in HbA1c results. Therefore there is a need of using same method and same unit to measure HbA1c. In order to rectify this problem, a uniform International Standardization of HbA1c has been developed in 1995 by International Federation of Clinical Chemistry (IFCC), under this method amixture of pure HbA1c and HbA1o were prepared for calibration of the reference method. Also cation exchange performed by HPLC is being considered as the most accepted and used reference assays.HbA1c was then defined as hemoglobin that is being irreversibly glycated at one or both N-terminals valines of the Beta chains [1].

Since the measurement of HbA1c according to IFCC is too specific, therefore only one molecular HbA1c species is being measured, thereby not considering non-HbA1c components in the final results. As a result HbA1c values achieved by using IFCC assays were 1.5 % to 2% lower than NGPS results that traced to the DCCT [62]. So this has lead laboratory workers in a fear of confusion occurring between two sets of HbA1c values and could have possibly resulted in least control of hyperglycemia [63]. In order to resolve this issue a “master equation”, was formulized in order to develop a strong relationship between all 3 designed comparison methods comprising of National Glycohemoglobin Standardization Programme of US(NSGP), Mono-s- Sweden and Japanese Society of clinical Chemistry [64]. This master equation allows IFCC to more expected HbA1c results, which

could be correlated with DCCT and UKPDS HbA1c results. In May 2007, a worldwide standardization of glycosylated HbA1c measurement was recommended by ADA, which states that IFCC reference system is the only valid system thathas put the standardization of HbA1c measurements into practice. Therefore recommending that HbA1c can be expressed under DCCT/UKPDS/NGSP unit represented in percentage (%age) and IFCC units i.e. mmol/lof Hb [65].

Factors Affecting HbA1c Results:

A series of clinical conditions are responsible that are known to affect the accuracy of HbA1c test, thus giving falsely elevated HbA1c values. Consequently this can delay as well as can change patient's whole diagnosis. The factors responsible for giving falsely HbA1c levels includes Increased life span of circulating Erythrocytes (RBC's) or erythrocyte turnover rate, decreased red cell survival, hemoglobinopathies ,alcoholism, iron deficiency and renal failure and hyperbillurubinemia. As we know that the HbA1c level is dependent upon the life span of RBCs. Hence the important factor that influences HbA1C values is erythrocyte turnover rate. Thus from the above fact it follows that higher HbA1c level is directly proportional to the increased erythrocyte circulation i.e. longer the erythrocyte circulation time, more will be the falsely elevated HbA1c levels. Another known condition that gives falsely elevated HbA1c results is the exposure of hemoglobin molecules to higher blood glucose in older RBC's, thus giving unexpected elevated HbA1c results[66].

Abnormal hemoglobin May also interfere with HbA1c results. So far as normal mechanism of HbA1c glycation is concerned, an adult HbA1o has to form HbA1c, but when abnormal hemoglobin is present, patient is likely to have falsely increased HbA1c levels.Iron deficiency is another known cause for increased HbA1c up to 20% but this condition can be reversed by providing iron supplements [67, 68, 69,70]. Falsely decreased HbA1c is seen in case of hemolytic anemia [71]. Also children's <18 years, acute pancreatic surgery patients taking drugs that increases blood glucose levels are some other factors that effects Hba1c levels. Falsely decreased HbA1c levels are seen when life span of erythrocytes is decreased i.e. increased hemoglobin turn over number. HbA1c may either falsely increase or decrease in case of pregnancy therefore HbA1c estimation is not done in case of gestational diabetes[66].

CHAPTER 2

Literature Review

In 1992 Daniel Singer and colleagues did a cross sectional study on 1045 patients in order to determine relationship between HbA1c and dyslipidemia. Significant positive correlations were found between HbA1c and lipid parameters. Results from the study found that Hba1c was significantly correlated to cardiovascular diseases among women other than men [72].

In 2002 Curt Rohlfing*et al*., defined a relationship between HbA1c and plasma glucose levels. Results were calculated by linear regression analysis done on the mean plasma glucose and HbA1c levels of 1439 subjects. All the participants produced a relationship between MPG (mmol/l) equal to (1.98% HbA1c). The time gaps like afternoon, evening, post lunch, pre-dinner, bed time showed higher correlation of MPG and HbA1c than the morning time time gaps (pre breakfast, post breakfast, pre –lunch)MPG was estimated by multiplying capillary blood glucose by 1.11[73].

In 2004Leve R, *et al.*,in a study of 100 pregnant women without gestational diabetes (early pregnancy) and 98 healthy pregnant women (late pregnancy) examined that HbA1c levels are significantly lower in early pregnancy. Also a control group of 145 healthy women of age 30years were investigated as a part of this population survey. In conclusion HbA1c levels were decreased in early pregnancy and further decrease was found in late pregnancy as compared to age matched non pregnant women. Normal HbA1c range in non pregnant women was 4.7-6.3%, in early pregnancy HbA1c was 4.5-5.7% and in late pregnancy HbA1c was further decreased up to 4.4-5.6% [74].

1n 2006, C.J.O. Sullivan, N.Hynes *et al*., determined the association between plasma HbA1c with increased post operative morbidity and mortality in patients that had undergone for vascular surgery. Patients were categorized into 4 groups based on plasma HbA1c level <6%, 6.1-7%, 7.1-8%. 2nd group consisted of non diabetic patients with HbA1c > 6 to 7% and a suboptimal group with HbA1c >7%. In case of patients who had plasma HbA1c >7% were again classified as having undiagnosed diabetes. Sub optimal HbA1c was found in 58% patients without diabetes. In suboptimal HbA1c, 6-7% had higher prevalence of overall 30-day- morbidity as compared to patients with HbA1c $\leq$ 6%.Results from the present study reveal that suboptimal HbA1c level >7% holds a prognostic significance in patients without diabetes that had undergone vascular surgery. [75]

In 2008 Lydin, LeslicKoren*et al.*,studied the effect of aging on HbA1c levels in individuals without diabetes. Across sectional survey on HbA1c was done across different age groups in non-diabetic patients from Framingham off spring study (FOS) and National health

&Nutritional Examination survey (2001-2004). By adjusting BMI, Sex, Age, fasting glucose a multi variation analysis was performed. it was found that HbA1c levels were positively associated with age in non-diabetic subjects.In FOC survey about 97% for HbA1c were 6.0% and 5.6% for non-diabetic individuals aged < 40 years and in NHANES survey 6.6% and 6.2% for aged >70 yrs with a peak value of 0.001(P <0.001). Since HbA1c association with age was similar in both the age groups therefore this study leads to conclusion that HbA1c levels are positively associated with age in non-diabetic subjects [76].

In 2009Amar Rashid and IqbalHaider did a cross sectional study on 100 type-2 Diabetic patients in order to determine serum lipid profile in controlled and uncontrolled type-2 diabetes. Statistically significant differences in serum lipid profile between both diabetic genders was observed comprising of 72 % were males and 28% were females.TG, TC, LDL-cholesterol were found higher in group 2 (uncontrolled diabetics) than in group 1(controlled diabetes) but HDL cholesterol was significantly lower in group 1 as compared to group 2. Hence these results leads to the conclusion those diabetic patients with good glycemic control had less risk of cardiovascular diseases than the poor glycemic group [77].

In 2010 K. Murugan, D.K. Shrivastava in Chhattisgarh region of In investigated a systematic survey on Glycosylated HbA1c and its association with obesity. Another important threat to health is obesity.It was found that there was no significant change in fasting blood glucose levels in obesity groups alone and diabetes mellitus associated with obesity (DM and obesity) groups marked increased blood sugar levels therefore indicating that obesity alone has no role in changing FBG levels and (DM and obesity) showed a significant rise in FBG levels. Further investigation found that a BMI of 25 kg/m2 has been associated with increased morbidity in patients having both diabetes and cardiovascular diseases and a BMI of > 30 kg/m^2 was found to be associated was found to be associated with morbidity and mortality that leads to diabetes, CVD and strokes. The study further predicted that diabetes associated obesity had elevated Hba1c levels. Hence this case study achieves a critical importance that HbA1c is the most reliable and widely accepted diagnostic path that would guide in the pathogenesis of diabetes associated obesity[78].

In 2010Chandershekher M Sultanpurand colleagues gave a comprehensive on HbA1c and its limitations. No doubt HbA1c is considered as the most accepted biomarker for diagnosis of diabetes mellitus. Nevertheless its more crucial to be aware about the possible interferences or limitations of HbA1c affect HbA1c interpretations. The limitations include various

pathological conditions such as haemoglobinopathies or abnormal number of hemoglobin. Erythrocyte turnover rate, use of certain drugs to treat malignancies. About 700 Hb variants which cause mutations in globin genes of hemoglobin results in amino acid substitution thus gives falsely high or low HbA1c results. Since the measurements of HbA1c is done by HPLC method but interferences of these Hb variants provides false results hence it was recommended to modify the method in order to the most accurate results [79]

In 2011 Iftikar Ahmad Asim Syed put a light on standardization of glycosylated HbA1c measurements. Different laboratories use various assays to measure glycosylated hemoglobin on the same sample. Such a lack of standardization results in wide variation of HbA1c results. To overcome this problem, IFCC had developed a new reference method that is used worldwide to measure HbA1c values in mmol/mol. Also NGPS derived HbA1c units in (%age) by using IFCC master equation. Thus HbA1c results are expressed in both % and mmol/l [80].

In 2012S. Appana Reddy *et al.*,in a review described clinical applications of Glycosylated Hemoglobin HbA1c, its history, biochemical process behind its formation, its standardization,various factors affecting HbA1c results,various cut of values obtained from different studies performed in India and Worldwide, correlation between HbA1c and diabetic complicationslike diabetic retinopathy, diabetic dyslipidemia, diabetic nephropathy, diabetic neuropathy [81].

In 2012Ikhlas K. Hamed*et a.*,analyzed the role of HbA1c as a diagnostic biomarker for predicting diabetic dyslipidemia in type 2- diabetic patients. Glycosylated HbA1c was estimated by HPLC. Blood serum was analyzed for Total Cholesterol, HDL, LDL and TG. Statistical data analyzed in a population of 450 diabetic patients gave a positive correlation between HbA1cand Total Cholesterol($p=0.000$), LDL cholesterol ($p=0.000$), LDL/HDL Cholesterol ($p=0.0001$) Patients with HbA1c values >7.0% had high risk of cardiovascular diseases [82].

In 2013 Abdul Rehman.A. Momin*et al.,* in a study of 100 T2DM patients and 100 age control groups examined the association among various lipid parameters Hba1c values and artherogenic ratios. Type 2 diabetic patients were divided into 2 groups based on their glycemic. Group consists of patients having HbA1c> 7.0% while as group 2^{nd} of those having HbA1c <7.0%. It was found that Patients with higher blood glucose had HbA1c≥ 7.0% and increased TC,LDL TG and VLDL cholesterol in patients with T2DM than control groups.

Atherogenic ratios TC/HDL and HDL/LDL differ in type 2 diabetic groups as compared to control groups.HDL cholesterol found was lower in type 2 diabetes. Patients with bad glycemic control (HbA1c ≤7.0%) significantly had increased levels of Total Cholesterol, LDL/HDL cholesterol ratios and decreased HDL/LDL Cholesterol as compared to the patients with good glycemic control (HbA1c≥7.0%). These clinical findings suggests the importance of glycemic control in order to prevent or control diabetic dyslipidemia and future risk of CVD especially in type 2 diabetes mellitus [83].

In 2013 Raja Reddy, Jayarama N and colleagues evaluated the relationship between HbA1c and lipid parameters in a total of 750 type-2 Diabetic subjects (males- 493 and females -257). Results were evaluated on the basis of statistical measurement.9.81% female diabetic patients were found to have increased values of FBG, TC, TG, HDL and LDL and decreased HDL levels. This study further suggested higher risk of CVDs in diabetic females as compared to diabetic males [84].

In 2013 Sasisekar T.V.D andShabanaconducted a study on 278 diabetic subjects. The main aim of this investigation was to evaluate the diagnostic value of HbA1c in order to predict the risk of CVDs. Data regarding age, duration of diabetes was taken. Venus blood was analyzed for estimation of FBG, TG, LDL, TC and HbA1clevels.High TC and LDL levels were found in females as compared to males. TG levels were raised in subjects having < 55 years of age as compared to those with > 55 years of age. Patients with >6 years duration of diabetes were found to have increased levels LDL, TG and TC- cholesterol levels but only TG and LDL levels were statistically significant. All parameters except HDL was higher in subjects with HbA1c≥ 7.0% as compared to another group with HbA1c≤ 7.0%. These findings suggest that HbA1c levels provide information about circulating lipids and thus can be used as a biomarker to predict diabetic dyslipidemia [85].

In 2014 YuthikaAgarwal, VipinGoyal, *et al.*, determined the influence of diabetic dyslipidemia in 100 type-2 diabetic population and 100 non- diabetic population of Haryana region of India. Fasting blood glucose, TC TG were significantly raised in diabetics as compared to control group. HDL, LDL, TG levels were significantly different in diabetic males as compared to diabetic females. 56% of high TG was most commonly present in diabetics. HDL alone 27% was most common dyslipidemia in diabetic subjects[86].

In 2015 Dr. A. Navanita Lakshmi, Dr.B. Kiranmani*et al.*, conducted a study on estimation of HbA1c and Micro-albuminuria levels in 50 individuals of both the genders and 15

metabolically healthy individuals of both the genders. Incidence of dyslipidemia was found in all unhealthy individuals. The incidence of micro albuminuria was found in smokers, alcoholics and hypersensitive individuals who were dyslipidemic[87].

In 2015 Syed Waseem Pasha, Faseeh K.M *et al*., studied the pattern of dyslipidemia 100 among type-2 diabetes mellitus patients of Mangalore region of India. 905 of the subjects had dyslipidemia, 84% of them had low levels (<40 mg/dl) oh HDL cholesterol. Patients with poor glycemic control (HbA1c>7.0%) had reduced HDL levels. Most common pattern of dyslipidemia in the study was low HDL followed by high TG cholesterol levels [88].

CHAPTER 3

Objectives of the study

OBJECTIVES OF THE STUDY:

1: To study Glycated Hemoglobin levels (HbA1c) in type 2 Diabetic patients.

2: To Compare and Correlate Glycated Hemoglobin levels with their lipid levels in type 2 Diabeticpatients and in normal subjects.

3: To Evaluate the association between Glycated Hemoglobin (HbA1c) and various lipid parameters.

CHAPTER 4

Plan of Works

TABLE: PLAN OF WORK

PLAN OF WORK.	
Introduction	Completed
Literature survey	Completed
Selection of patients	Completed
Study of patient history	Completed
Collection of sample	Completed
Collection of information about AECHITECH c System and AEROSET System.	Completed
Study of System manual	Completed
Operating Auto analyzer for estimation of HbA1c, blood sugar and lipid parameters	Completed
Evaluation of results	Completed
Representation of data	Completed

CHAPTER 5

Materials and Methods

Material and Methods:

The Study was conducted at Govt. Super Specialty Hospital Jammu, in the department of Biochemistry. 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 recruited for the study. The study was completed covering the period of 3 months from (January 23-to-April 24 -2015). 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 3 parameters i.e. HbA1c, blood glucose and lipid profile was 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. LDL cholesterol was estimated in only 2 samples so this parameter was excluded from the study. 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

Requirements:

Table no. 3

S.no	Materials
1	Syringes of 5ml
2	Tourniquet
3	Cotton and spirit
4	Test tube
5	Test tube stand
6	Refrigerator
7	Centrifuge machine
8	Auto analyzer (Architect c System and AEROSET System)
9	Cups and Cup carriers
10	Reaction vials
11	Pipettes for separating serum.

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 3 biochemical parameters: HbA1c, blood glucose and Lipid profile. Therefore separate testubes 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 into the centrifuge and was later used for analysis of blood glucose and lipid profile estimation. In case of HbA1c testing Whole blood sample was used for estimation.

Figure 1: Test tubes filled with blood samples. **Cup carriers holding cup filled with serum.**

Autoanalyzer: Architect c System and AEROSET System

Procedure, sampling, reagent delivery, mixing, processing and printing out results were automatically performed by Architect c System and AEROSET system. The instrument is a huge rectangular shaped electronic device that calculates and prints out the Concentration of Biochemical tests.

Figure 2: Auto analyzer (Architect c System and AEROSET System)

METHODOLOGY

Name:The MULTIGEN HbA1c Immunoassay

The MULTIGENT HbA1c assay was used for the quantitative i*nvitr*o measurement of percent HbA1c in human whole blood on the ARCHITECT c System and on theAEROSTAT SYSTEM. The assay measures the concentration of the HbA1c relative to the concentration of the total Hemoglobin.

PRINCIPLES OF PROCEDURE:

The assay consisted of two separate concentration measurements. The glycated hemoglobin and the Total Hemoglobin (THb). The two concentrations were used to determine the percent Hba1c or hemoglobin fraction. The individual concentration values of HbA1c and THb generated by the assay were used only for calculating the percent HbA1c or (hemoglobin fraction) and must not be used individually for diagnostic purposes. The whole blood specimens were first pre-treated with the MULTIGENT Hemoglobin Denaturant. The erythrocytes were lyzed and the hemoglobin was degraded by the proteolytic enzyme pepsin in order to form hemosylate. Both the Thb and HbA1c concentrations were determined from the same hemosylate.

Total Hemoglobin (THb)

The concentration of Total Hemoglobin was determined calorimetrically and is based on the method described by *Zander et al* ,. The hemosylate was added to the THb reagent which contains an alkaline solution of non-ionic surfactant. All the hemoglobin was converted into hematin. Resulting in a green solution. This conversion was measured in an end point reaction by using a wave length of 604nm. The absorbance of the sample was compared to a two-point calibration curve for total hemoglobin.

HbA1c

The concentration of HbA1c was measured immune turbid metrically using a microparticle agglutination inhibition method. The HbA1c antibody R1 contains a specific anti-HbA1c mouse monoclonal antibodies coupled to microparticle. The HbA1c agglutinator R2 contains several copies of the immunoreactive portion of HbA1c (hapten), that is covalently bound to a polymer. In absence of glycated HbA1c in the sample, the HbA1c hapten in the R2 agglutinator binds with the antibody coated micro particles in the R1 antibody. Since several immunoreactive haptens are available on the polymer. Cross linking and agglutination of the antibody-coupled microparticle can occur. This increase in the rate of agglutination and results in the increase in measured absorbance.

In the presence of glycated HbA1c in the sample, the HbA1c competes with the HbA1c hapten in the R2 agglutinator for binding-site of each HbA1c molecule. Thus HbA1c shows the rate of agglutination.The increase in HbA1c concentration in the sample in inversely proportional to the agglutination rate and measured absorbance. The absorbance is measured at 700 nm

The measured absorbance of the sample is compared to the measured absorbance of known HbA1c concentrations of a six –level calibration curve and the concentration of the sample is interpolated.

REAGENTS

Reagents Kit Details

REF 2K96-20 MULTIGENT HbA1c Reagent Kit is supplied as a liquid, ready-to-use, 4 reagent kit, which contains:

- HEMOGLOBIN DENATURANT 1×120ml
- R1 Total Hemoglobin (THb) 4 ×19ml
- R1 HbA1c denaturant 2×19ml
- R2 HbA1c denaturant 2×19ml

Estimated test per KIT: 300 approx.

Figure 3: HbA1c Reagent kit

Table 4: Ingredients contained in the HbA1c reagents.

Reagent	Reactive ingredients	Concentration
Hemoglobin denaturant	Pepsin (Procein)	0.01%
R1 (THb)	Sodium hydroxide	0.4%
R1 HbA1c	Micro particles coated with mouse antibodies to HbA1c	<0.1%
R2 HbA1c	**HbA1c hapten covalently attached to polymer**	**2ng/ml**

SPECIMEN COLLECTION AND HANDLING

Suitable specimens

Only whole blood specimens were collected by standard venipuncture technique into glass or plastic test tubes. Acceptable anticoagulants used are EDTA, heparin, lithium.

Note: The ARCHITECT *c Systems* and the AEROSET SYSTEMS do not provide the capability of verifying the sample type. Thus it is the responsibility of the operator to verify the correct sample type is used with the MULTIGENT Hb1Ac assay.

The whole blood specimens were lysed as a part of the pre-treatment process prior to analysis on the ARCHITECT *c Systems*or the AEROSET *System*.

SPECIMEN STORAGE:

Whole Blood: Analyze samples within 8 hours of collection. If testing was delayed, EDTA, lithium, heparin treated whole bold specimen were kept stable at 2-8 degree Celsius for 2 weeks.

Note: Frozen specimens must be thawed naturally at room temperature and mixed immediately prior to treatment. Do not freeze previously frozen specimens.

Assay Procedure:

Both the MULTIGENT HbA1c and THb assays were calibrated to determine HbA1c, expressed as percent (%) or mmol/L hemoglobin.

CALIBRATION

Both the HbA1c assay and the THb assay use REF 2K96-02 MULTIGENT HbA1c Calibrators supplied separately. The HbA1c Assay uses CAL 1-6, while the THb assays uses water blank, Supplied by the Auto-analyzer and CAL-1. All MULTIGENT HbA1c Assays are traceable to National Glycohemoglobin Standardization Program (NSGP) and International Federation of Clinical Chemistry (IFCC) reference methods.(9)

Percent HbA1c (NSGP)

The percent HbA1c is the HbA1c/THb ratio with a conversion factor to correlate the result with an NSGP- certified HPLC method.

HbA1c Fraction(IFCC)

The HbA1c fraction is the HbA1c/THb ratio expressed mmol/L hemoglobin utilizing IFCC traceable values.

RESULTS

The individual concentration measurements of THb and HbA1c were performed automatically by the Auto-analyzer**(ARCHITECT c System and the AEROSET system)** and were measured in g/dl or mmol/l.

The system was configured automatically to calculate the ratio of HbA1c to THb.

CONVENTIONAL Units: (NSGP Traceable)

The Calculation of the Percent HbA1c is generated by using the following equation that incorporates a factor to correlate the MULTIGENT HbA1c results to an NSGP –certified HPLC method.

$$\frac{\text{(HbA1c (g/dl)} \times 100)}{\text{Thb (g/dl)}} - 3 + (0.2 \times \text{THb (g/dl)}) = \%\text{HbA1c}$$

For example, the percent HbA1c for a specimen containing 1.000g/dl HbA1c and 1.40g/dl Thb is calculated as:

$$\frac{\text{(1.000g/dl} \times 100)}{\text{14.0G/dl}} - 3 + (0.2 \times 14.0)\ \text{THb (g/dl)} = 6.9\%\ \text{HbA1c.}$$

SI units (IFCC Traceable)

The calculation of the hemoglobin A1c fraction (HbA1c mmol/l) is generated using the following equation**.**

$$\frac{\text{(HbA1c (mmol/L)} \times 1000\ \text{mmol/mol}}{\text{THb (mmol/L)}} = 52\ \text{mmol/mol HbA1c}$$

For example, HbA1c mmol/L for a specimen containing 0.45mmol/L HbA1c and 8.68 mmol/L THb is calculated as

$$\frac{\text{(0.45 mmol/L)} \times 1000\ \text{mmol/mol}}{\text{8.68mmol/L}} = 52\ \text{mmol/LHb1Ac}$$

Table 5: HbA1c Reference range

Assay : Glycosylated HbA1C %		**Reference Ranges:**
		4-5.7% -Normal(No Diabetes)
		5.7-6.4% - Pre diabetes(Risk of Diabetes)
		6.5% good control
		≥6.5% Diabetes
		≥7.0% Diabetic complications

CHAPTER 6

Results

RESULTS

A total number of 50 individuals participated in the study. The demographical characteristics of the study population (n=50) were: females (n=27) and male (n=23). Participants age limit were 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 ≥ 7.0% and ≤ 7.0 %. Group 1 consists of patients with HbA1c ≥ 7.0% had extremely poor control on diabetes so they were named as diabetic dyslipidemic while on the other hand patients who had Hb1Ac ≤ 7.0% were kept under group 2 (good glycemic control). Results of this study depicts that HbA1c has a positive relation with lipid parameters and FBG but a negative correlation was found with HDL and HbA1c. 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 ± STD of total male and female subjects were 49.74± 15.25 and 48.70 ± 10.0. The mean value of HbA1c in females was 8.67 ± 10.0 which was slightly higher than males having mean HbA1c value of 8.21±2.12. Similarly the mean level FBG in females was 180.88±74.11 which was higher than males with mean level FBG 166.73±72.4. The mean value of TC in females was 195.5± 47±.38 which was higher than males 188.17±43.11. The mean TG in overall female population was 184.34 ± 76.09 that was found extremely higher than males 156.43± 62.36. Also mean LDL in females were 185.85±29.1 which higher than male population. The mean values of HDL in females were slightly high rather than males (table 6).

Table 6: Evaluation and Comparison Lipid profile parameters and HbA1c results of Total Malesand 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.Cho(mg/dl)	188.17± 43.11	195.5±47.38
LDL (mg/dl)	168.66±4.93	185.85±29.1
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

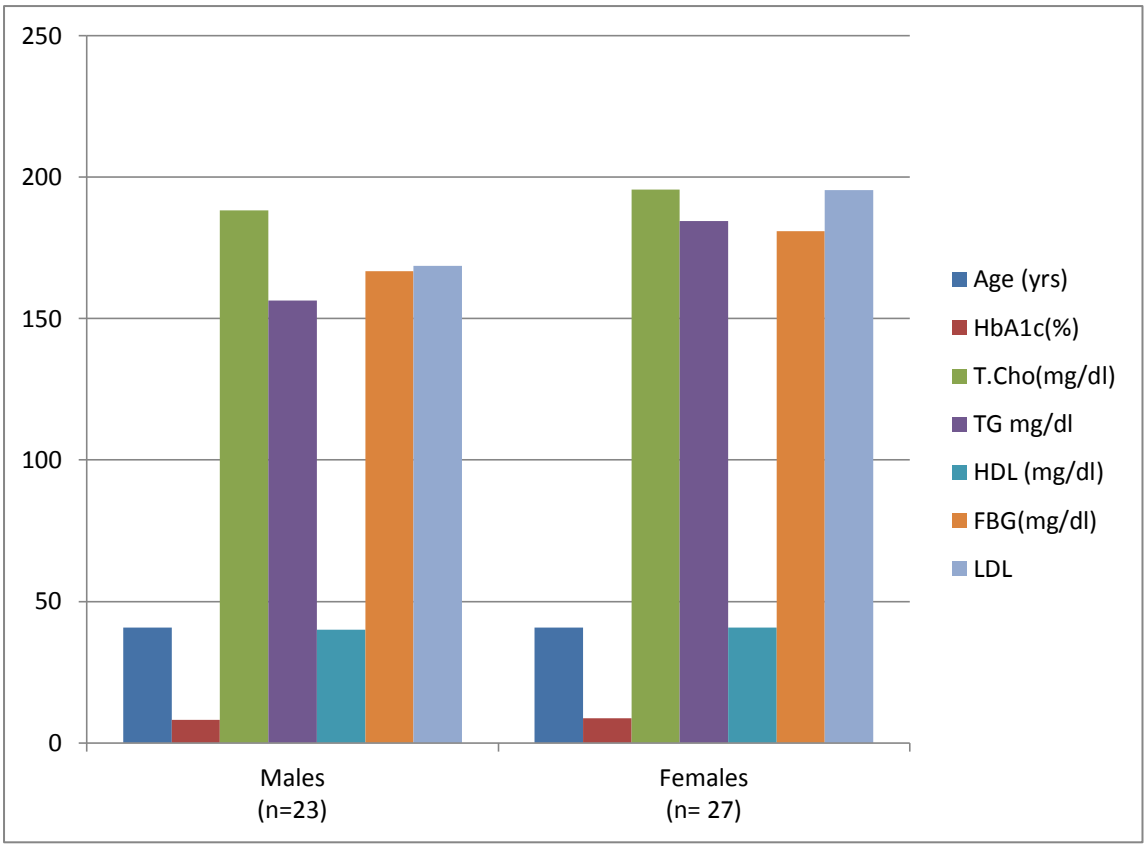

Figure 4: Lipid profile parameters and HbA1c results of Total 50 Males and Females subjects.

In the 1st part o the study all 50 subjects were divided into 4 groups named as diabetic dyslipidemic, diabetic, pre-diabetic and normal based on their various HbA1c cutoff values. Group 1 consists of 35patients with HbA1c ≥7.0 so they were kept under dyslipidemic category having high lipid profiles because of extremely poor glycemic control. The mean HbA1c of in case of diabetic dyslipidemia was 9.54 ± 1.85. In case of diabetic mean HbA1c was 6.6±0.14.In pre-diabetic mean HbA1c was 6.14±0.18 and in case of normal 5.1±0.33. The mean values of TC in diabetic dyslipidemia were 201.88± 48.25, in case of diabetic 152.8±49.84, in case of pre-diabetic 185.8±39.38 and in normal 170±14.26. The mean values of TG in diabetic dyslipidemia was 192±72.23, in diabetics it was 139.0±17.29, in per-diabetics mean TG was 137.4±53.83 and in normal subjects 134.4±4.5 Also the mean values of FBG in diabetic dyslipidemia was 200.25±72.34, in diabetics mean FBG 111±13.58 , in case of pre-diabetics mean FBG was 109.2± 16.05. Mean LDL in diabetic dyslipidemia was 198.36± 63.3, in diabetics, mean LDL cholesterol was157.76±51, in case of pre-diabetics mean LDL cholesterol was 115.12±46 and in case of normal population mean LDL cholesterol was 94.52±18.1 .Thus patients with high HbA1c ≥ 7.0% values had high FBG,TC, TG, LDL from rest of the groups but extremely low HDL cholesterol with a mean of 38.65±7.05 as compared to diabetic with a high mean value of HDL equal to 41.8±1.09,pre-diabetic had higher mean value of HDL than diabetics 43.2±0.83and normal group had the extremely high mean values of HDL cholesterol. With higher HbA1c values, the severity of Dyslipidemia increases in diabetic patients. Diabetic patients with elevated HbA1c and dyslipidemia are considered as a very high risk for cardiovascular diseases.

Table 7: 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.Cho(mg/dl)	201.88± 48.25	152.8±49.84	185.8±39.38	171±14.26
T.G (mg/dl)	192±72.23	139.0±17.29	137.4±53.83	134.4±4.5
LDL (mg/dl)	198.36±63.3	157.76±51	115.12±46.6	94.52±18.1
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

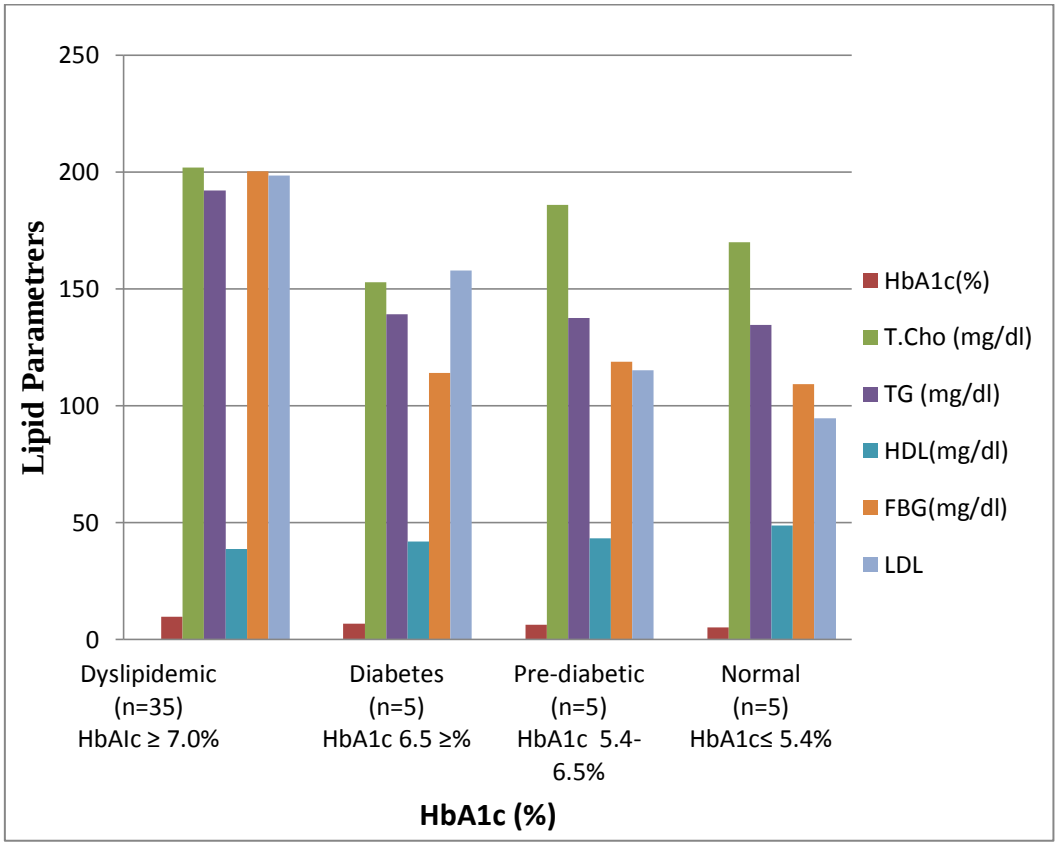

Figure 5: Graphical representation of GlycatedHbA1c levels and lipid Parameters in Dyslipidemic, Diabetic, Pre- Diabetic and in Normal Subjects.

In 2nd part of the study diabetic patients were classified into two groups based on HbA1c cut of values (≥7.0% and ≤7.0%). Groups 1 consists of Patients with HbA1c≥ 7.0% and Group 2 consist of patients with HbA1c≤ 7.0%.In table 8: Among 35 dyslipidemic individuals14 were males and 21 were females. The mean value of HbA1c in dyslipidemic individuals was 9.54±1.85 which was extremely higher than those having HbA1c ≤7.0 % i.e.5.94±0.68.The Mean LDL cholesterol in diabetic dyslipidemia was 198.36±63.3 which was again higher thangroup 2 with a mean LDL equal to 156.22±38. The mean value of TC in dyslipidemia was 201.88±48.25 which was higher than those having HbA1c ≤ 7.0% i.e. 169.5± 37.62. Similarly mean TG was found to higher in dyslipidemic patients 191.41±72.73 and lower in case of those having good control on diabetes i.e. their mean TG was 123.86± 35.08. Also mean FBG was 200.25±72.34 in dyslipidemic 144±18.53. But mean values of HDL in dyslipidemia were 38.65±7.05 which was less than those having good control on diabetes with a mean HDL of 44.53±4.22. (Refer to table no.8).

Table 8: 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. Cho (mg/dl)	201.88 ±48.25	169.5 ± 37.62
LDL(mg/dl)	198.36±63.3	156.22±38
HDL (mg/dl)	38.65 ±7.05	44.53 ± 4.22
FBG (mg/dl)	200. 25 ±72.34	114 ± 18.53

The above table represents comparison of lipid parameters in good and poor glycemic control. All the parameters were elevated from their normal ranges. But Group 1 participants with HbA1c ≥ 7.0% were more effectedthan group 2.

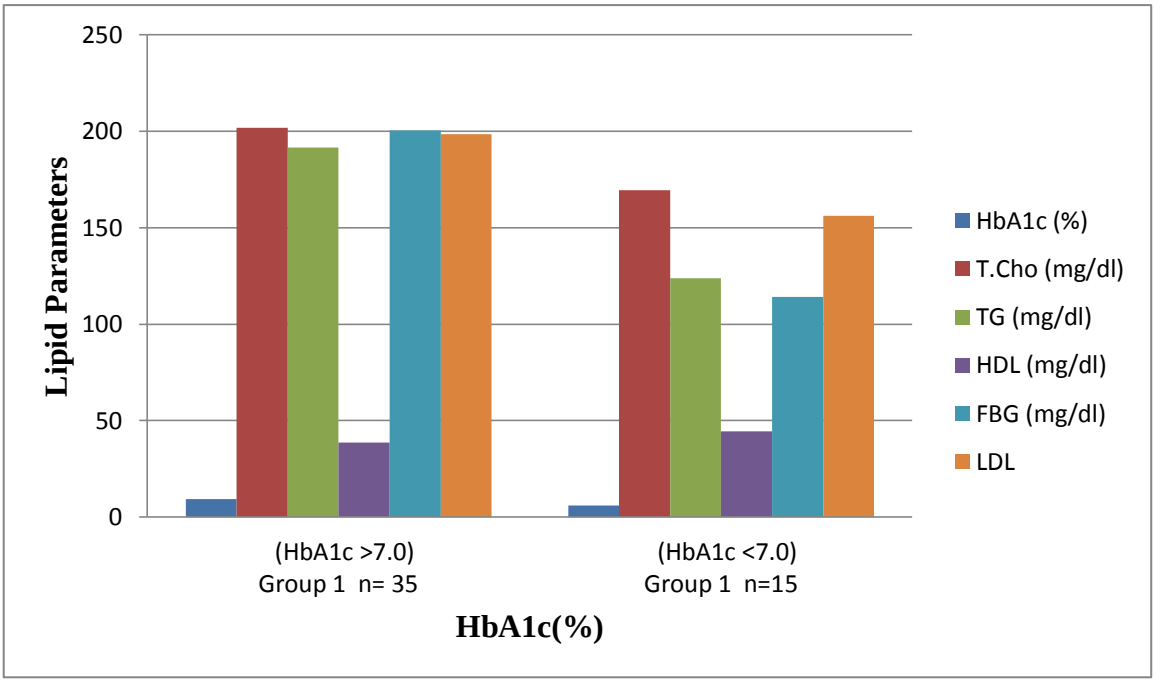

Figure6: Lipid Profile results based on glycemic control (HbA1c cut off ≥ 7.0% and ≤ 7.0%) inType -2 diabetic Patients.

Poor glycemic control -HbA1c ≥7.0 (n=35) Males 14 & Females 21

Good glycemic control – HbA1c ≤ 7.0% (n=15) Males 9 & females 6

Normal Values of lipid profile:

Total cholesterol - 100-200 mg/dl

HDL-cholesterol - 40 - 60 mg/dl

LDL cholesterol - upto 160 mg/dl

Triglycerides - 50- 150 mg/dl

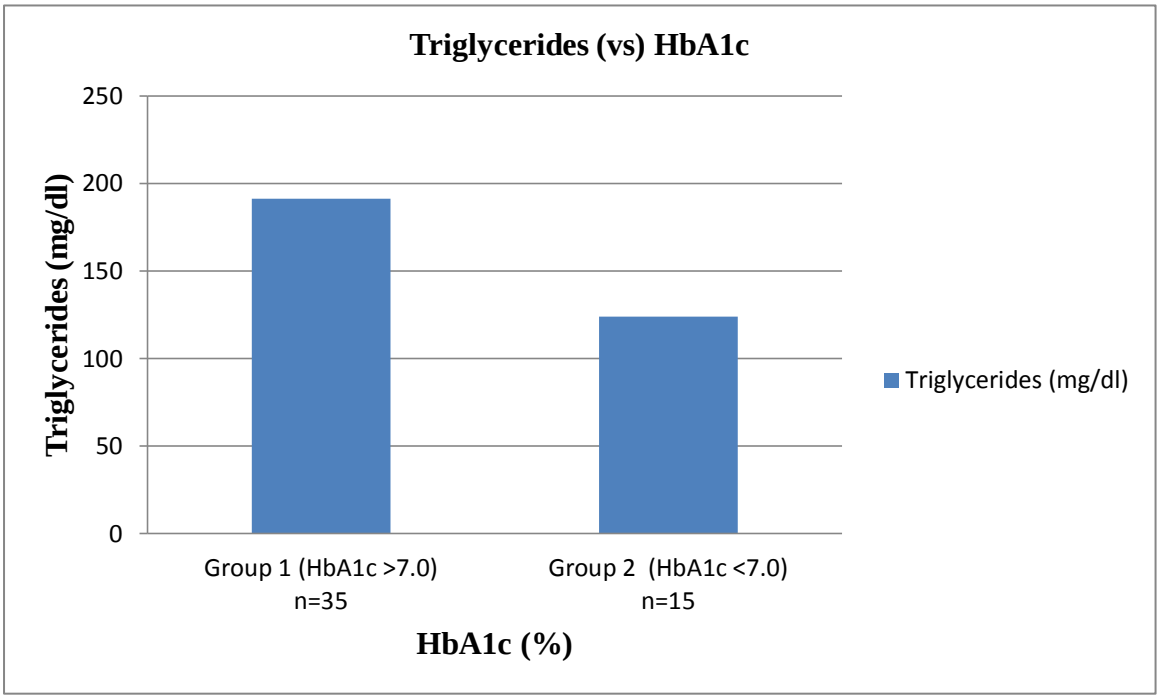

Figure 7: Graphical representation, showing correlation of Triglycerides withPoor glycemic control (HbA1c ≥7.0%) and Good glycemic control HbA1c ≤ 7.0%).

Group 1 - Hyper triglyceridemia.

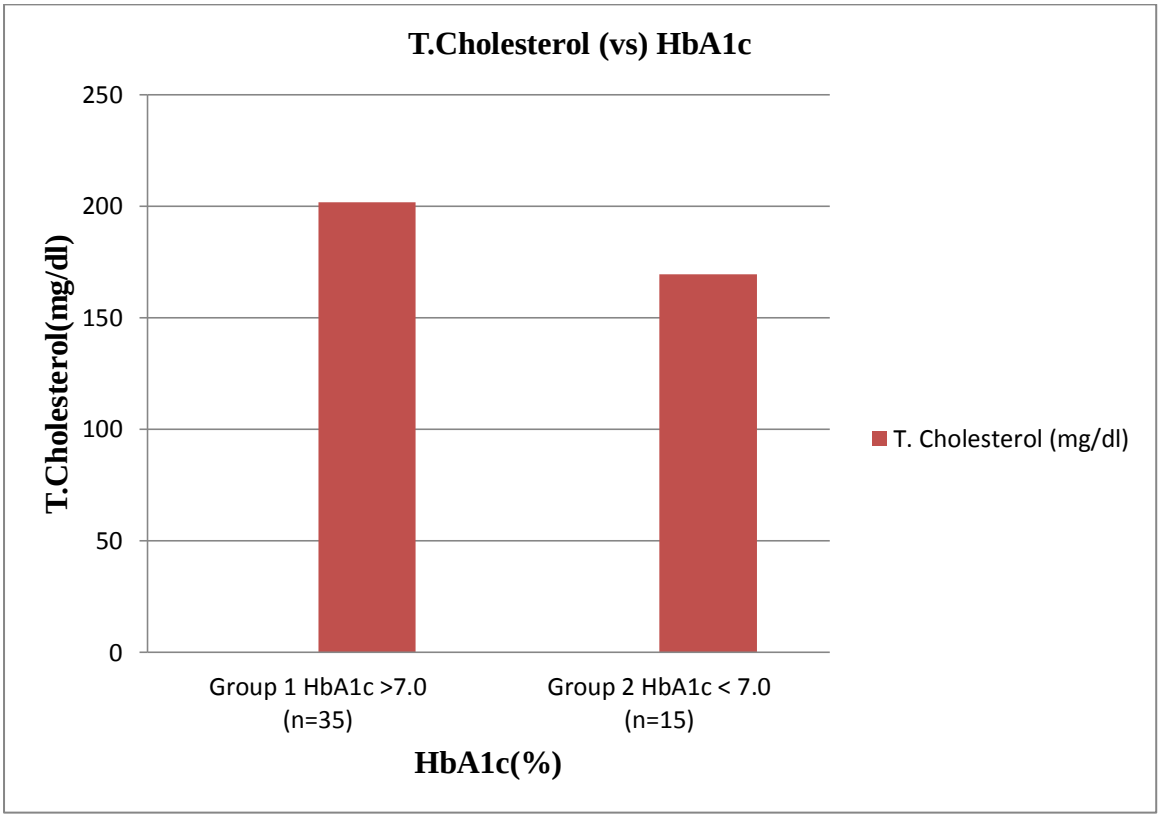

Figure 8: Correlation between HbA1c and Total cholesterol in poor and good glycemic control.

Group 1- hypercholesterolemia

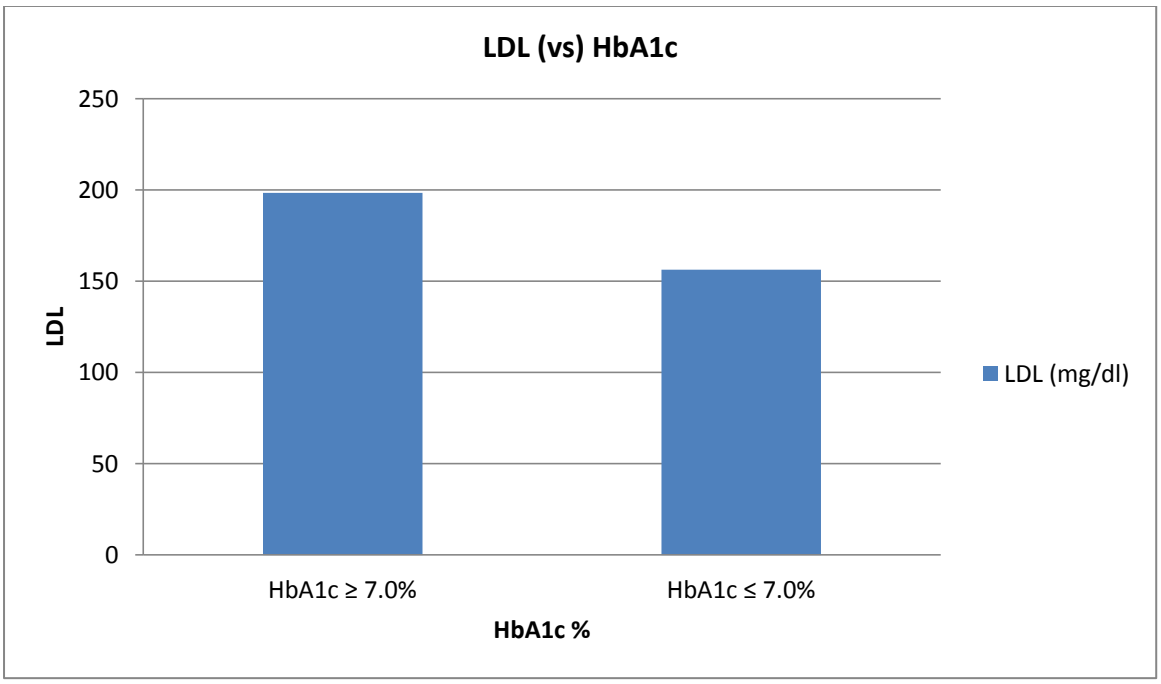

Figure 9: Correlation between LDL-cholesterol and HbA1c in poor and good glycemic control

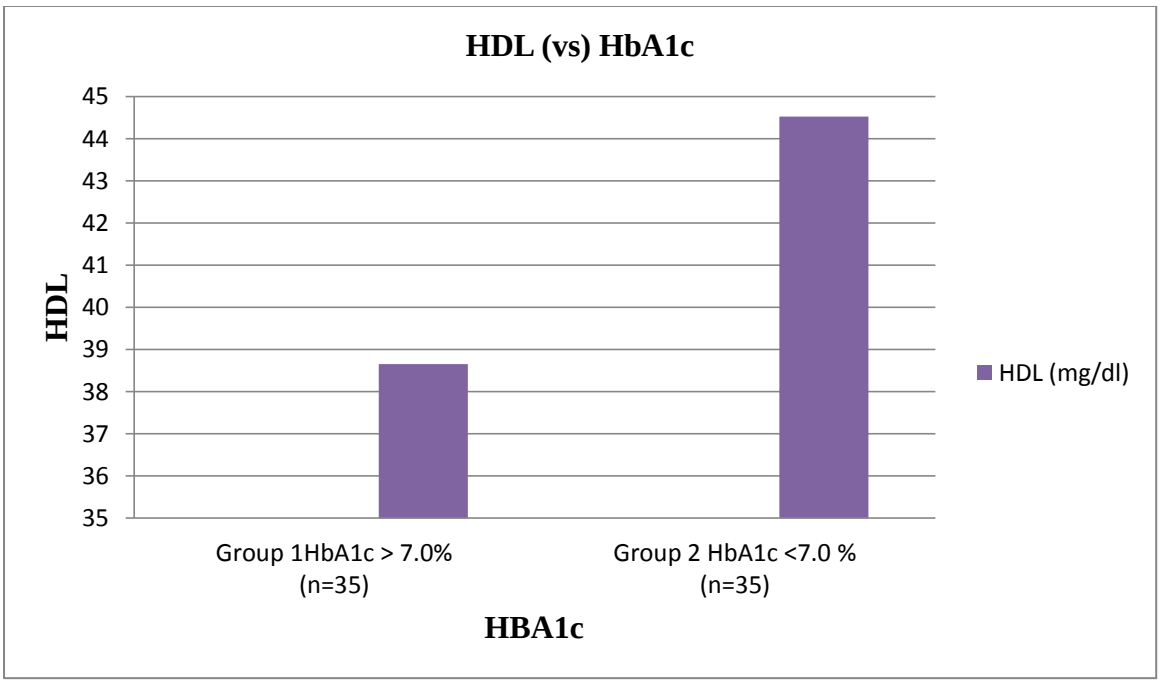

Figure 10: Graphical evaluation of HDL depicting a negative correlation between HbA1c and HDL in poor and good glycemic control.

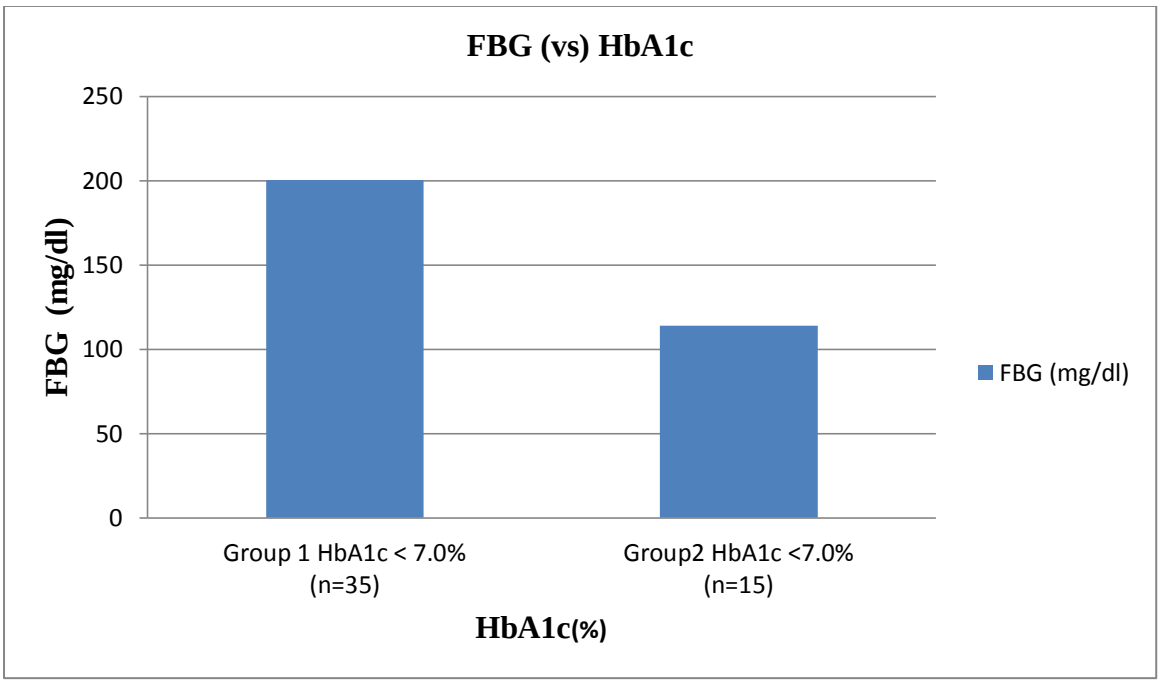

Figure 11: Comparison of FBG with HbA1c in poor and good glycemic control.

Group 1- Hyperglycemia.

CHAPTER 7

Discussion

In the present study a positive correlation of Hba1c with dyslipidemia in type-2 diabetes mellitus has been found. 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 [89,90,91]. The actual pathogenesis of diabetic dyslipidemia is not known so it still remains a challenge to find out the exact crux the of process; many evidences from the research studies suggests that insulin resistance has a central role in the development of diabetic dyslipidemia. The main cause of diabetic dyslipidemia is the increased free fatty acid that release from insulin resistant fat cells [92,93]. 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 andapolipoprotein B and cholesterol. Thus due to impaired ability of insulin to inhabit the free fatty acid release leads to increased production of hepatic VLDL cholesterol which inturn increases the degree of hepatic fat accumulation [89,94]. The features of Diabetic dyslipidemia can highly varied, however, the most common one's high triglyceride concentration, low HDL cholesterol, high total cholesterol, high LDL cholesterol, [95]. 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 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 (table 6). An observation from this study demonstrates a positive correlation between HbA1c and triglycerides, total cholesterol, LDL, FBG and HDL in both 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 6) .The mean Hb1A1c Fasting blood glucose were found higher in females than males, Among circulating lipids LDL and TGs were found extremely higher in females than the males . The mean HDL cholesterol was slightly higher in females than the males (fig 4). Diabetes confirms a marked increase in lipid

profile in both the genders but females were found to have increased susceptibility of cardiovascular diseases. However women's with diabetes were found to have increased risk of cardiovascular mortality [96].Diabetic women's are subjected to adverse changes to vascular function and other cardiovascular risk factors than diabetic men[97].The results of lipid profile showed that females diabetic patient had significantly higher levels of cholesterol, LDL ,triglycerides and fasting blood glucose and HbA1c levels other than males. Results of this study are in accordance with the studies performed by different scientists in the different areas[98,99].

In the present study diabetic patients were classified into 2 groups as per their glycemic index: group 1 consists of 35patients with HbA1c value ≥7.0% and second group consists of 15 patients with HbA1c value ≤ 7.0%.It was found that diabetic patients with HbA1c value ≥ 7.0% exhibited a major increase in TC, TG, FBG and significantly lower HDL cholesterol as compare to the patients with HbA1c value ≤ 7.0% (table 8 and fig no 6). Patients having HbA1c ≥ 7.0% were considered as diabetic dyslipidemic and those who had HbA1c was ≤ 7.0% were diabetic but maintaining a good glycemic control. Since LDL cholesterol was not checked in the patients that come for HbA1c so this parameter was excluded from the study but evidence from several studies shows that LDL cholesterol is also elevated in the diabetic patients. The present study also reveals high prevalence of hypercholesterolemia, hyper triglyceridemia, hyperglycemia and high LDL 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 shows a positive correlation was found between HbA1c and TG (Fig.7), HbAc1 and TC (Fig.8), HbA1c and LDL (figure 9), HbA1c and FBG (Fig 11) and a negative correlation was seen between HbA1c and HDL-cholesterol in both the groups (Fig 10). In this study diabetes confirms a markedly increased risk of cardiovascular events in both the groups; however group 1 with HbA1c ≥ 7.0% showed an extremely high risk of cardiovascular diseases. The results of this study are comparable to the study conducted by Ishfaq Ahmed *et al*., [89] have shown a high prevalence of dyslipidemia in type 2 diabetic subjects. High positive correlations between HbA1c and FBG have been found in the present study which is in conformity with the various studies (100). Results regarding dyslipidemia and HbA1c are also in accordance with the study conducted by khan *et al.,* (101).

The present study illustrates that most of the diabetic patients maintained a poor glycemic control that adversely affects their lipid profile and makes them prone to cardiovascular. This

study also confirms higher prevalence of dyslipidemia in diabetic patients than in non-diabetic patients. With the higher HbA1c the severity of dyslipidemia increases in diabetic patients. So diabetic patients with elevated HbA1c and dyslipidemia can be considered at a very high risk group for cardiovascular diseases. According to RohlfingCL ,*et al*., HbA1c levels ≤7.0% was said appropriate in order to reduce the risks of cardiovascular diseases. Improving glycemic control can considerably reduce the risk of cardiovascular diseases upto a great extent. Significant correlations found between HbA1c and various lipid parameters and significant differences between lipid parameters of two groups with (HbA1c ≥7.0 %and ≤ 7.0%) signifies that HbA1c can be used a potential biomarker in predicting dyslipidemia in type 2- diabetes mellitus in addition to glycemic control.

CHAPTER 8

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