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Evaluation of lipid profile and glycated haemoglobin in type 2 diabetic patients: A retrospective study

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Abstract---Background: Type 2 diabetes mellitus (T2DM) is a rapidly growing public health problem with significant implications for human health, living standards, the economy, and health-care systems around the world. Diabetic dyslipidemia is a risk factor for macrovascular (stroke, peripheral vascular disease, and coronary artery disease [CAD]) and microvascular (nephropathy, neuropathy, and retinopathy) disorders in T2DM patients. The level of circulating HbA1C is considered the gold standard of glycemic management, and it must be maintained in order to avoid T2DM problems. Aim : To investigate the association between glycated hemoglobin (HbA1C) and the lipid profile in patients with type 2 diabetes mellitus (T2DM) Methods and Materials: Between January 2021 to December 2021 researchers at the SNMMC, Dhanbad, Jharkhand, conducted a retrospective cross-sectional study. The study included 200 T2DM patients (138 females and 62 males), and the data was gathered through a review of the patients' computerised profiles. The electronic file system also contained biochemical data such as fasting plasma glucose (FPG), HbA1C, and lipid profile, as well as the patient's age, BMI, and gender. Only patients who saw their doctor on a regular basis and whose electronic records were complete were eligible.

Immunoassays were used for analysis of HbA1C while standardized automated high throughput enzymatic analyses were used for lipid profile. Results: The data of the participants was analysed by gender. BMI ($p=0.002$), HbA1C ($p=0.009$), triglycerides (TGs) ($p=0.001$), high-density lipoprotein cholesterol (HDL-C) ($p=0.002$), and low-density lipoprotein cholesterol (LDL-C) ($p=0.001$) were all substantially greater in females than in males. The study participants were divided into two groups based on their HbA1C levels (good glycemic index $\leq 7\%$ and poor glycemic index $>7\%$). Except for TGs ($p=0.020$) and HbA1C ($p=0.001$), there were no significant differences in any of the measures between the two groups. A correlation analysis of HbA1C and other factors revealed a significant association with TG ($r=0.16$, $p=0.020$), but no significant relationship with the other variables. HbA1C levels were connected with TGs ($p=0.020$) and were independent of age, BMI, TC, LDL-C, HDL-C, and FPG levels, according to the linear regression results. Conclusion: The glycated Hb was associated with TGs, and no significant association was found with age, BMI, TC, LDL-C, HDL-C and FPG levels.

Keywords---glycated hemoglobin, DMT2, glycemic control, dyslipidemia, lipid profile.

Introduction

Type 2 diabetes mellitus (T2DM) is a rapidly growing public health problem with significant implications for human health, living standards, the economy, and health-care systems around the world. According to the International Diabetes Federation (IDF), 425 million individuals in the globe have diabetes mellitus (DM), with 629 million DM patients expected by 2045 and 352 million people at risk of acquiring T2DM. Diabetic dyslipidemia is a risk factor for macrovascular (stroke, peripheral vascular disease, and coronary artery disease [CAD]) and microvascular (nephropathy, neuropathy, and retinopathy) disorders in T2DM patients.^{1,2}

According to Naqvi et al. (2017), dyslipidemia is one of the most common comorbidities associated with uncontrolled hyperglycemia in T2DM patients. In diabetics, glycated haemoglobin (HbA1C) levels are frequently evaluated to assess glycemic management. The goal is to lower the rate to below 7%. Multiple factors, including as sugar intake, exercise, and medication adherence, might alter HbA1C levels. According to several research, HbA1C could be used as a potential biomarker for predicting dyslipidemia and cardiovascular disease (CVD).^{3,4}

The level of circulating HbA1C is considered the gold standard of glycemic management, and it must be maintained in order to avoid T2DM problems. HbA1C levels are a key factor in determining the risk of diabetes-related complications and mortality, as well as glycemic control. In the literature, there are various contradictory findings, such as a Turkish study that identified a substantial association between total cholesterol (TC), LDL, triglycerides (TGs), and HbA1C, while others found no significant relationship. Likewise, whereas one

study found a substantial negative connection between HbA1C and LDL-C, others found the exact opposite.^{5,6}

Importantly, a recent study found a link between high TGs and HbA1C, leading to the conclusion that HbA1C could be a marker of TG levels and could predict CVD risk factors in T2DM. According to these studies, the association between HbA1C and lipid profile is not consistent. In order to clearly say whether HbA1C is a marker for dyslipidemia in diabetics, additional research is needed to understand the relative risks of acquiring dyslipidemia that are depending on HbA1C levels.^{7,8} The relationship between HbA1C and lipid profile in T2DM patients was explored in this study.

Materials and Methods

The protocol for this retrospective cross-sectional investigation was approved by the Research Ethics Committee at the SNMMC, Dhanbad. All participants signed a written informed permission form that covered the study and the publication of the results, and they were told about the study's purpose. The study subjects' confidentiality was preserved. Furthermore, this research was carried out in compliance with the Helsinki Declaration.

The information was gathered by evaluating the computerised profiles of the patients who took part in the study, which were stored in a medical electronic file system. The computerised file system also yielded biochemical data such as FPG, HbA1C, and lipid profile, as well as age and gender. Furthermore, patients were only chosen for the study provided they saw their doctors on a regular basis and their medical records were up to date in the system.

Instruments used in measurement of HbA1C

An immunoassay is a biochemical test that measures the presence or concentration of a macromolecule or a small molecule in a solution through the use of an antibody (usually) or an antigen (sometimes) was used. Immunoassays measure HbA1C specifically; antibodies recognize the structure of the N-terminal glycosylated amino acids (usually the first 4–10 amino acids) of the Hb β chain. Ion-exchange HPLC separates Hb species based on charge differences between HbA1C and other hemoglobins. The molecule detected by the immunoassay is often referred to as an "analyte" and is in many cases a protein, although it may be other kinds of molecules, of different sizes and types, as long as the proper antibodies that have the required properties for the assay are developed. Analytes in biological liquids were measured using immunoassays.

Immunoassays come in many different formats and variations. Immunoassays may be run in multiple steps with reagents being added and washed away or separated at different points in the assay. Multi-step assays are often called separation immunoassays or heterogeneous immunoassays. Some immunoassays can be carried out simply by mixing the reagents and sample and making a physical measurement. Such assays are called homogeneous immunoassays, or less frequently non-separation immunoassays.

The use of a calibrator is often employed in immunoassays. Calibrators are solutions that are known to contain the analyte in question, and the concentration of that analyte is generally known. Comparison of an assay's response to a real sample against the assay's response produced by the calibrators makes it possible to interpret the signal strength in terms of the presence or concentration of analyte in the sample. Immunoassays employ a variety of different labels to allow for detection of antibodies and antigens. Labels are typically chemically linked or conjugated to the desired antibody or antigen.

Instruments used for measurement of lipid profile

The measurement of plasma or serum cholesterol and triglycerides was carried out with the standardized automated high throughput enzymatic analyses, which achieved within and between run coefficients of variation of < 5%. These assays have been widely used since the 1980s, and have been standardized via the Center for Disease Control's Lipid Standardization program originally initiated by the National Heart, Lung, and Blood Institute for the Lipid Research Clinics Primary Prevention Trial. Our assays were standardized through the Centers for Disease Control Lipid Standardization Program.

Measurement of fasting blood sugar

A small device called a glucose meter or glucometer measures how much sugar is in the blood sample. The drop of blood you get with a finger prick is often enough to use on a test strip. A finger prick can be done with a special needle (lancet) or with a spring-loaded device that quickly pricks the fingertip.

Inclusion criteria

- Patients having a confirmed diagnosis of T2DM were chosen using criteria provided by the American Diabetes Association in 2007.
- Patients fulfilling the criteria like HbA1C 6.5 percent, FPG 126 mg/dl (7.0 mmol/l), 2-h plasma glucose 200 mg/dl (11.1 mmol/l) during an oral glucose tolerance test (OGTT), or random plasma glucose 200 mg/dl (11.1 mmol/l) as levels indicative of T2DM.

Exclusion Criteria

- Patients with CVD, thyroid abnormalities, renal issues, and other endocrinopathies,
- Patients on lipid-lowering medicines
- Patient with type 1 diabetes

Patients of both genders who visited the outpatient department between October 2019 and August 2020 were chosen. There were 200 patients who fit our criteria, and their histories and examination data on T2DM presenting symptoms, complications, treatment modalities, smoking and social drug use, and any other addictions were recorded. The sample size was calculated using OpenEpi version 3.

$$n=[DEFF*Np(1-p)]/[(d^2/Z^2_{1-\alpha/2}*(N-1)+p*(1-p)];$$

The prevalence of the problem was taken at 62.5% and the confidence level at 80%. The calculated sample size was 200. Anthropometric measurements (weight, height, and BMI), blood pressure, and laboratory results, including HbA1C, TC, TG, LDL-C, and HDL-C levels, were gathered from all patients. Blood samples were taken from all DM patients between 8:00 and 10:00 a.m. (12–14 h fasting), and plasma was utilised to estimate glucose levels. An autoanalyzer was used to determine the FPG, HbA1C, and lipid profile levels (Roche Modular P-800, Roche Diagnostics, Germany). We categorised the individuals' glycemic control as poor (HbA1C>7%) or good (HbA1C 7%) for analysis.

Statistical Analysis

The data was computed using SPSS version 21 (IBM Corp., Armonk, NY, USA). The data were found to be regularly distributed using the Shapiro–Wilk test. For comparison, the Student's t-test was utilised, and quantitative data were expressed as mean and standard deviation. The Pearson correlation coefficient was used to determine the relationship between distinct parameters, and an independent sample t-test was used to determine the mean difference between them. The connection between HbA1C and lipid profile, FPG, BMI, and age was determined using a linear regression test; the results were considered non-significant when the p-value was >0.050.

Statistically significant criteria

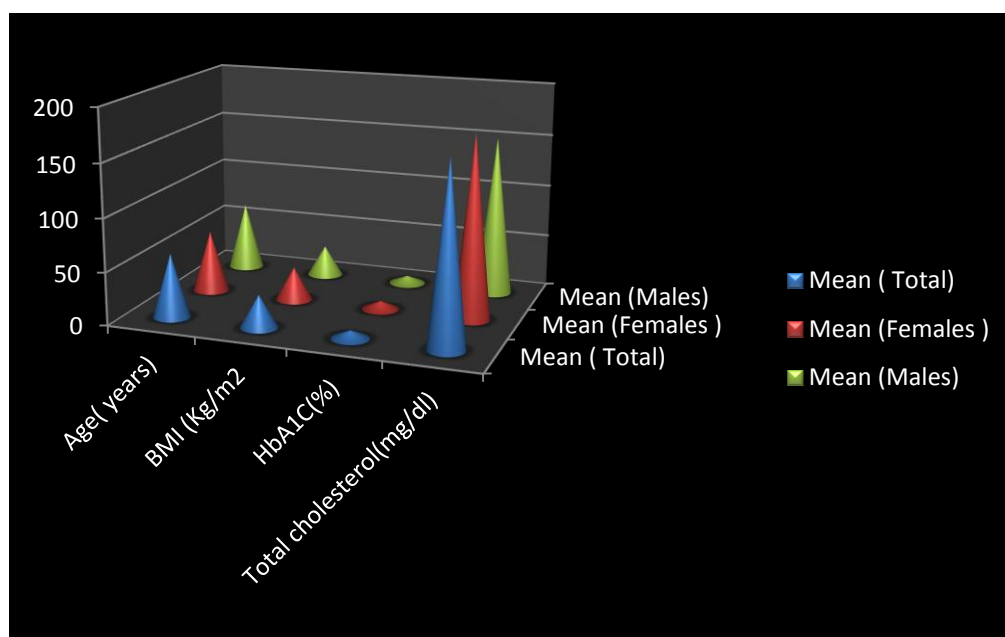
The connection between HbA1C and lipid profile, FPG, BMI, and age was determined using a linear regression test; the results were considered non-significant when the p-value was >0.050.

Results

A total of 200 T2DM patients were selected for the study (138 females and 62 males). The participants' basic characteristics were analyzed and compared according to gender (Table 1, Table 4, Graph 1 and Graph 3). The females had significantly higher values for BMI ($p=0.002$), TC ($p<0.001$), HDL-C ($p=0.002$), LDL-C ($p<0.001$) and HbA1C ($p=0.009$) compared to the males, while the age of the males was, on average, significantly higher than the females ($p<0.001$). There were 43.69% patients in the group with HbA1C levels <7%, and 56.31% subjects in the group with HbA1C levels >7%. There was no significant difference in any parameter except for TG level ($p=0.020$) and HbA1C ($p<0.001$) (Table 2, Table 5, Graph 2 and Graph 4). The Pearson correlation of HbA1C with other variables is shown in Table 3. A significant correlation of HbA1C was observed with TG ($r=0.16$, $p=0.02$) but there were no significant correlations with the other parameters. The results from a linear regression analysis indicated that the HbA1C values were associated with TG ($p=0.020$) and were independent of age, BMI, TC, LDL-C, HDL-C and FPG levels (Table 3).

Table 1: Gender-wise comparison of Age, BMI, HbA1C, total cholesterol of type 2 diabetes mellitus patients

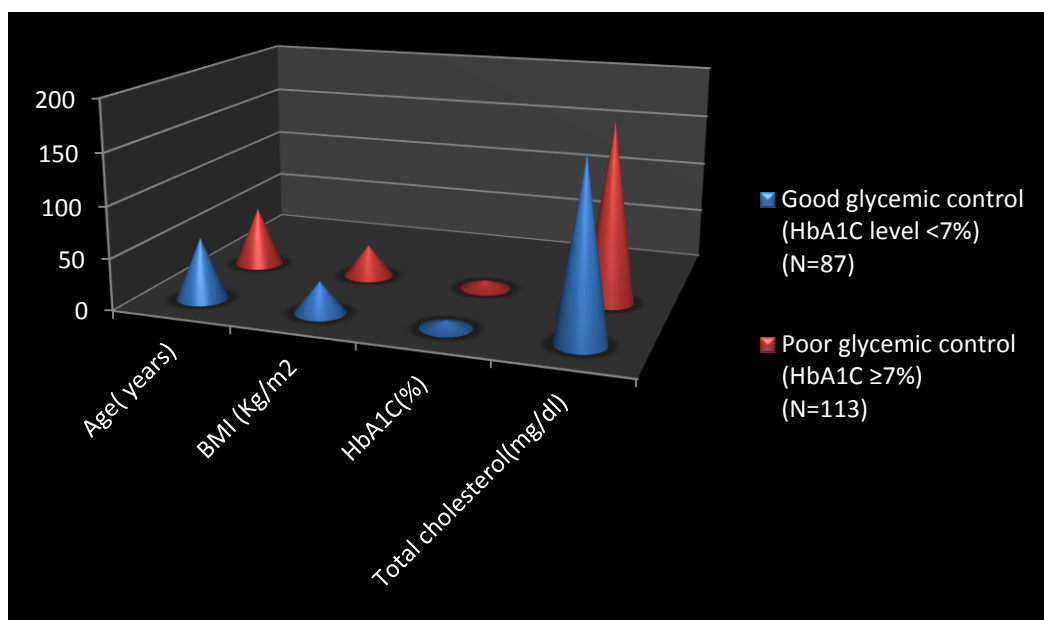
Parameters	Mean $\pm$ SD Total (N=200)	Mean $\pm$ SD Females (N=138)	Mean $\pm$ SD Males (N=62)	p-value
Age (yrs)	60.46 $\pm$ 13.54	58.53 $\pm$ 14.19	64.66 $\pm$ 10.99	<0.001*
BMI (Kg/m ²) *	30.8 $\pm$ 6.1	31.59 $\pm$ 6.56	29.08 $\pm$ 4.58	0.002
HbA1C(%)	7.65 $\pm$ 1.78	7.86 $\pm$ 1.86	7.20 $\pm$ 1.53	0.009*
Total cholesterol (mg/dl)	170.14 $\pm$ 40.9	174.40 $\pm$ 39.8	152.35 $\pm$ 40.2	<0.001*



Graph 1: Gender-wise comparison of Age, BMI, HbA1C, total cholesterol of type 2 diabetes mellitus patients

Table 2 : Comparison of Age, BMI, HbA1C, total cholesterol of type 2 diabetes mellitus patients according to their glycemic control

Parameter	Good glycemic control (HbA1C level <7%) (N=87)	Poor glycemic control (HbA1C $\geq$ 7%) (N=113)	p-value
	Mean $\pm$ SD	Mean $\pm$ SD	
Age (yrs)	61.6 $\pm$ 13.13	59.58 $\pm$ 13.85	0.29
BMI (Kg/m ²)	30.10 $\pm$ 5.45	31.34 $\pm$ 6.54	0.139
HbA1C (%)	6.13 $\pm$ 0.60	8.84 $\pm$ 1.47	<0.001
Total cholesterol (mg/dl)	162.80 $\pm$ 37.89	170.92 $\pm$ 43.31	0.154



Graph 2: Comparison of Age, BMI, HbA1C, total cholesterol of type 2 diabetes mellitus patients according to their glycemic control

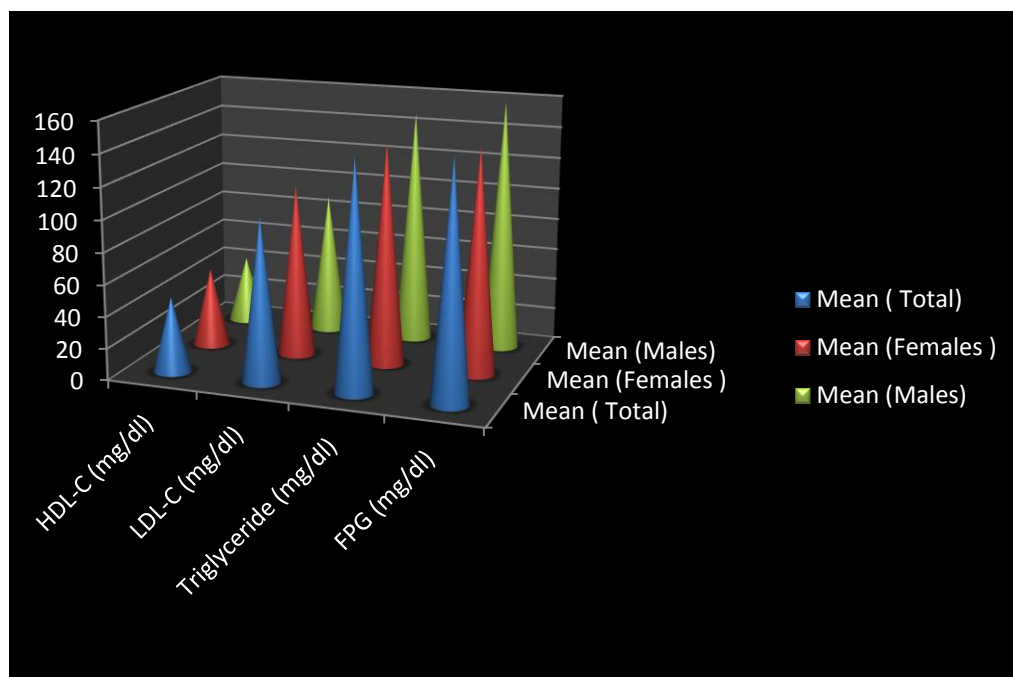
Table 3 : Correlation analysis (between HbA1C and age, BMI, FBS, and lipid parameters) and linear regression analysis of T2DM patients showing dependency of HbA1C on other variables

Parameter	Correlation		Regression analysis	
	Coefficient	p-value	Unstandardized Coefficients b	p-value
Age (yrs)	-0.066	0.346	-0.049	0.56
BMI (Kg/m ²)	0.035	0.614	0.00	0.63
Total cholesterol (mg/dl)	0.132	0.06	0.133	0.47
HDL-C (mg/dl)	-0.084	0.232	-0.113	0.12
LDL-C (mg/dl)	0.093	0.186	-0.553	0.54
Triglyceride (mg/dl)	0.164	0.02*	0.351	0.02*
FPG (mg/dl)	-0.092	0.191	-0.052	0.40

Table 4: Gender-wise comparison of HDL-C, LDL-C, Triglyceride, FPG of type 2 diabetes mellitus patients

Parameters	Mean $\pm$ SD Total (N=200)	Mean $\pm$ SD Females (N=138)	Mean $\pm$ SD Males (N=62)	p-value
HDL-C (mg/dl)	47.56 $\pm$ 16.2	49.88 $\pm$ 17.01	42.53 $\pm$ 13.14	0.002*
LDL-C (mg/dl)	103.24 $\pm$ 36.3	109.43 $\pm$ 36.34	89.32 $\pm$ 31.70	<0.001*
Triglyceride	143.48 $\pm$ 86.8	140.82 $\pm$ 77.94	148.79 $\pm$ 103.6	0.57

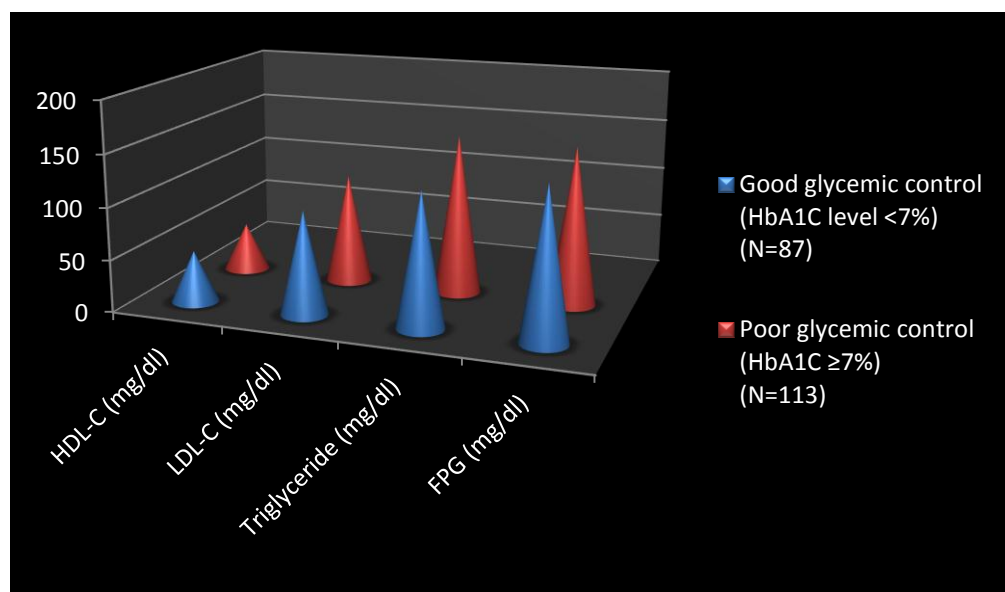
(mg/dl)				
FPG (mg/dl)	147.78±58.7	142.38±51.66	159.3±58.88	0.07



Graph 3: Gender-wise comparison of HDL-C, LDL-C, Triglyceride, FPG of type 2 diabetes mellitus patients

Table 5 : Comparison of HDL-C, LDL-C, Triglyceride, FPG of type 2 diabetes mellitus patients according to their glycemic control

Parameter	Good glycemic control (HbA1C level <7%) (N=87)	Poor glycemic control (HbA1C≥7%) (N=113)	p-value
	Mean ± SD	Mean ± SD	
HDL-C (mg/dl)	49.49±17.78	46.40±15.08	0.20
LDL-C (mg/dl)	99.38±37.89	105.95±34.41	0.21
Triglyceride (mg/dl)	128.42±70.85	154.11±96.65	0.02*
FPG (mg/dl)	144.36±56.88	152.28±55.44	0.32



Graph 4: Comparison of HDL-C, LDL-C, Triglyceride, FPG of type 2 diabetes mellitus patients according to their glycemic control

Discussion

HbA1C levels could be utilised as a biomarker to identify T2DM patients at risk of CVD and as a treatment guidance. Our findings show that HbA1C and TG have a substantial beneficial connection. Several previous studies have identified a favourable link between HbA1C and high TG levels, which is consistent with the findings of this study, though one other study found no correlation between HbA1C and TG. HbA1C is a direct sign of elevated TG and indirectly aids in estimating the risk of macro- and microvascular issues, according to the findings of this study and the others stated above.^{8,9}

Insulin resistance is thought to be the cause of dyslipidemia in T2DM patients. Increased TG levels in T2DM patients are caused by insufficient insulin secretion or function, which results in higher hepatic secretion of very low-density lipoprotein (VLDL) and late clearance of TG-rich lipoproteins, owing to increased substrate levels for TG synthesis.^{10,11} There was no link between HbA1C and TC or LDL-C in this investigation. Our findings are in line with those of another study, which found no significant association between these variables. However, these results are inconsistent with the results of numerous other studies that have stated a significant relationship between HbA1C and TC and LDL-C.^{12,13}

In addition, our findings reveal a statistically insignificant negative relationship between HbA1C and HDL-C. This is consistent with the findings of a few other research, but not with the findings of numerous other studies that found a significant negative connection between HbA1C and HDL-C. A positive connection between HbA1C and HDL-C has only been described in a few other studies.^{14,15} Our findings could be explained by the fact that our female participants had considerably higher HbA1C levels than the male participants, as well as the fact

that females normally had greater HDL-C levels than males. As a result, there was no discernible negative association. The linear regression study demonstrated a relationship between HbA1C levels and TGs ($p=0.020$), as well as the fact that the correlation is unaffected by age, BMI, TC, LDL-C, HDL-C, or FPG levels. HbA1C could potentially be a predictor of TG, TC, and LDL-C, according to Hussain et al (2017).^{16,17}

Females had considerably higher BMI, TC, LDL-C, HDL-C, and HbA1C values than males, according to the gender-wise comparison. Similar findings have been found in a few other investigations. However, in this situation, our findings differ from those of the other research in some aspects. One of the reasons for the gender-wise difference in lipid parameters could be the influence of sex hormones on the distribution of body fat that causes altered lipoprotein levels.^{18,19} The disparity in our findings could be attributed to differences in BMIs and ages between the two groups, as well as the length of time after DM diagnosis. Our participants had a BMI of more than 30, indicating that they were overweight. Obesity and physical inactivity have been linked to poor blood sugar regulation, according to Firouzi et al (2015).^{20,21}

The current investigation discovered that participants with HbA1C levels above 7% (bad glycemic control) had considerably greater TG levels than those with HbA1C levels below 7% (excellent glycemic control); however, no significant differences in the other parameters were discovered. Another study found that participants with HbA1C of more than 7% had significantly higher levels of TC, LDL-C, and TGs, as well as lower HDL-C, than those with HbA1C of less than 7%. Patients with strong glycemic control appear to have less dyslipidemia than those with poor glycemic control.^{22,23}

According to a recent study, the complexity of CAD tends to rise with age, high HbA1C, high LDL-C, elevated TGs, and decreased HDL-C levels. According to Hussain et al. (2017), HbA1C is not only a reliable glycemic index but also a predictor of dyslipidemia.^{24,25} The mean blood glucose level varies by approximately 35 mg/dl for every 1% increase in HbA1C readings above the usual threshold. Importantly, a one-percentage-point decrease in HbA1C lowers the risk of microvascular problems by 40%. The literature, on the other hand, shows that increased physical activity and lifestyle changes can help improve glycemic control and dyslipidemia.²⁶

Diabetes patients are advised to join a fitness programme and engage in regular exercise consisting of 30 minutes of moderate-intensity physical activity 4–6 times per week, with a minimum calorie expenditure of 200 Kcal. To minimise future difficulties, it is also recommended that every family physician be aware of the link between HbA1C and hyperlipidemia in T2DM patients and monitor their lipid profile and HbA1C at least twice a year. The variations we identified in our study in South Africa compared to studies from other areas of the world regarding the link between HbA1C and lipid profile parameters could be due to population variances, as dyslipidemia is already common in SA, even among non-diabetics.²² All of the participants in our study were on various anti-diabetic drugs. However, we were unable to evaluate the data by treatment modalities, and

it is possible that this patient grouping might have influenced the study's findings.²⁶

The study's strength is that we had all of the patients' biochemical data and ran a comparison, correlation, and regression analysis on it. The current investigation had several limitations, including the fact that it was a retrospective study with a small sample size and that patients' food habits, lifestyle patterns, time since diagnosis with DM, and duration of regular physical activity were unknown.

Conclusion

HbA1C was associated with TGs, while no significant associations were found with age, BMI, TC, LDL-C, HDL-C or FPG levels. Therefore, the use of HbA1C as a sign of dyslipidemia in our population should be undertaken with caution.

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