Type 2 diabetic patients and Hba1c as a potential biomarker for dyslipidemia occurrence

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ABSTRACT

Abnormalities of lipid metabolism are important risk factors for an increased incidence of diabetes-related complications including coronary heart disease, peripheral artery disease and stroke. One of the most important indicator of the risk of this complication is the increase of HbA1c and the abnormalities of lipid profile. The aim of this study was to trace back if there is an association of HbA1c increase and the increase of lipid profile in *type 2 diabetic* patients. The 50 sample from patients with *type 2 diabetes* and another 50 sample was taken as a control from non-diabetes (normal), blood was collected and serum HbA1c was measured by COBAS INTEGRA 400 PLUS. Total cholesterol, HDL-C, LDL-C and TG levels measured by using reagents kit of bio-labo in which an open system device based on enzymatic substrate reaction (BIOLABO-KENZAMAX Device) was used. Results shows there is a significant increase of HbA1c, total cholesterol, HDL-C and LDL-C in patients with *type 2 diabetes* in comparison with control. The difference between control and samples was statistically significant and the p-value for HbA1c ($p = 0.00$), cholesterol ($p = 0.002$), HDL-C ($p = 0.003$) and for LDL-C ($p = 0.001$). The negative associations between *type 2 diabetes* and *Triglycerides* were found in which p-value was 0.362. I conclude, the elevation level of HbA1c companied with elevation of *total cholesterol*, HDL-C and LDL-C in patients with *type 2 diabetes*. The measurement of HbA1c may take as a potential biomarker for predicting *dyslipidemia* in patients with *type 2 diabetes*.

Key words: *glycated hemoglobin* (HbA1c), *Type 2 diabetes mellitus* (T2DM), *Total cholesterol* (TC), HDL-C, LDL-C, *Triglycerides* (TG), *Cardiovascular disease* (CVD).

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INTRODUCTION

Globally, *type 2 diabetes mellitus* (T2DM) is a rapidly rising public health problem with a significant impact on human health, living standards, the economy and health care systems (Khan M., et. al., 2020). Statistics from the International Diabetes Federation (IDF) indicated that 425 million adults worldwide have diabetes mellitus (DM) and that by 2045, 629 million DM patients and 352 million people will be at risk of developing T2DM (Sylvain R. S., et. al., 2020).

T2DM patients are at risk for diabetic dyslipidemia, and this puts them at greater risk of developing macro-vascular disease (stroke, peripheral vascular disease, coronary artery disease (CAD) and micro vascular disease (nephropathy, neuropathy, and retinopathy) (Leon B. M., & Maddox T. M., 2015). Some studies have reported that in patients with T2DM, complications and the most common of which are those associated with uncontrolled hyperglycemia is dyslipidemia (Naqvi S., et. al., 2017). One of the measures used to monitor diabetes, by routinely measuring glycated hemoglobin (HbA1c) levels once every 3 months to monitor their blood sugar. The goal is to keep it below 7%. HbA1c levels are affected by several factors, including sugar intake, exercise and adherence to medication (Jafarian A., et. al., 2018 and Sherwani S., et. al., 2016). Some studies have reported that HbA1c could potentially be used as a potential biomarker for predicting dyslipidemia and cardiovascular disease (Sherwani S. I., et. al., 2016 and Sarkar S. M., 2017).

The level of circulating HbA1C is taken as the gold standard of glycemic control, and regulating it is imperative for avoiding T2DM complications. HbA1c values not only reflect glycemic control but are also the main factor in determining the risk of diabetes-related complications and mortality (Hussain A., et al. 2017 and Gebregziabher M., et. al., 2010). There are several conflicting results in the literature, such as a Turkish study that found a significant relationship between total cholesterol (TC), LDL, triglycerides (TGs) and HbA1c (Ozder A., 2014), while others reported a significant negative relationship between HbA1c and LDL-C (Alzahrani S. H., et. al., 2019). The aim of this study was to find out if there is an association between glycated hemoglobin and disorders of serum lipid profile in type 2 diabetic patients.

MATERIAL AND METHODS:

- **Samples collection**

5 ml of blood was withdrawn from the patients after a fasting of 8-9 hours. It was collected in the designated tubes. 3 ml in a white tube, free from additives such as anticoagulants, and there are types of them that add silicone or gel and 2 ml in an EDTA tube which contains an anticoagulant, the white tube was separated in Centrifuge for 5 minutes at 3000rpm shortly after collection and samples serum were collected. For Measurement of HbA1c, EDTA tube was used and COBAS INTEGRA 400 PLUS used to assess HbA1c.

- **HbA1c**

The anticoagulated whole blood specimen is hemolysis automatically on the Cobas Integra 400 plus analyzer with Cobas Integra Hemolyzing Reagent Gen.2. This method uses TTAB (Tetra-decyltrimethylamonium bromide) as the detergent in the Hemolyzing reagent to eliminate interference from leukocytes (TTAB does not lyse leukocytes). All hemoglobin variants which are glycated at the beta-chain N-terminus and which have antibody recognizable regions identical to that of HbA1c are measured by this assay.

- **TOTAL CHOLESTEROL**

Measurement of cholesterol in serum and plasma sample by manual procedure on a BIOLABO - KENZAMAX Device analyzer with bio-labo reagent. This method uses the enzymatic method. The absorbance is read at the wavelength of 505 nm.

- **HDL-CHOLESTEROL**

Measurement of HDL-CHOLESTEROL in serum and plasma sample by manual procedure on a BIOLABO-KENZAMAX Device analyzer with bio-labo reagent. This method uses the enzymatic method. The absorbance reading is done at a wavelength of 600 nm.

- **LDL-CHOLESTEROL**

The LDL value is calculated by a manual equation which is {LDL = CH – (HDL + TG/5)}. Or it is calculated using the Ewan automatic system after entering the results of cholesterol, triglycerides and HDL.

- **TRIGLYCERIDES**

Measurement of triglycerides in serum and plasma sample by manual procedure on a BIOLABO-KENZAMAX Device analyzer with bio-labo reagent. This method uses the enzymatic method. The absorbance is read at the wavelength of 505 nm.

DATA ANALYSIS

Statistical analysis was performed using Microsoft Excel 2007 and IBM SPSS version 23, and the statistical procedure was performed to compare the mean/mean of two independent groups (diabetes patients and normal individuals) to determine if there was a significant difference between them in the lipid profile test. The lipid profile tests were compared between diabetic patients and normal conditions using the T. test. Differences are expressed as mean ± standard deviation (SD). P-value ≤ 0.05 was considered statistically significant.

RESULTS

A total of 100 individuals aged from 20 to 70 years old was divided into two groups (50 people with type 2 diabetes and 50 non-diabetic as control group). Collected blood samples was used to find the association of rising HbA1c and lipid levels in patients with type 2 diabetes in comparison with control group (Non-diabetics).

1. **HbA1c**

Comparison showed between the HbA1c levels of diabetics type 2 and control. The result expressed as Mean ± SD. The HbA1c in comparison to patient's samples was 7.496 ± 0.826, and the average value of HbA1c for control was 5.448 ± 0.456 (table 1 &fig1).

Table 1. Statistical significance and the mean values of the HbA1c in T2DM and Non diabetic.

Tests	Mean	Std. Deviation	T Test	P – Value
HbA1c In Non–	5.448	0.456	–14.962	0.000*
HbA1c In Diabetic	7.496	0.826		

*Significant P ≤ 0.05

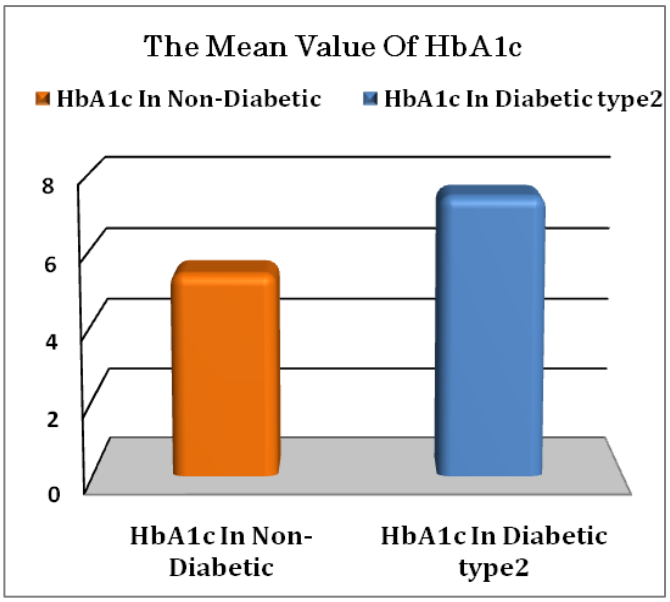

Figure 1: Mean values of HbA1c in type 2 diabetics and non–diabetic (p=0.00).

2. Total Cholesterol:

Total cholesterol in diabetic type 2 in comparison to non–diabetics (control). The result expressed as Mean ± SD. Diabetics samples 178.896 ± 53.180 and Control 145.938 ±38.032 (Table 2& fig2).

Table 2. Statistical significance and the mean values of the total cholesterol in T2DM and Non-diabetic individuals.

Tests	Mean	Std. Deviation	T Test	P - Value
Cholesterol In Non-Diabetic	145.938	38.032	-3.343	0.002*
Cholesterol In Diabetic type2	178.896	53.180		

*Significant P ≤ 0.05

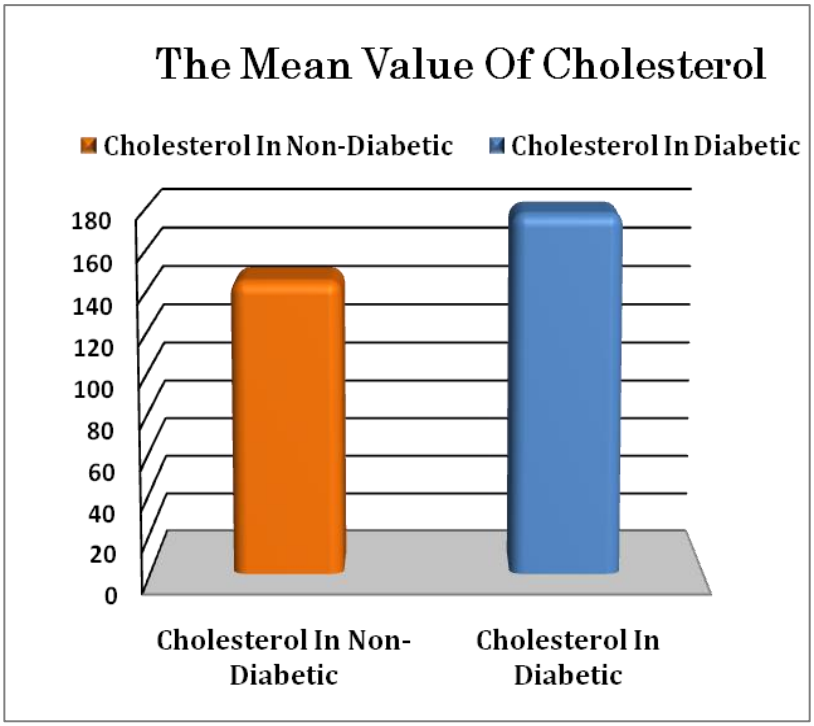

Figure 2: Mean values of Cholesterol in T2DM and non-diabetics (p=0.002).

3. HDL-C:

Comparison of HDL-C levels of diabetics type 2 and control. The result expressed as Mean ± SD. The HDL-C in patient's samples was 38.276 ± 9.183, and for the control was 91.834 ± 117.771 (table 3& fig3).

Table 3. Statistical significance and the mean values of the HDL-C in Non-diabetic and T2DM.

Tests	Mean	Std. Deviation	T Test	P - Value
HDL-C In Non-Diabetic	38.276	9.183	-3.179	0.003*
HDL-C In Diabetic type 2	91.834	117.771		

*Significant P ≤ 0.05

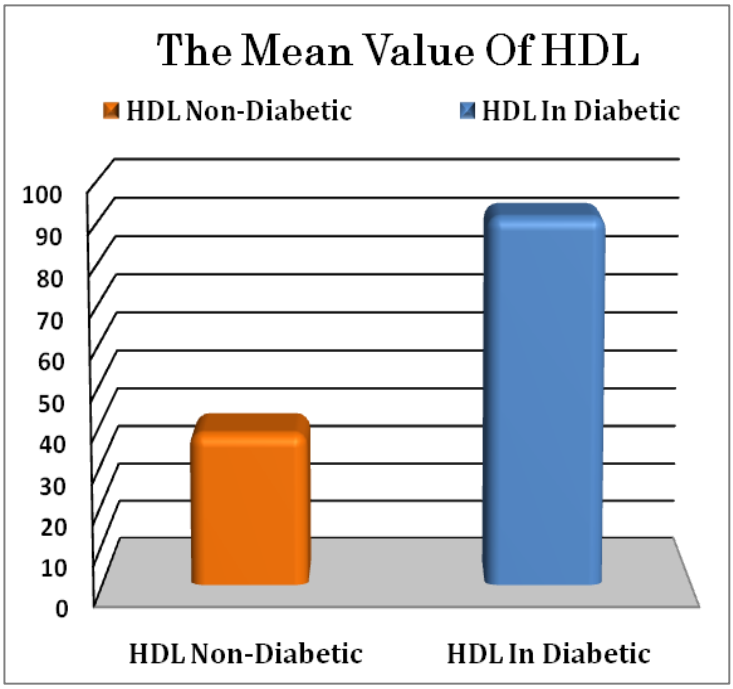

Figure 3: Mean values of HDL-C in diabetics type 2 and non-diabetics (p=0.003).

4. LDL-C:

Comparison of LDL-C in diabetics type 2 with control. The result expressed in mg/dl of the Mean ± SD. Sample LDL-C was 75.516 ± 25.266, and for the control was 103.26 ± 51.233 (Table 4& fig4).

Table 4. Statistical significance and the mean values of the LDL-C in T2DM and Non-diabetic.

Tests	Mean	Std. Deviation	T Test	P - Value

LDL In Non-Diabetic	75.516	25.266	-3.432	0.001*
LDL In Diabetic type 2	103.286	51.233		

***Significant P ≤ 0.05**

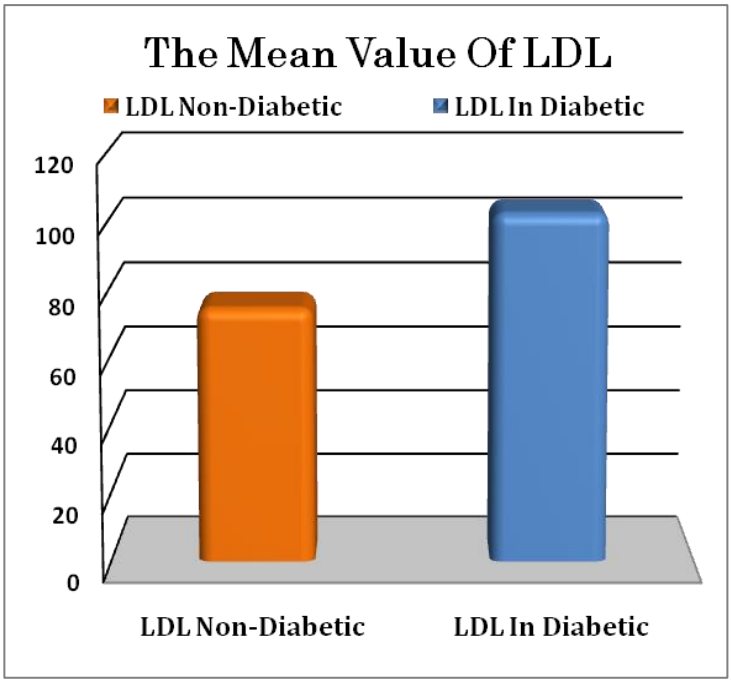

Figure 4: Mean values of LDL in diabetic type 2 and non-diabetic (p=0.001).

5. Triglycerides:

Comparison of triglycerides levels in diabetics type 2 with control. The result expressed in mg/dl of the Mean ± SD. The triglycerides in patient's samples was 115.368 ± 41.395, and the average value of triglycerides for control was 105.022 ± 71.657 (Table 5 & fig5).

Table 5: The mean values of the Triglyceride in T2D and Non-diabetic.

Tests	Mean	Std. Deviation	T Test	P - Value

Triglyceride In Non-Diabetic	115.368	41.395	0.920	0.362 **
Triglyceride In Diabetic type 2	105.022	71.657		

** **P > 0.05**

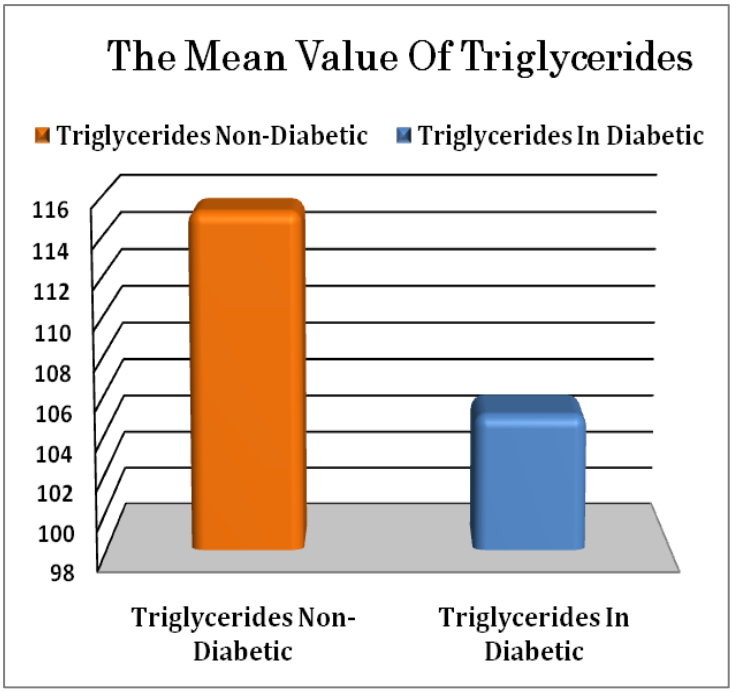

Figure 5: Mean values of Triglycerides in diabetic type 2 and non-diabetic.

DISCUSSION:

Patients with hyperlipidemia have a higher incidence of type 2 diabetes and abnormal glucose metabolism (Zhang L., et. al., 2008). Several studies have shown a significant increase in morbidity and mortality from type 2 diabetes and cardiovascular disease (Cui H.B., et. al., 2007 and Wang S.H., et. al., 2009). It was also proven that the formation of lipids in the blood is worse in diabetic patients than in non-diabetic individuals from different ethnic groups (Zhang L., et. al., 2008 and Zhang L., et. al., 2009).

This study showed an increase of HbA1c levels in patients with type 2 diabetes compared with the control group (figure 1). The increase of HbA1c was companied with increase of total Cholesterol (figure 2), high-density lipoprotein cholesterol (figure 3) and low-density lipoprotein cholesterol (figure 4). The difference of increase between sample and control group was statistically significant in which the p-values was less than 0.05. For HbA1c (P = 0.002), cholesterol (P = 0.002), HDL-C (P = 0.003) and LDL-C (P = 0.001). On the other hand, the Triglycerides (TG) measurements shows no difference in comparison with control level (the difference between sample and control was not significant) in which the p-value was greater than 0.05 (Table 5). This finding results was in line with other studies carried in the same assessment (Sherwani S. I., et. al., 2016 and Sarkar S. M., 2017). A study conducted in China 2018 found that long-term low levels of triglycerides in people with type 2 diabetes cause cardiovascular disease (Ren Y., et al., 2018).

Other studies have found that insulin increases the number of LDL-C receptors, so chronic insulin deficiency may be associated with a low level of LDL receptors, and this causes an increase in LDL particles and leads to an increase in the value of LDL-C in diabetes (Aclan Ozder, 2014). Another study found a significant relationship between total cholesterol (TC), LDL-C, triglycerides (TGs) and HbA1c (Ozder A., 2014), while others reported a significant negative relationship between HbA1c and LDL-C (Alzahrani S. H., et. al., 2019).

CONCLUSION

Significant correlation level of HbA1c with parameters of lipid profile suggests utility of HbA1c as a marker of dyslipidemia in addition to chronic hyperglycemia and hence should be analyzed accordingly. This study adds to the existing literature suggesting HbA1c as an indicator for dyslipidemia.

الملخص:

تعتبر اضطرابات أيض الدهون من العوامل الهامة في زيادة حدوث التعقيدات المنسوبة للسكري مثل مرض القلب التاجي وامراض الشرايين المحيطة و السكتة القلبية. من أحد أهم هذه المؤشرات المعقدة لهذا الخطر هو ارتفاع السكر التراكمي و مجموعة الدهون. يهدف هذا البحث الي تتبع ما اذا كان هناك علاقة لارتفاع السكر التراكمي و مجموعة الدهون عند مرضى السكري من النوع الثاني. تم أخذ 50 عينة من مرضى السكري النوع الثاني و 50 عينة أخرى من أشخاص أصحاء(ليس لديهم سكري) للمقارنة بين الفئتين حيث تم استخدام مصل الدم من الفئتين لقياس المعدل التراكمي باستخدام جهاز (Cobas integra 400plus). اجمالي الكولستيرول و والكولستيرول مرتفع الكثافة و الكولستيرول منخفض الكثافة و مستوى الدهون الثلاثية تم قياسهم باستخدام مواد شركة (Bio–labo) واستخدام أجهزة النظام المفتوح المعتمد على تفاعل الانزيم ومادته الفعالة (Biolabo–Kenzamax Device). أظهرت النتائج ارتفاع ذو دلالة احصائية لكل من ارتفاع السكر التراكمي و الكولستيرول الاجمالي و الكولستيرول مرتفع ومنخفض الكثافة لذا الأشخاص الدين لديهم السكري من النوع الثاني مقارنةً بالأشخاص الغير مصابين بهذا النوع. الفارق بين الشريحتين التي خضعت للدراسة كان ذو دلالة احصائية أي أن القيمة المحتملة كانت أقل من 0.05 (p–value ≤ 0.05) حيث كانت الدلائل الاحصائية للسكر التراكمي (p=0.00) والكولستيروت (p=0.002) وكولستيرول عالي الكثافة (p=0.003) والكولستيرول منخفض الكثافة (p=0.001) بينما العلاقة بين التراكمي والدهون الثلاثية وجدت سالبة اي انها ذات دلالة احصائية سالبة (p=0.362). نستخلص من هذه النتائج أن ارتفاع معدل السكر التراكمي قد اقترن بارتفاع في اجمالي الكولستيرول وكولستيرول مرتفع ومنخفض الكثافة عند مرضى السكري من النوع الثاني الامر الذي يمكن فيه اعتبار ارتفاع معدل التراكمي عند هذه الشريحة من مرضى السكري كمؤشر على حدوث اضطراب دهني متوقع.

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