

## RESEARCH PAPER

# The impact of severe hypertriglyceridemia on glycosylated hemoglobin test in type 2 diabetes mellitus

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### Abstract

**Background:** although the glycosylated hemoglobin (HbA1c) test is a valid test for diabetes diagnosis and evaluation of glycemic control, many factors are known to affect the results of the HbA1c test and make it reflect a false image of the glycemic control. One of these factors is hypertriglyceridemia.

**Objectives:** the study aims to assess the impact of severe hypertriglyceridemia (TG  $\geq$  1000 mg/dl) on HbA1c test results in type 2 diabetes mellitus.

**Method:** a retrospective study on two groups of adult patients with type 2 DM; were implemented in Faiha Diabetes, Endocrine and Metabolism Centre (FDEMC) in Basrah-Iraq. A total of 267 patients with type 2 DM and severe hypertriglyceridemia and a total of 313 patients with type 2 DM and normal triglycerides levels were included. Then the mean HbA1c of the patients with severe hypertriglyceridemia was measured in each FBS range and compared to the mean HbA1c of the patients with normal triglycerides levels in the same FBS range to determine the impact of severe hypertriglyceridemia on the readings of HbA1c in the diabetic patients.

**Results:** the mean HbA1c in both groups was compared in each FBS range and revealed that despite of recording nearly the same mean FBS, HbA1c was higher in patients with severe hypertriglyceridemia by 1.33% in the FBS range 130-149 mg/dl with a statistically significant p-value = 0.003. While it was lower in the FBS range 200-229 mg/dl, 230-259 mg/dl, 260-299 mg/dl, 300-349 mg/dl and 350-399 mg/dl by 1.02%, 0.85%, 1.77%, 2.29% and 2.23%, respectively with a statistically significant p-value that equal to 0.005, 0.034, < 0.001, < 0.001 and 0.039, respectively.

**Conclusion:** this study reveals that severe hypertriglyceridemia has a significant impact on HbA1c test results. The HbA1c values in diabetic patients with severe hypertriglyceridemia are unpredictable.

**Keywords:** HbA1c test, type 2 DM, severe hypertriglyceridemia.

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### Introduction

Glycosylated hemoglobin (HbA1c) is the percentage of hemoglobin A that has glucose linked to it. While the percentage is generally at a low level, in diabetes mellitus the increased blood glucose causes greater

hemoglobin binding, resulting in a higher HbA1c value.<sup>1</sup> Glycosylated hemoglobin (HbA1c) has long been recognized as a valid and reliable test for diabetes diagnosis and evaluating glycemic control in diabetic patients.<sup>2</sup> Its result reflects the mean glucose level during the previous 8 to 12 weeks. It has less pre-analytic and biological variability than other diabetes diagnostic tests, and it is more convenient for patients and doctors because no fasting is needed.<sup>3</sup> As a result, clinical

practice guidelines and societies have endorsed the HbA1c as the standard for determining whether a patient is in or out of the glycemic control range, as well as for guiding treatment decisions regarding adjustments in the treatment regimen.<sup>4</sup> However, its reliability is compromised in certain medical situations such as anemia, hemoglobinopathies, acute blood loss within three months, blood transfusion within three months, chronic ingestion of alcohol, salicylates, opioids, and high doses of vitamins A and C, chronic kidney disease, chronic liver disease, HIV on antiretroviral therapy, pregnancy, hypersplenism, hypertriglyceridemia and hyperbilirubinemia.<sup>5</sup> Not identifying these factors may influence the decision-making process and adversely affects the patients' health by intensifying the treatment and the risk of adverse events like hypoglycemia or wrongly de-intensify it.<sup>6</sup> There are four most used techniques for measuring HbA1c. They are immunoassay, ion-exchange high-performance liquid chromatography, boronate affinity high-performance liquid chromatography, and enzymatic assays.<sup>7</sup> The small intestine absorbs dietary triglycerides, which are then released into the lymph system before entering the systemic circulation as chylomicrons via the thoracic duct. Some of the triglycerides are removed from the chylomicrons by muscle and adipose tissues, and the remaining chylomicrons are taken up by the liver and processed into cholesterol-rich lipoproteins. Although the small intestine absorbs the majority of the triglycerides in the blood, the liver generates and secretes a little quantity.<sup>8</sup> Many causes can lead to hypertriglyceridemia including: obesity, overeating, diabetes mellitus and insulin resistance, excess alcohol intake, nephrotic syndrome and kidney failure, some types of familial hyperlipidemia like familial combined

hyperlipidemia (Type II hyperlipidemia), deficiency of lipoprotein lipase, hypothyroidism, systemic lupus erythematosus and medications like beta-blockers, anti-psychotics, bile acid sequestrants, estrogen, immunosuppressants, protease inhibitors, tamoxifen, glucocorticoids, thiazides and isotretinoin.<sup>9</sup> Hypertriglyceridemia must be diagnosed using fasting serum triglycerides levels. According to Endocrine Society, mild hypertriglyceridemia is defined as triglycerides level of 150-199 mg/dl, moderate hypertriglyceridemia is defined as triglycerides level of 200-999 mg/dl and severe hypertriglyceridemia is defined as triglycerides level of  $\geq 1000$  mg/dl.<sup>10</sup>

### **Patients and methods**

A retrospective study on two groups of adult patients (Age >18 years) with type 2 DM; those with normal triglycerides levels (TG < 150 mg/dl) and those with severe hypertriglyceridemia (TG  $\geq 1000$  mg/dl) were implemented in Faiha Diabetes, Endocrine and Metabolism Centre (FDEMC) in Basrah-Iraq. Data were collected from the electronic registry of patients who consulted Faiha Diabetes, Endocrine and Metabolism Centre (FDEMC). A total of 267 patients with type 2 DM and severe hypertriglyceridemia were collected from the electronic registry of patients who consulted to Faiha Diabetes, Endocrine and Metabolism Centre (FDEMC) from the 24<sup>th</sup> of August, 2008 to the 16<sup>th</sup> of September, 2021. A total of 313 patients with type 2 DM and normal triglycerides levels were collected from the electronic registry of patients who consulted FDEMC from the 18<sup>th</sup> of July, 2021 to the 16<sup>th</sup> of September, 2021. For both groups, we recorded patients' age, sex, body mass index, fasting blood sugar, glycosylated hemoglobin, Fasting triglycerides level, LDL,

VLDL, HDL, cholesterol, non-HDL and duration of diabetes. Both groups of patients were subdivided into sub-groups each one with its specific range of FBS. The ranges of FBS in these sub-groups were chosen to ensure good number of patients in each sub-group and nearly the same mean FBS in patients with normal triglycerides levels and those with severe hypertriglyceridemia in each range of FBS. Then the mean HbA1c of patients with severe hypertriglyceridemia was measured in each FBS range and compared to the mean HbA1c of patients with normal triglycerides levels in the same FBS range to determine the impact of severe hypertriglyceridemia on readings of HbA1c test in diabetic patients (type 2). Patients with conditions, other than severe hypertriglyceridemia, that affect or may affect the HbA1c test results (anemia, hemoglobinopathies, acute blood loss within three months, blood transfusion within three months, chronic ingestion of alcohol, salicylates, opioids, and high doses of vitamins A and C,

chronic kidney disease, chronic liver disease, HIV on antiretroviral therapy, pregnancy, hypersplenism, hypertriglyceridemia and hyperbilirubinemia)<sup>5</sup> have been excluded from the study. The Continuous variables have been summarized by using descriptive statistics presented as mean and SD. Categorical variables have been summarized by using counts and percentages. This study has considered the two-tailed probability values with ( $p \leq 0.05$ ) to be statistically significant. All statistical calculations were performed with IBM SPSS Statistics 26 for Windows.

## Results

Both groups (those with normal triglycerides levels and those with severe hypertriglyceridemia) were divided into sub-groups each one with its specific FBS range. The mean FBS and the mean HbA1c were measured in each sub-group, (Table-1).

**Table 1.** Variation of HbA1c in normal triglycerides and severe hypertriglyceridemia groups adjusted to FBS

FBS (Mg/dl)	Patients with normal triglycerides Levels					Patients with severe hypertriglyceridemia					P-value (FBS)	P-value (HbA1c)
	No.	Mean FBS	SD	Mean HbA1c	SD	No.	Mean FBS	SD	Mean HbA1c	SD		
72-129	68	111.6	12.5	7.16	1.47	8	114.0	18.6	8.16	1.95	0.629	0.083
130-149	56	138.8	5.6	7.81	1.28	12	138.0	6.0	9.14	1.66	0.696	0.003
150-169	48	158.6	5.3	8.51	1.24	17	158.7	4.4	8.92	1.60	0.947	0.279
170-199	46	183.02	9.22	9.30	0.95	33	186.4	9.0	9.53	1.53	0.111	0.426
200-229	28	214.6	7.9	10.82	1.31	36	215.5	8.6	9.80	1.47	0.666	0.005
230-259	27	242.07	9.76	11.57	1.07	36	245.78	9.62	10.72	1.81	0.138	0.034
260-299	22	281.0	10.7	12.45	0.78	36	279.8	11.4	10.68	1.57	0.68	<0.001
300-349	14	314.9	16.0	13.53	1.69	45	319.9	14.7	11.24	1.91	0.279	<0.001
350-399	4	373.25	23.36	13.83	2.77	18	371.39	15.26	11.6	1.59	0.379	0.039
400-513	0	-	-	-	-	26	446.58	38.16	12.63	2.2	-	-

P-value measured by independent t-test.

For the FBS range 72-129 mg/dl, 150-169 mg/dl, and 170-199 mg/dl, the mean FBS was nearly the same for both groups with no significant statistical difference. The mean HbA1c in patients with severe hypertriglyceridemia was higher than the mean HbA1c in patients with normal triglycerides levels by 1%, 0.41% and 0.23%, respectively, but it is not significant statistically. For the FBS range 130-149 mg/dl, the mean FBS was nearly the same for both groups with no significant statistical difference (P-value = 0.696), the mean HbA1c in patients with severe hypertriglyceridemia was higher than the mean HbA1c in patients with normal triglycerides levels by 1.33% with statistically significant p-value = 0.003. For the FBS range 200-229 mg/dl, 230-259 mg/dl, 260-299 mg/dl, 300-349 mg/dl and 350-399 mg/dl, the mean FBS was nearly the same for both groups with no significant statistical difference, the mean HbA1c in patients with severe hypertriglyceridemia was lower than the mean HbA1c in patients with normal triglycerides levels by 1.02%, 0.85%, 1.77%, 2.29% and 2.23%, respectively, with statistically significant p-value equals to 0.005, 0.034, < 0.001, < 0.001, and 0.039 respectively.

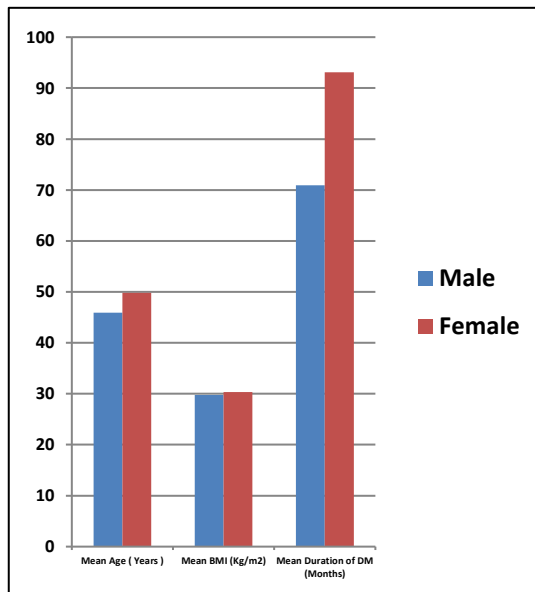


Fig 1. Diabetic patients (type 2) with normal triglycerides levels: Basic characteristics

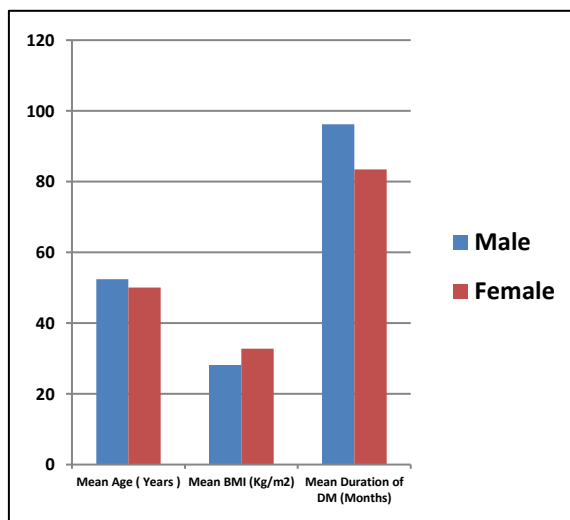
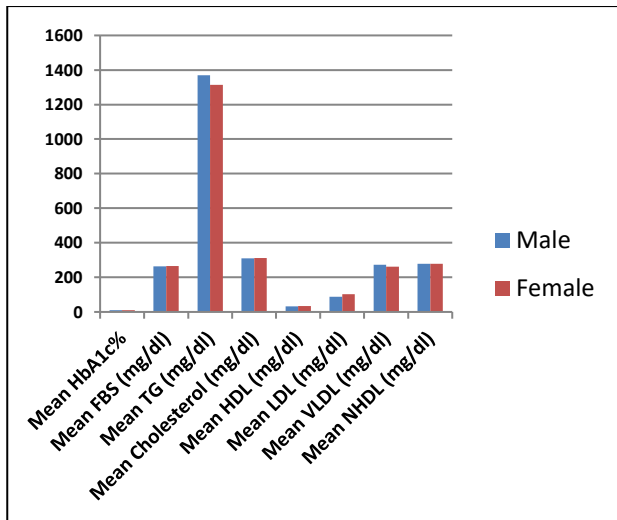
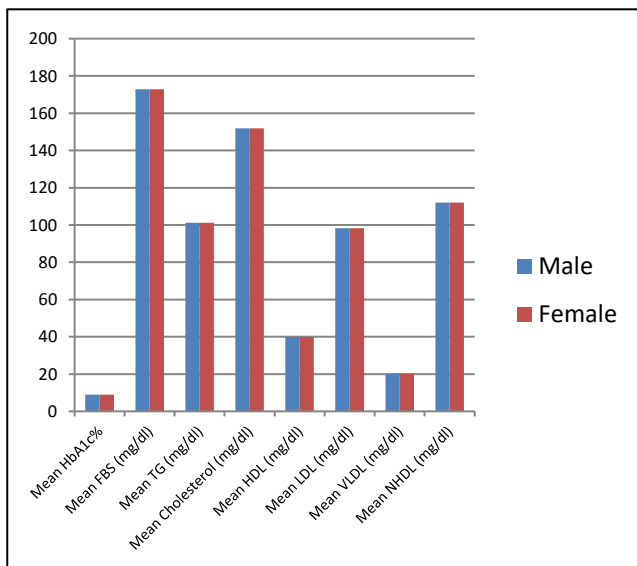


Fig 2. Diabetic patients (type-2) with severe hypertriglyceridemia: Basic characteristics



**Fig 3.** Diabetic patients (type 2) with normal triglycerides levels: Biochemical characteristics



**Fig 4.** Diabetic patients (type-2) with severe hypertriglyceridemia: Biochemical characteristics

## Discussion

There are few studies discussing the effects of hypertriglyceridemia on glycosylated hemoglobin (HbA1c) test. In the current study, it was found that severe hypertriglyceridemia has a significant impact on HbA1c test results in the

diabetic patients (type-2). The HbA1c values in the diabetic patients (type-2) with severe hypertriglyceridemia are unpredictable, they may be equal to, higher or lower than the actual values. There was no statistically significant difference between the mean HbA1c of diabetic patients (type-2) with severe hypertriglyceridemia and those with normal triglycerides levels in the FBS range 72-129 mg/dl, 150-169 mg/dl and 170-199 mg/dl. These findings are consistent with a study published on the 12<sup>th</sup> of February, 2019 by Rene Rodriguez-Gutierrez et al. in which they included 44 patients with hypertriglyceridemia (TG values 378-2986 mg/dl). Most of them had DM (77.3%) and 50% had type 2 DM. They measured the HbA1c values at the beginning of the study and then, after a rapid reduction of triglycerides levels, they measured HbA1c values again. They found that hypertriglyceridemia had no significant impact on HbA1c values.<sup>11</sup> In the FBS range 130-149 mg/dl, the mean HbA1c in the diabetic patients (type 2) with severe hypertriglyceridemia was higher than of those with normal triglycerides levels by 1.33% with a statistically significant p-value. In a study with a similar finding, Falko et al. published a case study of misleading HbA1c increase in a woman with type 2 DM and severe hypertriglyceridemia (23,000 mg/dl). They analyzed these findings further by washing out the patient's samples with saline solution to determine if severe hypertriglyceridemia was interfering with the HbA1c result because HbA1c did not accurately correspond with glycemic control (HbA1c =28.5% and FBS=400mg/dl). They reached the conclusion that HbA1c was falsely elevated when triglycerides levels were above 1750 mg/dl.<sup>12</sup> While the mean HbA1c in the diabetic patients (type 2) with severe hypertriglyceridemia was lower than of those

with normal triglycerides levels in the FBS range 200-229 mg/dl, 230-259 mg/dl, 260-299 mg/dl, 300-349 mg/dl and 350-399 mg/dl by 1.02%, 0.85%, 1.77%, 2.29% and 2.23%, respectively, with a statistically significant p-value. As matched to these findings, Garrib et al. reported a case of falsely low HbA1c. They detected a diabetic patient with an HbA1c of 4.8%, blood glucose monitoring of 200-325 mg/dl, and triglycerides level of 2388 mg/dl. After washing out the samples with saline solution, they re-evaluated the test. It showed a more accurate HbA1c for the glycemic control of the patient than in the prior trial (HbA1c=12.2%). Furthermore, they evaluated the HbA1c and triglycerides of 98 patients with diabetes and found that when triglycerides levels were >1320 mg/dl, HbA1c increased significantly in the washed samples (P=0.0001).<sup>13</sup>

#### **Limitations of the study:**

The small sample size may affect the results due to its impact on the statistical analysis. Also The retrospective nature of the study may not show the complete picture of the impact of severe hypertriglyceridemia on the readings of HbA1c. and Post-prandial glucose was not measured in this study and the comparison of HbA1c between the diabetic patients (type-2) with severe hypertriglyceridemia and those with normal triglycerides levels adjusted to the values of FBS alone.

**Conclusion & recommendation,** In the setting of elevated triglycerides, doctors face uncertainty when assessing HbA1c readings. Our results suggest that severe hypertriglyceridemia has a significant impact on HbA1c test results. The HbA1c values in diabetic patients (type-2) with severe hypertriglyceridemia are unpredictable. They may be equal to, higher or lower than the

actual values. Then we recommend that when assessing glycemic control of patients with type 2 DM, doctors should pay attention to the patients' triglycerides levels as severe hypertriglyceridemia may lead to falsely higher or lower HbA1c readings and hence gives a wrong estimation of the patients' glycemic control leading eventually to improper decisions regarding treatment regimes. Also doctors should rely more on repeated FBS and RBS readings instead of HbA1c tests to assess the control of diabetes in diabetic patients (Type-2) with severe hypertriglyceridemia.

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## تأثير ارتفاع الدهون الثلاثية الشديد على اختبار الهيموغلوبين الغليكوزيلاتي في داء السكري النوع الثاني

**المقدمة:** على الرغم من أن اختبار الهيموغلوبين الغليكوزيلاتي (HbA1c) هو اختبار صالح لتشخيص مرض السكري وتقييم السيطرة على نسبة السكر في الدم، فمن المعروف أن العديد من العوامل تؤثر على نتائج اختبار الهيموغلوبين الغليكوزيلاتي (HbA1c) وتجعله يعكس صورة خاطئة للتحكم في نسبة السكر في الدم. أحد هذه العوامل هو ارتفاع الدهون الثلاثية في الدم. **الغرض من الدراسة:** تقييم تأثير ارتفاع الدهون الثلاثية في الدم الشديد (الدهون الثلاثية  $\leq 1000$  مغ/ديسيلتر) على نتائج اختبار الهيموغلوبين الغليكوزيلاتي (HbA1c) في مرضى السكري (النوع الثاني).

**المرضى والاساليب:** دراسة مقطعية إسترجاعية على مجموعتين من المرضى البالغين المصابين بالسكري (النوع الثاني)، تم ادراج المرضى الذين لديهم مستوى طبيعي من الدهون الثلاثية (الدهون الثلاثية  $150 < \text{مغ/ديسيلتر}$ ) وأولئك الذين يعانون من ارتفاع شديد للدهون الثلاثية في الدم (الدهون الثلاثية  $\leq 1000$  مغ / ديسيلتر) في مركز الفحاء التخصصي للسكري والغدد الصم والايض في البصرة-العراق. تم جمع 267 مريضاً يعانون من السكري (النوع الثاني) وارتفاع الدهون الثلاثية في الدم الشديد و313 مريضاً يعانون من السكري (النوع الثاني) مع مستوى دهون ثلاثية طبيعي. تم تقسيم كلا المجموعتين من المرضى إلى مجموعات فرعية كل مجموعة مع مدى محدد من مستوى سكر الدم الصائم (FBS). ثم تم قياس متوسط الهيموغلوبين الغليكوزيلاتي (HbA1c) للمرضى الذين يعانون من ارتفاع شديد للدهون الثلاثية في الدم لكل مدى من مستوى سكر الدم الصائم (FBS)، ومقارنته بمتوسط الهيموغلوبين الغليكوزيلاتي (HbA1c) للمرضى الذين لديهم مستوى دهون ثلاثية طبيعي في نفس المدى من مستوى سكر الدم الصائم (FBS) لتحديد تأثير ارتفاع الدهون الثلاثية الشديد في الدم على قراءات الهيموغلوبين الغليكوزيلاتي (HbA1c) في مرضى السكري (النوع الثاني).

**النتائج:** تمت مقارنة متوسط الهيموغلوبين الغليكوزيلاتي (HbA1c) في كلتا المجموعتين (أولئك الذين لديهم مستوى دهون ثلاثية طبيعي وأولئك الذين يعانون من ارتفاع شديد في الدهون الثلاثية) في كل مدى من مستوى سكر الدم الصائم (FBS). وكشف أنه على الرغم من نفس متوسط سكر الدم الصائم (FBS) تقريباً، كان الهيموغلوبين الغليكوزيلاتي (HbA1c) أعلى في المرضى الذين لديهم ارتفاع شديد في الدهون الثلاثية بنسبة 1.33% في مدى سكر الدم الصائم 130-149 مغ/ديسيلتر مع قيمة P ذات أهمية إحصائية = 0.003، بينما كانت أقل في مدى سكر الدم الصائم 200-229 مغ/ديسيلتر، 230-259 مغ/ديسيلتر، 260-299 مغ/ديسيلتر، 300-349 مغ/ديسيلتر و 350-399 مغ/ديسيلتر بمقدار 1.02%، 0.85%، 1.77%، 2.29% و 2.23% على التوالي مع قيمة P ذات أهمية إحصائية تساوي 0.005، 0.034، 0.001، >0.001، 0.039، على التوالي. لم يكن هناك فرق معتد به إحصائياً بين متوسط الهيموغلوبين الغليكوزيلاتي (HbA1c) لكلا المجموعتين في مدى سكر الدم الصائم 72-129 مغ/ديسيلتر، 150-169 مغ/ديسيلتر و 170-199 مغ/ديسيلتر. بالإضافة الى ذلك، فإن الانحراف المعياري للهيموغلوبين الغليكوزيلاتي (HbA1c) في مجموعة ارتفاع الدهون الثلاثية الشديد كان بصورة عامة أعلى من الانحراف المعياري للهيموغلوبين الغليكوزيلاتي (HbA1c) في مجموعة مستوى الدهون الثلاثية الطبيعي مما يشير إلى أن البيانات منتشرة بشكل أكبر مع تباين أعلى في مستوى الهيموغلوبين الغليكوزيلاتي (HbA1c) في مجموعة ارتفاع الدهون الثلاثية الشديد.

**الاستنتاج:** كشفت هذه الدراسة أن ارتفاع الدهون الثلاثية الشديد لديه تأثير هام على نتائج اختبار الهيموغلوبين الغليكوزيلاتي (HbA1c). إن قيمة الهيموغلوبين الغليكوزيلاتي (HbA1c) في مرضى السكري (النوع الثاني) المصابين بارتفاع الدهون الثلاثية الشديد لا يمكن التنبؤ بها. قد تكون مساوية أو أعلى أو أقل من القيمة الحقيقية. **الكلمات المفتاحية:** اختبار HbA1c، النوع 2 DM، ارتفاع شحوم الدم الشديد.