Correlation of glycosylated haemoglobin (HbA1c) levels with subclinical atherosclerosis in patients with type 2 diabetes mellitus

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ABSTRACT

Background: The most common cause of mortality in people with diabetes is cardiovascular disease. The relation between glycosylated hemoglobin (HbA1c), a marker of glycemic control, and the development of subclinical atherosclerosis is debated. An acceptable indicator of subclinical atherosclerosis is the use of ultrasound to measure carotid intima-media thickness (CIMT).

Objective: To conduct a cross-sectional study exploring the correlation between HbA1c and subclinical atherosclerosis as reflected by the carotid intima-media thickness in patients with type 2 diabetes that had no history of an atherosclerotic cardiovascular disease (ischemic heart disease or cerebrovascular accident).

Methods: A total of 71, type 2 diabetic patients participated in this study. Demographic, anthropometric and laboratory measures of the participants were collected. CIMT values were measured by using a high-resolution ultrasound. Increased CIMT values were accepted as >0.9 mm. Participants were categorized into two groups according to CIMT values: a normal CIMT value of ≤ 0.9 mm group, and a high CIMT value of > 0.9 mm group. HbA1c and other collected variables were compared between normal and increased CIMT groups. Furthermore, subgroup analysis was carried out for patients with poor glycemic control (HbA1c>9%).

Results: The mean CIMT was 1.048 ± 0.14 mm, and approximately 79% of the included population had increased CIMT (> 0.9 mm). 49% of participants were poorly controlled (HbA1c > 9%) and 92% of those with poor glycaemic control had increased CIMT.

Correlation analysis of data belonging to participants with poor glycaemic control (HbA1c > 9%) showed a significant correlation between HbA1c levels and CIMT, with a correlation coefficient of 0.409 (P = 0.015).

Conclusion: This study revealed that HbA1c levels in patients with poor glycemic control are positively correlated with increased CIMT measures, which is an indicator of subclinical atherosclerosis (SCA). Moreover, increased age is found to be a predictor factor for the development of SCA and, consequently, adverse macrovascular outcomes in patients with T2D.

Keywords: T2DM, CIMT and HbA1c

العلاقة بين مستويات الهموغلوبين السكري مع تصلب الشرايين (دون السريري) في المرضى الذين يعانون من مرض السكري من النوع الثاني

المقدمة: تعتبر أمراض القلب والأوعية الدموية السبب الأكثر شيوعا للوفيات عند المرضى الذين يعانون من مرض السكري. إن العلاقة بين الهموغلوبين السكري (HbA1c) وهو أحد علامات السيطرة على سكر الدم، وتطور تصلب الشرايين دون السريري تعتبر قيد النقاش. وكمؤشرًا مقبولًا ل ölçذ تصلب الشرايين دون السريري هو استخدام الموجات فوق الصوتية لقياس سمك الطبقة البطانية (CIMT) للشريان السباتي.

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The introduction of diabetes mellitus is considered to be a major health problem worldwide, with 387 million people having diabetes worldwide, which is expected to rise to 592 million by 2035. In fact, diabetes is not only a condition with an increasing prevalence, but it is also a serious health problem. The International Diabetes Federation (IDF) published that diabetes led to approximately five million deaths in 2015. Moreover, the World Health Organization (WHO) considers that diabetes will be the seventh leading cause of death by 2030. Unfortunately, the diagnosis of type 2 diabetes mellitus (T2DM) is often not made for many years because hyperglycaemia, at early stages, is usually not severe enough to provoke symptoms of the disorder. Thus, patients with T2DM are at a high risk for developing complications early after the diagnosis. Strongly supported evidence shows that there is an increased risk of macrovascular complications (coronary artery disease, stroke and peripheral artery disease) in diabetes; the overall risk of cardiovascular disease (CVD) increases two- to fourfold. Also, despite a considerable improvement in diabetes management, deleterious cardiovascular outcomes still occur in a large proportion of patients with diabetes. Therefore, every attempt should be carried out to avoid these
complications by early detection of diabetes in high-risk groups, as well as by finding which risk factors can predict the development of such complications as early as possible and before they become obvious clinically. In type 1 diabetes mellitus, it has been proven that tight glycemic control has a protective effect against both macro- and microvascular complications, as shown by the results of the Diabetes Complications and Control Trial–Epidemiology of Diabetes Intervention Trial.\(^7\) However, in T2DM, it has been well established that glycosylated haemoglobin (HbA1c) levels, a marker of glycemic control, are closely related to the risk of microvascular complications and this finding led to the use of HbA1c in the diagnosis of diabetes by the International Expert Committee in 2009.\(^8\) Nonetheless, the issue of the correlation between glycosylated haemoglobin (HbA1C), a marker of glycemic control, and macrovascular outcomes has received considerable critical attention.\(^6\) Major studies around this topic, such as ACCORD trial,\(^9\) ADVANCE trial,\(^10\) and VADT,\(^11\) have failed to show that lowering HbA1c levels can reduce the incidence of macrovascular adverse effects. However, the ACCORD trial was stopped prematurely due to high mortality rate;\(^12\) therefore, their results cannot be considered. The VADT was a small study with a predominantly male sample (only three females were recruited), meaning it was not representative of the general population; in addition, male gender is considered as an independent risk factor for CVDs.\(^4\) Furthermore, the UK Prospective Diabetes Study (UKPDS) showed a statistically insignificant reduction in the CV endpoints after an intervention period of ten years.\(^13\) Surprisingly, the 10-year follow-up of UKPDS (UKPDS 80) showed that there was a statistically significant reduction in the incidence of myocardial infarction ten years after the intervention period had ended.\(^14\) However, an argument against this emerging benefit is that the growing number of events in both groups over this long period may have contributed to the change in analysis. As results from major studies about the benefit of reducing HbA1c on cardiovascular outcomes are variable, it raises the question about whether the HbA1C level is related to the development of macro vascular complication. In fact, atherosclerosis is the underlying disease process that leads to coronary artery disease, stroke, and peripheral artery disease. Atherosclerosis is a condition in which the fatty plaques where deposited inside the arteries.\(^15\) A well accepted non-invasive indicator of the development of the early stages of atherosclerosis (subclinical atherosclerosis (SCA)) is the measurement of carotid intima-media thickness (CIMT), which is calculated by using high-resolution B-mode ultrasonography.\(^16\) Therefore, and for a better understanding of the association between glycemic control and macrovascular complications, several epidemiological studies have investigated the correlation between HbA1c levels, as a marker of glycemic control, and CIMT measurements, as a predictor of SCA, which is the underlying process in the pathophysiology of macrovascular complications. Some of these studies have investigated this association in Asian populations (17-22), while others have done so in western populations (23-25). However, results from these studies showed conflicting findings, with some determining a positive correlation between HbA1c and CIMT, and others being unable to confirm this association.

**The aim of the study:** the aim of this study is to clarify the correlation between HbA1c levels and CIMT measurements in patients with T2DM and to determine other risk factors for cardiovascular disease, including lipids, blood pressure, smoking, obesity, male gender, and increasing age.
PATIENTS AND METHODS

Study Design and Population:
The study protocol had been approved by the scientific committee of the department of internal medicine and the ethical committee of College of Medicine, University of Basrah. All methods were carried out in accordance with guidelines and regulations followed at the College of Medicine and its Teaching Hospital (AL-Sadder Teaching Hospital). A cross-sectional observational study was conducted to answer the research question on the correlation between HbA1c levels and CIMT measurements.\[26\] From March 2016 to September 2016, a total of 111 patients were collected from AL-Sadder Teaching Hospital in Basrah city. All of them were outpatients. Patients included in this study were known cases of T2DM, and had no atherosclerotic cardiovascular disease (ASCVD), namely, ischemic heart disease or cerebral vascular accident. Forty patients were excluded because they had one or more of the exclusion criteria listed below. The study exclusion criteria were categorized as factors that may have influenced the accuracy of the HbA1c readings (27-28) and factors that may have affected the development of SCA. The former criteria are if a patient had one or more of the following conditions:

1. Iron deficiency anaemia, vitamin B12 deficiency, or hemoglobinopathies.
2. A recent blood transfusion.
3. Chronic kidney disease or chronic liver disease.
4. Hyperbilirubinemia.
5. Acute blood loss.
6. Alcoholism.

The use of statin belonged to the second category, as this may prevent or delay the development of SCA if taken at an appropriate dose. \[29\] Also the patients with history of ASCVD were excluded.

Ethics and Consent
Informed consent was obtained from all patients, and their identities remained anonymous during the entire study process.

Clinical Evaluation and Anthropometric Measurements
All patient demographics were recorded regarding age, gender, history of hypertension (on antihypertensive treatment or not) and smoking history. Participants were classified according to smoking history as current smokers who have smoked 100 cigarettes in their life and, currently, smoke any number of cigarettes, or non-smokers, including both former smokers and never smokers.\[30\] Self-reported information was taken regarding hypertension and smoking history. Height and weight of all included participants were measured, and body mass index (BMI) was calculated by the formula of weight/height\(^2\) (kilograms per square meter) and recorded for all included patients. In order to stratify them according to BMI, the patients were divided into those with BMI < 25 kg/m\(^2\), and other whose BMI \(\geq 25\) kg/m\(^2\) (overweight/obese)\[31\] to see the prevalence of increased CIMT in each category.

Blood Collection and Biochemical Parameters
Random blood samples were drawn for total cholesterol (TC), triglycerides (TG), and HbA1c assays. According to HbA1c level, the patients were divided into those with HbA1C level \(\leq 9\)% and those with HbA1c > 9%. We used 9% value of HbA1c as a cut-off point as glycemic control is considered to be poor if HbA1c value is more than 9%\[4\]. Lipid profile and HbA1c were measured using an automated biochemical instrument (Cobas Integra 400, Roche, Germany). The HbA1c determination is based on the turbidimetric inhibition immunoassay (TINIA)
Measurement of Carotid Intima-Media Thickness
A certified investigator examined common carotid arteries (CCAs) bilaterally by a B-mode ultrasound, using a high-resolution instrument (SonoSite Inc., Bothell, WA 98021 USA). Ultrasound measurements were performed according to Mannheim CIMT Consensus.[32] CIMT was measured on both CCAs, at a point located approximately 20 millimetres from the bulb. Three measurements were obtained from different sites of this segment, and the mean value of six measurements (of both sides) was used for analysis. A value up to 0.9 mm was accepted as a normal value of CIMT according to the European Society of Cardiology/European Society of Hypertension guidelines, [33] published in 2013. According to CIMT values, participants were divided into two groups: a normal CIMT value of ≤ 0.9 mm group, and a CIMT value of > 0.9 mm group, before collected variables were compared between the two.

Statistical Analysis
The statistical analysis of collected data used descriptive analysis in addition to an analytic approach to determine the relationship between the study output index (CIMT and the demographic, and predictor variables included in the study). Descriptive analysis includes determining the frequency (in tables) and pictorial presentation (bar charts, histograms, and pie charts). Continuous data is expressed as a mean with standard deviation, while categorical data is expressed as a frequency. Analytical tests used the chi-square and correlation coefficient using the Statistical Package for Social Science version 20 (SPSS-20) at a significance level of 0.05.

RESULTS
A total of 71 patients were recruited in the study. The response rate was (100%). The mean age was (52.7 ± 6.42) years. The gender distribution showed that 44 patients (61.97%) were females and the remaining (38.03%) were males (Figure-1).

![Gender Distribution](image)

**Fig 1. The gender distribution among T2DM patients (n=71)**

The mean BMI was 29.55 ± 4.72 Kg/m². About one-half of the patients (36 patients, 50.7%) were hypertensive, while only three patients (4.22%) were smokers. The minimum, maximum, mean and standard deviations of the measured indices of the participants are shown in (Table-1).

![Table 1](image)

**Table 1. Minimum, maximum, mean and standard deviation of the patients' variables.**

<table>
<thead>
<tr>
<th>The variable</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>Std. Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (in years)</td>
<td>40</td>
<td>70</td>
<td>52.70</td>
<td>6.422</td>
</tr>
<tr>
<td>Serum cholesterol (in mg/dl)</td>
<td>98</td>
<td>281</td>
<td>211.21</td>
<td>40.176</td>
</tr>
<tr>
<td>Serum triglycerides (in mg/dl)</td>
<td>60</td>
<td>485</td>
<td>206.76</td>
<td>86.472</td>
</tr>
<tr>
<td>BMI (in kg/m²)</td>
<td>20</td>
<td>48</td>
<td>29.55</td>
<td>4.723</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>6</td>
<td>13</td>
<td>9.24</td>
<td>1.553</td>
</tr>
<tr>
<td>CIMT (in mm)</td>
<td>0.7</td>
<td>1.5</td>
<td>1.048</td>
<td>0.148</td>
</tr>
</tbody>
</table>

Abbreviations: std. deviation: standard deviation; BMI: Body Mass Index; CIMT: Carotid Intima-Media Thickness.
The graphical presentation of both CIMT measures (Figure-2) and HbA1c values (Figure-3) were almost normal.

**Fig 2. Histogram distribution of CIMT measures.**

**Fig 3. Histogram distribution of HbA1c values**

The percentage of increased CIMT (> 0.9 mm) in the studied samples was about 78.8% (56 subjects out of a total 71 participants), as demonstrated in (Figure-4).

**Fig 4. The percentage of increased CIMT amongst T2DM patients**

There was no significant difference in CIMT measures between the two genders (Table-2).

**Table 2. Gender distribution and CIMT measures**

<table>
<thead>
<tr>
<th>Gender</th>
<th>Cimt (mm)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>&lt; 0.9 mm</td>
<td>7</td>
</tr>
<tr>
<td>Female</td>
<td>&gt; 0.9 mm</td>
<td>8</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>15</td>
</tr>
</tbody>
</table>

Chisq = 0.60, P = 0.438

When patients were divided according to BMI (i.e. those with BMI < 25 kg/m² and those with BMI of ≥ 25 kg/m²), a significant difference in CIMT was found between the two groups. Fifty three patients out of sixty four overweight / obese patients display increased CIMT (Chisq = 6.05, P = 0.014).

**Table 3. BMI and CIMT distributions amongst T2DM patients**

<table>
<thead>
<tr>
<th>BMI (KG/M²)</th>
<th>CIMT (mm)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 25</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>≥ 25</td>
<td>11</td>
<td>53</td>
</tr>
<tr>
<td>Total</td>
<td>15</td>
<td>56</td>
</tr>
</tbody>
</table>

Chisq = 6.05, P = 0.014
When the patients were divided according to hypertension history into two groups, no significant difference in CIMT measures was found between the two groups (Table 4).

Table 4. History of hypertension and CIMT distributions amongst T2DM patients

<table>
<thead>
<tr>
<th>History of hypertension</th>
<th>CIMT</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>≤ 0.9</td>
<td>&gt; 0.9</td>
</tr>
<tr>
<td><strong>HT</strong></td>
<td>8</td>
<td>27</td>
</tr>
<tr>
<td><strong>No HT</strong></td>
<td>7</td>
<td>29</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>15</td>
<td>56</td>
</tr>
</tbody>
</table>

*Chisq = 0.124, P = 0.72*

The patients were divided according to HbA1c level into two groups; the first group includes patients with HbA1C ≤ 9%, while the second one includes patients with HbA1C > 9%. The former group included 50.70% of patients, while 49.30% belonged to the latter, as illustrated by the pie chart in Figure 5.

A significant difference in CIMT between these two groups was found. Thirty two patients out of thirty five with poorly controlled diabetes (HbA1c > 9%) had increased CIMT (Chisq = 6.53, P = 0.010), as shown in (Table 5).

Table 5. HbA1c levels and CIMT measures distribution amongst T2DM patients

<table>
<thead>
<tr>
<th>HbA1c (%)</th>
<th>CIMT</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>≤ 0.9</td>
<td>&gt; 0.9</td>
</tr>
<tr>
<td>&gt; 9</td>
<td>3</td>
<td>32</td>
</tr>
<tr>
<td>≤ 9</td>
<td>12</td>
<td>24</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>15</td>
<td>56</td>
</tr>
</tbody>
</table>

*Chisq = 6.53, P = 0.010*

Correlation coefficient analysis between CIMT was nonsignificant. HbA1c correlation was 0.113 (P = 0.348), total cholesterol correlation was 0.15 (P = 0.904), TG correlation was 0.096 (P = 0.425), BMI correlation was 0.083 (P = 0.390). Interestingly, the only significant correlation found in this study was between CIMT and age, which was 0.359 (P = 0.002).

![Fig 5. The frequency and percentage of patients according to glycemic control](image-url)
Further analysis was carried out for subjects with poor glycemic control (HbA1c > 9%). The result of analysis of data belonging to this group showed a significant correlation between HbA1c levels and CIMT, with correlation coefficients of 0.409 and P value of 0.015, while other variables had no significant correlation.

### DISCUSSION
Diabetes mellitus is associated with serious complications. Both micro- and macrovascular complications of diabetes are associated with atherosclerosis. An acceptable indicator of subclinical atherosclerosis is the use of ultrasound to measure CIMT. In this study, the correlation between glycemic control, reflected by HbA1c, and the development of subclinical atherosclerosis, measured by the CIMT of CCAs, were investigated in patients with T2DM who have no history of ASCVD. This study showed that approximately 79% of patients with T2DM had increased CIMT values (> 0.9 mm), 49% of participants were poorly controlled (HbA1c > 9%), and 90% of our population were overweight/obese. 92% of those with poor glycemic control had increased CIMT (Chisq = 6.53, P = 0.010) and approximately 83% (53 subjects out of 64) of overweight/obese participants had increased CIMT (Chisq = 6.05, P = 0.014). Thus, there was a significant association between poor glycemic control and increased CIMT, as well as a significant association between high BMI (≥ 25 kg/m²) and increased CIMT. The correlation analysis of data initially was nonsignificant between HbA1c and CIMT (P = 0.348). The only significant correlation found in the initial analysis was between CIMT and age, with a value of 0.359 (P = 0.002). Nonetheless,
the subgroup analysis of data belonging to participants with poor glycaemic control (HbA1c > 9%) showed a significant correlation between HbA1c levels and CIMT, with a correlation coefficient of 0.409 (P = 0.015). Indeed, several cross-sectional studies have also investigated this correlation and many published studies on western or Asian populations support our findings. The Kora 4 study was a large population-based study on a western population and recruited participants with and without diabetes. The authors concluded that the relation between HbA1c and CIMT faded after adjustment for other cardiovascular risk variables. However, the mean HbA1c of those with known diabetes in the KORA 4 study was 6.9% ± 0.9, which is lower than the mean of our population. This supports the positive correlation of our study that appeared only in the subgroup analysis of data belonging to the poor glycaemic control (HbA1c > 9%) group. Similarly, no correlation was found between these two variables in a study conducted on an elderly population by Du HW et al. in which the mean HbA1c level was also lower than that of our population (6.9 ± 1). Moreover, they recruited old age people for whom the impact of diabetes on the development of cardiovascular events was significantly smaller than a younger age group, as found by a post Hoc analysis of participants included in two studies: (the Cardiovascular Health Study and the Atherosclerosis Risk in Communities study). Du HW et al. concluded that the presence of hypertension had a bigger impact than hyperglycaemia on the development of CIMT. Also, Olt et al. conducted their study on an Asian population with a mean HbA1c value of 8.6 ± 2, and found that there was no significant association between HbA1c levels and CIMT values. However, no effort was given to performing subgroup analysis according to glycemic control level as we did. In contrast, many studies have confirmed a significant positive correlation between high HbA1c levels and increased CIMT values. We can explain the results of the positive correlation between glycated haemoglobin and CIMT in participants with poor glycaemic control can be explained via two concepts. Firstly, as HbA1c is a glycated protein and regarded as a precursor for advanced glycation end products (AGEs) (37-38), it is considered an important factor in the pathogenesis of atherosclerosis in patients with diabetes. Secondly, HbA1c is closely related to fasting plasma glucose (FPG) levels, which are caused by insulin resistance more than insulin deficiency, with the former having been proven as an associated factor with atherosclerosis. Consequently, increased HbA1c may contribute to the development of CIMT through insulin resistance. However, the correlation coefficient of 0.4 found in this study is considered to be an intermediate correlation, i.e. not a strong correlation. This can be explained by the fact that postprandial glucose (PPG) levels, which are not reflected by HbA1c, as well as glucose instability with peaks and troughs, are independent risk factors for atherosclerosis and macrovascular complications.

**CONCLUSION AND IMPLICATIONS**

This cross-sectional study revealed that HbA1c levels in patients with poor glycaemic control are positively correlated with increased CIMT measures, which is an indicator of SCA. Moreover, increased age is found to be a predictor factor for the development of SCA and, consequently, adverse macrovascular outcomes in patients with T2DM. This study provided an implication for managing patients with diabetes, particularly those who are elderly, to prevent unfavourable cardiovascular outcomes. In Iraq, as a developing country, the rate of adequate glycemic control for patients with diabetes is still disappointingly low. Depending on our results, clinicians should aim to maintain HbA1c levels within normal range as a priority to avoid the development of cardiovascular events.
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Additional Information
Competing Interests
The authors declare that they have no competing interests (financial AND non-financial interests)