ABSTRACT
A total of 50 patients aged 35-75 years from Al-Sader Educational Hospital in Al-Najaf city was studied to determine the glycated hemoglobin risk factors with value creatinine and urea in serum and diabetic nephropathy. Diabetic patient were (35-45 years old) with HbA1c 7.9% (60mmol/mol). Patient were (45–55 years old), glycated hemoglobin (HbA1c) > 8.5%. Patients between (55–65 years were glycated hemoglobin (HbA1c) >10.5%. HbA1c levels, lipid profile, level of Creatinine and urea in serum, family history, BMI, blood pressure, disease severity, and complications were determined. Most patients developed some grade of retinopathy (examined by an ophthalmologist) except those with HbA1c 6.7% (50mmol/mol). Diabetic patients aged (55–65 years old) with HbA1c 7.6% (60mmol/mol). Patients aged 56–75 years old of glycated hemoglobin (HbA1c) >7% with poor glycaemia control ≥ 126mg/dL were assessed to classify diabetic retinopathy. HbA1c and GA are associated with nephropathy separately. Retinopathy and nephropathy may respond to different aspects of hyperglycemia. The GA found as a powerful indicator of microvascular complications same as HbA1c where long-term glycaemia is the risk factor.

Keywords: Diabetic nephropathy, Diabetic retinopathy, Glycated hemoglobin.

INTRODUCTION
More than 300 million individuals are affected by diabetes worldwide. One million new cases yearly in the USA only. Diabetic people are at high risk of developing diabetic retinopathy, nephropathy, and neuropathy that affect their life. Glycaemic control, age, disease duration, genetic factors, and smoking are the main risk factors. End-stage renal disease (ESRD) is due to diabetic nephropathy, which is common in western countries. Visual disability due to diabetic retinal disease is common among those of working age. Visual impairment can be treated through photocoagulation treatment. Glycated hemoglobin (hemoglobin A1c or HbA1c) level is used for long-term glycaemic control. Genetic and medical conditions factors influence HbA1c and its measurement, even with constant glucose levels. Adipose tissue is important in 2DM patients as the secretion of adipocytokines like leptin, TNFα, resistin, and adiponectin are implicated in insulin resistance and beta-cell dysfunction. Type 2 diabetes mellitus (DM) is primarily due to lifestyle factors (physical inactivity, smoking, alcohols, and pollutants) and genetics (especially first degree relatives). About 55% of T2DM patients are obese and increasing in children and adolescents. Recently, several genes were implicated with developing T2DM, including obesity genes. Long-lasting effects of diabetes mellitus cause many complications like diabetic nephropathy and high mortality. Diabetic retinopathy (DR) might cause blindness as a microvascular disorder with neuroretinal degeneration. Hemoglobin A1c is represented as a good marker for microvascular complications which is correlated with fasting plasma glucose. Recently, rapid, non-invasive and simple test (fundus-driven microperimetry) is used to measure retinal sensitivity in retina early functional changes detection.

MATERIAL AND METHODS
Patients who smoking, took anti-inflammatory agents, receiving hormone replacement therapy and with a history of macrovascular disease, were excluded.

Glycosylated Hemoglobin (HbA1c)
Glycosylated hemoglobin kit (Stanbio laboratory, USA) is used for quantitative colorimetric determination of glycohemoglobin in whole blood following the manufacturer’s recommended
Relationship of HbA1c Values to Retinopathy, Nephropathy, and Cardiovascular Disease

**Calculations**
For each standard and unknown, the ratio (R) of the Glycohemoglobin absorbance to the hemoglobin absorbance calculated as follows:

\[
R = \frac{A_{\text{glycosylated}}}{A_{\text{total}}} \\
\text{Glycohemoglobin (\%)} = \frac{R\text{ (unknown)}}{R\text{ (standard)}} \times 7.6
\]

**Measurement of Fasting Blood Glucose**
Serum glucose level was measured by glucose (Glucose-PAP) kit (Audit Diagnostics, Ireland) which was based on the following principle:

Glucose was oxidized (in the presence of glucose oxidase) to gluconic acid and hydrogen peroxide which converts phenol and 4-aminoantipyrine into a red quinine which is measured at 500nm. The intensity of the color of the red quinine produced is directly proportional to the quantity of glucose in the sample (Table 1).

**Procedure**
Reagent and specimens were put stand at room temperature.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Standard</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent 1</td>
<td>1 mL</td>
<td>1 mL</td>
</tr>
<tr>
<td>Sample</td>
<td>10 ul</td>
<td></td>
</tr>
<tr>
<td>Standard</td>
<td>10 ul</td>
<td></td>
</tr>
</tbody>
</table>

mix. let stand for 5 minutes at 37°C, the optical density (OD) and specify against reagent blank, measured at 500 nm

**Calculation**
Results calculated as the following:

\[
\text{Concentration of glucose} = \frac{\text{OD sample}}{\text{OD (standard)}}
\]

Standard concentration = 100 mg/dl

**RESULTS**

**Diabetes Mellitus Duration**
Figure 1 shows Diabetes mellitus duration divided into four categories, 35-45 years, 45-55 years, 55-65 years, 65-75 years. Found high diabetes retinopathy in 65-75 years old.

**Types of Retinopathy**
The patients were classified into four classes according to the worst eye (Figure 2).

**Parameters Associated with Higher Levels of HbA1c**
High level of HbA1c is significantly associated with the glycemic levels. A lower level of HbA1c is significantly associated with low HDL-C and total obesity. However, age, stroke, elevated TG, DM types, and hypertension have no influence on HbA1c level. HbA1c level and DM duration found significantly and negatively related with BMI (Table 2).

**Level of Urea with HbA1c**
Both HbA1c and Urea are affected significantly by the postprandial values, the pre-prandial values of urea, and the value at bedtime (Figure 3).

**Level Creatinine with HbA1c**
Serum Creatinine levels found higher than the normal range in patients. The mean of Creatinine level in serum blood (1.6 mg/dL) is affected by many factors like age, sex, and

**Table 1: Reagents composition**

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration in tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phosphate buffer</td>
<td>7.5 mmol/l</td>
</tr>
<tr>
<td>4-Aminophenazine</td>
<td>0.3 mmol/l</td>
</tr>
<tr>
<td>Phenol</td>
<td>1 mmol/l</td>
</tr>
<tr>
<td>Peroxidase</td>
<td>2000 U/l</td>
</tr>
<tr>
<td>Glucose oxidase</td>
<td>200,000 U/l</td>
</tr>
<tr>
<td>Preservatives</td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>5.56 mmol/l</td>
</tr>
</tbody>
</table>

**Figure 1: Distribution of diabetic patients in the study according to the duration of disease**
Relationship of HbA1c Values to Retinopathy, Nephropathy, and Cardiovascular Physical Status. In Figure 4 showing the relationship between Creatinine and HbA1c that is found significant.

**Legend:**
- **Normal**
- **Slight**
- **Mild/Moderate**
- **Proliferative**

**Figure 2:** Patients classified in each retinopathy groups (four groups)

**Table 2:** Study population general characteristics (n=50)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>56 ± 16</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>28.2 ± 6.2</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>137.7 ± 19.6</td>
</tr>
<tr>
<td>Pulse pressure (mmHg)</td>
<td>57 ± 18.3</td>
</tr>
<tr>
<td>Blood glucose (mg/dL)</td>
<td>199.3 ± 36.4</td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>197.3 ± 35.4</td>
</tr>
<tr>
<td>HDL-cholesterol (mg/dL)</td>
<td>101.1 ± 86.4</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>158.3 ± 100.3</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>7.4 ± 1.4</td>
</tr>
</tbody>
</table>

**DISCUSSION**

HbA1c and GA are associated with nephropathy separately. Retinopathy and nephropathy may respond to different hyperglycemia aspects as differential effects of HbA1c and GA. HbA1c and GA showed similar correlations with retinopathy and nephropathy, which support the study of Atherosclerosis Risk in Communities (ARIC) population, where GA found as a powerful indicator of these complications the same as HbA1c. Weak relation of both retinopathy and nephropathy with MBG and when GA or HbA1c levels are considered; indicating that the long-term glycaemia measurements is the risk factor for microvascular complications where HbA1c or GA levels showed strong association.
CONCLUSION
HbA1c and GA are associated with nephropathy separately. Retinopathy and nephropathy may respond to different aspects of hyperglycemia. GA found as a powerful indicator of microvascular complications same as HbA1c where long-term glycaemia is the risk factor.

ETHICAL CLEARANCE
The Research Ethical Committee at scientific research by ethical approval of both environmental health and higher education and scientific research ministries in Iraq

REFERENCES