



Original Article

## Correlation between *TCF7L2* gene expression and certain biochemical parameters in type 2 diabetes mellitus



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### المخلص

**أهداف البحث:** يعتبر جين "عامل الانتساخ-7" مثل 2 من الجينات المهمة التي وجد أنها مرتبطة بالاستعداد لمرض السكري من النوع الثاني في مختلف المجموعات العرقية. هدف هذا البحث هو التحقيق في العلاقة بين التعبير الجيني جين "عامل الانتساخ-7" مثل 2 وعدة مؤشرات كيميائية حيوية بين فئات عمرية مختلفة من مرضى السكري من النوع الثاني في عينة من السكان العراقيين.

**طرق البحث:** تم جمع 150 عينة دم بالتساوي من 3 مجموعات: مرضى السكري من النوع الثاني الشباب (10-35 سنة)، مرضى السكري من النوع الثاني المسنين (40-80 سنة)، ومجموعة ضابطة صحية (10-65 سنة). تم إشراك كلا الجنسين. تم إجراء تفاعل البلمرة المتسلسل في الوقت الفعلي لاستكشاف التعبير الجيني جين "عامل الانتساخ-7" مثل 2. تم إجراء الاختبارات الكيميائية الحيوية: نسبة الهيموجلوبين السكري، والدهون الثلاثية، والبروتين الدهني منخفض الكثافة، والبروتين الدهني عالي الكثافة. تم احتساب مؤشر كتلة الجسم. تم تحليل النتائج إحصائياً.

**النتائج:** كان لدى مرضى السكري من النوع الثاني سجلات أعلى فيما يتعلق بمؤشر كتلة الجسم، والدهون الثلاثية، والبروتين الدهني منخفض الكثافة، ونتاج البروتين الدهني عالي الكثافة أقل من مجموعة الضبط. هناك ارتباط إيجابي قوي بين نسبة الهيموجلوبين السكري ومؤشر كتلة الجسم، والدهون الثلاثية، والبروتين الدهني منخفض الكثافة. كان هناك ارتباط سلبي بين نسبة الهيموجلوبين السكري والبروتين الدهني عالي الكثافة. أظهر التعبير الجيني جين "عامل الانتساخ-7" مثل 2 اختلافاً كبيراً بين المرضى المسنين والشباب بنسبة 1.68 و 0.207 على التوالي. أظهرت هذه النتائج أن المرضى المسنين لديهم تعبير جيني أعلى مقارنة بالمرضى الشباب.

**الاستنتاجات:** تأثر التعبير الجيني جين "عامل الانتساخ-7" مثل 2 "بالعمر، وكانت السجلات الأعلى للمرضى المسنين من النوع الثاني من السكري. قد يؤثر ذلك على وظائف خلايا بيتا وإفراز الأنسولين.

**الكلمات المفتاحية:** الاختبار الكيميائي الحيوي، مرض السكري، التعبير الجيني؛ نسبة الهيموجلوبين السكري؛ السكري من النوع الثاني؛ جين "عامل الانتساخ-7" مثل 2

### Abstract

**Objectives:** The transcription factor 7-like 2 gene (*TCF7L2*) is associated with a predisposition to type 2 diabetes mellitus (T2DM) in different ethnic populations. This article investigated the relationship between *TCF7L2* gene expression and several biochemical indexes among different age categories of T2DM in a sample of the Iraqi population.

**Methods:** One hundred and fifty blood samples were collected from three groups: young T2DM (10–35 years), old T2DM (40–80 years), and healthy control (10–65 years) groups. Both sexes were enrolled. qPCR was performed to explore the expression of the *TCF7L2* gene. Biochemical tests were performed to assess hemoglobin A1C (HbA1c), triglyceride (TG), low-density lipoprotein (LDL), and high-density lipoprotein (HDL) levels. The body mass index (BMI) was calculated. The results were statistically analyzed.

**Results:** Patients with T2DM had higher BMI, TG, and LDL, and lower HDL than the control group. There was a strong positive correlation between hemoglobin A1C (HbA1c) and BMI, TG, and LDL and a negative correlation between HbA1c and HDL. Expression of the *TCF7L2* gene showed a significant difference between old and young patients by 1.68 and 0.207 fold, respectively.

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These results showed that old patients had higher gene expression than young patients.

**Conclusion:** *TCF7L2* gene expression was affected by age, with higher expression in old T2DM patients. This may influence beta cell functions and insulin secretion.

**Keywords:** Biochemical test; Diabetes mellitus; Gene expression; HbA1c; T2DM; *TCF7L2*

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## Introduction

Diabetes mellitus (DM) is a chronic metabolic disorder defined by elevated blood glucose levels resulting from insufficient insulin synthesis or impaired insulin function.<sup>1</sup> It affects approximately 537 million people worldwide. It is predicted to affect 10.2% by 2030 and 10.9% by 2045. The World Health Organization expects that by 2030, such a metabolic pandemic will emerge as the seventh major cause of mortality globally, affecting more than 400 million people.<sup>2</sup> DM is projected to have a global economic impact of \$745 billion by 2030 due to economic expansion, nutritional changes, and population aging.<sup>3</sup>

In Iraq, DM caused 7279 deaths or 4.24% of total deaths.<sup>4</sup> T2DM represents one of the most prevalent metabolic illnesses in the world.<sup>5</sup> It is caused by a combination of two main causes: improper insulin secretion by pancreatic beta cells and failure of insulin-sensitive tissues to respond to insulin.<sup>6</sup> Therefore, the molecular mechanisms involved in the synthesis and release of insulin and the insulin response in tissues must be extremely tightly controlled.<sup>7</sup> Consequently, the defects in any of the involved could result in T2DM. Genetic and environmental variables impact its development. Obesity, overeating, lack of exercise, and hereditary factors affect insulin production and resistance.<sup>7</sup> Based on twin family studies, T2DM complications have a 40% inheritance rate. In a study published in 2011, more than 36 genes were found to increase the risk of T2DM.<sup>8</sup>

Transcription factor 7-like 2 gene (*TCF7L2*) is one of the most well-studied genes for diabetic predisposition among many populations.<sup>9</sup> *TCF7L2* is a member of the T cell factor/lymphoid enhancer factor (TCF/LEF) family, with other members such as *LEF1*, *TCF7*, and *TCF7L1* in humans. The term “T cell factor 4 (TCF4)” was previously mistaken for transcription factor 4.<sup>10</sup> The *TCF7L2* gene is located on chromosome 10q25.3 and contains a transcriptional element of about 215.9 kb. This gene consists of 17 exons, 5 of which are alternative splicing units. It encodes a transcription factor that interacts with  $\beta$ -catenin and works within the Wingless (Wnt) signaling pathway. It regulates several cellular processes including cell morphology, proliferation, motility, and oncogenesis.<sup>11</sup> The gene *TCF7L2* has received considerable interest among researchers due to its significant genetic association with increased susceptibility to T2DM. This genetic link has

been shown to alter the function and survival of pancreatic beta cells.<sup>12</sup> *TCF7L2* is associated with metabolism, inflammation, and atherosclerosis.<sup>13</sup> It is considered a key locus associated with the risk of T2DM by acting as a downstream effector of the Wnt/ $\beta$ -catenin signaling pathway. *TCF7L2* risk variants interfere with the three mechanisms: glucose-stimulated insulin secretion, incretin-stimulated insulin secretion, and the conversion of proinsulin to insulin.<sup>14</sup>

Due to the importance of this gene in T2DM, the current study investigated its role in a sample of the Iraqi population and study age differences regarding gene expression.

## Materials and Methods

### Subjects and study design

The current study included the collection of 150 peripheral blood samples from patients with T2DM and healthy individuals as controls. These samples were equally divided into three groups: young T2DM patients (10–35 years), old T2DM patients (40–80 years), and healthy control (10–65 years) groups. Those patients were chosen randomly from private medical clinics in Baghdad, whereas the control group was randomly selected from apparently healthy individuals who did not have a diabetic history. All volunteers agreed to participate in this investigation after they were informed of the purpose of this study. American Diabetes Association criteria were followed by the physicians to diagnose T2DM if the fasting glucose was above 126 mg/dL (8.0 mmol/L). We included T2DM patients who had hemoglobin A1C (HbA1c) levels  $\geq 8\%$ . The exclusion criteria were pregnancy, alcoholism, liver disease, chronic renal infection, thyroid disease, and any endocrine disease.

### Collection samples

Five milliliters of vein blood were drawn from each participant under aseptic circumstances, and then treated as follows.

- 1- Two milliliters of blood were placed in an EDTA tube and stored at  $-20\text{ }^{\circ}\text{C}$  for the HbA1c test.
- 2- Two hundred and fifty microliters of blood from the first tube were added to an Eppendorf tube containing 750  $\mu\text{L}$  of Triazole LS reagent, gently mixed, and stored at  $-20\text{ }^{\circ}\text{C}$  for the gene expression test.
- 3- The remaining 3 mL of blood was added to a clot activator tube, left at room temperature ( $20\text{ }^{\circ}\text{C}$ – $25\text{ }^{\circ}\text{C}$ ) for a few minutes, and then centrifuged for 15 min at 3000 rpm/min. The upper serum was collected and stored at  $-20\text{ }^{\circ}\text{C}$  for subsequent biochemical tests.

### Biochemical tests

Routine clinical procedures were followed to perform the biochemical tests according to the manufacturer's instructions for each kit. These kits were: I-Chroma HbA1c Glycosylated Hemoglobin Kit (Biotech Med Co., Barcelona, Spain), and Triglycerides, Cholesterol MR, and HDL-Cholesterol kits (Linear Chemicals, Barcelona, Spain).

### Body mass index and low-density lipoprotein cholesterol measurements

The body mass index (BMI) index for each participant was determined as follows:

$$\text{BMI} = \text{mass (kg)} / \text{height (m)}^2$$

Total cholesterol was measured according to our previously published article.<sup>15</sup> Low-density lipoprotein cholesterol (LDL) was calculated in the present study according to the following Friedwald formula:<sup>16</sup>

$$\text{LDL} = \text{total cholesterol} - (\text{high-density lipoprotein [HDL]} + \text{triglyceride [TG]}/5)$$

### RNA extraction and cDNA synthesis

Total RNA was extracted using the Triazole Up Plus RNA Kit (TransGen Biotech Co., Ltd., Beijing, China), according to the manufacturer's instructions. Then the quantity and quality of the RNA samples were measured using the Nanodrop OneC spectrophotometer and the ratio of OD260/OD280 was measured for the total RNA samples. Then cDNA was prepared using the EasyScript One-Step gDNA Removal Kit and cDNA Synthesis Super Mix Reagent Kit (TransGen Biotech).

### Quantitative PCR

Quantitative PCR (qPCR) was conducted to measure the threshold cycle (Ct) for all cDNA samples using the Rotor Gene Real-Time PCR System (Qiagen, Germantown, MD, USA). Reaction mixtures were prepared by utilizing the 2xTransStart® Top Green qPCR Super Mix. Each reaction was performed twice and individually. The primers were obtained from Alpha DNA (Montreal, Canada) according to the sequences in Table 1. The housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) was used as a reference gene. The melting temperature (T<sub>m</sub>) for the forward and reverse primers was calculated according to the equation: T<sub>m</sub> (°C) = 2 (A + T) + 4 (G + C). The annealing temperature (T<sub>a</sub>) of the primers was estimated by the following equation: T<sub>a</sub> (°C) = T<sub>m</sub> - (2–5). We determined the lowest T<sub>m</sub> for both the forward and reverse primers by comparing their T<sub>a</sub>.<sup>17</sup>

### qPCR cycling protocol for *GAPDH* gene expression

The cycling profile for *GAPDH* qPCR was programmed after following the optimization circumstances that accommodate the thermal profile, as shown in Table 2.

### qPCR cycling protocol for *TCF7L2* gene expression

The cycling profile for *TCF7L2* qPCR was programmed after following the optimization circumstances that accommodate the thermal profile, as shown in Table 3.

### Statistical analyses

Data were statistically analyzed using SPSS 18.0 (SPSS Inc., Chicago, IL, USA). The results are expressed as the

mean ± standard deviation (SD). *P* < 0.05 was considered statistically significant. One-way analysis of variance and the chi-square test were used to statistically analyze the differences in means among the three groups. Duncan's multiple range test used different letters for statistically different means.

### Distribution of age groups

The current study included 150 blood samples that were divided into three groups: young diabetic patients, old diabetic patients, and a healthy control group, as shown in Table 4. The ages of the three groups were 22.5800c ± 7.73830, 57.7400a ± 9.28420, and 37.6400b ± 17.82312, respectively. There was a significant difference among these groups (*p* = 0.0001) (Figure 1).

### Results of the BMI

The BMI results for the three groups are shown in Table 5. There was a significant difference among the three groups (*p* = 0.001). The highest BMI level was for the elderly patients with a mean of 33.7645 ± 5.23186 kg/m<sup>2</sup>, followed by the young patients with a mean of 31.3372 ± 5.43931 kg/m<sup>2</sup>. The healthy control group had the lowest BMI level with a mean of 24.6428 ± 1.89878 kg/m<sup>2</sup>.

### Results of *TCF7L2* and *GAPDH* gene expression

*TCF7L2* gene expression was evaluated using qPCR. The level of gene expression was normalized to that of the *GAPDH* housekeeping gene and quantified using the 2<sup>-ΔΔCt</sup> and ΔCt values presented in Figures 2 and 3. The Livak Method<sup>19</sup> was used to quantify *TCF7L2* gene expression after normalization to the reference gene *GADPH*, among the patients and healthy control groups.

The gene expression of *TCF7L2* compared to the housekeeping gene *GAPDH*. The results were statistically analyzed and the fold expression of the *TCF7L2* gene is shown in Table 6.

Our results revealed a significant difference in *TCF7L2* gene expression between the control and patient groups (*p* = 0.0001). *TCF7L2* gene expression was 1.68-fold higher in old patients than in healthy controls, whereas there was a reduction in *TCF7L2* gene expression in the young patient group by 0.207-fold, as shown in Figure 4.

### Results of the biochemical tests

The results of the biochemical tests are shown in Table 7 and Figure 5. The results of the TG test for the three groups revealed a significant difference among the groups (*p* = 0.0001); the highest TG level was in the old patients with a mean of 273.9200 ± 182.19084 mg/dL, followed by the young patients with a mean of 193.0800 ± 82.83543 mg/dL, whereas the healthy control group had the lowest TG level with a mean of 89.5000 ± 14.57738 mg/dL. The LDL results in the three groups revealed a significant difference among the groups (*p* = 0.0001). The old and young groups had the highest LDL levels with means of

113.7600 ± 45.76629 and 110.1400 ± 43.42858 mg/dL, respectively. The healthy control group had the lowest LDL level with a mean of 73.4000 ± 9.24276 mg/dL. The HDL results revealed a significant difference among the groups ( $p = 0.03$ ). In contrast to the LDL results, the healthy control group had the highest HDL level with a mean of 44.0866 ± 3.57163 mg/dL, whereas the young and old groups had lower levels of HDL than the control groups with means of 40.3600 ± 10.93349 and 38.8180 ± 13.97984 mg/dL, respectively.

#### Results of correlation analysis of *TCF7L2* gene expression and other parameters

Correlation analysis for our results was done to determine the relationship between *TCF7L2* gene expression and each of the tested parameters (Table 8 and Figure 6). Our results showed the absence of any significant correlation between *TCF7L2* expression and the other parameters ( $p > 0.05$ ). Age showed a significant positive correlation with both BMI and TG ( $p < 0.01$ ). The results revealed highly significant positive correlations between BMI and HbA1c, TG, and LDL ( $p < 0.01$ ), whereas a significant negative correlation was observed between BMI and HDL ( $p < 0.01$ ). The results also confirmed that there was a significant positive correlation between HbA1c and each TG and LDL ( $p < 0.01$ ). TG was also significantly positively correlated with LDL and negatively correlated with HDL ( $p < 0.01$ ).

#### Discussion

The current study showed an increase in BMI level among Iraqi diabetic patients compared to the control group. These results are in accordance with a previous study showing that the Iraqi population suffers a steady increase in obesity and its health complications.<sup>8,20</sup> Middle Eastern countries (such as Iraq) have witnessed overweight and obesity spatially among adults. A previous study confirmed that the BMI cut-off point for the occurrence was 25.6 kg/m<sup>2</sup>,<sup>21</sup> which was consistent with our results. The prevalence of T2DM increases with increasing BMI.<sup>22</sup> Obesity itself causes a certain degree of insulin resistance. Adipocytes release non-esterified fatty acids, which are considered the main cause of insulin resistance and impairment of beta cell functions.<sup>23</sup> Ectopic fat distribution in the viscera alters fat metabolism and accelerates diabetes. Abdominal obesity is a greater metabolic risk factor than overall obesity. Adipose tissue factors can promote chronic inflammation, insulin resistance, and damage to pancreatic beta cells, leading to impaired glucose tolerance and diabetes progression.<sup>24</sup>

**Table 2: Thermal Profile of *GAPDH* gene expression.**

Step	Temperature (°C)	Time (s)	Cycle No.
Enzyme activation	94	60	1
Denaturation	95	5	35
Annealing	58	15	
Extension	72	20	
Dissociation	55–95		1

**Table 3: Thermal profile of *TCF7L2* gene expression.**

Step	Temperature (°C)	Time (s)	Cycle No.
Enzyme activation	94	30	1
Denaturation	94	10	35
Annealing	56	15	
Extension	72	20	
Dissociation	55–95		1

**Table 4: Age distribution for the three groups.**

Age groups	Young T2DM (n = 50)	Old T2DM (n = 50)	Control (n = 50)
<35 years	50 (100%)	0	27 (54%)
≥40 years	0	50 (100%)	23 (46%)
Mean ± SD	22.5800c ± 7.73830	57.7400a ± 9.28420	37.6400b ± 17.82312
Std. Error	1.09436	1.31298	2.52057
Maximum	35.00	85.00	70.00
Minimum	10.00	40.00	11.00
<i>p</i> -value	0.0001**		
Chi-square	0.004**		

\*\* High significant result ( $p < 0.01$ ).

**Table 5: BMI results for the three groups.**

BMI (kg/m <sup>2</sup> )	Young T2DM	Old T2DM	Control
18.5–24.9	2 (4%)	1 (2%)	25 (50%)
25–29.9	20 (40%)	10 (20%)	25 (50%)
≥30	28 (56%)	39 (78%)	0
Mean ± SD	31.3372b ± 5.43931	33.7645a ± 5.23186	24.6428c ± 1.89878
<i>p</i> -value	0.001**		

\*\* High significant result ( $p < 0.01$ ).

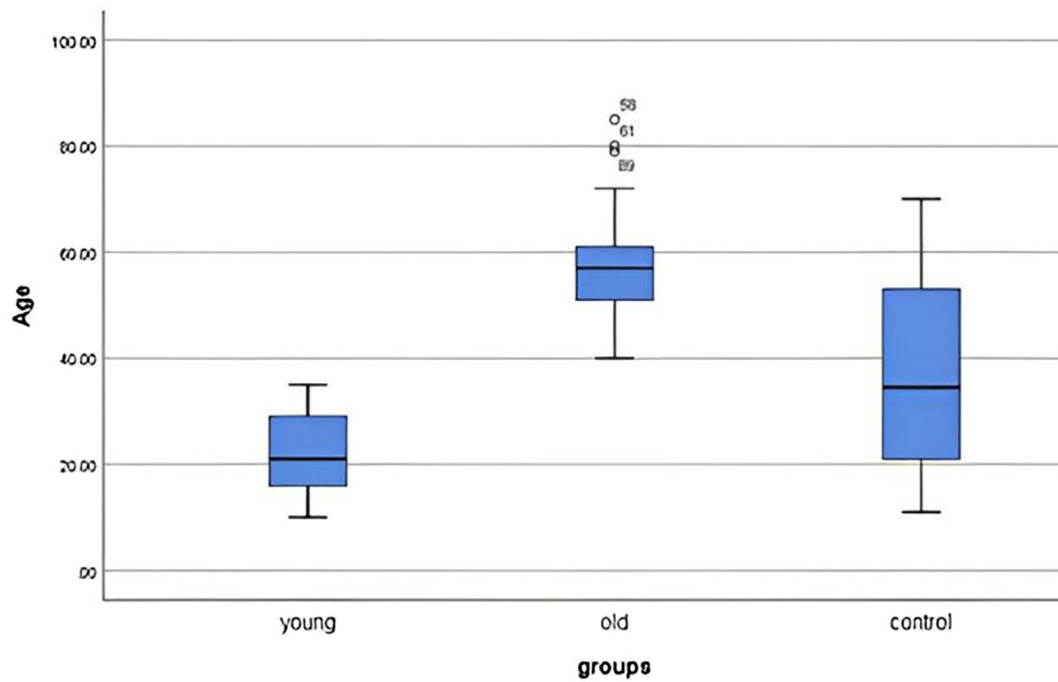
Recently, the *TCF7L2* gene has been suggested to play a role in obesity and T2DM.<sup>8</sup> Our results showed significant increases in HbA1c, TG, and LDL levels compared with

**Table 1: Gene expression primers.**

Gene	Primer	Primer sequence (5' → 3')	Length	P	Reference
<i>GAPDH</i>	Forward	GAAATCCCATCACCATCTTCCAGG	24	160	18
	Reverse	GAGCCCCAGCCTTCTCCATG	20		
<i>TCF7L2</i>	Forward	TGAAACCCAGATGTCACCAA	20	158	This study
	Reverse	AACGAAGATGGTCCCACTG	20		

P, Product size (bp)





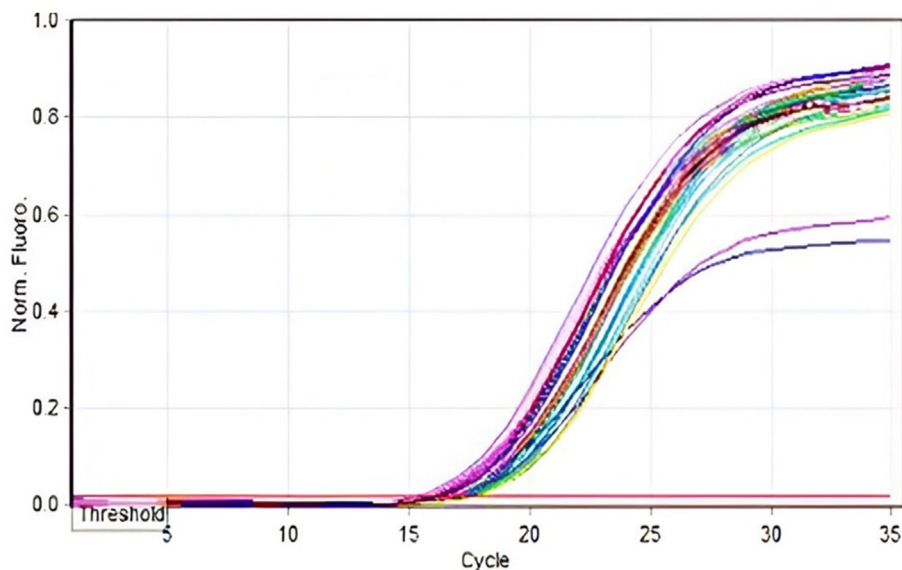
**Figure 1:** Mean age of the three groups (young T2DM patients, old T2DM patients, and healthy controls).

healthy controls. These results are consistent with those of Klisic et al.<sup>25</sup> who showed that an undesirable lipid profile was associated with T2DM, and recommended considering the lipid profile to predict high levels of HbA1c. The authors also advocated for the early detection of dyslipidemia (blood lipid level) and maintenance of normal lipid levels as a precautionary step to help with the diagnosis of diabetes.

Our results did not reveal a significant correlation between fold gene expression of *TCF7L2* and HbA1c concentration, which may have been due to the limited sample number. We found significant correlations between BMI and both TG and

LDL, which aligns with the fact that the rise in BMI is associated with dyslipidemia and diabetes.<sup>26</sup> Dyslipidemia is thought to be related to T2DM due to insulin resistance.<sup>27</sup> The causes of rising TG levels in T2DM are insufficient production or action of insulin.<sup>28</sup> Gluconeogenesis and glucagon-like peptide-1 (GLP-1) production are affected by the action of *TCF7L2*. This gene may cause T2DM by affecting lipid metabolism and adipogenesis.<sup>29</sup>

Our results on *TCF7L2* gene expression in older patients are consistent with those reported in a study conducted on human islets from diabetic patients, which showed a 5-fold increase in *TCF7L2* gene expression in T2DM.<sup>30</sup>



**Figure 2:** GAPDH amplification plots by qPCR.

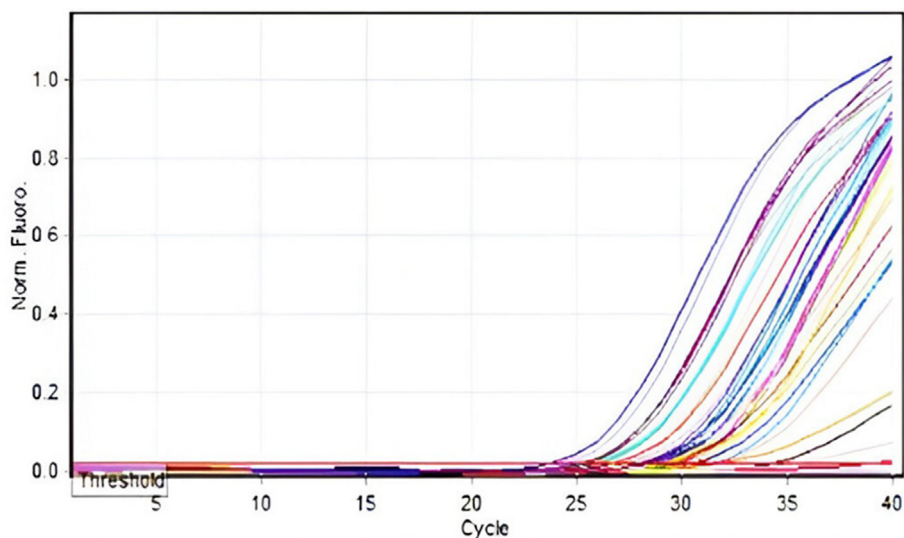


Figure 3: Amplification plots of *TCF7L2* genes by qPCR.

Table 6: Gene expression of *TCF7L2*.

	<i>TCF7L2</i> Ct Means	<i>GAPDH</i> Ct Means	$\Delta$ Ct	$\Delta\Delta$ Ct	Gene Expression Fold ( $2^{-\Delta\Delta Ct}$ )	<i>p</i> -value
Young T2DM	29.87	16.5794	13.29	2.27	0.207b	0.0001**
Old T2DM	26.90	16.6292	10.27	0.75	1.68a	
Control	27.88	16.8614	11.02	0	1.00a	

\*\* High significant result ( $p < 0.01$ ).

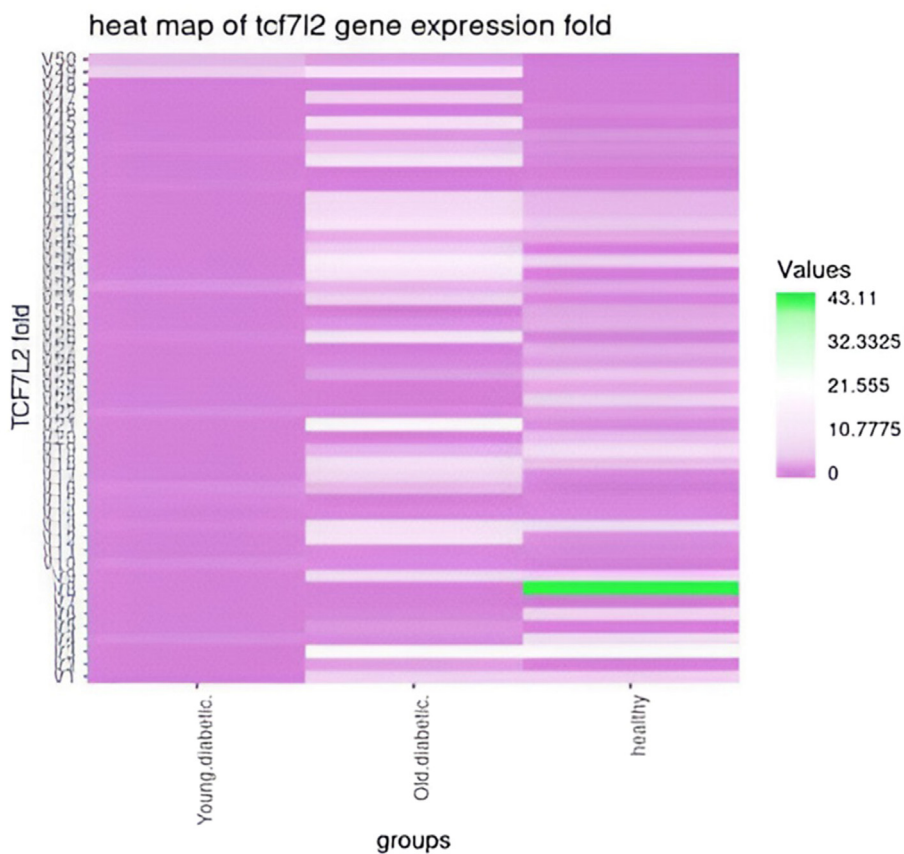


Figure 4: Heat map of *TCF7L2* gene expression for the three groups.

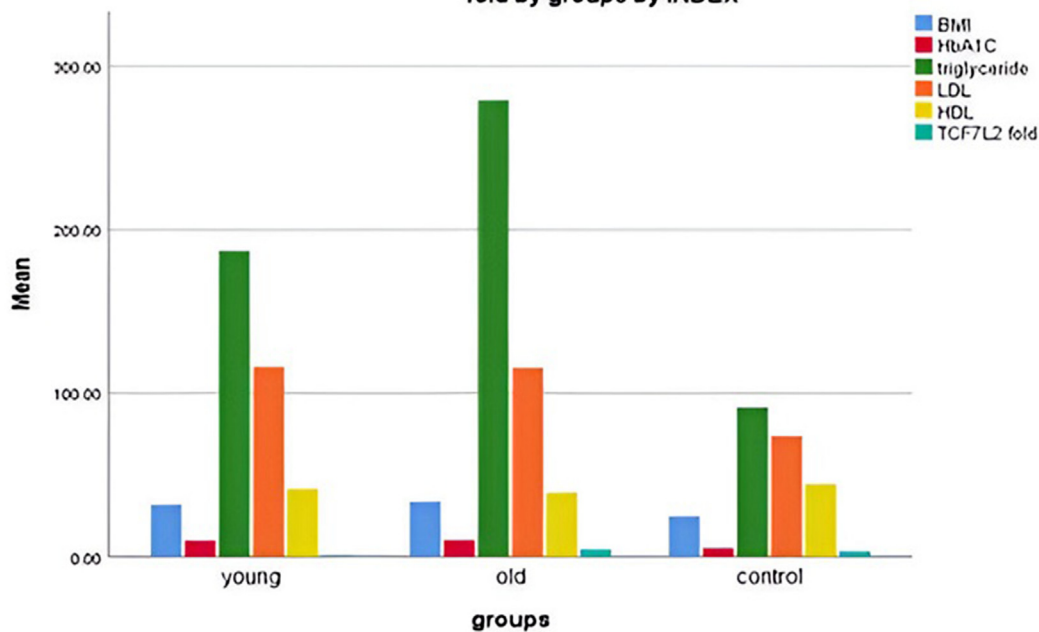
**Table 7: Results of the biochemical test results for the three groups.**

		N	Mean	SD	SEM	p-value
TG (mg/dL)	Young T2DM	50	193.0800b	82.83543	11.71470	0.0001**
	Old T2DM	50	273.9200a	182.19084	25.76568	
	Control	50	89.5000c	14.57738	2.06155	
LDL (mg/dL)	Young T2DM	50	110.1400a	43.42858	6.14173	0.0001**
	Old T2DM	50	113.7600a	45.76629	6.47233	
	Control	50	73.4000b	9.24276	1.30712	
HDL (mg/dL)	Young T2DM	50	40.3600a	10.93349	1.54623	0.037*
	Old T2DM	50	38.8180b	13.97984	1.97705	
	Control	50	44.0866a	3.57163	0.50510	

\* significant result ( $p < 0.05$ ).

\*\* High significant result ( $p < 0.01$ ).

**Clustered Bar Mean of BMI, Mean of HbA1C, Mean of triglyceride, Mean of LDL, Mean of HDL, Mean of TCF7L2 fold by groups by INDEX**



**Figure 5:** Means of the tested parameters.

*TCF7L2* plays a crucial role in the regulation of glucose in the blood. It encodes a transcription factor that is expressed in the fetal pancreas and regulates the Wnt signaling pathway through GLPs. Genetic alteration in *TCF7L2* may influence insulin resistance by modulating GLP-1. Saxena et al.<sup>31</sup> showed that modification of the *TCF7L2* gene increases the risk of developing T2DM by altering insulin secretion. The overexpression of this gene could impair beta cell functions.<sup>30</sup>

*TCF7L2* polymorphisms are significantly linked to T2DM susceptibility in Caucasian and Asian populations.<sup>32</sup> These polymorphisms cause T2DM in several ways such as impairment of insulin secretion, defects in glucose-induced glucagon suppression, abnormal insulin processing, and enhancement of liver glucose release during fasting.<sup>33</sup> Our results also revealed a significant decrease in *TCF7L2* gene expression in young patients, which may be related to the decrease in insulin resistance in these patients compared with older patients due to the efficiency of beta cells, which

decrease with duration of T2DM.<sup>34</sup> Younger patients are expected to have better beta cell proliferation and function compared with older patients.

The exact mechanism of how *TCF7L2* gene expression is regulated during the development of T2DM has not been completely discovered, but many previous studies have focused on its role in beta cells and insulin production rather than insulin resistance. A study conducted by Shu et al.<sup>35</sup> showed opposite regulation between the level of mRNA and protein levels of *TCF7L2* in T2DM. There was a reduction in *TCF7L2* protein expression at the same time as an increase in *TCF7L2* mRNA expression due to post-transcriptional regulation. The authors confirmed that the increase in *TCF7L2* mRNA in T2DM is a consequence of beta cell function.

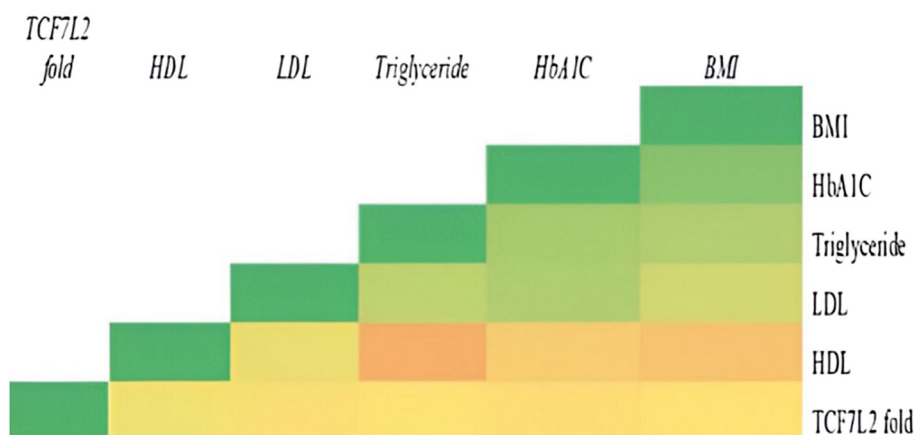
Post-transcriptional regulation is disrupted in T2DM through inhibition of translation by the accumulation of unfolded protein. Beta cells can process and fold large amounts of protein (about 5% of the total protein is insulin).

**Table 8: Correlation of *TCF7L2* gene expression with the biochemical tests.**

		Age	BMI	HbA1c	TG	LDL	HDL	<i>TCF7L2</i> fold
Age	r	1	0.252	0.054	0.284 <sup>a</sup>	0.008	-0.082	0.104
	p		0.002	0.514	0.000	0.924	0.317	0.204
BMI	r	0.252 <sup>a</sup>	1	0.655 <sup>a</sup>	0.411 <sup>a</sup>	0.236 <sup>a</sup>	-0.217 <sup>a</sup>	0.004
	p	0.002		0.000	0.000	0.004	0.008	0.958
HbA1c	r	0.054	0.655 <sup>a</sup>	1	0.479 <sup>a</sup>	0.430 <sup>a</sup>	-0.149	-0.031
	p	0.514	0.000		0.000	0.000	0.069	0.703
TG	r	0.284 <sup>a</sup>	0.411 <sup>a</sup>	0.479 <sup>a</sup>	1	0.339 <sup>a</sup>	-0.374 <sup>a</sup>	0.016
	p	0.000	0.000	0.000		0.000	0.000	0.842
LDL	r	0.008	0.236 <sup>a</sup>	0.430 <sup>a</sup>	0.339 <sup>a</sup>	1	0.096	-0.037
	p	0.924	0.004	0.000	0.000		0.243	0.656
HDL	r	-0.082	-0.217 <sup>a</sup>	-0.149	-0.374 <sup>a</sup>	0.096	1	0.061
	p	0.317	0.008	0.069	0.000	0.243		0.458
<i>TCF7L2</i> fold	r	0.104	0.004	-0.031	0.016	-0.037	0.061	1
	p	0.204	0.958	0.703	0.842	0.656	0.458	

Correlation coefficient (r), significance (p-value).

<sup>a</sup> Significant correlation at the 0.01 level (2-tailed).



**Figure 6:** Heat map of Pearson's correlation for all data.

There are specific requirements for beta cells to function properly. Stress or accumulation of antioxidant molecules could alter post-transcriptional regulation. These facts are in accordance with our results showing increased *TCF7L2* mRNA levels in old patients since stress increases with age. Previous studies have shown that the increase in *TCF7L2* mRNA expression compensates for the lack of *TCF7L2* protein. The presence of this protein is essential for the functioning and presence of beta cells.<sup>36</sup>

The loss of *TCF7L2* protein results in a decrease in the GLP-1 receptor (GLP-1R) and gastric inhibitory polypeptide (GIP)-stimulated AKT phosphorylation, as well as a decrease in the phosphorylation of AKT-mediated forkhead box 1 and nuclear exclusion. The interaction between *TCF7L2* and GLP-1R/GIP receptor expression is essential in the regulation and survival of beta cells and thus controls insulin production. Any disturbance in this relationship could alter glucose homeostasis and insulin production.<sup>35</sup> However, age represents a main risk factor for T2DM, due to the decline in beta cells with disease duration. In addition, insulin secretion decreases with age.<sup>37</sup> Higher glucose-stimulated insulin secretion has been observed in younger patients (<40 years)

compared to older patients, which correlates with the ability of beta cells to generate ATP.<sup>38</sup>

#### Limitations and future research

The main limitation of this study was the limited sample size, due to the reliance on personal funding. We recommend examining a larger sample size and examining *TCF7L2* single nucleotide polymorphisms.

#### Conclusion

*TCF7L2* gene expression was affected by age. Old T2DM patients had the highest score, which may influence beta cell functions and insulin secretion.

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### Conflict of interest

The authors have no conflicts of interest to declare.

### Ethical approval

The Ethics Committee of the Genetic Engineering and Biotechnology Institute of Baghdad, Iraq approved this study (Ref No. EC/1310-A) on May 15, 2022.

### Author contributions

AHY and AMA conceived and designed the study. AHY conducted the research, provided the research materials, and collected and organized the data. AHY analyzed and interpreted the data. AHY wrote the initial and final drafts of the article and provided logistical support. All authors have critically reviewed and approved the final draft and are responsible for the content and similarity index of the manuscript.

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