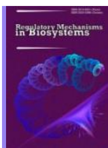

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Association of TCF7L2 gene expression with clinical and biochemical markers in type 2 diabetes mellitus patients in Babylon Province

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T2DM is a long-term, progressive metabolic disease characterized by elevated blood glucose levels brought on by insulin resistance and a decline in pancreatic β-cell function. Our research aimed to examine the correlation between the levels of parameters (FBS, HbA1c, TG, TC, HDL, LDL, and VLDL) and the genetic expression of the TCF7L2 gene in patients with T2DM (G2) in comparison to the control group (G1). There were 200 participants in the study: 100 patients with T2DM (G2) and 100 ethnically matched controls (G1). The T2DM patients were nonsmokers between the ages of 45 and 60 who attended the Marjan Teaching Hospital, Specialized Diabetes Center in Babylon Governorate, Iraq. Together with the genetic expression analysis of the TCF7L2 gene, which was confirmed to be pertinent to the clinical symptoms and biochemical features of the present investigation, specific tests were performed for the biochemical parameters of FBS, HbA1c, TG, TC, HDL, LDL, and VLDL. The study found that there was a significant difference between the two study groups in the levels of FBS, HbA1c, TC, and the gene expression level of the TCF7L2 gene. The mean values for the parameters of FBS, HbA1c, TC, HDL, LDL and VLDL were high in the T2DM group (G2) compared to the control group (G1). By studying the Pearson correlation, a correlation was found between FBS and HbA1c, between FBS and TG, and between HbA1c and TG, where the Pearson correlation value was significant. The study concluded that low gene expression of TCF72 is associated with high FBS, HbA1c, and TG levels in patients with T2DM, and that there is a correlation between clinical parameters in patients with T2DM..

Keywords: type 2 diabetes mellitus disease; T2DM; TCF7L2.

Introduction

Type 2 diabetes mellitus (T2DM) is a complex, lifelong metabolic disease marked by chronic high blood glucose due to resistance of tissue to the action of insulin and the slow failure of pancreatic β-cells. At first, insulin output stays the same or goes up, but the body becomes less sensitive to insulin in skeletal muscle, fat, and the liver (Galicia-Garcia et al., 2020). This results in reduced glucose absorption, heightened hepatic gluconeogenesis, and higher blood glucose levels. With time, β-cells experience functional deterioration, leading to insufficient insulin production to satisfy metabolic requirements. Type 2 diabetes mellitus (T2DM) is multifactorial, exhibiting significant correlations with obesity, central adiposity, sedentary lifestyle, increasing age, and genetic susceptibility (Cunningham et al., 2021). The pathophysiological pathways include lipotoxicity, glucotoxicity, low-grade inflammation, oxidative stress, and changes in gut macrobiotic, all of which contribute to insulin resistance and β-cell dysfunction (Thipsawat, 2021). T2DM often progresses gradually and might stay asymptomatic for extended periods. Symptoms manifest as polyuria, polydipsia, weariness, fluctuations in weight, hazy eyesight, and delayed wound healing (Ruze et al., 2023). Improper management of persistent hyperglycaemia leads to microvascular consequences (retinopathy, nephropathy, and neuropathy) and macrovascular issues (atherosclerotic cardiovascular disease, stroke, and peripheral artery disease).

Lifestyle modification comprising a balanced diet, consistent exercise, and weight reduction constitutes the fundamental basis of treatment (Bellary et al., 2021). Pharmacological alternatives include metformin as the primary therapy, supplemented by medicines such as sulfonylureas, thiazolidinedione, DPP-4 inhibitors, SGLT2 inhibitors, and GLP-1 receptor agonists, used either individually or in combination.

Insulin treatment may be necessary in advanced stages (Farmaki et al., 2020). Transcription Factor 7-Like 2 (TCF7L2) is one of the genes most closely linked to type 2 diabetes mellitus (T2DM) and is essential for glucose metabolism. The transcription of genes related to insulin production, secretion, and β-cell survival is regulated by TCF7L2 (Younus & Al-Faisal, 2024). Hyperglycemia may result from overexpression or certain polymorphisms (like rs7903146) that increase β-cell death and hinder glucose-stimulated insulin production. Incretin hormones (GLP-1, GIP) are modulated by TCF7L2, which increases the release of insulin in response to oral glucose (Del Bosque-Plata et al., 2022).

Reduction or modification of TCF7L2 activity reduces incretion responsiveness, a characteristic deficit of type 2 diabetes. Insulin resistance worsens due to impaired gluconeogenesis and lipid metabolism caused by abnormal TCF7L2 expression in the liver and adipose tissue (Aboelkhair et al., 2021). Although altered TCF7L2 gene expression affects beta cell function, incretin signalling, and hepatic glucose metabolism in T2DM patients, the specific mechanisms connecting this changed expression to the illness remain ambiguous. Controversy persists over the mechanisms by which changed TCF7L2 gene expression induces beta cell failure, and the precise histological patterns of TCF7L2 expression and its role in insulin production remain unclear.

The interplay of genetic variants, epigenetic control, and environmental factors affecting TCF7L2 activity remains unclear. This gap hinders the translation of genetic discoveries into tailored therapy strategies and the establishment of dependable biomarkers for the early identification of disease risk in T2DM. The research aims to investigate gene expression using qPCR technology to assess the impact of T2DM on TCF7L2 gene expression in comparison to the control group (G1).

Materials and methods

The research included 200 individuals, with 100 diagnosed with type 2 diabetes mellitus (G2) and the remaining 100 being healthy (G1). The study started on February 2, 2025, and ended on April 16, 2025, the date of sample collection. The ages of the participants ranged from 45 to 60 years. This age group was adopted because it is the age group most affected by type 2 diabetes. Other age groups and

samples of smokers were excluded to prevent the effects of smoking and the effects resulting from age influencing the study results. Also, the participants in the T2DM group of patients (G2) did not suffer from other diseases that were not addressed in this study. The purpose of this was to exclude the effect of complications of these diseases, thus making the study more accurate and specific to studying the effect of T2DM disease only. 5 mL of venous blood was collected from participants in study groups G1 and G2 who were attending Marjan Teaching Hospital, Specialized Diabetes Center in Babylon Governorate, Iraq, and the clinical examinations for the study were performed in the same hospital. The serum was then spun in a centrifuge at room temperature for about ten minutes to separate it (4000 x g). The complete blood and serum were kept at –20 °C until they were needed for other tests. Using the Beckman Coulter Au 480 device (Beckman Coulter, USA), the first part of the study included direct, specialized evaluations of several biological parameters, such as FBS, HbA1c, TG, TC, HDL, LDL, and VLDL.The TCF7L2 genetic expression test was the subject of the second study section.

For the purpose of RNA analysis, a 5 mL blood sample was taken from each participant in the two study groups (G1 and G2). The RNA Extraction Kit (ADDBio/Korea) was used to extract genomic RNA from blood at a concentration of 20 μg/mL or less. After extracting RNA, a NanoDrop instrument was used to test the absorbance at 260–280 nm to find out how much RNA there was and how pure it was to determine the standard metrics that are based on the Quantus Fluorometer's results. Next, the RNA samples were evaluated using Quantus techniques. The range of concentration and purity is 1.7–1.9. The RNA Integrity Number (RIN) was evaluated using the underlying process method before and after processing. RNA fragments were separated by capillary electrophoresis using an Agilent 2100 Bioanalyzer. Separating RNA fragments created an electropherogram, which displays them as peaks along a signal intensity axis. Using the prominence and ratio of the key ribosomal peaks (28S, 18S, and 5S), a unique RIN algorithm analyzed this electropherogram to detect and assess rRNA peaks.

Increasing the ratio of these peaks to the total RNA area reduces degradation. The electropherogram shows degradation products as tiny pieces or "anomalies" that it recognizes and measures. Features in the fast and post portions of the trace are evaluated by the algorithm, ranging from 1 (extremely deteriorated) to 10 (intact); the program automatically assigns a RIN based on these findings. The RNA samples were of extremely good quality and appropriate for genetic study, as shown by the RNA Integrity Number (RIN) of 8.2 to 8.5. The extraction techniques were more accurate, and the observed changes in gene expression were caused by biological reasons rather than RNA degradation. An ADDBio/Korea kit and a Promega (USA) QuantusTM Fluorometer are used for the cDNA synthesis.

Primers were used for amplification of the retrieved cDNA. Using a real-time qPCR device (BioRad, USA), the GAPDH gene was identified as 5'-GTCTCCTCTGACTTCAACAGCG-3' (Forward) and 5'-ACCACCCTGTTGCTGTGTAGCCAA-3' (Reverse); the adiponectin gene was identified as 5'-GCCAGCTCCTAGCCGTAGACT CTGCTG -3' (Forward) and 5'-GGAGGTCTGTGATGAAAGAGG CC-3' (Reverse). For BioRAD (USA), thermal conditions were performed. The ADDBio/Korea cDNA synthesis kit may be used to create the cDNA that is extracted from total RNA.

The adiponectin and GAPDH gene copy numbers were calculated using the Ct values obtained from the genomic DNA sample. The most common and easiest way to figure out the chances that the target and reference genes are amplified with the same 100% efficiency is to utilize the same method. Before employing the Livak technique, the target and reference genes must have the same amplification efficiency. Transcript levels utilizing TCF7L2 data were normalized using the following calculation in accordance with the ΔΔCt method:

ΔCT (test) = CT (target, test) – CT (ref, test)

ΔCT (control) = CT (target, control) – CT (ref, control)

ΔΔCT= ΔCT (test) – ΔCT (control)

2-ΔΔCT = [(CT gene of interest – CT internal control) sample A — (CT gene of interest – CT internal control) sample B)]

2-ΔΔCT = ratio of normalized expression

E% = 10^{-1} x slope (Initial conc) – 1.

Descriptive statistics revealed a statistically significant association (P-value < 0.05) between the RT-qPCR amplification of TCF7L2 and the housekeeping gene (GAPDH) as well as data normalization in the two groups after ensuring the normal distribution of data. The results of the present study are expressed as percentages or as mean ± standard deviation (SD). Pearson correlation analysis was performed between TCF7L2 expression and clinical biochemical markers used in the study. We used SPSS version 27 (SPSS Inc., Chicago, IL, USA) to analyze the mean differences between two groups for quantitative variables. Statistical analyses on clinical biological markers were conducted using GraphPad Prism version 9 (GraphPad Software, San Diego, CA, USA). The data were expressed as mean ± standard deviation (SD) with a significance threshold of P < 0.05 (ANOVA).

Results

This analysis examined the parameter tests of the control group (G1) and T2DM patients group (G2) (Table 1). Fasting blood sugar is represented by FBS, glycated hemoglobin A1c by HbA1c, triglycerides by TG, total cholesterol by TC, high density lipoprotein by HDL, low density lipoprotein by LDL, and very low density lipoprotein by VLDL. Table 1 shows the clinical characteristics. This experiment employed age matching to reduce the discrepancies in parameter values that came from big variations in age.

Table 1
The clinical and biochemical characteristics of the study groups (G1 and G2) (mean ± SD)

Parameters	Groups		P-value
	G1-control	G2-T2DM	
FBS, mg/dL	99.2 ± 8.5	316.4 ± 22.2	0.0001
HbA1c, mmol/mol	4.76 ± 0.69	11.17 ± 2.56	0.0001
TG, mg/dL	148 ± 31	260 ± 35	0.049
TC, mg/dL	183 ± 16	194 ± 11	0.460
HDL, mg/dL	45.3 ± 6.9	48.3 ± 10.9	0.507
LDL, mg/dL	89.2 ± 14.8	102.4 ± 16.6	0.324
VLDL, mg/dL	36.2 ± 9.5	43.2 ± 13.3	0.483

The relative quantification analysis of TCF7L2 gene expression in the control group (G1) and the T2DM patients (G2) yielded the findings shown in Table 2. GAPDH gene amplification in G1 and G2 is compared in Figures 1 and 2, whereas TCF7L2 gene amplification in G1 and G2 is compared in Figures 3 and 4.

Table 2
The comparison between the gene expression TCF7L2 gene as compared with the housekeeping (GAPDH) gene

Gene	Groups		T-test	P-value	$2^{-\Delta\Delta ct}$	Efficiency, %
	G1-control (mean ± SD)	G2-, β-TM (mean ± SD)				
TCF7L2	18.42± 1.13	25.17± 2.34	25.71	0.002	0.034	90–96

Table 3
The correlation between TCF7L2 gene expression and biomarkers (FBS, HbA1c, and TG) in the T2DM group (G2)

	Correlation	TCF7L2	FBS	HbA1c
FBS	Pearson correlation	–0.168	–	–
	Sig. (2-tailed)	0.114	–	–
HbA1c	Pearson correlation	–0.168	1.000	–
	Sig. (2-tailed)	0.114	0.0001	–
TG	Pearson correlation	0.023	0.256	0.256
	Sig. (2-tailed)	0.832	0.015	0.015

A number of parameters (FBS, HbA1c, and TG) had significantly different mean values, according to the analysis; the T2DM patient group (G2) had significantly higher values than the control group (G1) (P < 0.05), while the parameters (TC, HDL, LDL, and VLDL) were non-significant (P > 0.05).

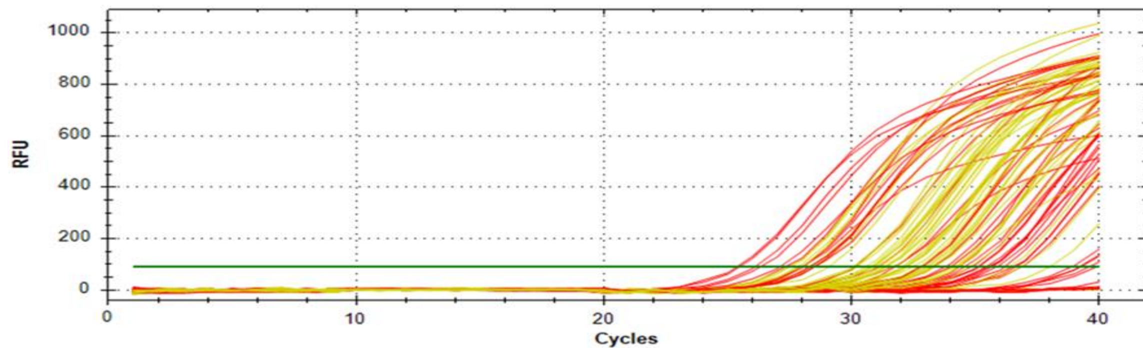

Fig. 1. Amplification curve of housekeeping (GAPDH) gene expression in T2DM patients' group (G2, red line) and control individuals (G1, yellow line)

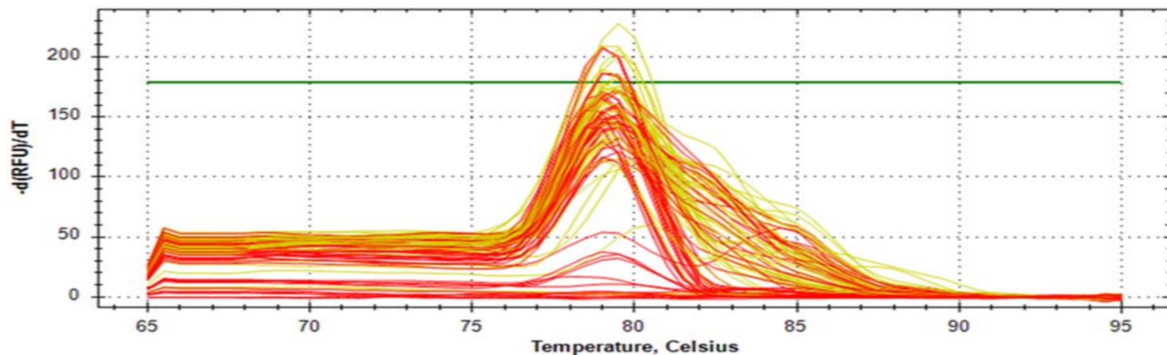

Fig. 2. Disassociation melting curve analysis of housekeeping (GAPDH) gene expression in T2DM patients' (G2, red line) and control (G1, yellow line)

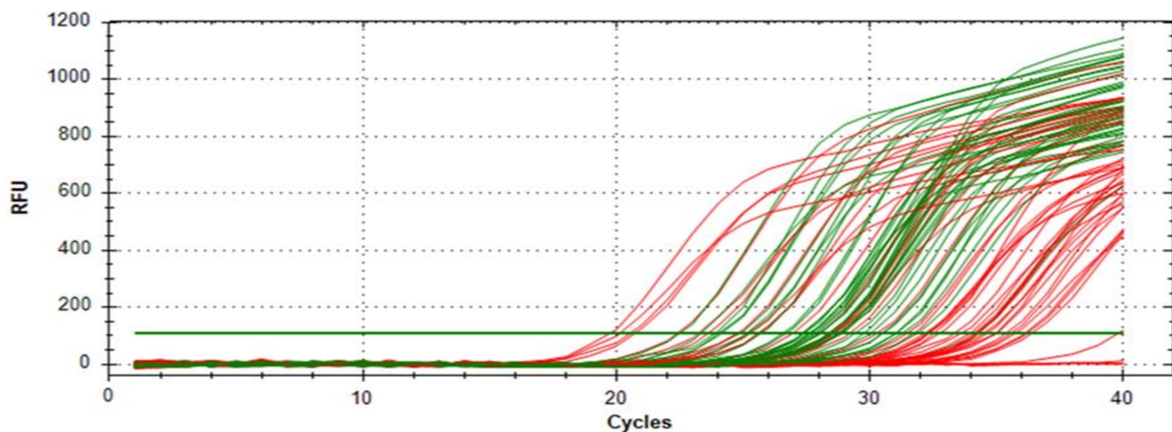

Fig. 3. Amplification curve of the expression of the gene of interest (TCF7L2) in T2DM patients' group (G2) depicted in red: green indicates the control group (G1)

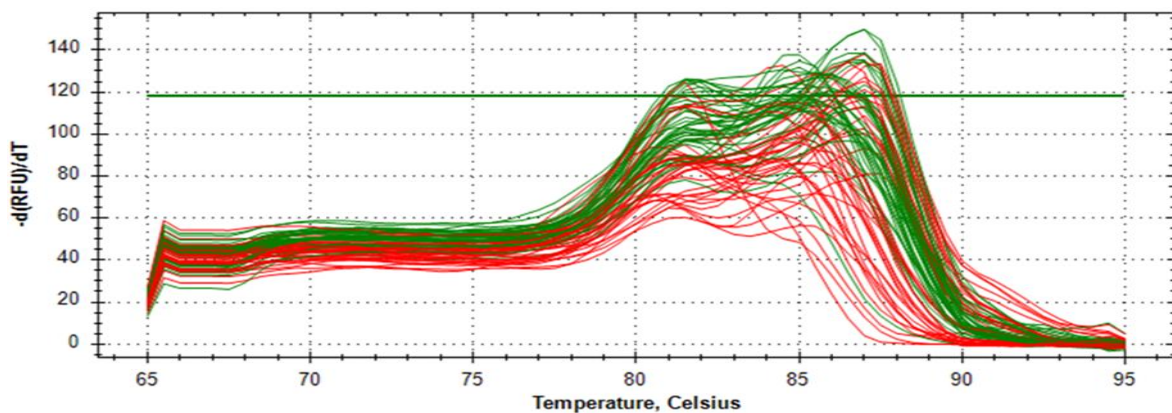

Fig. 4. Disassociation melting curve analysis with the samples tested for the gene of interest (TCF7L2) in T2DM patients' group (G2, green line) and controls (G1, red line)

Discussion

The current research supports the findings of Ajami et al. (2020), who found elevated FBS levels in T2DM patients compared to controls. In individuals with T2DM, elevated FBS is caused by a combi-

nation of decreased pancreatic β-cell function and insulin resistance. The liver still produces glucose during a fast by hepatic gluconeogenesis and glycogenolysis, but this production is not inhibited because of insulin resistance. At the same time, β-cell malfunction prevents them from secreting enough insulin to combat the rise in blood sugar (Ajami et al., 2020). Chronic inflammation, elevated free fatty acids, and genetic variables (such as TCF7L2 variations) exacerbate β-cell dysfunction and insulin resistance. Accordingly, inadequate insulin action and unchecked hepatic glucose production cause T2DM patients to have consistently elevated FBS (Nazari et al., 2021). The present study confirms the results of Sartore et al. (2023), who discovered that T2DM patients had higher HbA1c levels than controls. Chronic hyperglycemia during 8–12 weeks is shown in a high HbA1c inT2DM. It happens as a result of reduced pancreatic β-cell insulin production and insulin resistance in muscle, liver, and adipose tissue. The insulin-resistant liver increases blood glucose levels during fasting by continuing gluconeogenesis and glycogenolysis (Sartore et al., 2023). In addition to the inability of the peripheral tissues to adequately absorb glucose, β-cell malfunction hinders the production of sufficient compensatory insulin. HbA1c is the result of non-enzymatic hemoglobin glycation caused by persistently high glucose. Hyperglycemia is further aggravated by genetic variables (such as TCF7L2 polymorphisms), obesity, chronic inflammation, and lipotoxicity, which results in consistently high HbA1c values and indicates inadequate long-term glycemic management (Kidwai et al., 2020). The results of the present study corroborate those of Sciali et al. (2021), who discovered that T2DM patients had higher TG levels than controls. The main causes of elevated triglycerides (TG) in people with T2DM are insulin resistance and changes in lipid metabolism. In adipose tissue, insulin typically inhibits lipolysis and encourages lipid storage; in type 2 diabetes, resistance causes an increase in the release of free fatty acids (FFA) into the bloodstream (Scicali et al., 2021). Increased FFAs promote the production of hepatic triglycerides and the release of very-low-density lipoproteins (VLDLs) (Liu et al., 2022). Additionally, TG clearance from plasma is decreased by decreased lipoprotein lipase activity. Chronic inflammation and hyperglycemia also disrupt lipid metabolism. Obesity makes these consequences worse, especially central adiposity. Patients with T2DM often have hypertriglyceridemia, which raises their risk of atherogenic dyslipidemia and cardiovascular disease (Chamroonkiadtikun et al., 2020). High total cholesterol (TC) in individuals with T2DM is mostly caused by insulin resistance, poor lipid metabolism, and prolonged hyperglycemia. Furthermore, dyslipidemia is made worse by decreased HDL production and lipoprotein lipase activity. Disrupted cholesterol control is a result of obesity, long-term low-grade inflammation, and altered adipokine production (such as leptin and adiponectin). Together, these variables cause atherogenic hypercholesterolemia, which raises the cardiovascular risk in people with T2DM (Ragheb et al., 2020). Recent study corroborates the results of Aktas et al. (2020), which indicated increased HDL levels in individuals with T2DM relative to controls. Gene mutations that increase HDL synthesis or decrease its clearance, such as those in the CETP, APOA1, or LIPC genes, may be the cause of high HDL cholesterol (HDL-C) in T2DM. HDL levels may also be raised by certain drugs, such as fibrates, niacin, or some SGLT2 inhibitors (Aktas et al., 2020). Even when HDL is increased, T2DM may diminish its functional quality, which lessens its ability to prevent cardiovascular disease (Yuge et al., 2023). The results of the present study corroborate those of Klimentidis et al. (2020), who discovered that T2DM patients had higher LLDL levels than controls. The main causes of elevated low-density lipoprotein (LDL) cholesterol are insulin resistance and hyperglycemia, however there are other contributing factors as well. Insulin resistance decreases the amount of LDL removed from the bloodstream via affecting hepatic LDL receptor function. Apolipoprotein B-100 is glycated by hyperglycemia, which changes the structure of LDL particles and makes them more atherogenic while also making it more difficult for receptors to recognize them (Klimentidis et al., 2020). Furthermore, hepatic synthesis of very-low-density lipoprotein (VLDL), a precursor to LDL, is enhanced by elevated free fatty acid flow from adipose tissue in insulin-resistant conditions. Increased tiny, dense LDL particles, which are more oxidative reactive and have a longer residence period in plasma, are another aspect of dyslipidemia in type 2 diabetes (Bonilha et al., 2021). The current study corroborates the results of Carlier et al. (2020), which indicated increased VLDL levels in T2DM patients relative to controls. The main causes of elevated very-low-density lipoprotein (VLDL) levels are poor VLDL degradation and increased hepatic VLDL synthesis (Carlier et al., 2020). Type 2 diabetes is characterized by insulin resistance, which increases the flow of free fatty acids to the liver, promoting the synthesis of triglycerides and the subsequent assembly and release of VLDL. Delays in VLDL removal from circulation are also caused by decreased activity of lipoprotein lipase, an enzyme essential for VLDL breakdown (Bragg et al., 2022).

The study's findings demonstrated that the mean values of the TCF7L2 gene's genetic expressions varied between the first group (G1), which comprised healthy controls, and the second group (G2), which comprised T2DM patients (Table 2). Compared to the control group (G1), the T2DM patient group's (G2) P-values were considerably lowers ($P < 0.05$).

The amplification curve depicts the expression levels of the GAPDH in two cohorts: Type 2 diabetes mellitus patients (G2, shown by red lines) and healthy control subjects (G1, indicated by yellow lines), as seen in Figure 1. The x-axis represents the number of PCR cycles, whilst the y-axis displays the relative fluorescence units (RFU), indicating the quantity of amplified product. The horizontal threshold line on the figure indicates the point when fluorescence markedly exceeds the background, facilitating the determination of the cycle threshold (Ct) value. The control group (G1) demonstrates earlier amplification, with the majority of curves above the threshold between around 18 and 28 cycles, indicating elevated initial expression of GAPDH (Camacho-Jiménez et al., 2023). The patient group (G2) exhibits delayed amplification, with curves often intersecting the threshold between 25 and 35 cycles, indicating reduced GAPDH expression. The decreased expression of GAPDH, a housekeeping gene usually maintained at consistent levels, in T2DM patients may indicate more extensive disturbances in cellular function or metabolic activities linked to the illness (Carvajal-Agudelo et al., 2022). The dissociation (melting) curve analysis shown in Figure 2 assesses the specificity of the amplified GAPDH gene product in both type2 diabetes mellitus patients (G2, red lines) and control subjects (G1, yellow lines).Temperature in degrees Celsius is shown on the x-axis, while DNA melting behavior is indicated by the y-axis, which exhibits the rate of change in fluorescence with temperature (−d(RFU)/dT). Both groups show a single, distinct peak at around 80 °C, indicating that little primer-dimer formation or non-specific amplification occurred and that a consistent, particular amplicon was produced (Kose, 2024). The GAPDH qPCR amplification's specificity in both cohorts is further supported by the alignment of melt peaks between G1 and G2. The consistency of the amplicon sequence and product length across samples is further supported by the comparable melting temperatures, which further support the validity of GAPDH as a reference gene in this experimental configuration (Kwon et al., 2021). The expression of the target gene, TCF7L2, in people with T2DM (G2, red lines) as opposed to healthy controls (G1, green lines) is shown in the amplification curve. The number of PCR cycles is shown by the x-axis, and the quantity of amplified product is indicated by the relative fluorescence units (RFU) on the y-axis. Amplification curves that pass the threshold early indicate greater starting expression of the target gene, much like in a typical qPCR study (Jing et al., 2021). Higher expression levels of TCF7L2 are shown by the control group (G1, green) in Figure 3, which shows early amplification and often crosses the threshold between ~20 and 28 cycles. In contrast, the T2DM patient group (G2, red) has a discernible amplification delay, surpassing the threshold around 28–35 cycles, indicating somewhat lower gene expression levels (Jing et al., 2021). Given the gene's known involvement in glucose metabolism and insulin secretion pathways, the clear distinction between the two groups points to a downregulation of TCF7L2 in T2DM patients. These findings could suggest that the pathogenesis of T2DM involves deregulation of TCF7L2. The dissociation (melting) curve analysis shown in this figure evaluates the

specificity of qPCR amplification for the TCF7L2 gene in samples from T2DM patients (G2, green lines) and healthy controls (G1, red lines), as seen in Figure 4. The temperature in degrees Celsius is shown by the x-axis, and the rate of DNA strand separation as temperature increases is shown by the y-axis, which shows the negative derivative of fluorescence with respect to temperature (−d(RFU)/dT). Both groups exhibit a prominent peak at 84–87 °C, signifying the generation of a distinct and uniform TCF7L2 amplicon across all samples. The existence of a single peak and the lack of numerous peaks or shoulders indicate the high specificity of the used primers, with no notable non-specific products or primer-dimers present (Krawczyk et al., 2025). The uniformity in melting temperature across the patient and control groups verifies that the amplified product is congruent in sequence and size throughout. This melt curve analysis confirms the validity of the qPCR findings for TCF7L2 expression and corroborates the trustworthiness of the previous amplification data comparing gene expression levels in T2DM patients and healthy persons. The correlation study that used Pearson's correlation method found a relationship between the clinical parameters, as it found a correlation between FBS and HbA1c, between FBS and TG, and between HbA1c and TG, where the Pearson correlation value was influential (P-value < 0.05), as shown in Table 3.

Conclusion

This findings suggest that downregulation of TCF7L2 gene expression is associated with elevated FBS, HbA1c, and TG in T2DM patients, supporting its role as a potential molecular biomarker for early detection of diabetes in adults.

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