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Study of transcription factor 7-like 2 (TCF7L2) gene polymorphism in cirrhotic patients with diabetes

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Abstract

Patients with chronic liver disease (CLD) as chronic hepatitis C (CHC) are at high risk of diabetes type 2 (T2D). Genetic factors are suggested to modulate diabetes development in cirrhotic patients. TCF7L2 gene has been reported to be associated with type 2 diabetes, but the association of TCF7L2 with cirrhotic patients with diabetes is unclear. We aimed to study the TCF7L2 gene polymorphisms (rs 290487) in cirrhotic patients with diabetes.

Method The study was assessed on 25 cirrhotic patients with type 2 diabetes who were compared to 25 cirrhotic HCV patients (nondiabetic), 25 diabetic type 2 patients, and 25 age- and gender-matched healthy control groups. After the collection of relevant clinical data and basic laboratory tests, single-nucleotide polymorphism (SNP) in the TCF7L2 gene (rs290487) was performed by a real-time PCR technique.

Results Cirrhotic patients with diabetes presented significantly poorer liver function, higher incidence of cirrhotic complications, and higher glucose levels compared with cirrhotic nondiabetic patients. The TCF7L2 rs290487 TT variant showed significantly increased diabetes risk in cirrhotic patients compared with CC and CT genotypes.

Conclusions TCF7L2 rs290487 polymorphism could be associated with increased diabetic risk in cirrhotic patients.

Keywords Liver cirrhosis, Chronic hepatitis C, Diabetes mellitus, TCF7L2, Single-nucleotide polymorphism

Introduction

The liver has a major role in the control of glucose homeostasis in the body. The association between chronic liver disease (CLD) and diabetes mellitus (DM) is known since long. Chronic liver disease (CLD) such as chronic hepatitis C is a major risk factor for decreased glucose tolerance and development of type 2 diabetes (T2D) [1, 2]. In its turn, diabetes not only exacerbates the clinical course of cirrhosis in chronic viral hepatitis but also increases the risk of complications after liver transplantation and reduces life expectancy [3, 4].

Several processes have been involved in the development of insulin resistance (IR) in chronic hepatitis C (HCV) patients. Insulin generally carries out its biological effects through the phosphorylation of insulin receptor substrates 1 and 2 (IRS-1 and IRS-2) [5]. Hepatitis

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C virus (HCV) core upregulated suppressor of cytokine signaling (SOCS) 3 which causes ubiquitination of IRS1 and IRS2 impairing their expression [6]. Besides, HCV significantly blunted the activation of two downstream targets that are critical for most of the metabolic effects of insulin: phosphoinositide 3-kinase (PI3-kinase) and Akt (Protein Kinase B, a downstream target of PI3-kinase) [7].

Also, inflammatory response to HCV is demonstrated to be implicated. Tumor necrosis factor alpha (TNF-α) and IL-6 can induce insulin resistance by activating Serine phosphorylation of IRS-1 [8]. Serine/threonine (Ser/Thr) phosphorylation of IRS-1 inhibits its association with the insulin receptor, which in turn inhibits tyrosine phosphorylation of IRS-1 and promotes degradation. In addition, TNF-α can directly cause sensitization of beta cells to the toxic effects of free radicals [9]. Further, TNFα downregulates glucose transporter type 4 (GLUT4), insulin-regulated glucose transporter, and mRNA expression in muscle and adipose tissues [10].

Genetic factors were reported to have a great influence on the development of diabetes. Genome-wide association study (GWAS) revealed transcription factor 7-like 2 (TCF7L2, also known as TCF4) as the most significant type 2 diabetes candidate gene [11].

TCF7L2 is located on chromosome 10q25.2-q25.3. The gene contains 19 exons and has autosomal dominant inheritance, and SNP rs290487 is located close to the 3′ end of the gene [12]. TCF7L2 plays a master role in regulating insulin biosynthesis, secretion, and processing [13]. Moreover, TCF7L2 through the Wnt signaling pathway is essential for the proliferation of the pancreatic epithelium and islet proliferation. It can directly bind to multiple gluconeogenesis-associated genes and is involved in hepatic glucose metabolism [14]. In this study, we aim to study TCF7L2 gene polymorphisms in cirrhotic patients with type 2 diabetes.

Subjects and methods

Patients

The present case–control study was conducted in the National Liver Institute, Menoufia University, in the period from October 2019 to October 2020. A total of 100 Egyptian subjects, 25 cirrhotic patients with type 2 diabetes who were compared to 25 cirrhotic HCV patients (nondiabetic), 25 diabetic type 2 patients, and 25 age- and gender-matched healthy control groups, were enrolled in the study. The study was conducted according to ethical standards for human experimentation (Helsinki Declaration). The ethics committee of the National Liver Institute (NLI) approved the protocol (NLI Institutional Review Board (IRB) number; 00003413), and written consent was filled and signed by all participants.

The diagnosis of cirrhosis was based on clinical findings as well as imaging studies (abdominal ultrasound) and laboratory results. The severity of cirrhosis was graded according to calculated values of FIB-4 and APRI scores:

- FIB4 score: Age (y)×AST (IU/L)/platelet count (×109/L)×√ALT (IU/L)) [15].
- APRI score: (AST/upper limit of the normal AST range) X 100]/Platelet Count [16].

Diabetes diagnosis was based on the 2019 World Health Organization as fasting blood glucose of ≥ 7 mmol/L (126 mg/dl) or non-fasting blood glucose of ≥ 11.1 mmol/L (200 mg/dl) confirmed on at least 2 occasions or need for anti-diabetic medicines.

Patients with causes of liver cirrhosis other than chronic HCV infection as chronic HBV infection, metabolic liver diseases, and alcoholic liver diseases were excluded. The study was approved by the local ethics committee of the Menoufia University. Informed consent was taken from both the patients and control group subjects before the beginning of the study.

Routine laboratory investigations

After the collection of relevant clinical data, basic laboratory tests were performed including complete blood counts (Sysmex XT-1800i, Sysmex Corporation, Kobe, Japan), liver function tests in the form of aspartate aminotransferase (AST), alanine aminotransferase (ALT), albumin, and total and direct bilirubin. Fasting blood glucose level, hemoglobin A1C (HbA1C), hepatitis serology, and serum α-fetoprotein level were also assessed (Cobas 6000; Roche Diagnostics, Mannheim, Germany). Prothrombin concentration and international normalized ratio (INR) were measured (Sysmex CS-1600; Sysmex Europe GmbH, Norderstedt, Germany). HCV-RNA levels were detected using COBAS AmpliPrep/COBAS TaqMan (Roche Diagnostics Ltd., Germany) with a detection limit of 15 IU/ml.

DNA extraction and genotyping

Genomic DNA was isolated from the EDTA-anticoagulated whole blood of each subject using the QIAamp DNA Blood mini kit (QIAGEN, Hilden, Germany).

The genotypes of TCF7L2 gene (rs 290,487) SNP polymorphism were determined by real-time PCR (TaqMan®: C___1349543_10, Applied Biosystems, Thermo Fisher Brand, Foster City, USA) on Qiagen Rotor GENE Q real-time PCR system (Qiagen GmbH, Hilden, Germany). The

PCR reaction was carried out using a TaqMan universal master mix (Applied Biosystems, Foster City, USA) at a probe concentration of 2X). The probes were labeled using the fluorescent dyes VIC and FAM, each specific for one of the available alleles (C or T), and the genotypes were classified as homozygote allele (CC), heterozygote (CT), and homozygote allele (TT) for rs290487. The reaction was performed in a total reaction volume of 20 µL containing 5µl template DNA, 0.5µl TaqMan assay 20k, 10µl PCR genotyping Master Mix (2X), and 4.5µl water, nuclease-free. Under the following conditions, an initial denaturation step at 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 60°C for 1 s. Then, the final extension at 60°C for 5 min was carried out. As a negative control, a PCR mix without a DNA sample was used to ensure a contamination-free PCR product.

Statistical analysis

Results were collected, tabulated, and statistically analyzed by a statistical package SPSS version 20 (Armonk, NK; IBM corporation). Data was expressed into two phases: descriptive (number, percentage, mean, and standard deviation) and analytical study (chi-square test, Mann–Whitney test, Kruskal–Wallis test, ANOVA test followed by post hoc test (Dunn's multiple comparisons test), and Fisher's exact test, odds ratio (OR), and confidence interval (CI) test) were used. p value < 0.05 was considered statistically significant.

Results

Demographic and laboratory data of the studied groups

There was no significant difference among the four studied groups in terms of age and gender distribution. The clinical parameters of the group of patients are summarized in Table 1. It was noticed that there were significantly poorer liver function tests in cirrhotic patients with DM compared to cirrhotic nondiabetic patients in addition to diabetic noncirrhotic patients.

In addition, cirrhotic patients with diabetes had significantly advanced stages of fibrosis as evidenced by elevated FIB-4 score and APRI score compared to cirrhotic non-diabetic patients ($p = 0.001$ and 0.013, respectively) and diabetic noncirrhosis group ($p > 0.001$). Also, cirrhotic patients with diabetes had significantly elevated fasting blood glucose levels and HbA1C levels compared to cirrhotic nondiabetic patients ($p > 0.001$) and a significant increase in HbA1C levels compared to diabetic noncirrhotic patients ($p = 0.001$). There was no significant difference between cirrhotic diabetic patients and diabetic patients without cirrhosis regarding fasting blood glucose level (Table 1).

TCF7L2 (rs 290,487) genotype distribution and allele frequency among studied groups

The distribution of genotypes and allele frequencies of rs290487 (C/T) is presented in Table 3. CC genotype was significantly more predominant in the control group (44%) and cirrhotic nondiabetic group (40%) than in the diabetic noncirrhotic (16%) and cirrhotic diabetic groups (12%). Heterozygous CT showed a significantly higher prevalence in cirrhotic diabetic patients (72%) and diabetic noncirrhotic patients (64%) compared to the cirrhotic and control groups (56% and 52%, respectively), $p = 0.045$, with T allele significantly elevated in diabetic noncirrhotic patients (52%) and cirrhotic diabetic patients (52%) compared to control (30%) and cirrhotic patients (32%), $p = 0.028$ (Table 2).

Table 3 shows the calculation of the odds ratio which showed that the TT genotype displayed a significant statistical risk for DM more than the CC "reference group" between the diabetic patient group and control as well as cirrhotic patient groups ($p = 0.046$ and 0.049, respectively), with OR (95% CI) 13.75 and 12.5 riskier for DM than CC "reference group". The T allele was significantly increased in diabetic patients compared to the control and cirrhotic patient group ($p = 0.025$ and 0.043, respectively), with OR (95%CI) 2.528 and 2.302 riskier than the C allele.

Also, cirrhotic diabetic patients had a significantly higher incidence of TT with OR (95% CI) 14.667, $p = 0.038$ compared to the control. The T allele showed OR (95%CI) 2.53, $p = 0.025$, and this was associated with the dominant model (CC vs. CT + TT), $p = 0.012$ but not the recessive model (CC + CT vs. TT), $p = 0.349$.

At the same time, the calculation of the odds ratio showed that the TT genotype displayed a significant statistical risk for DM more than the CC "reference group" between cirrhotic diabetic patients and cirrhotic nondiabetic patients ($p = 0.047$) with OR (95% CI) 13.33 riskier than CC "reference group." Additionally, CT was significantly predominant ($p = 0.043$) with OR (95% CI) 4.29 riskier compared to the same group. The T allele is riskier for DM than the C allele [OR (95%CI) 2.302] with significantly higher incidence in cirrhotic diabetic patients compared to cirrhotic only patients ($p = 0.043$) and significantly associated with the dominant model (CC vs. CT + TT), $p = 0.024$, not the recessive model (CC + CT vs. TT), $p = 0.349$.

Meanwhile, statistically, there are no significant differences in genotype distribution and allele frequency between cirrhotic diabetic patients with and diabetic patients without cirrhosis ($p > 0.05$) as the allelic distribution of the C allele was similar within the two groups; however, the T allele frequency was associated with increased diabetes risk compared to other groups.

Table 1 Comparison between the different studied groups according to clinical data

	Control ($n=25$)	Cirrhotic ($n=25$)	DM ($n=25$)	Cirrhotic diabetic ($n=25$)	*p*	Multiple comparisons	
Gender							
Male	15 (60%)	17 (68%)	13 (52%)	19 (76%)	(0.324)		
Female	10 (40%)	8 (32%)	12 (48%)	6 (24%)			
Age (years)							
Mean ± SD	57.8 ± 6.2	56.8 ± 8	59.6 ± 5.1	59 ± 5.3	(0.419)		
Median (Min.–Max.)	58 (46–71)	57 (47–76)	59 (49–68)	59 (48–69)			
ALT (U/L)						p1 < 0.001*	p4 < 0.001*
Mean ± SD	19.6 ± 3	55.1 ± 15.2	19.3 ± 3.3	53.2 ± 20.5	(< 0.001*)	p2 = 0.876	p5 = 0.001*
Median (Min.–Max.)	20 (12–26)	49 (42–110)	19 (12–25)	45 (35–125)		p3 < 0.001*	p6 < 0.001*
AST (U/L)						p1 < 0.001*	p4 < 0.001*
Mean ± SD	14.7 ± 3.15	77.7 ± 18.7	14 ± 2.8	85.5 ± 34	(< 0.001*)	p2 = 0.755	p5 = 0.915
Median (Min.–Max.)	14 (10–20)	75 (56–145)	14 (10–18)	68 (47–166)		p3 < 0.001*	p6 < 0.001*
Albumin (g/dl)						p1 < 0.001*	p4 < 0.001*
Mean ± SD	4.6 ± 0.3	3.5 ± 0.8	4.3 ± 0.4	2.5 ± 0.55	(< 0.001*)	p2 = 0.338	p5 = 0.001*
Median (Min.–Max.)	4.6 (3.8–5.1)	3.9 (1.9–4.8)	4.2 (3.8–5)	2.4 (1.5–3.8)		p3 < 0.001*	p6 < 0.001*
Total bilirubin (mg/dl)						p1 < 0.001*	p4 = 0.001*
Mean ± SD	0.65 ± 0.2	1.7 ± 1.4	0.8 ± 0.2	3.2 ± 2	(< 0.001*)	p2 = 0.166	p5 = 0.049*
Median (Min.–Max.)	0.7 (0.3–1.1)	1.2 (0.3–6.1)	0.8 (0.5–1.2)	3.2 (0.6–8.2)		p3 < 0.001*	p6 < 0.001*
Direct bilirubin (mg/dl)						p1 < 0.001*	p4 = 0.017*
Mean ± SD	0.3 ± 0.2	0.8 ± 0.75	0.4 ± 0.15	1.5 ± 1.2	(< 0.001*)	p2 = 0.224	p5 = 0.039*
Median (Min.–Max.)	0.3 (0.1–0.6)	0.5 (0.1–3.5)	0.4 (0.2–0.7)	1 (0.2–5)		p3 < 0.001*	p6 < 0.001*
WBC ($\times 10^3$/cmm)							
Mean ± SD	6.8 ± 1.1	6.5 ± 1.15	7 ± 0.8	7.35 ± 1.5	(0.074)	> 0.05 (NS)	> 0.05 (NS)
Median (Min.–Max.)	6.7 (5–9.1)	6.1 (5–9.2)	7 (5.5–8.2)	7.5 (4.8–9.5)			
Platelets ($\times 10^3$/cmm)						p1 < 0.001*	p4 < 0.001*
Mean ± SD	327 ± 63.8	136.9 ± 40.8	456.9 ± 69.7	113.5 ± 34.7	(< 0.001*)	p2 < 0.001*	p5 = 0.428
Median (Min.–Max.)	310 (240–450)	125 (85–230)	460 (400.0–510.0)	102 (85–210)		p3 < 0.001*	p6 < 0.001*
Hb (g/dl)						p1 < 0.001*	p4 = 0.225
Mean ± SD	14.5 ± 1.5	12.8 ± 1.8	13.6 ± 1.05	11.6 ± 1.1	(< 0.001*)	p2 = 0.111	p5 = 0.022*
Median (Min.–Max.)	15.4 (12.4–16.3)	12.4 (10.2–14.9)	13.2 (12.5–15.4)	12 (10.2–13.4)		p3 < 0.001*	p6 < 0.001*
INR						p1 < 0.001*	p4 < 0.001*
Mean ± SD	1 ± 0	1.3 ± 0.2	1 ± 0.03	1.5 ± 0.2	(< 0.001*)	p2 = 0.998	p5 = 0.002*
Median (Min.–Max.)	1 (1–1)	1.3 (1–1.7)	1 (1–1.1)	1.4 (1.1–2)		p3 < 0.001*	p6 < 0.001*
Fasting blood glucose (mg/dl)						p1 < 0.001*	p4 < 0.001*
Mean ± SD	79.5 ± 7	106 ± 9.5	206.8 ± 27.2	201.9 ± 21.7	(< 0.001*)	p2 < 0.001*	p5 < 0.001*
Median (Min.–Max.)	80 (70–96)	110 (88–115)	200 (175–298)	200 (170–230)		p3 < 0.001*	p6 = 0.780
HbA1C %						p1 = 0.235	p4 < 0.001*
Mean ± SD	4.8 ± 0.5	5.4 ± 0.4	10.5 ± 1.4	9.3 ± 1.3	(< 0.001*)	p2 < 0.001*	p5 < 0.001*
Median (Min.–Max.)	4.7 (4–5.8)	5.4 (4.6–5.9)	10.2 (8.6–13)	8.9 (7.6–12.2)		p3 < 0.001*	p6 = 0.001*
FIB-4 score						p1 < 0.001*	p4 < 0.001*
Mean ± SD	0.6 ± 0.1	4.8 ± 2	0.4 ± 0.1	6.4 ± 2.15	(< 0.001*)	p2 = 0.975	p5 = 0.001*
Median (Min.–Max.)	0.6 (0.3–0.9)	4.3 (2.3–11)	0.4 (0.3–0.6)	5.8 (2.3–13.2)		p3 < 0.001*	p6 < 0.001*
APRI						p1 < 0.001*	p4 < 0.001*
Mean ± SD	0.12 ± 0.03	1.5 ± 0.55	0.08 ± 0.02	2 ± 0.8	(< 0.001*)	p2 = 0.993	p5 = 0.013*
Median (Min.–Max.)	0.11 (0.06–0.19)	1.48 (0.70–2.79)	0.08 (0.04–0.11)	1.74 (0.8–4.15)		p3 < 0.001*	p6 < 0.001*

p p value for comparing between the studied, *p p* value for comparing between the studied groups, *p1 p* value for comparing between control and cirrhotic, *p2 p* value for comparing between control and DMT2, *p3 p* value for comparing between control and cirrhotic diabetic, *p4 p* value for comparing between cirrhotic and DMT2, *p5 p* value for comparing between cirrhotic and cirrhotic diabetic, *p6 p* value for comparing between DMT2 and cirrhotic diabetic

* Statistically significant at $p < 0.05$

Table 2 TCF7L2 genotyping and allele distribution of the studied groups

TCF7L2	Control ($n=25$)		Cirrhosis ($n=25$)		DM ($n=25$)		Cirrhotic diabetic ($n=25$)		χ^2	*P*
	No	%	No	%	No	%	No	%		
Genotypes										
CC®	11	44.0	10	40.0	4	16.0	3	12.0	12.249*	$^{MC}p=0.045$*
CT	13	52.0	14	56.0	16	64.0	18	72.0		
TT	1	4.0	1	4.0	5	20.0	4	16.0		
Dominant model										
CC®	11	44.0	10	40.0	4	16.0	3	12.0	9.921	$^{MC}p=0.019$*
CT+TT	14	56.0	15	60.0	21	84.0	22	88.0		
Recessive model										
CC+CT	24	96.0	24	96.0	20	80.0	21	84.0	4.829	$^{MC}p=0.234$
TT	1	4.0	1	4.0	5	20.0	4	16.0		
Alleles										
C®	35	70.0	34	68.0	24	48.0	24	48.0	9.124*	0.028*
T	15	30.0	16	32.0	26	52.0	26	52.0		

χ^2 Chi-square test, *MC* Monte Carlo, *p p* value for comparing between the studied groups

* Statistically significant at $p \leq 0.05$

Relation between TCF7L2 genotypes with different parameters in the cirrhotic diabetic patient group.

HbA1C was found to be significantly increased in patients with TT genotype of TCF7L2 compared to CT & TT genotypes ($p = < 0.001$). Also, fasting blood glucose was elevated in patients with TT genotype compared to CC and CT genotypes, but was not statistically significant ($p = 0.203$). On the other hand, we could not find statistical significance between different genotypes regarding liver function and markers of fibrosis (FIB4 and APRI scores) (Table 4).

Relation between TCF7L2 genotypes with fasting blood glucose and HbA1C in the diabetic patient group

Additionally, there was a statistically significant difference between TCF7L2 genotypes regarding fasting blood glucose ($p = 0.046$) and HbA1C ($p = < 0.001$) among the diabetic patients group (Table 5).

Discussion

The liver is the central metabolic organ and plays a key role in glucose homeostasis. Glucose intolerance occurs in many cirrhotic patients, and subsequently, diabetes manifests clinically as liver function deteriorates [3]. In the current study, we aimed to study TCF7L2 rs 290,487 polymorphism in cirrhotic patients with diabetes.

The effect of diabetes on the clinical outcome of chronic hepatitis C and cirrhosis has been detected. We found that the cirrhotic patients with DM had poorer liver function and higher incidence of cirrhotic complications manifested by elevated FIB-4 score and APRI score compared to cirrhotic nondiabetic patients and diabetic noncirrhotic patients.

These results were in accordance with García-Compeán et al. and Kumar (2018) who showed that diabetes has clinical implications in chronic liver disease as chronic hepatitis C patients. Liver function deteriorates with an increased rate of complications of cirrhosis, decreased 5-year survival rate, and increased risk of hepatocellular carcinoma. The major complications of cirrhosis associated with diabetes include hepatic encephalopathy (HE), spontaneous bacterial peritonitis, sepsis, variceal hemorrhage, renal dysfunction, and increases in morbidity and mortality of liver cirrhosis [17, 18].

Also, these findings agreed with the previous study by Liu et al. (2016) which reported that the presence of DM and liver cirrhosis in the same patient represents a double pathological insult for the liver, increasing the risk of decompensating events, morbidity, and mortality [19]. Coman et al. (2021) in their study reported that liver cirrhotic patients with diabetes are at higher risk for hepatic encephalopathy. DM also increases the risk of variceal hemorrhage and contributes to elevated portal pressure and variceal re-bleeding, while uncontrolled DM is associated with an increased risk of bacterial infections. DM also increases the risk of HCC and contributes to adverse liver transplantation outcomes [20].

Mechanisms by which diabetes may deteriorate liver function giving rise to adverse outcomes were suggested. It may increase fibrosis and inflammation through the

Table 3 Study the risk of development of DM in different genotypes and alleles

TCF7L2	DM vs. control		DM vs. cirrhotic		Cirrhotic diabetic vs. control		Cirrhotic diabetic vs. DM		Cirrhotic diabetic vs. cirrhotic	
	OR (95% C.I)	*p* value	OR (95% CI)	*p* value	OR (95% CI)	*p* value	OR (95% CI)	*p* value	OR (95% CI)	*p* value
Genotypes										
CC®	Ref	–	Ref		Ref	–	Ref	–	Ref	–
CT	3.385 (0.870–13.166)	0.072	2.857 (0.731–11.171)	0.124	5.07 (1.176–21.914)	0.023*	1.50 (0.29–7.74)	0.697 (FE)	4.29 (0.99–18.59)	0.043*
TT	13.750(1.207 – 156.66)	0.046*(FE)	12.500(1.089–143.439)	FEp = 0.049*	14.67(1.16–185.24)	0.038*(FE)	1.067 (0.15–7.82)	1.000 (FE)	13.33 (1.05–169.57)	0.047*(FE)
Dominant model										
CC®	Ref	–	Ref		Ref	–	Ref	–	Ref	–
CT + TT	4.125 (1.092–15.586)	0.031*	3.500 (0.921–13.307)	0.056	5.76 (1.36–24.36)	0.012*	1.40 (0.28–7.00)	1.000 (FE)	4.89 (1.15–20.79)	0.024*
Recessive model										
CC + CT	Ref	–	Ref	-	Ref	–	Ref	–	Ref	–
TT	6.00 (0.647–55.664)	0.189(FE)	6.000 (0.647–55.664)	FEp = 0.189	4.57 (0.47–44.17)	0.349 (FE)	0.76 (0.18–3.25)	1.000 (FE)	4.57 (0.47–44.17)	0.349(FE)
Alleles										
C®	Ref	–	Ref	-	Ref	–	Ref	–	Ref	–
T	2.528 (1.112–5.744)	0.025*	2.302 (1.021–5.190)	0.043*	2.53 (1.11–5.74)	2.53 (1.11–5.74)	1.00 (0.46–2.19)	1.000 (FE)	2.30 (1.02–5.19)	0.043*

FE Fisher's exact, *OR* odds ratio, *CI* confidence interval, *LL* lower limit, *UL* upper limit, *p p* value for comparing between the studied groups

* Statistically significant at $p \leq 0.05$

®Reference group

Table 4 Relation between TCF7L2 SNP with laboratory investigation in cirrhotic diabetes ($n=25$)

Liver function	TCF7L2			*P*
	CC ($n=3$)	CT ($n=18$)	TT ($n=4$)	
ALT(U/L)				
Median (Min.–Max.)	47 (44–63)	45.5 (38–125)	39.5 (35–49)	0.229
AST (U/L)				
Median (Min.–Max.)	67 (58–107)	82 (47–166)	65.5 (56–68)	0.315
Albumin (g/dl)				
Mean ± SD	2.1 ± 0.5	2.6 ± 0.6	2.55 ± 0.5	0.415
Total bilirubin (mg/dl)				
Median (Min.–Max.)	3.4 (1.5–5.5)	3.5 (0.6–8.2)	1.6 (1.6–1.8)	0.245
Direct bilirubin (mg/dl)				
Median (Min.–Max.)	1.3 (0.6–3.5)	1.55 (0.2–5)	0.8 (0.5–0.85)	0.356
INR				
Mean ± SD	1.6 ± 0.3	1.4 ± 0.25	1.5 ± 0.15	0.640
FIB-4 score				
Mean ± SD	6.6 ± 2.2	6.55 ± 2.35	5.3 ± 0.85	0.546
APRI				
Mean ± SD	2.2 ± 0.9	2.1 ± 0.85	1.4 ± 0.3	0.568
Fasting blood glucose(mg/dl)				
Mean ± SD	182.3 ± 6.7	200.7 ± 21.8	221.75 ± 10.7	0.203
HbA1C				
Mean ± SD	8.2 ± 0.5	9.1 ± 1	11.4 ± 0.7	< 0.001*
Significance among groups	P1 = 0.311, P2 = 0.001*, P3 = 0.001*			

H H for Kruskal–Wallis test, *F F* for ANOVA test, *p p* value for comparing between different categories, *p1 p* value for comparing between CC and CT, *p2 p* value for comparing between CC and TT, *p3*, *p* value for comparing between CT and TT

* Statistically significant at $p \leq 0.05$

Table 5 Relation between TCF7L2 genotypes with fasting blood glucose and HbA1C in the diabetic group

	TCF7L2			*F*	*p*
	CC ($n=4$)	CT ($n=16$)	TT ($n=5$)		
Fasting blood glucose(mg/dl)					
Min.–Max	180.0–198.0	175.0–298.0	200.0–245.0	3.543*	0.046*
Mean ± SD	187.0 ± 7.70	207.75 ± 30.26	219.60 ± 19.32		
Median	185.0	202.0	210.0		
HbA1C					
Min.–Max	8.60–9.90	8.60–11.90	11.50–13.0	12.088*	< 0.001*
Mean ± SD	9.03 ± 0.59	10.26 ± 1.05	12.34 ± 0.63		
Median	8.80	10.10	12.30		

F F for ANOVA test, *p p* value for comparing between different categories

* Statistically significant at $p \leq 0.05$

activity of pro-inflammatory and fibrogenic adipokines such as tumor necrosis factor-alpha, tumor growth factor beta-1, resistin, leptin, hepatic growth factor, and adiponectin [21]. In addition, immunosuppression induced by diabetes in cirrhotic patients may also be involved in mortality by increasing the incidence of infections [22].

In support of our findings, several prospective and retrospective surveys indicated that the survival rate was significantly lower in cirrhotic diabetic patients than in cirrhotic non-diabetic subjects [21, 23].

It has been reported that diabetes is more frequent in patients with hepatitis C virus-related cirrhosis than those with hepatitis B virus-related cirrhosis, because of the direct role of the hepatitis C virus in insulin resistance and pancreatic β cell dysfunction. HCV may also interfere with glucose metabolism indirectly by causing peripheral insulin resistance in the muscle tissue of chronic HCV patients [24].

As mentioned above, the most strongly associated T2DM locus resides within the TCF7L2 gene. Our study demonstrates a significant association between the TCF7L2 gene and type 2 diabetes. Previous articles mainly reported the significant association between the TCF7L2 gene and type 2 diabetes [25–28].

Our findings revealed that TCF7L2 (rs 290,487) TT genotype is associated with increased levels of fasting blood glucose and glycated hemoglobin A1C. These results agreed with a study conducted by Shokouhi et al. that selected three functional SNPs in the TCF7L2 gene

to explore the potential relationship of susceptibility to T2DM and found that T allele of rs290487, rs12255372, and rs7903146 polymorphisms of TCF7L2 are associated with T2DM per se or metabolic traits related to this disease [29]. This is in contrast to another study Li et al. 2019 that found that the genotype CC of rs290487 was significantly associated with an increased hazard of T2D compared to CT + TT ($p < 0.001$, OR = 1.579) [30].

In addition, the current study provided evidence that the type 2 diabetes susceptibility gene TCF7L2 was associated with diabetes in cirrhotic patients. TCF7L2 rs290487 TT and CT genotype significantly increased diabetes risk in cirrhotic diabetic patients versus the control group and cirrhotic non-diabetic patients compared to CC genotypes. This result was consistent with a previous study by Shokouhi et al. which revealed that rs290487 TCF7L2 gene T allele was significantly associated with cirrhotic diabetic patients [29].

Also, in support of our findings, a previous study led by Ling et al. showed a significant association between diabetes in cirrhotic patients and different SNPs in the TCF7L2 gene and noted that two SNPs were differentially distributed between cirrhotic diabetic patients and cirrhotic nondiabetic patients. The multivariate logistic analysis showed that TCF7L2 rs290487 and rs6585194 polymorphisms were independently associated with diabetes in cirrhotic patients. The TCF7L2 rs290487 TT variant homozygote showed much higher insulin resistance and significantly increased diabetes risk in cirrhotic patients compared with CC and CT genotypes [14].

It is worth noting that diabetic patients had significantly increased platelet count (thrombocytosis) compared to the control group ($p < 0.001$), cirrhotic patient ($p < 0.001$), and cirrhotic diabetic patients ($p < 0.001$). In this issue, Kraakman et al. 2017 uncover a previously unknown link between hyperglycemia and enhanced platelet production and reactivity.

The authors demonstrated that high blood glucose levels trigger the neutrophil release of S100 calcium-binding protein A8/A9 (S100A8/ A9), which binds to the receptor for advanced glycation end products (RAGE) on Kupffer cells, ultimately leading to increased thrombopoietin (TPO) production in the liver. TPO causes megakaryocyte proliferation and increased platelet production [31]. Another study by Akinsegun et al. and Rodriguez et al. revealed a higher mean platelet count for diabetics on treatment than for nondiabetic controls [32, 33]. This indicates that follow-up of platelet count is essential in diabetic patients to exclude thrombotic complications.

Our investigation had some limitations. Firstly, in this study, we only investigated one functional SNP in the TCF7L2 gene, which might not be present in a widespread view of the genetic variability of TCF7L2 in the Egyptian population. Secondly, the number of participants included was moderate, so the statistical power of the present study might be limited. Finally, environmental risk factors may be different between the Egyptian population and others. Thus, the risk of T2DM is likely to be influenced by gene–gene and gene–environment interactions to various degrees. The association of TCF7L2 polymorphism with T2DM risk may differ in different ethnic group studies.

In conclusion, our findings suggest that rs290487 SNP of the TCF7L2 gene is associated with an increased risk of diabetes susceptibility in cirrhotic patients. Further studies with larger sample sizes are needed to identify the possibility to use this polymorphism as a potential genetic marker for T2DM in cirrhotic patients. This would help to identify cirrhotic patients who may be at greater risk of developing diabetes, which would contribute to decreasing the risk of decompensating events, morbidity, and mortality.

Abbreviations

CI	Confidence interval
CLD	Chronic liver disease
DM	Diabetes mellitus
HCV	Chronic hepatitis C
HE	Hepatic encephalopathy
IRS-1	Insulin receptor substrate-1
mRNA	Messenger ribonucleic acid
OR	Odds ratio
PI3-kinase	Phosphoinositide 3-kinase
SNP	Single-nucleotide polymorphism
T2D	Diabetes type 2
TNF—α	Tumor necrosis factor-α
TPO	Thrombopoietin

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Authors' contributions

All authors cooperated in the conceptualization, design of the work, data curation, resource detection, formal analysis, interpretation of the data, the creation of new software used in the work, validation and methodology, and revision of the manuscript.

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Availability of data and materials

All data are available upon request.

Declarations

Ethics approval and consent to participate

All procedures performed in our study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Informed consent was obtained from all individual participants included in the study.

Consent for publication
All authors are in agreement with the content of the manuscript.
On behalf of my colleagues, we have pleasure in dealing with your journal. I would like to thank you and I hope that our research work is under your kind care and observation.

Competing interests
The authors declare that they have no competing interests.

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