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Impact of *IL-28B* gene polymorphism on chronic hepatitis-C patients progression with diabetes and non-diabetes

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Abstract

Background: Chronic hepatitis C (CHC) is a silent viral infection; however, elevated mortality and morbidity rates are noted in Egypt due to its adverse effects. The augmented incidence of diabetes in patients with viral C infection may be owing to glucose intolerance, high BMI, senility, and inherited factors.

Purpose of the study: Little information is available about the connection between interleukin-28B (*IL-28B*) genotype in disease progression among CHC patients with diabetes. Thus, we aimed to assess the association between *IL-28B* genotype (rs12979860) in CHC with type 2 diabetes mellitus (T2DM) versus those without diabetes in disease progression among Egyptian patients.

Results: CC genotype was significantly lower in diabetics than in non-diabetics (13.7% vs. 36.3%). While (CT/TT) were significantly higher in diabetics than in non-diabetics (CT 58.8% vs. 43.7%), (TT 27.5% vs. 20%) ($p=0.03$) and likewise alleles ($p=0.04$). Multivariate logistic regression analysis was significant with viral load $p<0.001$, alanine aminotransferase (ALT) $p<0.001$, genotype CC versus TT $p=0.04$ & T2DM $p=0.03$.

Conclusion: CC genotype might be used as a protective factor and TT genotype as a risk factor in disease progression among CHC patients with T2DM. Additionally, viral load, ALT & T2DM might interplay as predictors of disease severity. Detecting the genetic factors can be helpful in predicting and preventing the complications of diabetes associated with the hepatitis C virus (HCV).

Keywords: *IL-28B*, T2DM, CHC, Fibrosis, PCR–RFLP

Background

IL28B belongs to type III of the interferon (IFN) family and displays antiviral activity by persuading a subgroup of IFN-stimulated genes (ISGs) [1]. Single nucleotide polymorphisms (SNPs) structure is one of the most critical sorts of the human genome [2]. SNPs close to the *IL-28B* genotype encoding interferon lambda 3 (IFN-λ3) have been identified to be firmly connected with spontaneous and treatment eradication of HCV infection [3]. One of these SNPs, rs12979860, was essential in expecting the goals of HCV resolution as it determines the liver impact expression of ISGs [4, 5]. Consequently, allelic variants of the *IL-28B* polymorphism may be associated with the competence of the inflammatory practice during HCV infection and with the tools that HCV embraces to spurt abolition by innate and adaptive immunity [6]. Patients with cirrhosis had a higher TT and CT genotype of rs12979860 [7].

The association between *IL-28B* polymorphisms and the development of liver fibrosis is yet doubtful, and its possible mechanisms are unclear. The polymorphisms

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expressed in inflammatory cytokines may accelerate liver fibrosis progression. It was assumed that *IL-28B* genotypes have a role in the assessment of inflammation and the degree of fibrosis [8].

Hepatitis C virus is the most popular cause of acute and chronic liver inflammatory diseases and leads to hepatic fibrosis, cirrhosis, and hepatocellular carcinoma (HCC). It is considered that about 150–200 million people have been infected by HCV worldwide and about 85% are in the chronic stage [9].

Egypt has a very high prevalence of HCV [10]. According to an Egyptian demographic survey from 2008 to 2012, 14.7% of the Egyptians had HCV antibodies [11], and 9.8% were in the active stage [12]. More than 90% of Egyptian patients are HCV genotype 4 [13]. In a more recent study, an estimated prevalence of anti-HCV antibodies in Cairo, the capital of Egypt, was 19.8% [14].

The severity of hepatic disease associated with HCV varies extensively, as does the percentage of progression on the way to cirrhosis. The latter seems to depend on host-associated cofactors, like older age, gender, alcohol intake, increased BMI, immune status, and the presence of associated infections. T2DM is one of these cofactors, and there is a probable relation between CHC and the augmented prevalence of T2DM [15].

Diabetes mellitus (DM) is characterized by prolonged hyperglycemia and impaired carbohydrates, lipid, and protein metabolism due to insulin secretion or insulin action impairment. Among different types of diabetes, T2DM is a very common form of disease that has been predicted as a heterogeneous group of metabolic and multifactorial disorders. It is a growing epidemic worldwide that is associated with serious complications and decreases both the lifespan and quality of life [16].

Even at the period of insulin resistance (IR) which occurs at the beginning before T2DM comes up, the course of hepatitis C is known to be changed in these prediabetic patients. Although people may have IR without HCV, important scientific and investigational statistics propose that HCV plays a vital role in its pathogenesis. This feature is significant because IR looks to quicken the progress of chronic hepatitis C [9].

T2DM prevalence in Egypt was 15.6% [17]. In a meta-analysis study, the ultimate global prevalence of T2DM among CHC was 19.7% with the highest rank of both T2DM and CHC prevalence in Africa and Asia, followed by North America and then Europe [18]. According to case–control studies, 21% to 50% of patients with CHC are diabetics, which was expressively higher than that in the whole populace [9]. Possibly, HCV infection encourages IR in a way that is not completely understood [19].

In Egypt, little is known about factors directed to CHC progression and CHC relation to T2DM, so it hypothesized in this study that the *IL-28B* gene polymorphism might impact the progression of liver fibrosis in patients infected with HCV. However, it seems to be different when infected HCV patients become diabetic. Thus, the study aimed to assess the link between *IL-28B* genotype rs12979860 in CHC disease progression with T2DM versus those without diabetes among Egyptian patients.

Methods

Type of the study

This is a cross-sectional case–control study.

Sample size

The sample size was calculated by MedCalc software according to the prevalence of CHC, power of 80%, and alpha error α (0.05) to be 153 with an addition of 5% as compensation for missed follow-up or refusal of the cases [20].

Target population

There is enrollment of 160 CHC patients attending the Hepatology unit at University Hospital. They were defined by detectable serum anti-HC antibodies and HCV ribonucleic acid (RNA) and diagnosed on clinical, biochemical, and radiological bases as previously described by Dienstag et al. [21].

Inclusion criteria

Two main groups: Group (1) included 80 CHC patients without diabetes at the time of sample collection, and group (2) included 80 CHC patients with T2DM diagnosed according to American Diabetes Association ADA 2018 [22]. Each group was further sub-divided by using transient elastography (TE) Fibro-Scan into 40 patients with Fibrosis [F1-2] and 40 patients with compensated cirrhosis [F3-4].

Exclusion criteria

Excluded patients with a history of decompensated liver disease (variceal bleeding, ascites, encephalopathy and jaundice), autoimmune hepatitis, concomitant other viral hepatitis infection (e.g., hepatitis A or B), concomitant metabolic liver disease, in addition to drug addiction, chronic alcohol intake, and pregnant ladies were also excluded from the study.

All included subjects were subjected to:

- Full history taking
- Complete clinical examination and body mass index (BMI) calculation.
- Abdominal ultrasound & Fibro-Scan 502 machine (EchoSense) was used to assess stages of liver stiff-

ness that were expressed in kilopascal kPa, ranging from 0—≤5 kPa for (F0), 5—7.1 kPa for (F1), 7.1—9.5 kPa for (F2), 9.5—12.5 kPa for (F3). Finally, for the diagnosis of cirrhosis > 12.5 kPa (F4) [23].

- Laboratory tests: Hepatitis C virus antibody (HCV-Ab), hepatitis B surface antigen (HBs Ag), fasting blood sugar (FBS), postprandial blood sugar, glycated hemoglobin (HbA1c), liver enzymes (ALT and AST), prothrombin time, international normalization ratio (INR), serum bilirubin, serum albumin, α fetoprotein, kidney function tests, complete blood count CBC, as well as, plasma HCV RNA which was analyzed by using the Roche COBAS Taq Man HCV Test.
- Genotyping of *IL-28B* rs12979860

IL-28B rs12979860 genotyping was performed by polymerase chain reaction-restriction fragment length polymorphism (PCR–RFLP) as follows:

Deoxyribonucleic acid (DNA) extraction

A blood sample of 2 ml was withdrawn under complete aseptic measures from all participants of the study and was dispensed in a sterile tube containing ethylenediamine tetra acetic acid (EDTA). All the reagents were highly purified analytical PCR materials. All the tubes, tips pipettes used for DNA extraction were DNase, RNase-free tubes to avoid contamination purchased from Gentra (Minneapolis. USA). DNA was isolated using the genomic blood DNA extraction Mini Kit purchased from INTRONBIO according to the manufacturer's instructions. DNA quantitation and purity assessment were done using Milton Roy Spectronic 3000 Array.

Polymerase chain reaction "PCR" detection of the *IL-28B* rs12979860 gene

Reagents and kits used included Tris–acetate–EDTA (TAE) buffer prepared by dissolving 4.84 g Tris (base) into 500 ml distilled water, then 1.142 ml of glacial acetic acid was added and mixed for 5 min, and then 0.75 g EDTA was liquefied, pH was adjusted to 8.5 with pH meter, and the whole volume completed to 1 L with distilled water. Loading dye was prepared by dissolving 25 mg bromophenol blue, 250 µl xylene cyanol, and 40 g sucrose into 100 ml of 50 mM EDTA (storage temperature 4 °C). The stock solution of ethidium bromide was prepared by dissolving 5 mg of ethidium bromide in 5 ml distilled water (stored in dark bottle). The 3% agarose gel was prepared by dissolving 6 g agarose in 200 ml 1X TAE buffer while heating and stirring until it boils and the agarose is completely dissolved. The liquid was cooled to 65 °C, and then 10 µl of ethidium bromide was added before pouring into the adjusted gel dish. Primers of *IL-28B* gene rs12979860 (purchased from Fermentas) were: forward 5'-GCTTATCGCATACGGCTAGG-3' and reverse 5'-AGGCTCAGGGTCAATCACAG3'PCR.

The kit comprised a prepared PCR Master Mix (dream Taq polymerase). The recommended amount of size marker DNA ladder to load on the gel was 2-3 µl per lane. BstU-I restriction enzyme and 10X fast digest green Buffer (100 µl) were purchased from Fermentas.

PCR was performed in a final volume of 20 µl that contained: 2X PCR master mix 10 µl, Primers (2.5 µM or 1/40 of dilution 100 µM stock): 1 µl of each primer, genomic DNA: 5 µl and deionized water: 3 µl. The amplification was carried out using DNA thermal cycler 480, PERKINELMER (Norwalk, CT 06,856, USA), Serial No. P 16,462 according to manufacturer instruction.

The restriction digestion reaction was performed using restriction endonuclease BstU-I. This reaction was done in 25 µl, a total volume that contained: 10 X buffer 2.5 µl, 10 U/µL BstU-I 1 µl, PCR product 15 µl, and deionized water 6.5 µl. Then, this mixture was incubated at 37 °C for 1 h. The digested PCR product 4 µl was combined with 4 µl loading dye and 1 X buffer solution to form a total volume appropriate for the electrophoresis gel comb used. All digested PCR products were electrophoresed using a submarine gel electrophoresis system (Pharmacia Biotech by SEMKO AB, Sweden) and submarine chamber (Maxicell, EC360, M-E-C apparatus Co. Florida, USA). The gel was photographed under ultraviolet trans-illumination (Heralab GmbH laborgerate trans-illuminator, Germany). The digested fragments were 135, 82, and 25 bp for the C allele and 160 and 82 bp for the T allele variants.

Statistical analysis

Data were coded and entered using SPSS (Statistical Package for the Social Sciences) version 24. Quantitative data were summarized by using mean and standard deviation. Mann–Whitney test was used for non-normally distributed quantitative variables. Qualitative data were summarized by using frequency and percentage. Fisher's exact test and the Chi-square test (χ^2) for categorical variables. Kruskal–Wallis test is a one-way ANOVA test used to determine the statistically significant differences between two or more groups. Multiple logistic regression analysis were done. The threshold of significance is fixed at a 5% level (P-value). P-values less than 0.05 were considered as statistically significant, p value > 0.05 insignificant, and $p < 0.001$ highly significant.

Results

One hundred sixty patients known with CHC were included in the study: 107 males (66.8%) and 53 females (33.2%), and their age ranged from 20 to 70 years with mean ± SD (42.6 ± 9.7 years). They were divided into two

groups CHC with diabetes and CHC without diabetes. Age showed a significantly higher difference in the diabetic with liver cirrhosis versus non-diabetic with cirrhosis ($p<0.001$). Moreover, BMI showed a significantly higher difference in diabetes with fibrosis versus non-diabetes with fibrosis ($p=0.001$) (Table 1).

CT genotype expression represented (51.25%) as shown in Table 2, while almost nearby comparable values in genotype CC versus TT (25% vs. 23.75%). The frequency of alleles in CHC (fibrosis vs. cirrhosis) is presented in Fig. 1, which shows the highest C allele frequency in non-diabetic patients with fibrosis and highest T allele frequency in diabetic patients with cirrhosis. Genotypes distributions were statistically significant on comparing diabetes group versus non-diabetes group ($\chi^2=6.14$, $p=0.03$) and likewise alleles ($\chi^2=2.48$, $p=0.04$); however, the expression of the genotype was insignificant inside the same group, whether diabetic or non-diabetic regarding the different grades of fibrosis ($p>0.05$) (Table 3).

As demonstrated in Table 4, a highly significant difference has been detected between the different genotypes and viral load that was identified by PCR, with lower viral load in the CC genotype and the higher in the TT genotype ($p=0.003$) as well as ALT, in which the genotypes were highly significant in relation to ALT ($p<0.001$). Also, higher results were recorded with genotype TT regarding mean ± SD for ALT (71.58 ± 33.83) and viral load by PCR (578,060 ± 1,454,550). The least significant difference "LSD" between PCR and each genotype was highly significant, especially between CC versus TT [CC vs. CT $p=0.034$], [CC vs. TT $p=0.009$] & [CT vs. TT $p=0.01$].

Table 5 depicts rank of different factors affect CHC disease progression, with the highest rank of viral load and ALT. Viral load [$p<0.001$ OR = 11.2 95% CI (5.6–29.4)], ALT [$p<0.001$ OR = 9.5 95% CI (4.5–16.3)], then genotype CC versus CT [$p=0.02$ OR = 4.3 95% CI (2.5–11.5)], followed by T2DM [$p=0.03$ OR = 4.2 95% CI (2.3–10.9)], then genotype CC versus TT [$p=0.04$ OR = 3.4 95% CI (2.1–7.1)], and finally age [$p=0.04$ OR = 2.7 95% CI (1.7–5.3)]. T2DM and age may interplay collectively as predictors of disease severity among diabetic group, or independently in that manner mentioned before.

Table 1 Demographic characteristic regarding fibrosis degree (fibrosis versus cirrhosis) in non-diabetic and diabetic group

Variable	Liver fibrosis		Significance	Liver cirrhosis		Significance
	GI a no-diabetes ($n=40$)	GII a diabetic ($n=40$)	*p* value	GI b no-diabetes ($n=40$)	GII b diabetic ($n=40$)	*p* value
Age (years)						
Mean ± SD Range	39.45 ± 9.07 23–58	41.55 ± 7.57 24–57	0.26 (U test)	40.79 ± 11.65 20–64	57.58 ± 4.39 45–70	< 0.001*** (U test)
BMI: (Kg/m^2)						
Mean ± SD Range	25.69 ± 2.42 22–34	27.57 ± 2.74 20–32	0.001*** (U test)	24.98 ± 3 20–31	25 ± 2 22–29	0.97 (U test)
Sex						
Male *n* (%)	22 (55%)	28 (70%)	0.58 (χ^2 test)	32 (80%)	31 (77.5%)	0.78 (χ^2 test)
Female *n* (%)	18 (45%)	12 (30%)		8 (20%)	9 (22.5%)	

***Highly significant $p<0.001$ U: Mann–Whitney test

Table 2 Comparison of IL28B genotype and allele between non-diabetic and diabetic

Variable	Group I a & b Non-diabetic ($n=80$)	Group II a & b Diabetic ($n=80$)	Total ($n=160$)	χ^2	*p* value	OR (95%CI)
Genotype						
CC	29 (36.3%)	11 (13.7%)	40 (25%)	6.14	0.03*	1
CT	35 (43.7%)	47 (58.8%)	82 (51.3%)			3.01(1.31–6.88)
TT	16 (20.0%)	22 (27.5%)	38 (23.7%)			2.02(0.79–5.18)
Allele						
C	94 (58.7%)	76 (47.5%)	85 (53.1%)	2.48	0.04*	1.45 (1.09–2.5)
T	66 (41.3%)	84 (52.5%)	75 (46.9%)			

X^2 = Chi-square test *: significant $p<0.05$ OR: odds ratio, CI: confidence interval

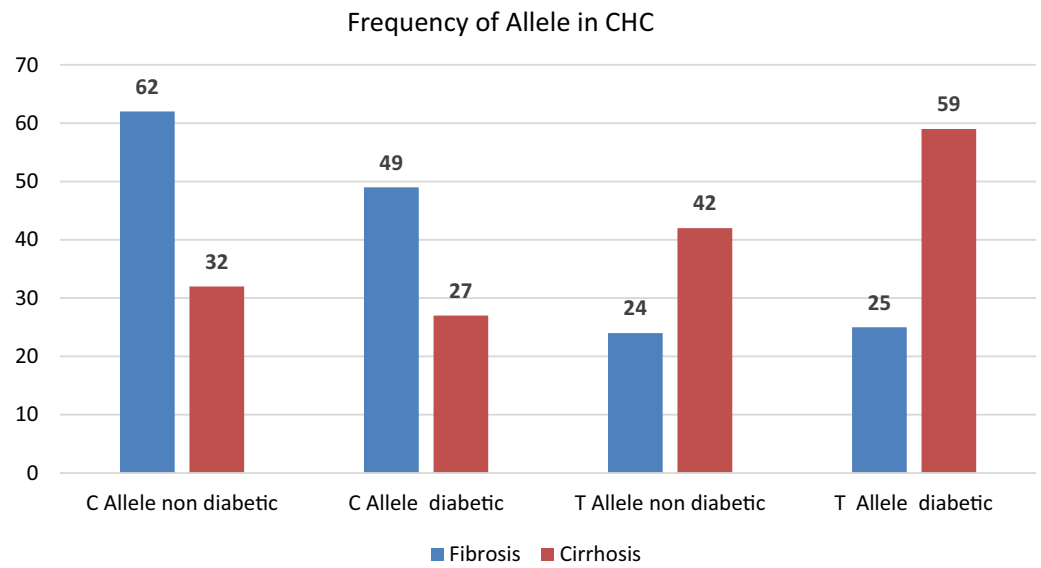

Fig. 1 Frequency of allele in chronic hepatitis C (fibrosis vs cirrhosis). This figure shows numerical distribution of C and T Alleles in fibrosis versus cirrhosis in both diabetic and non-diabetic CHC patients. It was noticed that T alleles were more frequent in diabetic patients with cirrhosis and more hepatic sequels, contrasted with C alleles which were more frequent in non-diabetic patients with fibrosis

Table 3 IL28B genotype expression in non-diabetic and diabetic groups according to grades of fibrosis

Variable		CC	CT	TT	χ^2	P
G1 a and b non-diabetic ($n=80$)	Fibrosis ($n=40$)	18 (45.0%)	15 (37.5%)	7 (17.5%)	2.65	0.27*
	Cirrhosis ($n=40$)	11 (27.5%)	20 (50%)	9 (22.5%)		
	OR(95%CI)	1	2.18 (0.71–6.78)	2.1 (0.52–8.79)		
GII a and b diabetic ($n=80$)	Fibrosis ($n=40$)	6 (15.0%)	26 (65.0%)	8 (20.0%)	2.26	0.32*
	Cirrhosis ($n=40$)	5 (12.5%)	21 (52.5%)	14 (35.0%)		
	OR(95%CI)	1	0.97 (0.22–4.36)	2.1 (0.39–11.87)		

X^2 = Chi-square test *: nonsignificant = $p > 0.05$

Table 4 Relation between IL28 B genotype and ALT / and viral load by PCR for the all studied patients

Parameter	CC ($n=40$)	CT ($n=82$)	TT ($n=38$)	K	*p*
ALT(U/L) Mean ± SD Median Range	37.35 ± 26.02 30 10–143	54.49 ± 27.21 50.5 14–161	71.58 ± 33.83 63 15–175	34.39	< 0.001***
PCR (IU/mL) Mean ± SD Median Range	153,050 ± 399,946 31,000 1600–1,800,000	1,081,300 ± 2,754,340 90,000 1200–16,200,000	578,060 ± 1,454,550 380,000 3200–7,500,000	11.45	0.003*

ALT: alanine aminotransferase PCR: polymerase chain reaction SD: standard deviation K = Kruskal–Wallis test ***: highly significant

Least significant difference (LSD) of ALT between genotypes: (CC versus CT $p = 0.002$ CC versus TT $p = < 0.001$. CT versus TT $p = 0.004$). LSD of PCR between genotypes: (CC versus CT $p = 0.034$. CC versus TT $p = 0.009$. CT versus TT $p = 0.01$)

Table 5 Multivariate logistic regression analysis for significant predictors of CHC disease progression

Variable	B	S.E	OR	95.0% C.I		χ^2	*p*	Rank
				Lower	Upper			
Age > 48	1.27	0.42	2.76	1.76	5.34	6.74	0.04**	6
BMI > 25	0.81	0.43	0.79	0.354	1.791	0.53	0.61*	7
T2DM	2.98	0.45	4.28	2.31	10.94	6.89	0.03**	4
Genotype CC versus CT	2.11	0.49	4.35	2.56	11.55	8.09	0.02**	3
Genotype CC versus TT	1.82	0.56	3.45	2.12	7.14	7.12	0.04**	5
ALT > 45 U/L	3.12	0.54	9.56	4.56	16.34	11.22	< 0.001***	2
Viral load > 1,000,000 IU/ml	4.1	0.62	11.27	5.63	29.45	12.03	< 0.001***	1

B = coefficient of regression, S. E = standard error, OR: odds ratio, CI: confidence interval, X^2 = Chi-square test, *nonsignificant $p > 0.05$ **significant $p < 0.05$ ***highly significant $p < 0.001$

Discussion

Interleukin-28B genotyping profile is expressed in HCV-infected patients into favorable CC genotype and unfavorable TT genotype. The former CC genotype might be defensive against disease progression and development of diabetes, while the later TT genotype might be a risk factor for the appearance of T2DM, fibrosis, and cirrhosis.

In this study, 160 patients who were positive for chronic HCV, 107 males and 53 females, aged between 20 and 70 years, were divided into two groups; 80 CHC patients without diabetes at the time of sample collection and 80 CHC patients with T2DM and were subdivided by Fibro-Scan into mild and advanced fibrosis.

As shown in Table 1, age showed a significantly higher difference in diabetes with compensated cirrhosis versus non-diabetes with compensated cirrhosis ($p < 0.001$), and that *may be attributed to* the fact that included diabetes patients were older than the non-diabetes with range [45–70 years versus 20–64 years] and mean ± SD (57.58 ± 4.39 vs. 40.79 ± 11.65; $p < 0.001$, respectively); this factor could potentially contribute to the development of T2DM and disease progression in advanced fibrosis.

BMI showed a significantly higher difference in diabetes patients with fibrosis than non-diabetes with fibrosis ($p = 0.001$) because people with T2DM usually suffer from fatness and show an inactive manner of living [24]. This matched with studies on T2DM in healthy individuals as well as diseased patients with genotypes other than HCV genotype 4 [25]. The amplified prevalence of diabetes in chronic liver disease is limited to HCV, and this relationship may be due to glucose intolerance in these patients [26]. Furthermore, high BMI, getting old and genomic factors such as a family history of T2DM, may lead to the development of T2DM and make difficulties in evaluating the pathogenic role of the virus C as a causative organism which enhances the development of T2DM [26].

Table 2 demonstrates that more than half of included patients have CT genotype expression. Although many studies had been done in *IL-28B* (rs12979860) in Egypt and around the world [27–30], the analysis of the prevalence of different genotypes CC, CT, and TT of *IL-28B* rs12979860 among all populations diseased and healthy is scarce and needs more evaluation. However, a study done by Thomas et al. reported the distribution of the C allele showed a lower frequency among Africans than Europeans and was more prevalent in Asians [31].

In the non-diabetic group, the genotype CC represented (36.3%), CT (43.7%), and TT (20%); these results are near to those of Bakr et al. and similar to El-Awady et al. [32, 33]. On the other hand, the CC genotype was significantly lower in CHC patients with diabetes (13.7%). In contrast to, the non-CC genotypes (CT/TT) showed a higher significant difference in CHC patients with diabetes than without diabetes [CT 58.8% vs. 43.7%, TT 27.5% vs. 20%, respectively] ($p = 0.03$, and $\chi^2 = 6.14$).

When we compared the allele frequency between two groups in this study, the favorable C allele was more in the non-diabetes group (58.7%) than in the diabetes group (47.5%). In contrast, the non-favorable T allele was lower in the non-diabetes (41.3%) compared to the diabetes group (52.5%) with statistically significant difference ($p = 0.04$, and $\chi^2 = 2.48$) (Table 2). These results were in concurrence with El-Awady et al. [33].

The level of CC genotype in the non-diabetes group was higher in fibrosis than in compensated cirrhosis [45.0% vs. 27.5%, respectively], but statistically insignificant ($p = 0.27$). At the same time, the level of CC genotype in the diabetes group was the worst either with fibrosis and/or compensated cirrhosis [15%, vs. 12.5%, respectively]. So, the reduction in CC genotype had been linked to the stage of fibrosis and the presence of diabetes. On the contrary, the non-CC genotypes were higher in the diabetes group with fibrosis and compensated cirrhosis but statistically insignificant ($p = 0.32$), as presented in Table 3.

Diabetes is accompanied by increased fibrosis in patients with HCV, but possibly it is due to the frequent occurrence of diabetes in patients with cirrhotic liver disease [34]. The association of T2DM with the increased disease severity may be due to inflammation caused by the activity of the virus, duration of the disease, insulin secretion, insulin resistance, and the effect of other risk factors of T2DM [35].

The relation of T2DM and IL-28B genotype and the degree of severity of fibrosis in CHC patients with CC genotype compared to patients with non-CC genotypes (CC 12.5% vs. CT 52.5% vs. TT 35%, respectively) is shown in Table 3. The reduction in favorable CC genotype was substantial in the diabetic group with compensated cirrhosis, opposite to the increasing unfavorable TT genotype in the same diabetic group. Nonetheless, we cannot judge whether this connection between diabetes and genotype was primary or secondary to the effect of CHC on the liver and general metabolism.

Chang et al. and Eurich et al. tried to comprehend the role of *IL-28B* rs12979860 genotype in the progression of cirrhosis in CHC patients. They detected that the unfavorable TT genotype in *IL-28B* rs12979860 had been linked with disease progression and possibly HCC [34, 36]. However, Agundez et al. and De la Fuente et al. found that the *IL-28B* rs12979860 genotype distribution was similar in CHC patients and that of patients with HCC [37, 38]. Marabita et al. concluded that the *IL-28B* genotype did not influence developing cirrhosis, whereas factors like age, male gender, and HCV genotype 3 are triggers to hasten disease progression [39].

A significant relation has been detected between the ALT level and the genotype; the CC genotype showed the lowest level of ALT in comparison to the CT/TT genotype ($p<0.001$), as presented in Table 4. ALT levels were increased in the TT genotype and mixed CT genotype, which were elevated in the diabetes group with cirrhosis. LSD between ALT and each genotype was highly significant [CC vs. CT $p=0.002$], [CC vs. TT $p=<0.001$] & [CT vs. TT $p=0.004$].

On the contrary, Agundez et al. found a higher level of ALT with the CC genotype patients [37]. This opposite relation might be attributed to the different HCV genotype 1 and the effect of viral genotype on ALT level [40]. Also, *Al-Swaff* reported the high ALT values were mainly present in the early and late stages of inflammation [41].

A different viral genotype 1 was included in their studies than the common genotype 4 in our country, alcohol drink, and alcoholic cirrhosis, which could make different results as assessed by *Schreiber* et al., who detected a low predictive value of IFN-λ3 genotypes regarding HCV genotype 2 and 3 in their study. Alcohol drink and alcoholic cirrhosis still could present in those with CHC, and their results showed an effect of the non-favorable TT genotype on fibrosis progression [42].

Both ALT and HCV RNA viral load by PCR were much lower in patients with *IL-28B* genotype CC compared to other genotypes. In other words, the patients with non-CC genotype are associated with more inflammatory changes. These results conflicted with the results of the study by Tadeusz et al., who found no correlation between the viral load and the genotype [CC, CT, or TT] of the patients [43].

In the present study, the reduction in CC genotype in diabetes group versus non-diabetes group may be explained by the multivariate logistic regression analysis that showed different cofactors together combined with hepatitis C such as viral load, ALT, *IL-28B* genotypes, diabetes and age, might interplay as predictors for CHC disease progression from fibrosis to cirrhosis among diabetes group collectively, [viral load $p<0.001$], [ALT $p<0.001$], [genotype CC vs. CT $p=0.02$], [genotype CC vs. TT $p=0.04$], [T2DM $p=0.03$] and [age $p=0.04$], or independent predictors of disease severity which is highly significant with viral Load & ALT ($p<0.001$), then genotype CC versus CT ($p=0.02$) and T2DM ($p=0.03$) in that manner consequently as shown in Table 5.

Strengths of the study

It is a new innovative study and a rare one in Egypt that studies the role of genetics in CHC progression and development of T2DM in CHC patients. This study is a preliminary study that pinpoints the importance of genetics in assessing either disease development (T2DM) or disease progression (CHC).

Limitations of the study

Regarding our study's constraints, the study is specific to a certain governorate. It cannot be generalized all over Egypt or even beyond the research setting at certain universities and hospitals. The study was done on a small sample size of patients that was limited to the power of the study. Also, no documents to decide precisely whether diabetes occurred before or after HCV disease, as most patients with diabetes and HCV may stay undiagnosed for a time and also, not all CHC patients developed diabetes. The Egyptian populations are infected mostly with HCV genotype 4 more than 90% [13]; so, we can consider this research applicable for genotype 4 and not an extension to any other research directed to a specific racial group other than Egyptians. Finally, the homeostatic model assessment "HOMA" test for assessing β-cell function and IR was not asked.

Conclusion

The association between *IL28B* (rs12979860) and CHC disease progression among T2DM patients indicates that genetic pathways may stand beyond the development of diabetes in HCV-infected patients other than IR. Viral load, genotypes CT and TT, and diabetes may interact collectively in CHC disease progression and add to rising ALT.

Abbreviations

ALT: Alanine aminotransferase; BMI: Body mass index; CHC: Chronic hepatitis C; DNA: Deoxyribonucleic acid; HCC: Hepatocellular carcinoma; HCV: Hepatitis C virus; IL-28B: Interleukin-28B; INF: Interferon; IR: Insulin resistance; ISGs: Interferon stimulated genes; LSD: Least significant difference; PCR–RFLP: Polymerase chain reaction-restriction fragment length polymorphism; RNA: Ribonucleic acid; SNPs: Single nucleotide polymorphisms; T2DM: Type 2 diabetes mellitus.

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Authors' contributions

Authors including SAA, NAA, RMA, and AAB collected patients' samples, clinical data, biochemical results, and elastograms. Statistical analysis, interpretation of data, and writing the manuscript were done by SAA, NAA, AAK, FR, SSA, and DG. All authors have read, revised, and approved the final version of manuscript.

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Availability of data and materials

Available.

Declarations

Ethics approval and consent to participate

The work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans. The study protocol was approved by the institution's review board of Faculty of Medicine, Zagazig University, and a written informed consent was taken from all participants before the study. The committee's reference number is not available.

Consent for publication

Not applicable.

Competing interests

The authors declare no conflict of interest.

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