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ORIGINAL RESEARCH ARTICLE

Could insulin receptor H1085H C > T single nucleotide polymorphism predict insulin resistance in type 2 diabetic and chronic hepatitis C virus patients in Egypt?

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Abstract

Background: Insulin-receptor (INSR) is an $\alpha_2\beta_2$ heterotetramer disulfide-linked trans-membrane glycoprotein and a family member of tyrosine kinase receptors. It mediates the pleiotropic actions of insulin regulating glucose homeostasis. It is encoded by a single gene: INSR gene. The INSR gene comprises 22 exons. Exons 17–22 encode the tyrosine kinase domain, and mutations in this region impair the function of the insulin receptor that may cause insulin resistance and hyperinsulinemia. Single nucleotide polymorphism with C > T substitution at His 1058 position of INSR (rs 1799817) located in exon 17 was considered to be involved in insulin resistance. Insulin receptor might be counter-regulated by degradation, differential expression, or modification by phosphorylation in cells expressing HCV core protein. HCV infection eventually leads to liver steatosis and fibrosis, increased oxidative stress, and peroxidation, all of which trigger a cascade of inflammatory responses, thus contributing to the development of insulin resistance. The present retrospective case-control aimed to study INSR H1085H C > T (rs 1799817) SNP in Egyptian patients suffering from chronic HCV infection with DM. The current study was conducted on two hundred and two participants of 100 males and 102 females, divided as follows: the control group (group I) included 50 apparently healthy volunteers of comparable age, sex, and socioeconomic status as patients groups, group II included 50 type 2 DM patients without HCV infection, group III included 52 chronic HCV infected patients without DM, and group IV included 50 HCV patients with DM.

Results: HOMA-IR and QUICKI index was significantly higher in the patient groups (groups II, III, and IV) than in controls ($P < 0.001$, $P = 0.019$, and $P < 0.001$, respectively). It was significantly lower in patients of group III than in patients of groups II and IV with $P < 0.001$ for both. DM patient group without HCV infection (group II) and HCV with DM (group IV) showed a significant decrease in CC genotypes and a significant increase in TT genotypes than the controls ($P < 0.001$, $P = 0.018$, respectively). HCV patients with DM (group IV) had the highest frequency of heterozygous genotype (CT) (50%). HCV-infected patients with T2DM (group IV) also showed a significantly higher frequency of minor allele (T) (35%) than controls (20%), and a lower frequency of the wild allele (C) (65%) than controls (80%).

Conclusion: The mutant allele "T" of INSR H1085H C > T (rs 1799817) SNP might be associated with an increased risk of developing insulin resistance and T2DM.

Keywords: Hepatitis C virus, Type 2 diabetes mellitus, INSR H1085H C > T SNP

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Background

Hepatitis C virus (HCV) infection, a common chronic liver disease, is a major public health problem. It was estimated that more than 185 million persons (2–3% of the world population) are HCV infected; of them, around 71 million are chronically infected individuals and 34 million are chronically infected with HCV genotype 4. Before the era of directly acting antiviral agents (DAAS), Egypt had the highest prevalence of HCV worldwide and ranked 5th among all countries for the burden of disease from viral hepatitis. Genotype 4 represents over 90% of HCV cases in Egypt [1, 2].

Hepatitis C viral eradication after antiviral treatment can lead to an improvement in insulin resistance and a reduction in the incidence of diabetes mellitus after the end of therapy. This offers a strong argument in favor of a causal relationship between HCV and DM [3].

Several hypotheses have been proposed to the mechanisms underlying HCV-mediated insulin resistance. HCV infection eventually leads to liver steatosis and fibrosis, increased oxidative stress, and peroxidation, all of which trigger a cascade of inflammatory responses, thus contributing to the development of insulin resistance [4].

Insulin receptor (INSR) is an $\alpha_2\beta_2$ heterotetramer disulfide-linked trans-membrane glycoprotein and a family member of tyrosine kinase receptors. It mediates the pleiotropic actions of insulin regulating glucose homeostasis [5, 6].

The insulin receptor is encoded by a single gene: INSR gene. The INSR gene is located on the short (p) arm of chromosome 19 and comprises 22 exons. Exons 17–22 encode the tyrosine kinase domain, and mutations in this region impair the function of the insulin receptor that may cause insulin resistance and hyperinsulinemia. Single nucleotide polymorphism (SNP) with C > T substitution at His 1058 position of INSR (rs 1799817) located in exon 17 was considered to be involved in insulin resistance [7, 8]. Insulin receptor might be counter-regulated by degradation, differential expression, or modification by phosphorylation in cells expressing HCV core protein [9].

SNP in the INSR was among the most frequently studied candidate genes in DM. To the best of our knowledge, no much data are available on INSR H1085H SNP in HCV-infected populations with DM.

Methods

The present research is a retrospective case-control study aimed to study INSR H1085H C > T (rs 1799817) SNP in Egyptian patients suffering from chronic HCV infection with DM. Our study was conducted on two hundred and two participants of 100 males and 102 females, divided as follows: the control group (group I) included 50 apparently healthy volunteers of comparable age, sex, and socioeconomic status as patients groups, group II included 50 type 2 DM patients without HCV infection, group III included 52 chronic HCV infected patients without DM, and group IV included 50 HCV patients with DM.

Exclusion criteria include patients with hepatocellular carcinoma (HCC) or any other malignancy, coexisting viral infection like hepatitis B surface antigen-positive patients, renal failure, liver failure, chronic pancreatitis, polycystic ovarian disease (PCOD), pregnant females, and those taking medications that affect insulin resistance including glucocorticoids were excluded from the study.

1. Full history was taken from all subjects.
2. Full physical examination and anthropometric measurements including body mass index [10] and waist to hip ratio (WHR) [11] were done.
3. Ultrasound abdomen [12]
4. The following laboratory investigations were done including determination of fasting and postprandial serum glucose, glycated hemoglobin (HbA$_1$C), triglycerides, cholesterol (total, high-, and low-density lipoprotein fractions), urea, creatinine, uric acid levels, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and gamma-glutamyl transferase (GGT) activities [13]. Insulin level was determined [14], and then the Homeostatic Model Assessment of Insulin Resistance (HOMA IR) [15] and Quantitative Insulin Sensitivity Check (QUICKI) index [16] were calculated. Polymerase chain reaction (PCR) [17] for HCV for groups III and IV, HCV antibodies [17] for groups I and II, and hepatitis B surface antigen [18] for all groups were done.
5. Genomic DNA was extracted from peripheral blood leukocytes by using ion-exchange column chromatography. Genotyping of INSR H1085H (rs 1799817) using polymerase chain reaction (PCR-RFLP) technique with restriction enzyme PmlI was done [19]. The details of molecular studies which had been done in the current study are as follows:

DNA extraction from peripheral blood leucocytes [19]

The genomic DNA extraction from peripheral blood leukocytes was carried out using column-based commercial genomic DNA extraction kits Thermo Scientific Gene JET^{TM} Whole Blood Genomic DNA Purification Mini Kit #K0781 (Fermentas-Thermo, USA).

Then assessing the integrity of the extracted genomic DNA by agarose gel electrophoresis (AGE), the eppendorf containing the checked purified DNA in the elution buffer was tightly capped and stored till the time of PCR at − 20 °C.

Polymerase chain reaction amplification using specific primers [19]

A set of primers flanking the SNP region were used for the detection of insulin receptor H1085H C > T (rs 1799817) single nucleotide polymorphisms using the following reagent:

1) DreamTaq PCR Master Mix (2X) # K1071 (200 runs of 50 μl) Thermo Scientific™
2) DNA gel Loading Dye (6X) # R0611: Thermo Scientific™
3) Primers: A pair of primers for each gene H1085H C > T (rs 1799817) was used. The lyophilized primers were supplied by Invitrogen, UK, which were reconstituted by addition of sterile nuclease-free water as follow:

Insulin receptor H1085H C > T (rs 1799817)

For the forward sense sequence, 195 μl was added and 225 μl for the reverse sense sequence to give a final concentration of 100 pmoles/μl for each and stored at − 20 °C. It was diluted to give a final concentration of 10 pmoles/μl.

Primers for insulin receptor H1085H C > T (rs 1799817)

Forward sense sequence: (INSR-G01)

5′-CCA AGG ATG CTG TGT AGA TAA G-3 (Tm (thermodynamic) = 64 °C)

Reverse antisense sequence: (INSR-F12)

5′-TCA GGA AAG CCA GCC CAT GTC-3 (Tm (thermodynamic) = 66 °C)

Protocol of amplification

In a 0.2-ml eppendorf tube placed on ice, the following reagents were added and mixed.

DreamTaq PCR Master Mix (2X)	12.5 μl
Forward primer	0.5 μl (10 pmol)
Reverse primer	0.5 μl (10 pmol)
Extracted DNA	1 μl
Water nuclease-free	10.5 μl

The total volume was 25 μl, and then the tubes were transferred to the thermal cycler (Quanta Biotech, UK) where the PCR conditions were adjusted (Table 1).

The amplified products were separated on 2% agarose gel electrophoresis after adding a loading dye as follows: 2 μl loading dye added to 10 μl of amplified DNA.

Table 1 Thermal cycler condition of insulin receptor H1085H C > T (rs 1799817)

Phases	Cycle number	Temperature (°C)	Time
Amplification			
• Denaturation		93	45 s
• Annealing	35	56	30 s
• Extension		72	45 s
Final extension	1	72	7 min

Restriction digestion of PCR products using PmlI for insulin receptor H1085H C > T (rs 1799817) [19]

Restriction includes endonuclease enzymes, PmlI enzyme for insulin receptor H1085H C > T (rs 1799817), targeting specific sequence of the amplified DNA product for detection of INSR H1085H C > T (rs 1799817) gene polymorphisms.

Reagents

For *INSR* H1085H C > T (rs 1799817)

– Eco72I (PmlI) (10 U/μl) # ER0361: restriction enzyme recognized CAC^GTG sites and cut best at 37 °C in Tango buffer. PmlI was used for insulin receptor H1085H C > T (rs 1799817) polymorphism.
– 10× buffer tango for Pm1I containing 33 mM tris-acetate (PH7.9), 10 mM magnesium acetate, 66 mM potassium acetate, and 0.1 mg/ml BSA.

Protocol for restriction enzymes

Eco72I (PmlI) for insulin receptor H1085H C > T (rs 1799817) polymorphism In an eppendorf tube, the following reagents were mixed.

Water (nuclease-free)	18 μl
10× tango buffer	2 μl
Amplified PCR product	10 μl
Restriction enzyme, PmlI	2 UI

Thus, the total volume was 32 μl. They were mixed gently, spin down for few seconds, and incubated at 37 °C for 2 h then the digested products were separated on agarose gel electrophoresis after adding loading dye as follows: 2 μl of loading dye was added to 10 μl of restricted DNA products.

Agarose gel electrophoresis for the amplified and digested PCR products using

1) Gene Ruler ^{TM} 50 bp DNA ladder (Thermo Fisher Scientific), the DNA fragments ranged from 50 to 1000 bp. It contains two reference bands: 250 and 500 bp.
2) Tris borate EDTA (TBE) buffer (10×) stock solution: working TBE (1×) buffer was prepared by measuring 100 ml stock solution and completed to 1 l of distilled water.
3) Agarose gel (wt/vol) 2.5% then 100 volts was applied for about 45 min till the loading dye had migrated to near the end of the plate. The DNA will migrate towards the anode.

– A negative control, water (nuclease-free) and positive control, and previous read samples were allowed to run in case of amplified product detection.
– In the case of pm1I INSR-restricted products, a PCR product was allowed to separate with each run to identify unrestricted products.
– Ultraviolet transillumination (302 nm) was used for the visualization of the DNA bands.

Results

For insulin receptor H1085H C > T (rs 1799817) polymorphism

The amplified DNA leads to the formation of 317 bp amplicon. When a C allele was present in PmlI SNP (rs1799817) within the exon 17 of the *INSR* gene, the PmlI enzyme yielded 274 and 43 bp fragments on the agarose gel. The PCR products remained uncut (317 bp) in the presence of the restriction enzyme (PmlI), confirming the presence of a T allele in this locus.

So, in the amplified product of H1085H, CC genotype appears as two bands of 274 bp and 43 bp fragments and CT genotype appears as 317 bp, 274 bp, and 43 bp fragments while TT genotype appears as a single uncut 317 bp fragment (Figs. 1 and 2)

There was no significant difference in both age ($P = 0.183$) and sex ($P = 0.101$) between the four studied groups.

Regarding BMI, it was significantly higher in diabetic patients without HCV infection (group II) and in HCV patients with DM (group IV) than controls, and group IV than group III ($P < 0.001$ for all).

Regarding WHR, it was significantly higher in group II and group IV than controls, in group III and group IV than group II, and in group IV than group III ($P < 0.001$ for all).

Serum levels of AST, ALT, and GGT were significantly higher in groups III and IV than controls and group II ($P < 0.001$ for all). There was no significant difference between serum activities of AST and GGT in group II and controls ($P = 0.063$ and $P = 0.703$, respectively). Serum activity of ALT was significantly increased in group II than controls ($P = 0.032$), but this was of no significance as the serum level of ALT was within the normal range in both groups. There was no significant difference as regard AST, ALT, and GGT activities between group IV and group III.

Fasting serum (FSG), postprandial serum glucose (PPSG), and glycosylated hemoglobin (HbA$_1$C%) were significantly higher in diabetes mellitus patients in groups II and IV than in controls with $P < 0.001$ (Table 2).

There was no significant difference between patients of group III and controls regarding FSG, PPSG, and HbA$_1$C%. HbA$_1$C% was significantly higher in patients of group II than in patients of group IV ($P = 0.005$) (Table 2).

Also, fasting insulin was significantly higher in groups II, III, and IV than in controls with $P < 0.001$. And it was significantly lower in patients of group III than in patients of group II and group IV with $P = 0.002$ and $P < 0.001$, respectively. And it was significantly higher in patients of group IV than in patients of group II with $P < 0.001$ (Table 2).

Regarding HOMA-IR, it was significantly higher in groups II, III, and IV than in controls with $P < 0.001$, $P = 0.019$, and $P < 0.001$, respectively. And it was significantly lower in patients of group III than in patients of groups II and IV with $P < 0.001$ for both (Table 2).

Regarding QUICKI index, it was significantly lower in patient groups II, III, and IV than in controls with $P < 0.001$. And it was significantly higher in patients of group III than in diabetic patients in groups II and IV with $P < 0.001$. It was significantly lower in patients of group IV than in patients of group II with $P = 0.009$ (Table 2).

It was noticed that the observed frequencies of the different genotypes of INSR H1085H C > T polymorphism were compared to the expected frequencies, according to the Hardy-Weinberg equilibrium, in each of the studied groups. All the studied groups showed $P > 0.05$ (after performing chi-square test) indicating an agreement with Hardy-Weinberg equilibrium.

The INSR H1085 C > T genotype distribution was significantly different between the four studied groups ($P < 0.001$). DM patient group without HCV infection (group II) and HCV with DM (group IV) showed a significant decrease in CC genotypes and a significant increase in TT genotypes than the controls ($P < 0.001$, $P = 0.018$, respectively). There was a significant increase in
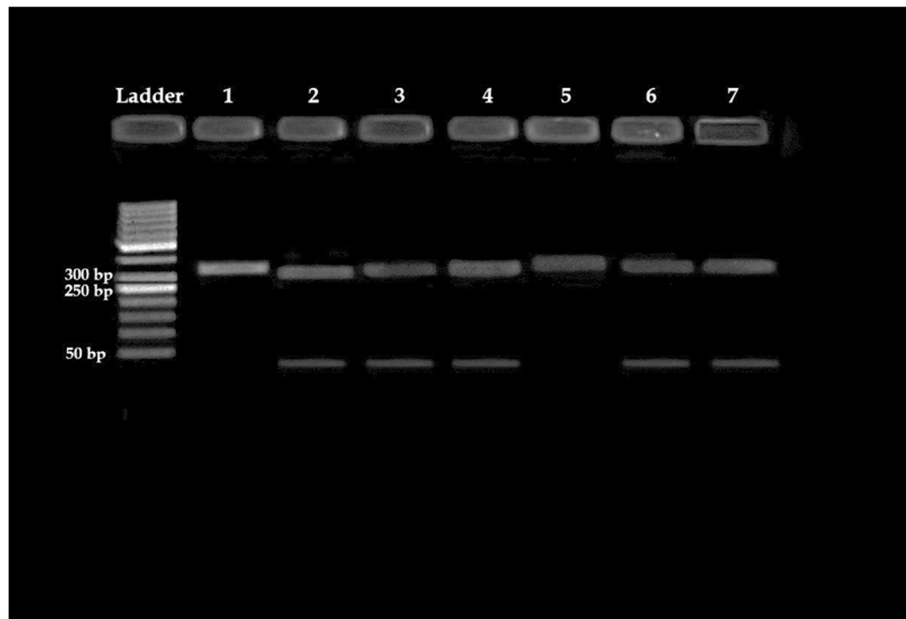
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Fig. 1 Agarose gel electrophoresis (2.5 %) of PmII-digested PCR product for *INSR* H1085 C > T SNP (rs 1799817). DNA ladder of 50 bp interval (50–1000 bp), lane 1: 317 bp DNA amplicon of *INSR* C > T; lanes 2–4 and lanes 6 and 7: CC; lane 5: TT

TT genotypes in DM patients without HCV infection (group II) than HCV patients (groups III and IV) ($P <$ 0.001 for both). On the contrary, there was no significant difference in genotype distribution between HCV-infected patients without DM (group III) and controls or between HCV-infected patients without DM (group III) and HCV-infected patients with DM (group IV) ($P = 0.131$ and $P = 0.658$, respectively). It was noticed that the highest frequency of wild genotype of INSR polymorphism (CC) was in controls (60%), while

the lowest one was in DM patients without HCV infection (group II) (10%). The highest frequency of homozygous mutant genotype (TT) was in DM patients without HCV (62%), whereas the mutant genotype was absent in controls. HCV patients with DM (group IV) had the highest frequency of heterozygous genotype (CT) (50%) (Table 3, Fig. 3).

The allelic frequency was significantly different between the four studied groups ($P < 0.001$). Groups II and IV showed a significant increase in T allele than
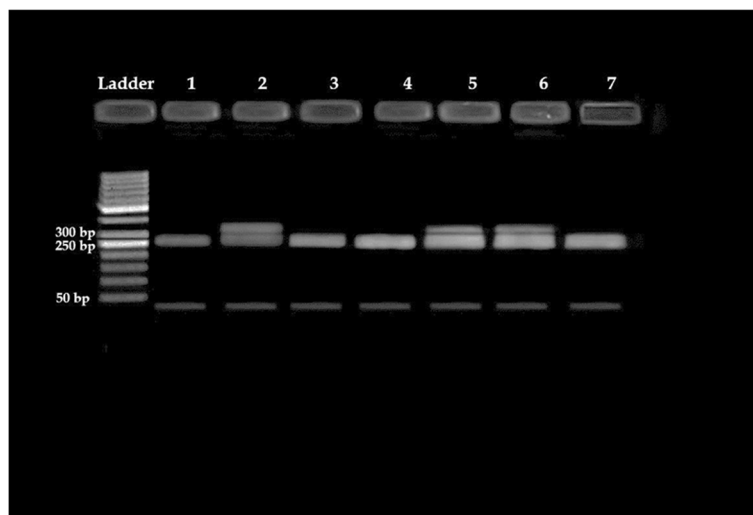

Fig. 2 Agarose gel electrophoresis (2.5 %) of PmII-digested PCR product for *INSR* H1085 C > T SNP (rs 1799817). DNA ladder of 50 bp interval (50–1000 bp), lanes 1, 3, 4, and 7: CC; lanes 2, 5, and 6: CT

Table 2 Statistical significance of fasting serum glucose level (FSG) (mg/dl), postprandial serum glucose (PPSG) (mg/dl), HbA$_1$C (%), fasting insulin (μIU/ml), and HOMA-IR and QUICKI index in the studied groups

	Group I (n = 50)	Group II (n = 50)	Group III (n = 52)	Group IV (n = 50)	Test of sig.	P
FSG (mg/dl)						
Min.–Max.	75.0–99.0	129.0–322.0	59.0–100.0	83.0–766.0	H = 143.378	< 0.001*
Mean ± SD	86.3 ± 5.62	195.2 ± 51.82	82.31 ± 11.46	182.68 ± 106.6		
Median	87.0	189.0	80.0	168.50		
$p_{\text{Group I}}$		< 0.001*	0.239	<0.001*		
Sig. bet. Grps		$p_1 < 0.001^*, p_2 = 0.132, p_3 < 0.001^*$				
PPSG (mg/dl)						
Min.–Max.	85.0–130.0	108.0–391.0	77.0–145.0	85.0–540.0	F = 72.836	< 0.001*
Mean ± SD	102.3 ± 12.9	207.3 ± 70.44	101.6 ± 17.87	223.62 ± 81.85		
Median	99.0	201.0	95.0	210.0		
$p_{\text{Group I}}$		< 0.001*	1.000	< 0.001*		
Sig. bet. Grps		$p_1 < 0.001^*, p_2 = 0.447, p_3 < 0.001^*$				
HbA$_1$C (%)						
Min.–Max.	3.80–5.50	6.20–13.70	3.80–5.40	5.40–12.20	F = 145.963	< 0.001*
Mean ± SD	4.68 ± 0.49	8.75 ± 2.01	4.58 ± 0.47	7.89 ± 1.42		
Median	4.80	8.10	4.50	7.75		
$p_{\text{Group I}}$		< 0.001*	0.974	< 0.001*		
Sig. bet. Grps		$p_1 < 0.001^*, p_2 = 0.005^*, p_3 < 0.001^*$				
Fasting insulin (μIU/ml)						
Min.–Max.	1.20–10.10	5.40–77.0	2.40–25.30	10.0–514.2	H = 121.986	< 0.001*
Mean ± SD	5.75 ± 2.65	17.09 ± 16.22	9.19 ± 3.65	40.68 ± 72.89		
Median	5.65	11.0	9.30	18.65		
$p_{\text{Group I}}$		< 0.001*	< 0.001*	< 0.001*		
Sig. bet. Grps		$p_1 = 0.002^*, p_2 < 0.001^*, p_3 < 0.001^*$				
HOMA-IR						
Min.–Max.	0.30–2.20	2.70–49.0	0.50–5.30	4.60–65.10	H = 157.864	< 0.001*
Mean ± SD	1.23 ± 0.55	9.14 ± 11.09	1.81 ± 0.73	12.76 ± 11.04		
Median	1.30	5.10	1.95	7.40		
$p_{\text{Group I}}$		< 0.001*	0.019*	< 0.001*		
Sig. bet. Grps		$p_1 < 0.001^*, p_2 = 0.055, p_3 < 0.001^*$				
QUICKI index						
Min.–Max.	0.34–0.49	0.23–0.33	0.30–0.43	0.21–0.31	F = 150.524	< 0.001*
Mean ± SD	0.38 ± 0.04	0.30 ± 0.03	0.35 ± 0.02	0.28 ± 0.02		
Median	0.37	0.30	0.35	0.29		
$p_{\text{Group I}}$		< 0.001*	< 0.001*	< 0.001*		
Sig. bet. Grps		$p_1 < 0.001^*, p_2 = 0.009^*, p_3 < 0.001^*$				

controls ($P < 0.001$, $P = 0.018$, respectively). There was no significant difference in allelic frequency between HCV-infected patients without DM (group III) and controls ($P = 0.106$) or between group III and group IV ($P = 0.428$) (Table 3, Fig. 4).

Testing the dominant model of inheritance revealed a statistically higher frequency of exposed genotypes "CT&TT" among the diabetics (group II) when compared to the control group (90% vs 40%, respectively; $P < 0.001$), with a substantial increase in insulin resistance and DM risk among the exposed group (CT and TT), with a crude odds ratio of 13.5 (95% confidence interval, 4.569–39.889) when compared to unexposed group (CC). After logistic regression analysis, the odds ratio was adjusted for BMI, age, and sex to be 12.238 (95% confidence interval, 3.041–49.239) ($P < 0.001$).

Table 3 Comparison between the four studied groups according to genotype distribution of INSR H1085H C > T (rs 1799817) polymorphism

Parameters	Group I (n = 50)		Group II (n = 50)		Group III (n = 52)		Group IV (n = 50)		χ^2	P
	N	%	N	%	N	%	N	%		
INSR H1085H C > T										
CC homo wild	30	60.0	5	10.0	24	46.2	20	40.0	84.746*	< 0.001*
CT hetero	20	40.0	14	28.0	25	48.1	25	50.0		
TT homo mutant	0	0.0	31	62.0	3	5.8	5	10.0		
Sig. bet. Grps	$p_1 < 0.001*$, $^{MC}p_2 = 0.131$, $p_3 = 0.018*$, $p_4 < 0.001*$, $p_5 < 0.001*$, $^{MC}p_6 = 0.658$									
C	80	80.0	24	24.0	73	70.2	65	65.0	76.146*	< 0.001*
T	20	20.0	76	76.0	31	29.8	35	35.0		
Sig. bet. Grps	$p_1 < 0.001*$, $p_2 = 0.106$, $p_3 = 0.018*$, $p_4 < 0.001*$, $p_5 < 0.001*$, $p_6 = 0.428$									

χ^2 chi-square test, *MC* Monte Carlo, *p* p value for comparing between the different groups, common letters are not significant (i.e., different letters are significant), p_1 p value for comparing between group I and group II, p_2 p value for comparing between group I and group III, p_3 p value for comparing between group I and group IV, p_4 p value for comparing between group II and group III, p_5 p value for comparing between group II and group IV, p_6 p value for comparing between group III and group IV, *Sig. bet. Grps* significance between groups
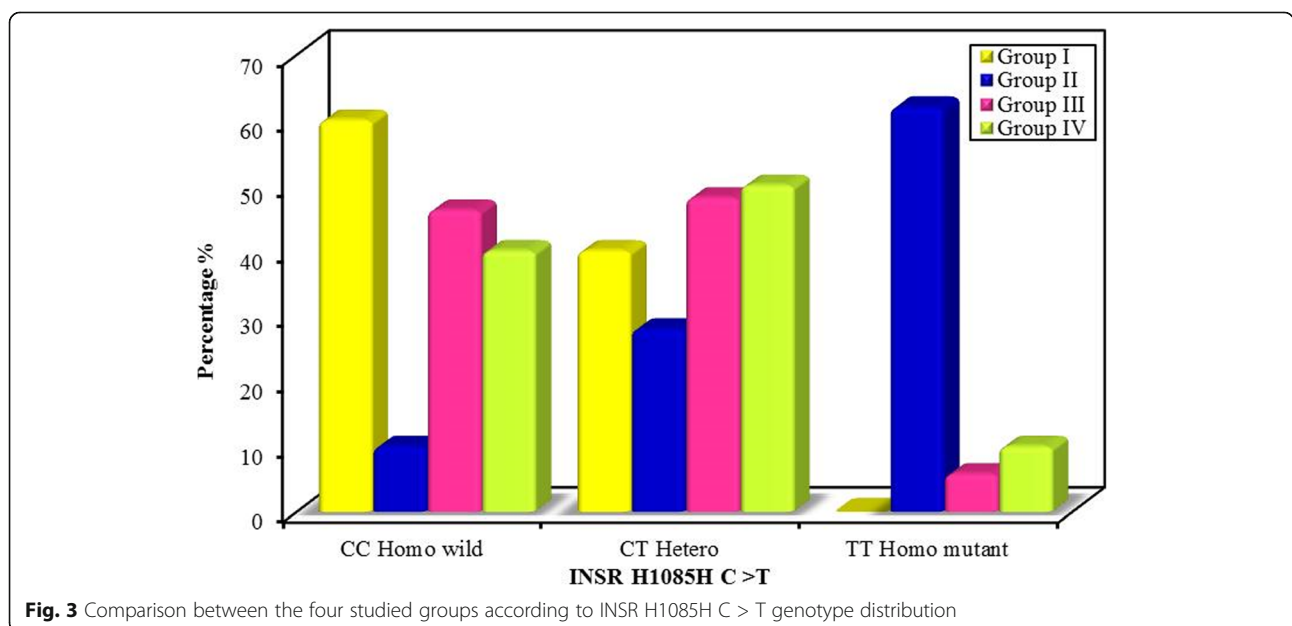*Statistically significant at $p \le 0.05$

Testing the recessive model, where the exposed group was considered to be composed of only subjects with the homo-mutant TT genotype, was not applicable as the odds ratio cannot be estimated because of the absence of (TT) genotype among the control subjects.
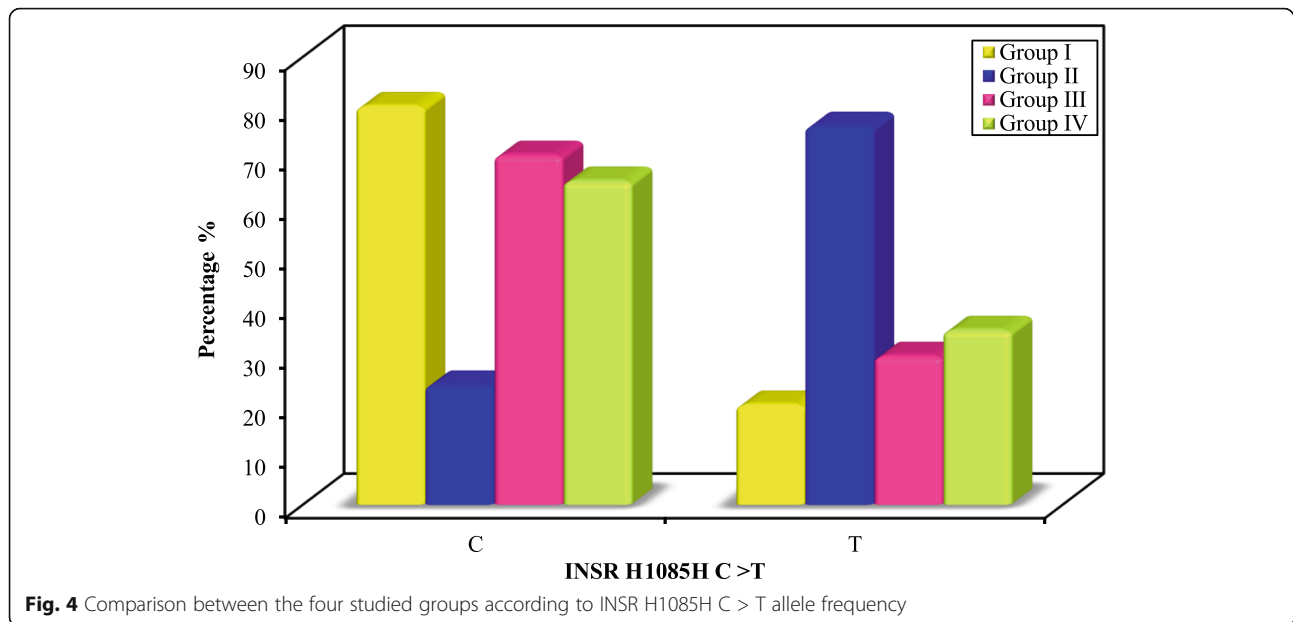
Comparing between groups I and III, no statistically significant difference was found in the risk of development of IR and DM according to INSR H1085H C > T genotypes between exposed and unexposed groups in both dominant and recessive modes of inheritance.

Testing the dominant model of inheritance revealed a statistically higher frequency of exposed genotypes "CT&TT" among the HCV-infected patients with DM (group IV) than the control group (60% vs 40%, respectively; P = 0.046) with an apparently increase in insulin resistance and DM risk among the exposed group (CT and TT) with a crude odds ratio of 2.250 (95% confidence interval, 1.011–5.008) than unexposed group (CC). After logistic regression analysis, the odds ratio was adjusted for BMI, age, and sex to be insignificant, 15.120 (95% confidence interval, 0.841–271.824) (P = 0.065), meaning that genotype exposure by itself was insignificant, but with other confounders like BMI, the risk of IR and DM increased.

Testing the recessive model, where the exposed group was composed of only subjects with the homo-mutant

Fig. 3 Comparison between the four studied groups according to INSR H1085H C > T genotype distribution

Fig. 4 Comparison between the four studied groups according to INSR H1085H C > T allele frequency

TT genotype, was not applicable, and odds ratio cannot be estimated because of the absence of (TT) genotype among the control subjects.

Diabetic patients (groups II and IV) were divided into controlled (with HA1c ≤ 7), fairly controlled (with HA1c = 7.1–9), and uncontrolled (with HA1c > 9). In group II, 20% of patients were controlled, 44% were fairly controlled, and 36% were uncontrolled. In group IV, 34% of patients were controlled, 44% were fairly controlled, and 22% were uncontrolled. We studied INSR H1085 C > T genotype distribution in controlled versus uncontrolled DM patients. There was no statistically significant difference regarding INSR H1085 C > T genotype distribution between controlled and uncontrolled DM patients in group II ($P = 0.774$) and group IV ($P = 0.572$).

Discussion

Type 2 diabetes mellitus is found to be 1.8- to 2.5-fold higher in patients with chronic HCV infection and two- to threefolds more prevalent in HCV than in HBV infection worldwide. HCV eradication after antiviral treatment could lead to an improvement in insulin resistance and a reduction in the incidence of diabetes mellitus after the end of therapy. This offers a strong argument in favor of a causal relationship between HCV and DM [3].

Several hypotheses have been proposed to explain the mechanisms underlying HCV-mediated insulin resistance. Insulin receptor (INSR), a family member of tyrosine kinase receptors, mediates pleiotropic actions of insulin regulating glucose homeostasis. The main action of its activation is inducing glucose uptake. The decrease in insulin receptor signaling leads to hyperglycemia and all the sequelae of type 2DM [4–6].

Insulin receptor might be counter-regulated in cells expressing HCV core protein which may contribute, at least in part, to the induction of insulin resistance in hepatocytes expressing HCV core protein [9].

The study of INSR H1085H C > T SNP and its effect on insulin resistance is important. Unfortunately, it was not studied frequently. Moreover, it was studied mainly on females with polycystic ovary syndrome (PCOS) giving importance to the current study.

From the current study, it could be concluded that the mutant allele "T" was associated with a statistically significant increased risk of developing insulin resistance and DM.

Also, results of the present study revealed a statistically significant higher frequency of exposed genotypes (TT and CT) among patients of DM without HCV infection (group II) 90% than the control group 40% with a substantial increase in insulin resistance and DM risk among the exposed group (CT and TT) than the unexposed group (CC).

This association between INSR H1085H (rs 1799817) SNP, insulin resistance, and DM risk among diabetic patients without HCV infection (group II) remained after performing logistic regression analysis to adjust for some important insulin resistance confounding factors.

On the contrary, HCV-infected patients with DM (group IV) showed a statistically higher frequency of exposed genotypes (TT and CT) among them than the control group. After logistic regression analysis, the odds ratio was adjusted for BMI, age, and sex to be insignificant.

In accordance with the present work, Mukherjee et al. [20] studied INSR H1085 H C > T polymorphism on Indian females by direct sequencing and found that the polymorphic genotype (CT and TT) was significantly associated with PCOS in lean women ($P = 0.004$).

Tucci et al. on Canadian population and Tucci et al. [21] on Caucasian population had found an association between rs1799817 and PCOS.

Siegel et al. [22] on Americans argued for the first time that the INSR gene itself took part in the development of insulin resistance as the main cause of PCOS.

Two genome-wide studies (GWAS) by Chen et al. [23] and Shi et al. [24] reported an association of rs1799817 of INSR gene in PCOS women with an increase in the frequency of uncommon T allele in lean PCOS women with body mass index [(BMI) < 27 kg/m^2] than lean controls.

Kumar et al. [25] found an increased frequency of INSR H1085H T allele in PCOS patients from China and India.

Moreover, to overcome small sample sizes, two meta-analysis studies were done: Ioannidis et al. [26] and Feng et al. [8]. They showed no significant association between INSR H1085H polymorphism and PCOS.

Mutib et al. [27] had also found an association of INSR H1085H polymorphism and PCOS, indices of insulin resistance, and dyslipidemia in Iraqi women using restriction fragment length polymorphism (RFLP-PCR) but unlike the current study, they found that CC genotype frequency was higher in PCOS patients whereas TT genotype was higher in control women. These differences in phenotypic expression could be due to the frequency of distribution of genotypes TT, CT, and CC across the population where the effect of genotype influences suppression or increased expression of phenotypic change.

Unlike the current work, some studies reported no association of PmlI (rs1799817) polymorphism in the INSR gene, insulin resistance, and PCOS risk including Urbanek et al. [28], Tehrani et al. [29], Bagheri et al. [19], and Xu et al. [30] on Han Chinese. Moreover, Thangavelu et al. [31] showed also a negative association between polymorphism and PCOS women, but the genotypes influenced the phenotypic expression as women with TT genotype of H1085H showed an increase in insulin level. Zhu et al. [32] showed also no association with INSR H1085 polymorphism and DM or diabetic nephropathy in Chinese Han population. On the contrary to the current study, Bodhini et al. [33] found that "T" allele was significantly lower in the DM subjects with temporary significant protection against diabetes as it became insignificant after adjustment of P value by other confounders.

The discrepancy between the current work and these studies may be caused by population stratification; using the classic case-control design would have the limitation of associations between allelic variants in candidate genes and disease might have arisen on account of population stratification by ethnicity or environmental factors. Also, most of the studies were conducted on females only. Another reason may be using different techniques to determine the SNP that differs in method performance.

Although HCV infection is an important risk factor for insulin resistance and diabetes mellitus, the current work found no significant association between INSR H1085H C > T polymorphism and HCV-induced IR and DM. However, no previous studies regarding the INSR H1085H SNP and the risk of insulin resistance and DM or even the risk of HCV-induced DM in Egyptians were done, giving importance to the current study.

Conclusions
From this study, it could be concluded that the INSR H1085H (rs 1799817) polymorphism might be a contributing risk factor for the development of type 2 diabetes mellitus as established by the high frequency of minor allele (T) in type 2 diabetic patients without HCV infection. We could also conclude that increased frequency of mutant allele (T) than wild allele (C) in INSR H1085H polymorphism in type 2 diabetic patients with BMI < 25 kg/m^2 might be risk factors for type 2 diabetes mellitus even in subjects with normal body weight. Finally, we were able to assure that BMI has its role as an independent risk factor for the development of type 2 diabetes mellitus as in our study, there was a significant increase of body mass index in type 2 diabetics without HCV infection and HCV-infected patients with type 2 diabetes mellitus.

In the light of the current study conclusion and its limitation, we are recommending the study of other related gene polymorphisms that might contribute to the development of type 2 diabetes in HCV patients, to study the relation of INSR H1085H to the response of different antidiabetic drugs, to assess of gene expression of INSR in HCV infected patients which may be useful for early detection of type 2 diabetes in these patients, and to conduct the study on larger sample size and in other ethnic HCV cohorts are needed to validate the present study. Finally, we are recommending regular screening for type 2 diabetes mellitus in HCV patients.

HBV: Hepatitis B virus; HCV: Hepatitis C virus; HOMA-IR: Homeostatic model assessment for insulin resistance; IL-6: Interleukin-6; INSR: Insulin receptor; IR: Insulin resistance; Kb: Kilo bite; KDa: Kilo Dalton; KRLB: Kinase regulatory loop binding domain; LH: Luteinizing hormone; MFA: Minor frequency allele; mRNA: Messenger RNA; NGS: Next-generation sequencing; NO: Nitric oxide; NTR: Non-translated region; PCOS: Polycystic ovarian syndrome; PI-3 kinase: Phosphoinositide-3 kinase; PIP-3: Phosphatidylinositol (3, 4, 5)-trisphosphate; PKC: Protein kinase C; PPSG: Postprandial serum glucose; QUICKI: Quantitative insulin sensitivity check index; RER: Rough endoplasmic reticulum; RFLP: Restriction fragment length polymorphism; RNA: Ribonucleic acid; ROS: Reactive oxygen species; Rpm: Revolutions per minute; Rs: Reference SNPs (ID); RSP: Residue signal peptide; RT-qPCR: Real-time quantitative polymerase chain reaction; RTK: Receptor tyrosine kinase; SH2: Src homology 2; SNPs: Single nucleotide polymorphisms; T2DM: Type 2 diabetes mellitus; TC: Total cholesterol; TGN: Trans-Golgi network; TNF-α: Tumor necrotizing factor-α; WHR: Waist hip ratio; β-cell: Beta-cell

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