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Single Nucleotide Polymorphism of Interleukin-28β Subunit Genes Predict Host Susceptibility to Hepatitis C virus (HCV) Infection among Iraqi Patients

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Abstract

Hepatitis C virus (HCV) is a liver disease that affects14 million people. Feasible research was conducted for identifying the genotypes and allele frequency of some single nucleotide polymorphisms (SNPs) of the IL-28β genes and their predictive role in disease incidence in Iraqi patients. The SNPs (rs28416813, rs4803219, rs11881222, and rs8103142) of IL-28β have been associated with susceptibility to several diseases. Ninety eight (98) HCV patients were included in this research; with average age ± SE (42.28 ± 3.44) years. Also, 80 healthy people (with average age ± SE (29.40 ± 2.84) years) were included as a control group. The SNPs were detected by allele-specific PCR (polymerase chain reaction) using specific primers. The results showed that IL-28β rs28416813 CC genotype was increased in HCV patients significantly while rs4803219 CC and CT genotypes were increased in the HCV patients significantly and insignificantly, respectively. Moreover, rs4803222 GA and AA genotypes frequencies were elevated in HCV patients insignificantly and significantly, respectively. Finally, IL28β rs8103142 gene polymorphism displayed overrepresentation of CT and TT genotypes incidence in hepatitis C patients highly significantly and non-significantly, respectively, as compared to control. The current data suggested that IL28β genetics may have an effect on susceptibility with HCV infection in Iraqis.

Keywords: Hepatitis C virus, Interleukin-28β, single nucleotide polymorphism.

تنبؤ تعدد أشكال النوكليوتيدات المفردة لجينات تحت وحدات الانترليوكين-28 بيتا بقابلية الاشخاص للإصابة بفيروس التهاب الكبد الوبائي نمط سي (HCV) بين المرضى العراقيين.

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الخلاصه

فيروس التهاب الكبد الوبائي سي (HCV) هو مرض كبدي يصيب 14 مليون شخص. تم إجراء البحث لتحديد الأنماط الجينية وترددات الأليل لبعض تعدد أشكال النوكليوتيدات المفردة (SNPs) لجينات IL-28β ودورها التنبئي في حدوث المرض لدى المرضى العراقيين. ارتبطت SNPs rs28416813 و rs4803219 و rs11881222 و rs8103142 من IL-28β بالقابلية للإصابة بالعديد من الأمراض. تم تضمين ما

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مجموعه 98 مريضا بفيروس التهاب الكبد C بمتوسط عمر 42.28 ± 3.44 سنة في هذا البحث . كذلك تم تضمين 80 شخصا سليما (بمتوسط عمر 29.40 ± 2.84 سنة) كمجموعة سيطرة. تم الكشف عن SNPs بواسطة اختبار تفاعل البلمرة المتسلسل الخاص بالأليل باستخدام بادئات متخصصة. أظهرت النتائج أن النمط الجيني CC rs28416813 IL−28β زاد في مرضى التهاب الكبد الوبائي بشكل ملحوظ بينما زادت الأنماط الجينية CC rs4803219 و CT في مرضى HCV بشكل معنوي وغير معنوي على التتالي. علاوة على ذلك ، فإن ترددات الأنماط الجينية GA rs4803222 و AA ارتفعت في مرضى التهاب الكبد الوبائي بشكل معنوي وغير معنوي. وأخيرا ، أظهر تعدد الأشكال الجيني IL28β rs8103142 تعبيرا مفرطا لحدوث الأنماط الجينية CT و TT في مرضى التهاب الكبد C كان معنويا وغير معنويا مقارنة بالسيطرة. تشير البيانات الحالية إلى أن جينات IL28β قدت على القابلية للإصابة بعدوى فيروس التهاب الكبد C لدى العراقيين.

Introduction:

Hepatitis C virus (HCV) has infected approximately 70 million people worldwide. Only a minority of individuals (20–30%) can clear the virus spontaneously in the acute phase of infection while most patients develop a persistent infection. These patients are at substantial risk of developing liver inflammation, fibrosis, cirrhosis, and hepatocellular carcinoma (HCC) [1].

Interferons (IFNs) have critical antiviral activities and immune regulatory functions in infections and autoimmunity. These IFN functions are mediated by inducing the expression of various IFN-stimulated genes (ISGs) [2].

The type III IFN family is composed of four members: IFN-λ1(IL-29), IFN-λ2(IL-28A), IFN-λ3(IL-28β), and the recently identified IFN-λ4 [3,4].

Type III IFN responses to infections or autoimmunity are predominantly observed in epithelial barriers, including the gastrointestinal and respiratory tracts [5]. Early IFN-λ antiviral activity limits infection and prevents triggering other systemic immune responses and inflammation [6]. The genes encoding three IFN- λ family members are clustered on human chromosome 19 (region 19q13 + 13). This location differs from that clustered on chromosome 9 of the type I IFN family. Like the IL-10 gene family, there are several exons in IFN- λs, and six exons in IFN-λ2 and IFN-λ3, and five exons in IFN-λ1 in comparison with type I IFNs, which encoded inside a single exon. IFN-λ2 and IFN-λ3 are almost identical, sharing 96% amino acid similarity, while IFN- λ1 is 81% homologated to IFN- λ 2/3 [7].

Mutation of any unusual nucleotide sequence alteration is usually, but not always, an attribute that causes illness. A difference in the DNA sequence occurring in a population at or above a frequency of 1 percent is called polymorphism [8].

Several studies have shown the function of IL28β-upstream SNPs on chromosome 19 coding for IFN- λ 3 (rs12979860, rs8099917, rs12980275, and rs8103142) in predicting mediated treatment and spontaneous clearance of HCV infection [9].

This study examined four SNPs (rs28416813, rs4803219, rs11881222, and rs8103142) of IL-28β genes in HCV patients to understand their susceptibility role in the disease.

Materials and methods

Patients

Ninety-eight consecutive patients with Hepatitis C virus (HCV) were included in this study to determine SNPs of IL-28β. They were admitted to Special Nursing Home Hospital in Baghdad between December 2018 and January 2019. These patients were of average age ± SE (42.28 ± 2.97) years [10]. Also eighty apparently healthy volunteers included in this study as a control group, with average age ± SE (29.40 ± 2.84) years. Viral load was determined in their plasma by real-time PCR. None of them had a history of any underlying autoimmune or

chronic infectious disease [11]. This prospective study was carried out after obtaining the requisite ethics committee permission from the Department of Biology (University of Baghdad) to approve the study protocol (BEC/1019/001 in 1/10/2019) and to get patients' consent.

Molecular detection of HCV

The nucleic acid was isolated from whole EDTA blood using a rapid blood genomic RNA extraction kit (Acrogene/ USA); the standard procedure recommended by the manufacturer was employed. The isolated RNA was subjected to PCR for the purpose of cDNA synthesis and amplification by Sacace, Italy kit. The PCR protocol included Six reaction tubes to perform PCR of extracted calibrators with dilution (from 8,00 log IU/ml to 1,00 log IU/ml). The PCR reaction tube consisted of fifty µl of calibrator, RNA sample, negative control and positive control with ten µl of internal control was introduced into each sample. The PCR cycling conditions are mention in table 1. The PCR products were associated with the generation of a fluorescence signal measurable in FAM/Green channel (for IC) or in Joe/Yellow/HEX channel (for HCV RNA), resulting in a sigmoid growth curve (log scale) (Figure1).

Table 1: PCR thermocycler for amplification protocol

Stage	Temp. ˚C	Time	Fluorescence detection	Cycle repeats
Hold	50	15 min.	-	1
Hold	95	15 min.	-	1
Cycling	95	5s	-	5
	60	20s	-	
	72	15s	-	
Cycling 2	95	5s	-	40
	60	30s	FAM,JOE/HEX/CY3	
	72	15s	-	

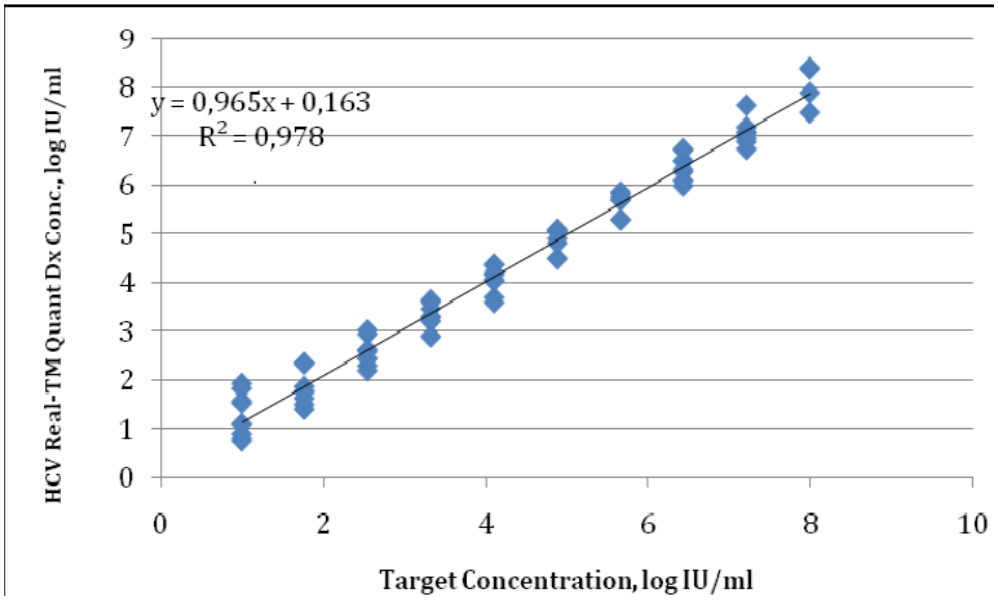

Figure 1: Standard curve obtained by Excicycler Real-Time PCR.

IL-28β gene SNPs

Four SNPs of IL-28β encoding genes; (rs28416813, rs4803219 rs11881222, and rs8103142) were selected on the basis of their minor allele frequency (MAF ≥ 10%) (https://www.ncbi.nlm.nih.gov/snp/). An allele-specific PCR method was employed to detect the SNPs using specific primers (Table 2). Geneious software (version 10.2.2) was used to design the primers [12]. After the DNA isolation, PCR amplification was carried out. The PCR mixture (20µL) consisted of 1.5µL DNA, 1µL of each primer, 5µL Master Mix (Intron company, Korea), and 11.5 µL of nuclease-free water. The PCR conditions included initial denaturation at 94 °C (5 min), followed by 40 cycles of denaturation at 94°C (35s), annealing for 35s (the temperatures given in Table 2), and extension at 72 °C(35 s). This was followed by a final extension at 72°C for 10 min. The PCR products were electrophoresed on 1.5% agarose gel at 5 V/cm2 for 60 min. and stained with diamond dye. The migrating PCR products alongside a pattern of 100 bp DNA ladder were then visualized using gel documentation system

Statistical analysis

The sample size was estimated using G*Power 3.1.9.4 [13]. Hardy-Weinberg equilibrium (HWE) evaluation of SNP genotype frequencies were done by Pearson's Chi-square test. Odds ratio (OR) and its 95% confidence interval (CI) were used to assess SNP-disease association, and the significant differences were determined by two-tailed Fisher exact probability (p). Bonferroni correction was applied to correct the p-value due to multiple comparisons. A corrected p (pc) ≤ 0.05 was considered significant. The statistical package SPSS (version 19.0) and Winpepi software (version 11.65) were employed to carry out these analyses. SHEsis software was used to estimate haplotype frequencies and linkage disequilibrium (LD) between SNPs. The LD was expressed as LD coefficient (D') [14].

Table 2: Forward and reverse primers for IL-28 β gene SNPs and their PCR condition protocol.

SNPs	Primers sequences	PCR Conditions	PCR product length
Interleukin-28β SNP rs28416813	C- allele specific primer: F1: **5-CTGAGGGAATGCAGAGGC-3** G- allele specific primer: F2: **5-CTGAGGGAATGGAGAGGC-3** Common revers primers: **5-TGGAATCCTCTTGGGAACATAC-3**	**An initial denaturation at** 94 ˚C for 5 min. -Then, 30 cycles; each cycle consisted of denaturation at 94˚C for 30s, annealing at 61˚C for 30s and extension at 72˚C for 30s. -A final extension at 72 ˚C for 10 min.	488 bp
Interleukin-28β SNP rs4803219	C- allele specific primer: F1: **5-ATCCTGACAGAAGGGCA-3** T- allele specific primer: F2: **5-ATCTCTGACAGAAGGGCA-3** Common revers primers: **5-GTGGAATCCTCTTGGGAACATAC-3**	**An initial denaturation at** 94 ˚C for 5 min. -Then, 30 cycles; each cycle consisted of denaturation at 94 ˚C for 30s, annealing at 62.4˚C for 30s and extension at 72 ˚C for 30s. -A final extension at 72 ˚C for 10 min.	206 bp
Interleukin-28β SNP rs11881222	A- allele specific primer: F1: **5- TGTGGTCAGGTAGGAGCA-3** G- allele specific primer: F2: **5- TGTGGTCAGGTGGGAGCA-3** Common revers primers: **5-GCAGGGACTGACTCATGTTT-3**	**An initial denaturation at** 94 ˚C for 5 min. -Then, 30 cycles; each cycle consisted of denaturation at 94 ˚C for 30s, annealing at 62.4˚C for 30s and extension at 72 ˚C for 30s. -A final extension at 72 ˚C for 10 min.	380bp

Interleukin-28β SNP rs8103142	C- allele specific primer: F1: **5-GCACCTGCAGTCCTTCA-3** T- allele specific primer: F2: **5-GCACTTGCAGTCCTTCA-3** Common revers primers: **5-TGCTCCTACTGCAGGGA-3**	**An initial denaturation at** 94 ˚C for 5 min. -Then, 30 cycles; each cycle consisted of denaturation at 94 ˚C for 30s, annealing at 62.3˚C for 30s and extension at 72 ˚C for 30s. -A final extension at 72 ˚C for 10 min.	200bp

Results and Discussion

IL-28β SNP rs28416813 current findings distinguished two alleles (G and C), which corresponded to three genotypes (GG, GC, and CC) Figures (2, 3, 4, 5).

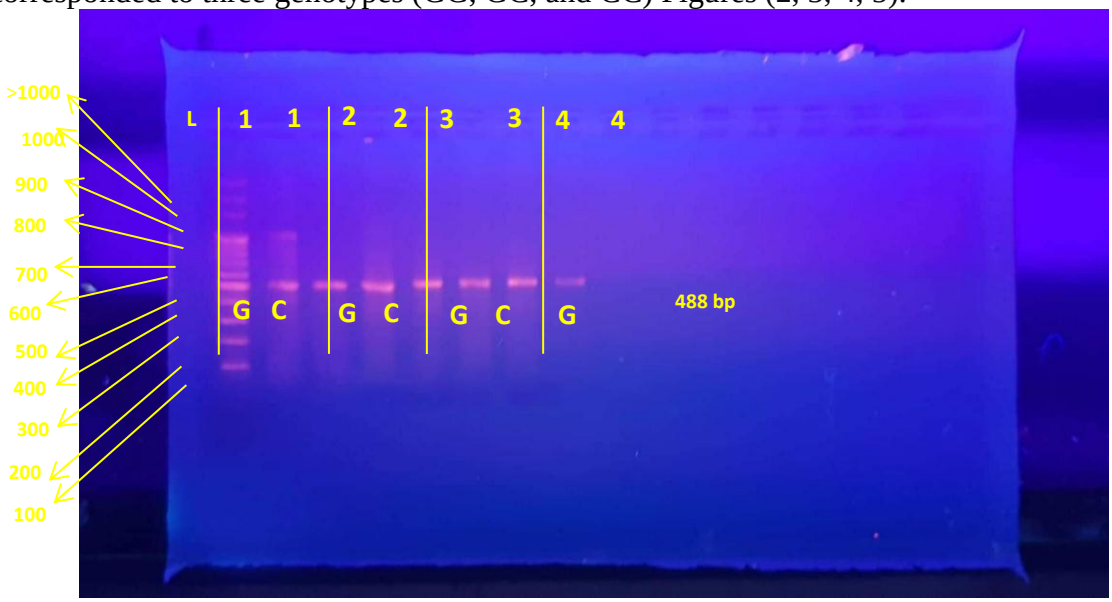

Figure 2: Gel electrophoresis of DNA-PCR amplified products (488 bp) for the SNP rs28416813 G/C on 1.5% agarose (5 V/cm2 for 60 min) showing genotypes for four samples. Sample 1: GC, Sample 2: GC, Sample 3: GC, Sample 4: GG

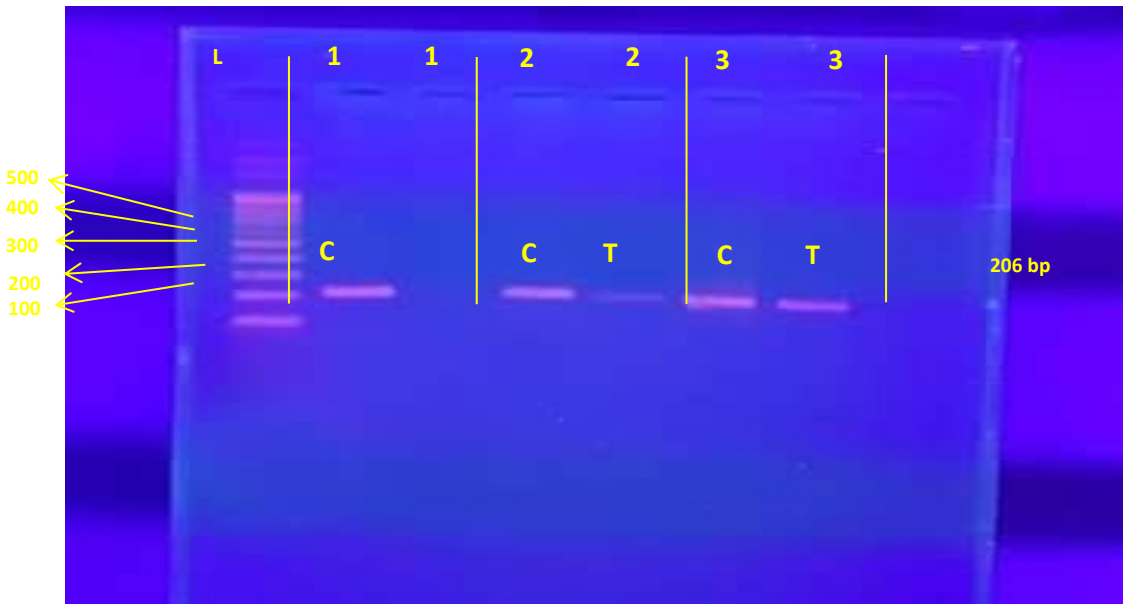

Figure 3: Gel electrophoresis of DNA-PCR amplified products (206 bp) for the SNP rs4803219 C/T on 1.5% agarose (5 V/cm2 for 60 min) showing genotypes for three samples. Sample 1: CC, Sample 2: CT, Sample 3: CT.

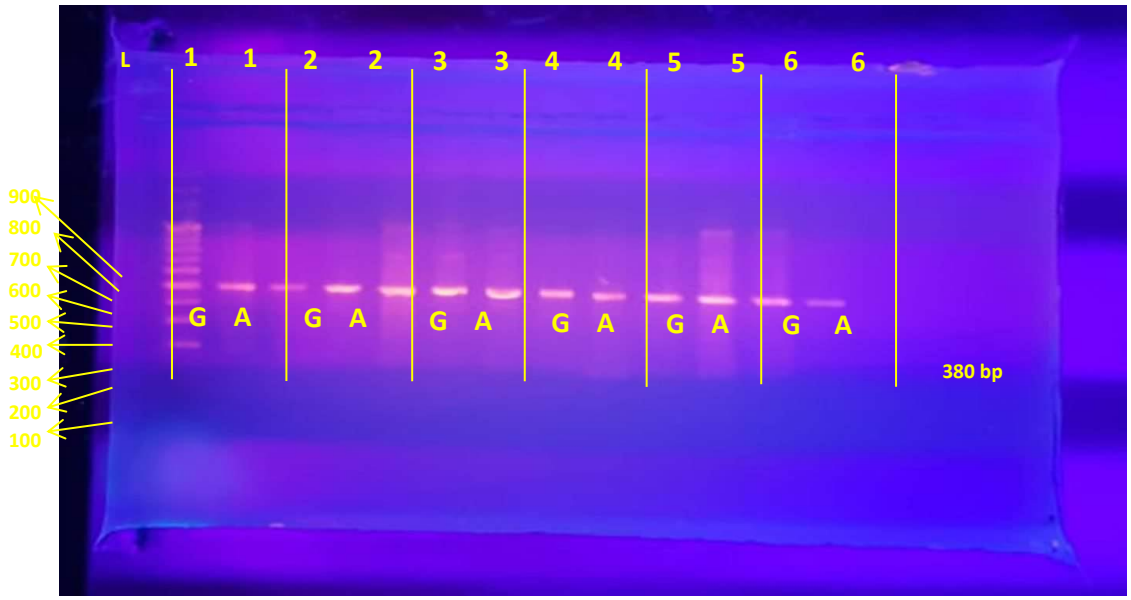

Figure 4: Gel electrophoresis of DNA-PCR amplified products (380 bp) for the SNP rs11881222 G/A on 1.5% agarose (5 V/cm2 for 60 min) showing genotypes for six samples; all of them with GA.

Figure 5: Gel electrophoresis of DNA-PCR amplified products (200 bp) for the SNP rs8103142 C/T on 1.5% agarose (5 V/cm2 for 60 min) showing genotypes for seven samples. Sample 1: CT, Sample 2: CT, Sample 3: CT, Sample 4: CT, Sample 5: CT, Sample 6: CT, Sample 7: CC.

There were no major differences in HCV patients and control groups regarding the frequencies observed and predicted of the genotype. Thus, there was no deviation from the Hardy-Weinberg (H-W) equilibrium (Table 3). The comparison between the patients and control groups showed an important finding, in which IL-28β CC genotype was significantly increased in HCV patients (30.7% vs. 10%; P = 0.036). Such a difference was associated with an odds ratio (OR) value of 3.86. In contrast, the genotypes GG and GC were non-significantly decreased (28.5% vs. 30%; OR=0.91; P = 1.0 and 40.8% vs. 60%; OR=0.48; P = 0.137) respectively. However, in terms of allele frequencies, no significant difference between patients and control groups was observed at p=1.55.

Table 3: Genotyping and allele frequency of rs28416813 between patients and control groups.

Genotypes	Hardy-Weinberg frequency								OR	95% CI	P
	Patients (98)				Control (80)						
	Observed		Expected		Observed		Expected				
	No.	%	No.	%	No.	%	No.	%			
GG	28	28.5	24.0	24.50	24	30.0	28.80	36.0	0.91	(0.37-2.24)	1.0
GC	40	40.8	48.0	48.97	48	60.0	38.40	48.0	0.48	(0.21-1.11)	0.13
CC	30	30.7	26.0	26.53	8	10.0	12.80	16.0	3.86	(1.18-12.59)	**0.03**
Total	98	100.0	98.0	100.0	80.0	100.0	80.00	100.0			
P-HWE	0.2589				0.1138						
Allele frequency											
G	96 (49%)				96 (60%)				0.64	(0.37-1.12)	0.15
C	100 (51%)				64 (40%)				1.56	(0.89-2.73)	0.15

The present study of IL-28β SNP rs4803219 identified two alleles (C and T), which corresponded to three genotypes (CC, CT, and TT). There were no significant differences between the frequencies observed and expected of the genotype in HCV patients and control groups. So, there was no variance from the equilibrium (Table 4).

The CC and CT genotypes were increased in HCV patients, however, the first was significant (42.85% *vs.* 12.5%; OR=5.07; P = $2.4x10^{-3}$) and the other was non-significant (53.0 *vs* 52.5; OR=1.06; p=1.0. In contrast, the genotype TT was significantly decreased (4.15% *vs.* 35.0%; OR=0.08; P = $1.7x10^{-4}$). Concerning the allele frequencies, the allele C was significantly increased (69% *vs*. 39%; OR=3.48; P = $3.4x10^{-5}$) in patients as compared to the control group while allele T was significantly decreased (31.0% *vs.* 61.0%; OR=0.29; P = $3.4x10^{-5}$) (Table 4).

Table 4: Genotyping and allele frequency of rs4803219 between HCV patients and control groups

Genotypes	Hardy-Weinberg frequency								OR	95% CI	P
	Patients (98)				Control (80)						
	Observed		Expected		Observed		Expected				
	No.	%	No.	%	No.	%	No.	%			
CC	42	42.85	47.62	48.59	10	12.5	12.02	15.02	5.07	(1.72-14.92)	**$2.4x10^{-3}$**
CT	52	53.00	40.78	41.62	42	52.5	37.96	47.47	1.06	(0.47-2.42)	1.0
TT	4	4.15	9.60	9.79	28	35.0	30.02	37.52	0.08	(0.02-0.36)	**$1.7x10^{-4}$**
Total	98	100.00	98.00	100.00	80	100.0	80.00	100.00			
P-HWE	0.0637				0.5026						
Allele frequency											
C	136 (69%)				62 (39%)				3.48	(1.95-6.22)	**$3.4x10^{-5}$**
T	60 (31%)				98 (61%)				0.29	(0.16-0.51)	**$3.4x10^{-5}$**

Checking for the equilibrium of Hardy-Weinberg (H-W) in the recent study regarding SNP rs11881222 revealed that HCV patients showed a significant difference in genotyping distribution (P = 0.00039). Such a discrepancy was observed because of variations between the observed and predicted frequencies of GG, GA, and AA genotypes. The homozygous genotype GG, in particular, was observed at a frequency of 2.09 percent, whereas the predicted frequency was 13.79 percent. The heterozygous (GA) genotype was observed at a frequency of 69.69%, whereas its predicted frequency was 45.68%. Nevertheless, no such variations were found in the control group, where the observed and predicted frequencies of the genotypes were in good agreement with H-W equilibrium (Table 5).

In table 5, the findings of IL28β rs4803222 G/A gene polymorphism showed overrepresentation of GA heterozygote genotype frequency and AA homozygote genotype frequency, non-significant and significant respectively, in hepatitis C patients versus control group (69.69% vs. 62.5%; OR=1.4; CI=0.59-3.34; p=0.504) and (28.22% vs. 10%; OR=3.5; CI=1.07-11.49; p=0.038), respectively. On the other hand, the GG genotype was significantly reduced in patients as compared to the control group (2.09% vs. 27.50%; OR=0.05; CI= 0.01-0.43; $P=4.4x10^{-4}$). Regarding the allele levels, allele G was significantly reduced (37% vs. 59%; OR=0.41; CI=0.23-0.72; $P=2.9x10^{-3}$) relative to controls in patients, while allele A was significantly increased (63% vs. 41%; OR=2.45; CI=1.39-4.32; $P=2.9x10^{-3}$)

Table 5: Genotyping and allele frequency of rs11881222 between patients and control groups.

Genotypes	Hardy-Weinberg frequency								OR	95% CI	P
	Patients (98)				Control (80)						
	Observed		Expected		Observed		Expected				
	No.	%	No.	%	No.	%	No.	%			
GG	2	2.09	13.75	13.79	22	27.50	27.62	34.52	0.05	0.01-0.43	**$4.4x10^{-4}$**
GA	68	69.69	44.62	45.68	50	62.50	38.78	48.47	1.40	0.59-3.34	0.504
AA	28	28.22	39.63	40.53	8	10.00	13.60	17.02	3.50	1.07-11.49	**0.038**
Total	98	100.00	98.00	100.00	80	100.00	80.00	100.00			
P-HWE	0.00039				0.06712						
Allele frequency											
G	72 (37%)				94 (59%)				0.41	0.23-0.72	**$2.9x10^{-3}$**
A	124 (63%)				66 (41%)				2.45	1.39-4.32	**$2.9x10^{-3}$**

Examination of the Hardy-Weinberg (H-W) equilibrium of current research regarding SNP rs8103142 revealed that patients with HCV exhibited an important difference in the dissemination of genotyping (P = 0.0000003). Such a disagreement was detected because of the dissimilarities between the observed and predicted occurrences of the CC, CT, and TT genotypes. The homozygous genotype CC, in particular, was observed at the occurrence of 6.12 percent, whereas the expected frequency was 24.51 percent. The heterozygous genotype (CT) was seen at 85.71% while its expected frequency was 48.95%. However, no such disparities were originated in the control group, where the observed and predicted frequencies of the genotypes were in good arrangement with the equilibrium of H-W (Table 6).

The current study of IL28β rs8103142 gene polymorphism displayed overrepresentation of CT heterozygote genotype incidence and TT homozygote genotype rate, high significant and non-significant, respectively, in hepatitis C patients as compared to the control group, (85.71% vs. 52.5%; OR=5.56; p = $5.6x10^{-4}$) and (8.17% vs. 2.5%; OR = 3.39; p = 0.377) respectively. Relatively speaking, the CC genotype was significantly reduced in patient more than in the control group (6.12% vs. 45.0%; OR=0.08; P=$2.6x10^{-5}$). In terms of the proportions of the alleles, allele C was significantly reduced in patients as compared to controls (49% vs. 71%; OR=0.39; P=$2.3x10^{-3}$), while allele T increased significantly (51% vs. 29%; OR=2.55; P=$2.3x10^{-3}$).

The present search is consistent with a previous Egyptian study by Zainab *et al.* [15], who mentioned that the risk frequency of IL-28β rs8103142 CT genotype in HCV-infected subjects was significantly higher than the CT genotype in the control group (61.8% vs. 44.0%; P= 0.00). In contrast, the T allele frequency was substantially higher than the C allele in the healthy control group as compared to the HCV-infected subjects (p < 0.001; OR = 2.05; 95 % CI = 1.27–3.31). Nevertheless, there is a variation between the current study and the Egyptian study, in which the beneficial genotypes of IL-28β rs8103142 TT was significantly higher than the genotypes of CT and CC in controls comparative to HCV-infected subjects (p<0.001). Accordingly, the patients, who are heterozygous to CT genotype, are at higher risk of HCV infection than those carrying the homozygous TT genotype, whereas the homozygous CC genotype is considered a protective factor.

Table 6: Genotyping and allele frequency of rs8103142 between patients and control groups

Genotypes	Hardy-Weinberg frequency								OR	95% CI	P
	Patients (98)				Control (80)						
	Observed		Expected		Observed		Expected				
	No.	%	No.	%	No.	%	No.	%			
CC	6	6.12	24.02	24.51	36	45.00	40.62	50.77	0.08	0.02-0.29	**$2.6x10^{-5}$**
CT	84	85.71	47.97	48.95	42	52.50	32.77	40.97	5.56	2.04-15.11	**$5.6x10^{-4}$**
TT	8	8.17	26.01	26.54	2	2.50	6.61	8.26	3.39	0.37-30.78	0.377
Total	98	100.00	98.00	100.00	80	100.00	80.00	100.00			
P-HWE	0.0000003				0.0750534						
Allele frequency											
C	96 (49%)				114 (71%)				0.39	(0.22-0.70)	**$2.3x10^{-3}$**
T	100 (51%)				46 (29%)				2.55	(1.43-4.55)	**$2.3x10^{-3}$**

The findings of a study done by of Chen *et al.* [16] agree with the recent study in that they revealed that there was an association between IL28β genetic variations and the sustained virological response. The genotypes of CC, CC, AA and CT of rs28416813, rs4803219, rs11881222 and rs8103142, respectively, were more in patient with HCV than the control subjects, therefore, patients, who are carrying these genotypes, are at higher risk of HCV infection than those carrying other genotypes whereas the alleles risk of the previously mentioned SNPs were C, C and A allele except SNP rs8103142, whose risk factor was not recorded.

In another study by Clausen *et al.* [17], it is appeared that the rs11881222 AG genotype (OR, 0.4; 95% CI, 0.2–0.8) and the coding rs8103142 CT genotype (OR, 0.3 ; 95% CI, 0.1–0.7) were related to a reduced clearance rate of hepatitis C virus. These results may support

the current outcomes, in which the genotype CT of SNP rs8103142 is considered a risk factor of Hepatitis C virus infection.

Two-Locus Haplotypes

Two-locus haplotypes and linkage disequilibrium (LD) coefficient (D') were estimated between SNPs of *IL-28β* genes (rs28416813, rs4803219, rs11881222, and rs8103142) in hepatitis C virus infection patients and of the control group. There was a weak LD between the SNPs in the patients and control groups, as suggested by the estimated D' value. The SNPs rs11881222 and rs8103142 were an exception, in which the D' value was 0.731 and 0.999 in HCV patients and control groups, respectively, (strong LD) (figure 6 and 7).

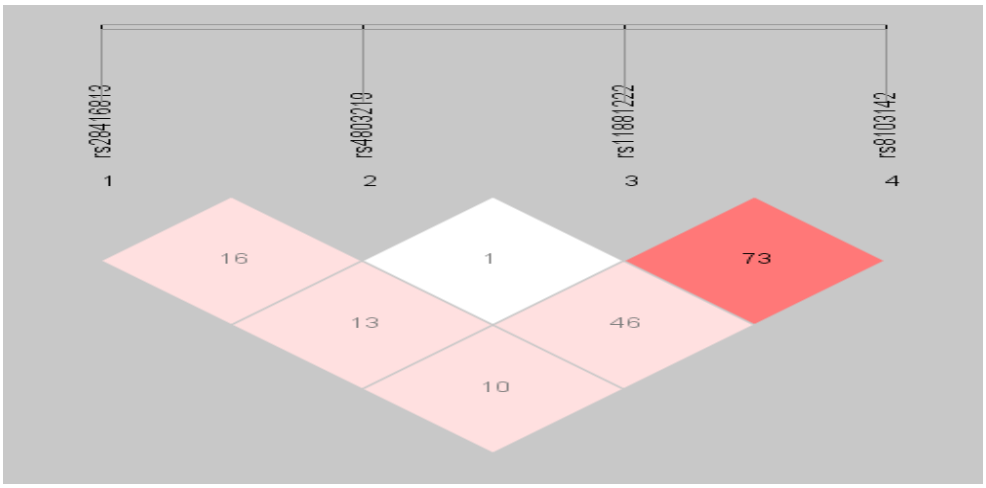

Figure 6: Pairwise analysis depicting linkage disequilibrium coefficient (D′) among SNPs of *IL-28β* (rs28416813, rs4803219, rs11881222, and rs8103142)% in hepatitis C virus infection patients.

	rs4803219		rs11881222	rs8103142
rs28416813	0.169	0.134	0.106	
rs4803219		-	0.020	0.462
rs11881222	-	-	0.731	

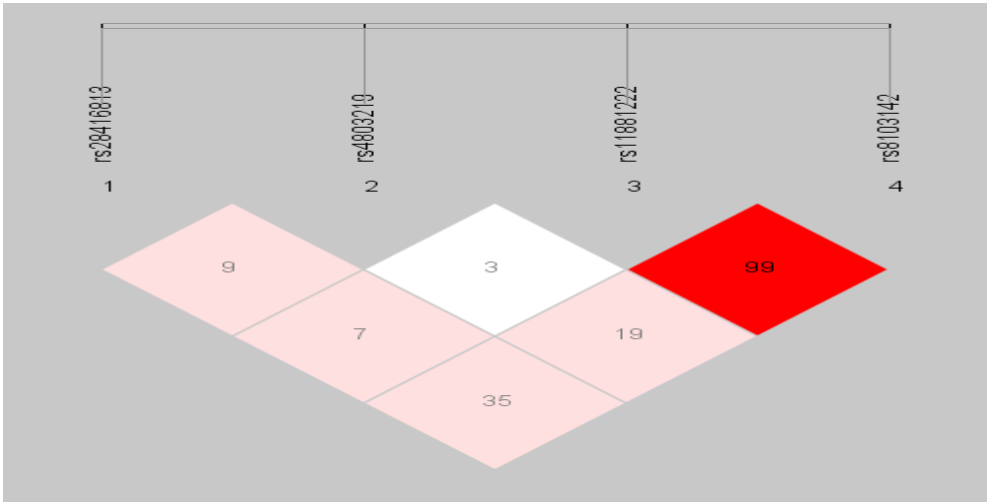

Figure 7: Pairwise analysis depicting linkage disequilibrium coefficient (D′) among SNPs of *IL-28β* (rs28416813, rs4803219, rs11881222, and rs8103142)% in the control group.

	rs4803219	rs11881222	rs8103142
rs28416813	0.091	0.078	0.352
rs4803219	-	0.033	0.196
rs11881222	-	0.999	

The estimated two-locus haplotype frequencies showed some significant variations between the patients and control groups. The haplotypes C-C-A-T of SNPs (rs28416813, rs4803219, rs11881222, and rs8103142) revealed a significantly increased frequency in patients (C-C-A-T: 9.16% *vs*. 0%; $p = 0.0049$) and in G-C-A-T: 19.91% *vs*. 0%; $p < 0.001$). In contrast, (C-C-G-C, C-T-G-T and G-C-G-T) haplotypes showed significantly decreased frequencies in patients ($p = 0.0254$, 0.0003, 0.0128, respectively). However, G-T-A-C and G-T-G-C haplotypes frequency was highly significantly decreased in patients (1.58%, 0.51% *vs*. 25.41, 15.39%; $p = 0.000$ and 0.0001 respectively). The associated ORs were 0.0484 (95%CL: 0.0093-0.2519) and 0.0291 (95%CL:0.0017- 0.4829), respectively, (Table 7). The current data suggested that IL28β genetics may have an effect on the susceptibility to HCV infection in Iraq.

Table 7: Estimation of two-locus haplotype frequencies between SNPs of IL-28β (rs28416813, rs4803219, rs11881222, and rs8103142) genes of hepatitis C virus infection patients and of the control group.

Gene	Haplotype	%		OR (95% CI)	*p*
		Patients (N = 96)	Control (N = 80)		
IL-28β	C-C-A-C	11.78	8.57	1.4646[0.5432,3.9486]	0.4488
	C-C-A-T	9.16	0.0000	-	**0.0049**
	C-C-G-C	0.0000	5.01	-	**0.0254**
	C-C-G-T	9.99	5.97	1.7962 [0.5785,5.5769]	0.3056
	C-T-A-C	5.34	3.04	1.8502 [0.3943,8.6828]	0.4290
	C-T-G-C	13.25	4.90	3.0527 [0.9484,9.8263]	0.0514
	C-T-G-T	0.0000	12.51	-	**0.0003**
	G-C-A-C	9.12	4.22	2.3402 [0.6474,8.4595]	0.1837
	G-C-A-T	19.91	0.0000	-	**0.0000**
	G-C-G-C	7.41	4.70	1.6691 [0.4650,5.9912]	0.4279
	G-C-G-T	1.63	10.27	0.1484 [0.0269,0.8196]	**0.0128**
	G-T-A-C	1.58	25.41	0.0484 [0.0093,0.2519]	**0.0000**
	G-T-A-T	3.62	0.0000	-	0.0818
	G-T-G-C	0.51	15.39	0.0291 [0.0017,0.4829]	**0.0001**
	G-T-G-T	4.21	0.0000	-	0.0600

IL-28β: Interleukin-28 beta; OR: Odds ratio; CI: Confidence interval; p: Two-tailed Fisher's exact probability.

Conclusions

The results explain the important role played by the genotypes or alleles of IL-28β gene SNPs in increasing the risk of developing Hepatitis C virus infection or protection against it.

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