


ORIGINAL ARTICLE

Association of interferon gamma gene polymorphism and susceptibility to hepatitis C virus infection in Egyptian patients: A multicenter, family-based study

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Key words

gene polymorphism, hepatitis C virus susceptibility, interferon gamma, intrafamilial, viral clearance.

Accepted for publication 3 October 2017.

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Declaration of conflict of interest: None.

Financial support: This research was funded by Science & Technology Development Foundation (STDF), Project No. 1784 (TC/2/Health/2009/hep-1.3).

Funding support: This research was funded by Science & Technology Development Foundation (STDF) Project NO.1784 (TC/2/Health/2009/hep-1.3).

Abstract

Background and Aim: Polymorphisms in some genes may influence the persistence of hepatitis C virus (HCV) infection, clinical outcome, HCV replication, and liver damage. This study was conducted to investigate the role of the interferon gamma (IFN- γ) gene at (+874 T/A, -764 G/C, -179 C/A) single-nucleotide polymorphisms (SNPs) and its receptor (IFN- γ R2) at (rs 2786067 A/C) SNP in the susceptibility of Egyptian families to HCV infection with high-resolution techniques.

Methods: In total, 517 Egyptian families, with 2246 subjects, were recruited to this study from the Upper and Lower Egypt governorates and were classified into three groups: 1034 patients with chronic hepatitis C virus, 108 subjects with spontaneous virus clearance (SVC), and 1104 subjects as a healthy control group. All subjects were genotyped for (+874 T/A, rs2430561, -764 G/C, rs2069707, -179 C/A, rs2069709, and rs 27860067, A/C) SNPs of the IFN- γ gene using the allelic discrimination real-time polymerase chain reaction technique and were confirmed using sequence-based typing.

Results: The carriage of T allele of (+874) IFN- γ is a risky allele and was significantly higher in chronic hepatitis C more than other two groups (odds ratio [OR]: 2.6646, $P < 0.0002$). On the other hand, the C allele of (-764, rs2069707) is a protective allele and was higher in SVC than the other two groups (OR: 0.2709, $P < 0.0001$). However, both (-179 C/A, rs 2069709) and (rs 27860067, A/C) SNPs are not polymorphic enough to be studied in the Egyptian population.

Conclusions: HCV infection is associated with the T allele of (+874 rs2430561), while SVC of HCV is associated with the C allele of (-764, rs2069707) of the IFN- γ gene.

Introduction

Hepatitis C virus (HCV) infection is one of the main causes of chronic liver disease worldwide.¹ The prevalence of HCV infection is estimated to be about 3% in the world.² It was reported that around 15% of the Egyptian population has chronic hepatitis C virus. Over 90% of the infections have been reported to be HCV genotype 4.^{3,4}

The virus entry is associated with an interferons (IFNs) release within the cell.⁵ IFNs, especially interferon gamma (IFN- γ), which is a secretory protein mainly produced by T and natural killer cells, counteract the viral replication either by direct inhibition or via activation of the immunoregulatory mechanisms responsible for the control of HCV infection.⁶

Previous sequence analysis revealed several polymorphisms within the IFN- γ gene.^{7,8} These polymorphisms are located in different sites along the IFN- γ gene, including (+874 T/A, -764 C/G, -179 G/T) loci.⁹⁻¹¹ However, the (+874 T/A, rs2430561) single-nucleotide polymorphism (SNP) can influence IFN- γ expression.⁷ Expression of IFN- γ is genetically controlled, and the presence of alleles T and A at the +874 position from the translation start site is related to high and low IFN- γ expression, respectively, which in turn influences the activity of HCV.^{8,9,12}

The SNP (rs2069707 C/G) of the IFN- γ gene is located in the proximal promoter region at position -764, next to the binding motif for HSF1.¹⁰ Some data support the hypothesis that the

SNP variant -764 G is an important genetic marker for treatment response and spontaneous recovery in HCV infection.^{13,14}

Other studies demonstrated that IFN- γ SNPs appear to affect IFN- γ expression, such as rs2069709, which is a G to T transition at position -179 bp in the promoter region.¹¹ Several studies on (-179 G/T, rs2069709) showed that SNP has been implicated in several autoimmune and chronic inflammatory conditions. However, none of them has been shown to be related to HCV infection.¹⁵

Impaired function of IFN- γ receptors might lead to increased production of IFN- γ , as seen in patients with complete IFN- γ receptor deficiencies.^{16,17} The identification of a cluster of IFN- γ R2 variants, which are strongly associated with liver fibrosis progression in CHCV infection, underlines the role of IFN- γ in the development of liver fibrosis that may pave the way for new treatments.¹⁷

Aim of the study

This study was conducted to investigate the association of the IFN- γ gene, and its receptors, polymorphism with HCV infection outcomes (either susceptibility or clearance) in Egyptian families with high-resolution techniques.

Subjects and methods

Study population. This multicenter study was carried out in the Molecular Genetic Unit, Faculty of Medicine, Mansoura University between 2011 and 2016. We included Egyptian HCV patients and their families or close household contacts from different populations, including upper, middle, and lower Egypt. Each family was selected based on the following: at least one positive HCV patient as an index patient, one positive HCV household member and one negative HCV member with no history of any liver complications or disorders.

In total, 517 Egyptian families in this study were recruited from Dakahlia, Cairo, and Assuit governorates. Their families included 2246 subjects: 1034 CHC cases (cases with positive polymerase chain reaction [PCR] HCV >6 months), 1104 control group cases (cases with negative PCR HCV), and 108 spontaneous virus clearance (SVC) group cases, who demonstrated HCV antibody positive but HCV-RNA negative in two successive samples at least 6 months apart. The HCV-positive group in the family was selected using criteria that included HCV positive by PCR RNA for >6 months, adults (over 18 years) of both genders, and any stage of HCV-related liver diseases.

Exclusion criteria of index cases included: patients coinfected with HIV or HBV (HBV core antibodies), patients positive for anti-HCV antibodies, and no detectable HCV-RNA in

the serum. Patients with autoimmune hepatitis, hepatocellular carcinoma (HCC), and metabolic liver diseases were also excluded. Healthy household controls were included in this study using the following inclusion criteria: individuals of both genders and age > 18 years; first- and second-degree consanguinity to the index case; living and sharing usual family activity with the index case; having no serological evidence of HCV, HBV, or HCC; and having no history of liver disease. Each participant was subjected to routine clinical and laboratory investigations by a clinician in addition to PCR HCV RNA analysis to confirm HCV infection, and genetic polymorphism of four snips of the IFN- γ gene ($+874$ T/A, rs2430561, -764 G/C, rs2069707, -179 C/A, rs2069709, and rs27860067, A/C) were performed for all subjects as well.

Written informed consent was obtained from each participant. The study was reviewed and approved by the institution's human research committee.

Laboratory methods

Genomic DNA extraction from peripheral blood.

Genomic DNA extraction from peripheral blood was performed for all subjects using a commercial Qiagen DNA isolation kit (QIAmp DNA Mini kit; Qiagen, Hilden's, Germany) following the manufacturer's instructions. The DNA was assessed using a NanoDrop spectrophotometer (NanoDropTM2000/2000c spectrophotometer, Thermo Scientific, CA, USA) and 2% ethidium bromide-stained agarose gel to confirm their integrity.

IFN- γ ($+874$ T/A, rs2430561) polymorphism.

Polymorphism of IFN- γ ($+874$ T/A, rs2430561) was genotyped using amplification-refractory mutation system (ARMS)-PCR and confirmed using sequence-based typing (SBT) (Table 1).

Genotyping of IFN- γ ($+874$ T/A, rs2430561) polymorphism using ARMS-PCR technique.

The T and A polymorphism sequences were identified using a specific primer synthesized to cover a 24-bp region for each allele. The primers were designed using primer 3 plus (v 4.1; ABI Universal PCR Master Mix, Applied Biosystems, Foster City, CA, USA). ARMS-PCR was performed in a total volume of 20 μ L primer containing 1 μ L of generic primer (100 pmol/ μ L; 5' TCAACAAAGCTGATACTCCA 3'), 1 μ L of specific A primer (100 pmol/ μ L, 5' TTCTTACAACA-CAAAATCAAATCA 3'), or 1 μ L of specific T primer (100 pmol/ μ L, 5' TTCTTACAACACAAAATCAAATCT 3'). In addition, internal control of the growth hormone gene (GH) primers was used with each sample as follows: 1 μ L of forward (10 pmol/ μ L, 5' GCCTTCCCAACCATTCCCTTA 3'), 1 μ L of reverse (10 pmol/ μ L, 5' TCACGGATTTCTGTTGTGTTTC 3'), 4 μ L of dNTP (2 mM), 2.4 μ L of MgCl₂ (25 mM), 3 μ L of buffer (10 \times), 0.5 μ L

Table 1 SNPs selected within the IFN- γ gene and its receptor (IFN- γ R2)

Gene	Cytogenetic region	Marker	Position	Location	SNP (alleles)	Methods of PCR technique
IFN- γ	12q15	rs2069707	-764	Promotor	G/C	TaqMan Allelic discrimination Real Time
		rs2069709	-179	Promotor	C/A	TaqMan Allelic discrimination Real Time
		rs2430561	$+874$	Intron 1	T/A	ARMS and DNA sequencing
IFN- γ R2	21q22.11	rs27860067	—	Exon 3	A/C	TaqMan Allelic discrimination Real Time

ARMS, amplification-refractory mutation system; IFN- γ , interferon gamma; PCR, polymerase chain reaction; SNP, single-nucleotide polymorphism.

of Taq DNA polymerase (5 U/ μ L), 4.1 μ L of sterile MilliQ H₂O, and 2 μ L of genomic DNA.

ARMS-PCR was performed in a thermal cycler 7500 real time PCR system (Applied Biosystems, Foster City, CA, USA) with the following cycles: initial denaturation, 95°C (10 min); 40 cycles of denaturation, 95°C (1 min); annealing, 47°C (30 s); and extension, 72°C (1 min), followed by final extension, 72°C (10 min) and 4°C (hold temperature). ARMS-PCR-amplified products were subjected to 2% agarose gel electrophoresis, stained with ethidium bromide, and visualized on an ultraviolet transilluminator.

Typing of IFN- γ (+874 T/A, rs2430561) polymorphism using SBT technique. The IFN- γ (+874 T/A, rs2430561) polymorphism gene was typed using the SBT technique. Amplification was first determined using the ARMS-PCR method.⁹ The PCR reaction was obtained using a thermal cycler (Applied Biosystems, 2720). A cycle sequence was performed for the purified PCR product in the forward direction by the reverse primer using Big Dye Terminator Cycle Sequencing Kits (version 3.0) according to manufacturer's instructions. Sequenced products were then separated by capillary electrophoresis (Applied Biosystems 310 Genetic Analyzer). Sequences were analyzed with dedicated software.

Genotyping of (rs2069707, rs2069709) SNP of IFN- γ gene and (rs 27860067) SNP of IFN- γ R2 gene using allelic discrimination RT-PCR technique. The oligonucleotide sequences flanking these SNPs were designed as primers for TaqMan allelic discrimination. The allele-specific probes were labeled with a fluorescent dye (VIC and FAM) and used in the allele typing of each DNA sample with real-time (RT) PCR reaction on the apparatus (Applied Biosystems, model 7500) using ready-made fluorescein-amidite-labeled SNP primers and probes (purchased from Applied Biosystems) (Table 1).

For 20 μ L reaction volume, we used the following amounts of reagents: 10.0 μ L of TaqMan Universal Master Mix II (2 \times) + 1.0 μ L SNP Genotyping Assay Mix (20 \times) + 1.0 μ L DNA template + 8.0 μ L RNase free water + 20.0 μ L total volume. This volume is multiplied by the number of replicates in

the run. RT-PCR was performed in a thermal cycler (Applied Biosystems, 2720) with the following cycles: holding step at 95°C for 10 min, then 40 cycles of the denaturation step at 95°C for 15 s and the annealing/extension step at 60°C for 1 min.

Statistical analysis. Data were computed and statistically analyzed using SPSS software program (IBM Corp. Released 2012, Version 21.0. IBM SPSS Statistics for Windows; Armonk, NY, USA). We used chi-square and Fisher's exact tests to compare qualitative variables. Hardy-Weinberg equilibrium was assessed in each group separately using χ^2 tests.

The allele carriage is defined as the number of individuals carrying at least one copy of a specific allele. Allelic frequencies are defined as the number of occurrences of the test allele divided by the total number of alleles in the group. The odds ratio (OR) at a 95% confidence interval (CI) of a specific allele carriage was calculated, compared with no carriage of the target allele, using Med Calc software (Med Calc statistical software version 16.4.3. Med Calc (software bvba, Ostend, Belgium)). The difference was considered significant if $P \leq 0.05$. The Bonferroni-corrected P value (P_c) is an adjustment made to P values when several dependent or independent statistical tests are being performed simultaneously on a single dataset.¹⁸

Results

The clinical and laboratory characteristics of the studied groups (SVC, CHC, and Negative control groups) are shown in Table 2. The distribution of allele frequencies of current (rs2430561 and rs2069707) SNPs of the IFN- γ gene was within the HWE. Heterozygosity and polymorphic information content (PIC) in all study groups was enough to conduct statistical analysis for Egyptians (Table 3). The SNP rs2069707 of the IFN- γ gene and rs 27860067 of the IFN- γ R2 gene were not polymorphic in Egyptians, and all individuals were genotyped as AA and CC, respectively.

IFN- γ (+874 A/T) SNP was genotyped in Egyptian families with HCV using ARMS-PCR and SBT technique as shown in Table 3. The other SNPs were genotyped using TaqMan Allelic Discrimination protocol (Fig. 1). The distribution of the IFN- γ (+874 A/T) polymorphism genotype was 10.81% AA, 51.5%

Table 2 Clinical and laboratory characteristics of the studied groups

	SVC group (108)	HCV-positive group (CHC) (1034)	HCV-negative control group (1104)
Gender: M/F	71/37	620/414	488/616
	Mean (\pm SD)	Mean (\pm SD)	Mean (\pm SD)
Age	43 (10.5)	40.7 (15.3)	32.4 (23.7)
S. bilirubin (mg/dL)	0.85 (0.2)	1.5 (0.8)	0.8 (0.2)
S. albumin (g/dL)	4.3 (0.44)	3.8 (0.6)	4.4 (0.5)
SGOT = AST (IU/L)	22 (5.2)	49 (10)	17 (6)
SGPT = ALT (IU/L)	19 (4.9)	52 (11)	19 (8)
ALP (U/L)	62 (18.2)	77.8 (15.9)	55 (18.9)
AFP (ng/mL)	8.2 (4.2)	25 (16.8)	7 (3.1)
ANA (U)	0.5 (0.18)	0.7 (0.18)	0.6 (0.25)
S. creatinine (mg/dL)	0.9 (0.18)	0.8 (0.35)	0.6 (0.39)

S. bilirubin (N: <1.1 mg/dL); S. albumin (N: 3.9–5.1 g/dL); SGOT, aspartate aminotransferase (N: < 40 IU/L); SGPT, alanine aminotransferase (N: < 45 IU/L); ALP, alkaline phosphatase (N: 37–116 U/L); AFP, alpha fetoprotein (N: < 10 ng/mL); ANA, antinuclear antibodies (N: < 1.1 U); S. creatinine (N: < 1.4 mg/dL).

CHC, chronic hepatitis C virus; SVC, spontaneous virus clearance.

Table 3 Distributions of IFN- γ (rs2430561 and rs2069707) SNPs among the study groups.

SNP	rs 2430561/(+874) (A/T)							rs 2069707/(–764) (G/C)						
	Genotypes			MAF (%)	PIC (%)	HET (%)	Hardy–Weinberg $\times 2/p$	Genotypes			MAF (%)	PIC (%)	HET (%)	Hardy–Weinberg $\times 2/p$ (%)
	AA (%)	AT (%)	TT (%)					GG (%)	GC (%)	CC (%)				
SVC group	51	31	18	34	30	31	7.455/0.0085	56.48	28.72	14.8	30	34	29	1.157/0.543
CHC group	11	51	38	63	36	52	3.786/0.051677	85.66	13.77	0.57	7	13	14	0.0012/0.9718
Control group	35	52	13	39	20	52	3.820/0.050626	86.26	13.04	0.70	7	12	13	0.5118/0.47435

CHC, positive hepatitis C virus; HET, heterozygosity; IFN- γ , interferon gamma; MAF, minor allele frequency; PIC, polymorphic information content; SNP, single-nucleotide polymorphism; SVC, spontaneous viral clearance.



Figure 1 +874 IFN- γ amplification-refractory mutation system-polymerase chain reaction (ARMS-PCR) gel. Part I: Ethidium bromide-stained 2% agarose gel for single-nucleotide polymorphism (SNP) IFN- γ (+874 A/T). PCR-ARMS product: (A) First well is a DNA marker of 100 bp. (B) Second and third wells are blank samples for forward 01 and forward 02 that has deionized water instead of the sample template. (C) Sample (1) has only two DNA bands for one well, one of them at 426 bp of human growth hormone (HGH) internal control and the other band at 261 bp of +874 A allele; however, the well of the T allele did not work, so these samples are negatives for the +874 T allele. (D) Sample (2) has one DNA band of each well at 426 bp of (HGH) internal control and only one band at 261 bp of +874 A allele, with the absence of T allele band, so these samples are negatives for the +874 T allele. (E) Samples (3,7,8) have two bands for each well, one of which is of 261 bp, indicating the presence of the +874 A allele or +874 T allele, so these samples are positives for A and T alleles. The second band is the internal control (HGH), which is of 426 bp. (F) Samples (4,5,6) have one DNA band of each well at 426 bp of (HGH) internal control and only one band at 261 bp of +874 T allele with the absence of A allele band, so these samples are negatives for the +874 A allele. Part II: IFN- γ (+874 A/T) SNP using sequence-based typing technique. Lane (a): Representative sequence chromatographs of IFN- γ (+874T) intron 1 of homozygote sample TT. Lane (b): Representative sequence chromatographs of IFN- γ (+874A) intron 1 of homozygote sample AA. Lane (c): Representative sequence chromatographs of IFN- γ (+874T/A) intron 1 of heterozygote sample TA. IFN- γ , interferon gamma.

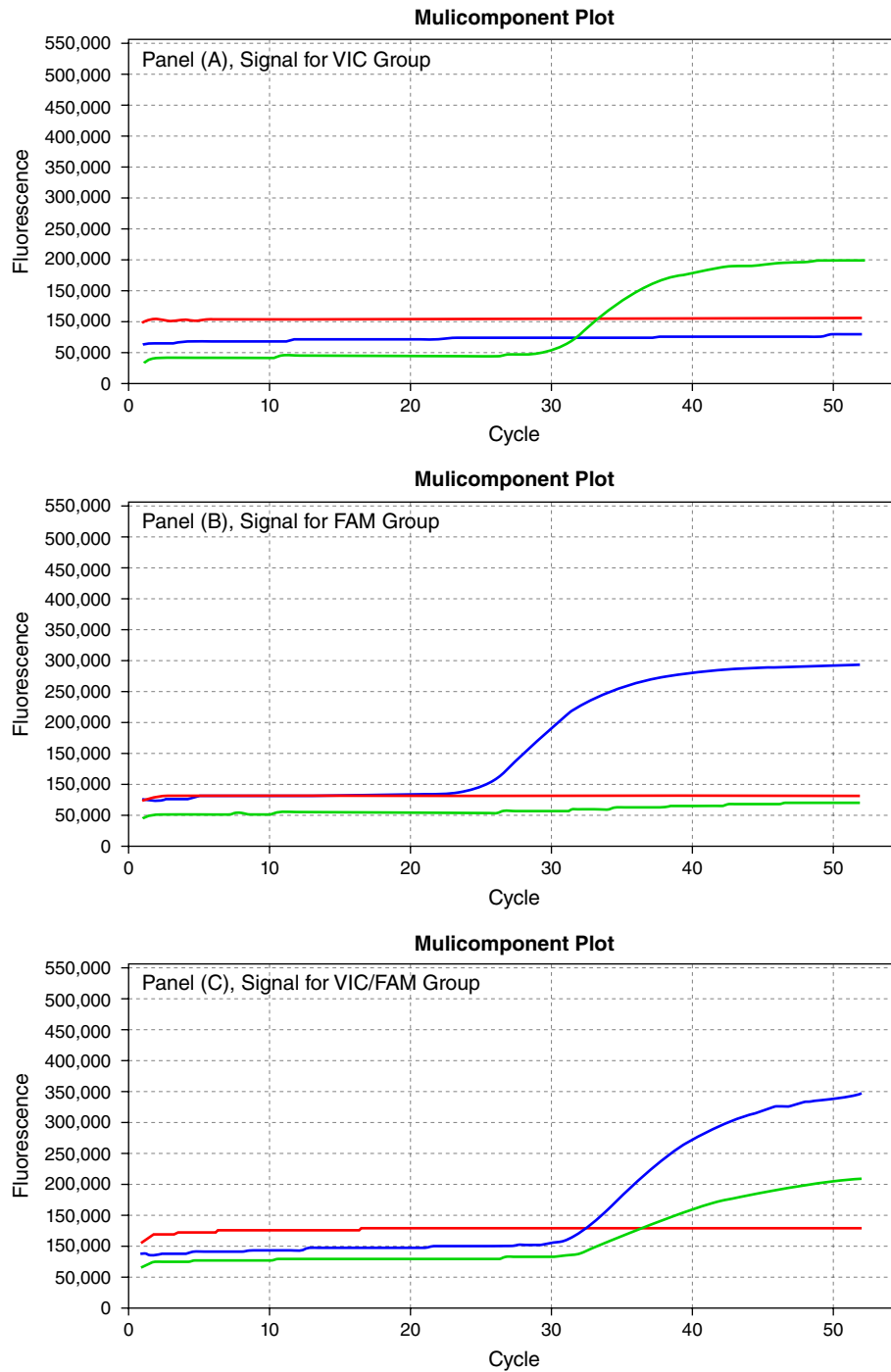


Figure 2 Real-time polymerase chain reaction charts for different genotypes of (-764 G/C) single nucleotide polymorphism of the interferon gamma gene. VIC, ROX and FAM are immunochemistry dyes. (a) Homozygote (GG): FAM; ROX; VIC. (b) Homozygote (CC): FAM; ROX; VIC. (c) Heterozygote (GC): FAM; ROX; VIC.

AT, and 37.77% TT in HCV-infected patients, whereas in HCV-negative patients, the distribution was 34.8% AA, 51.9% AT, and 13.3% TT. In the SVC group, the distribution was 50.9% AA, 30.6% AT, and 18.5% TT (Table 3).

IFN- γ genotyping for IFN- γ (-764 G/C, rs2069707) SNP was performed for common alleles using allelic discrimination

with a RT-PCR technique, as shown in Figure 2. The distribution of the IFN- γ (-764 G/C) polymorphism genotype was 56% GG, 15% CC, and 29% GC in SVC group, whereas in HCV-infected patients, the distribution was 86% GG, 0.6% CC, and 14% GC. In HCV-negative patients, the distribution was 86% GG, 0.7% CC, and 13% GC (Table 3).

Table 4 Association of minor allele of rs2430561 (T) and rs2069707 (C) of IFN- γ polymorphism among the study groups

	rs 2430561 (+874 T)		rs 2069707 (-764 C)	
	OR (95% CI)	<i>P</i> (<i>P_c</i>)	OR (95% CI)	<i>P</i> (<i>P_c</i>)
SVC <i>versus</i> control	0.6741 (0.3914–1.1611)	0.155 (NA)	0.2709 (0.1798–0.4083)	0.0001 (0.0001)
SVC <i>versus</i> CHC	2.6646 (1.5888–4.4688)	0.0002 (0.0006)	0.2709 (0.18844–0.4321)	0.0001 (0.0001)
CHC <i>versus</i> control	9.7905 (7.3818–12.9853)	0.0001 (0.0003)	0.95980 (0.07565–1.4349)	0.95980 (NA)

CHC, positive hepatitis C virus; CI, confidence interval; IFN- γ , interferon gamma; NA, not applicable; OR, odds ratio; *P*, significant level; *P_c*, corrected *P* value; SVC, spontaneous viral clearance.

Comparing the allele carriage of at least one copy of minor allele of each SNP revealed that the carriage of allele T IFN- γ +874 T/A, rs2430561 was associated with increased risk in HCV patients compared to that of noninfected exposed persons (OR = 9.7905, 95% CI 7.3818–12.9853, *P* < 0.0001). The T allele was also associated with HCV-positive patients compared with individuals with SVC (OR = 2.6646, 95% CI = 1.5888 to 4.4688, *P* = 0.0002) (Table 4). There was no significant difference between the carriage of T allele in SVC and HCV-negative patients (OR = 0.6741, 95% CI = 0.3914–1.1611, *P* = 0.1551), suggesting that the A allele may have a role in protection against HCV infection, as shown in Table 4.

Analysis of the frequency of IFN- γ -764 GC, rs 2069707 alleles revealed that the C allele was a protective allele, and a highly statistical significant difference (*P* < 0.0001) was found between the SVC and HCV-positive groups (OR = 0.2709, 95% CI = 0.18844–0.4321) and between the SVC and HCV-negative groups (OR = .27090, 95% CI = .17980 –0.4083). However, on comparing the HCV-positive and HCV-negative groups, there was no statistical significant difference (*P* = 0.9598), as shown in Table 4.

Discussion

IFN- γ is a key regulatory cytokine that plays a pivotal role in the defense mechanisms against viral infection in addition to its fibrogenic activity.^{19,20}

Because pro-/anti-inflammatory cytokines play a key role in the development of liver injury, genomic scanning for SNPs in the genes of several important inflammatory mediators needs to be further investigated, which could help to identify patients at markedly increased risks of live virus disease progression and could guide the design of individualized treatment strategies for hepatitis C infection.^{10,12,21,22}

However, the pattern of association between the IFN- γ +874 T/A gene polymorphism and viral hepatitis is inconclusive.²³ Based on this polymorphism, three genotypes are possible: T/T, T/A, and A/A.²⁴ T-to-A polymorphic sequence at position +874 in the IFN- γ gene (+874 IFN- γ) might be associated with disease susceptibilities.²⁵ Our study was conducted to determine allele frequencies in the IFN- γ gene at position +874 among Egyptian patients and to evaluate the association of IFN- γ , (rs2430561) gene polymorphisms with HCV infection outcomes in the Egyptian population.

Our results found that the T allele of IFN- γ +874 rs2430561 is significantly higher in CHCV patients than the SVC and control patients; therefore, they are associated with increasing risk of HCV infection. While the A allele had a higher

frequency in HCV-negative and SVC groups, they were associated with a decreasing risk of HCV infection.

Our results support some studies' results which found that the number of TT genotype individuals was significantly elevated when compared to TA genotype subjects.^{21,26,27} Bouzgarrou *et al.* conducted a study on Caucasian individuals and found a significantly higher rate of TT and TA genotypes in patients with cirrhosis and HCC, respectively.²⁸

A study conducted on patients from Southern Iran with genotypes 1, 2, and 3 demonstrated no association between IFN- γ +874 polymorphisms and HCV infection outcomes. However, clearance was associated only with haplotype (A allele at +874 loci and G allele at +2109 loci),²⁹ whereas liver cirrhosis was reported to be associated with the T allele at position +874 of the IFN- γ gene in HCV-infected Taiwanese patients.³⁰

In contrast to our results, a study conducted on Caucasian patients who underwent liver transplantation for end-stage liver disease due to HCV infection revealed that the IFN- γ +874 polymorphism genotypes are not related to liver fibrosis progression in recurrent hepatitis C patients.²⁰

Another study on Asian and Caucasian populations discovered that the T allele is a protective gene in liver disease and is more powerful than the A allele, where the +874 AA genotype is associated with a 1.350-fold increased risk of hepatitis virus-related disease, especially in the Asian population; the IFN- γ +874 TT genotype increases the level of IFN- γ production; and the AA and TA genotypes result in a decrease in IFN- γ production.²³ On the other hand, it was reported that the IFN- γ +874 AA genotype was associated with an increased risk of mild and/or moderate/severe chronic hepatitis and cirrhosis, but the +874 TA genotype was associated with reduced risk, although the IFN- γ +874 T/A alleles did not differ between any of the groups.³¹ When a comparison between populations from different ethnic backgrounds was made, it was found that the frequency of the IFN- γ genotype A/A was associated with low expression in CHC patients and was significantly higher in African Americans than in Caucasian or Cuban Americans.³⁰ In Irish individuals, there was no significant difference in the frequency of genotypes associated with polymorphisms of the IFN- γ gene between individuals with viral clearance or those with persistent HCV infection and Irish women.²⁴

IFN- γ +874 T/A polymorphism may affect the development of HCV infection through different mechanisms. First, the IFN- γ +874 T/A genotype TT, which produces a high level of IFN- γ , aids the host's antiviral defense system. In contrast, the AA and TA genotypes result in low IFN- γ production, potentially increasing the risk of hepatitis virus infection.⁹ Second, as described earlier, IFN- γ binds to a specific cell-surface receptor

(IFN- γ R), which plays a significant role in multiple types of cancers and stimulates cell-signaling pathways (JAK-STAT). The IFN- γ +874 T/A polymorphism leads to the dysfunction of IFN- γ R, potentially increasing the risk of liver diseases.²² In addition, the DNA sequence containing the +874 T allele is the preferential binding site for the NF- κ B transcription factor.¹² If the NF- κ B pathway is affected, it may lead to oxidative damage, which can also increase the risk of cancer, liver cirrhosis, and HCC.⁹ As this transcription factor induces IFN- γ expression, the +874 T allele is associated with high IFN- γ expression in opposition to low expression of the A allele.¹²

The second chosen SNP on the first gene of IFN type II was (-764 G/C, rs2069707) of the IFN- γ gene. The regulation of IFN- γ gene transcription involves a large number of transcription factors and multiple regions of the gene,³² including an NF- κ B binding motif³³ next to the -764 C/G polymorphism region.³⁴ The SNP (rs2069707 C/G) of the IFN- γ gene is located in the proximal promoter region at position -764 next to the binding motif for HSF1.

Comparing the distribution of (-764 G/C, rs2069707) alleles between the three groups revealed that the carriage of C allele was significantly higher in SVC than the CHC and control groups, and this was in agreement with the study by Azam *et al.*, who found that there was a strong association between the IFN- γ genotype rs2069707GG and the spontaneous clearance of HCV.¹⁴

Others stated that the -764 G allele was associated with a higher level of IFN- γ than the -764 C allele, and no mutation was detected in the -764 position.³⁵

Another study reported that the association of the -764 G alleles with spontaneous recovery was not as strong as the association with treatment response.¹⁰

The third chosen SNP on the first gene among IFN type II was (-179 G/T, rs2069709) of the IFN- γ gene, which is not polymorphic in the Egyptian population, and all genotyped subjects were CC.

Several studies on (-179 G/T, rs2069709) SNP has been implicated in several autoimmune and chronic inflammatory conditions. However, none of them has been shown to be related to HCV infection.¹⁵

The last IFN- γ SNP was its receptor (rs 27860067, A/C) of the IFN- γ R2 gene; IFN- γ R2 is a protein-coding gene that has been located on the human chromosome 21q22.11. IFN- γ R2-deficient patients present, according to the cellular responses to IFN- γ , either complete (undetectable response to IFN- γ) or partial (residual response to IFN- γ) IFN- γ R2 deficiency.³⁶

This SNP was not detected in the blood of any participant recruited to our study. No minor A allele was detected in any of the analyzed samples as all the genotyped subjects were CC, and this means that this marker is not polymorphic in Egyptian populations. Further studies are still required to confirm these results.

Sample sizes, population admixture, differences in ethnic backgrounds, differences in treatment protocols, and different criteria for selection of target population may all contribute to these conflicting results and discrepancies. In addition, interactions between factors such as age, gender, ethnic background, and geography may lead to various effects of cytokine gene polymorphisms in different population samples.³⁶

Our study included a substantial number of cases and controls pooled from different areas, which greatly increased the statistical power compared with other studies, which provides explanations for the inconsistencies observed in previous studies.

Acknowledgment

The authors thank the Medical Biochemistry Department and Medical Experimental Research Centre (MERC), Mansoura Faculty of Medicine, for their kind help and support in the completion of routine laboratory work.

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