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Original Article

Epstein–Barr virus and Interleukin-28B polymorphism in the prediction of response to interferon therapy in hepatitis C patients

Abdel-Rahman N. Zekri [a],*, Dina Abdullah [a], Ahmed Osman [b], Mahmoud N. El-Rouby [a], Naglaa Zayed [c], Gamal Esmat [c], Wafaa Elakel [c], Hanan Abdel Hafez [c]

[a] Virology and Immunology Unit, Cancer Biology Department, National Cancer Institute, Cairo University, Cairo, Egypt
[b] Biochemistry Department, Faculty of Science, Ainshams University, Cairo, Egypt
[c] Endemic Medicine and Hepatology Department, Faculty of Medicine, Cairo University, Cairo, Egypt

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ABSTRACT

Background and study aims: In chronic hepatitis C virus (HCV), viral and host factors are known to be predictors for anti-viral therapy. IL-28B genotype strongly influences treatment outcome, while Epstein–Barr virus (EBV) co-infection could accelerate the course of chronic HCV infection. This study was conducted to assess whether EBV co-infection adds to the predictive value of IL-28B.

Patients and methods: A total of 105 patients with chronic HCV were classified according to their response to treatment into two groups: 38 sustained virological responders (SVRs) and 67 nonresponders (NRs). Collected sera at baseline and follow-up (FUP) were used for assessing EBV antibodies by enzyme-linked immunosorbent assay (ELISA) and the expression of EBV genes (BNLF-1, BZLF-1, and EBER-2) by polymerase chain reaction (PCR). Collected peripheral blood was used for detecting IL-28B rs.12979860 single-nucleotide polymorphism.

Results: Regarding IL-28B genotype frequencies, a significant difference ($p = 0.003$) was observed between SVRs (C/C = 51.4%, C/T = 48.6%, T/T = 0%) and NRs (C/C = 25%, C/T = 55%, T/T = 20%). On assessing EBV infection at baseline and FUP, it was found that 61% and 55% were positive, respectively, with no significant difference between SVRs and NRs. As for anti-viral capsid antigen (VCA) antibodies, the NRs had significantly higher baseline anti-VCA immunoglobulin M (IgM) levels than SVRs ($p = 0.01$). While FUP anti-Epstein–Barr nuclear antigen-1 (EBNA-1) IgG reported a significant decline within SVR patients ($p = 0.02$), neither baseline nor FUP anti-VCA IgG levels showed a statistically significant viral response. Finally, on comparing EBV markers with CC versus CT and TT genotypes, it was found that FUP anti-VCA IgG levels were significantly increased in CC genotype ($p = 0.003$).

Conclusion: Interleukin-28B polymorphism could be a possible predictor of response to pegylated interferon/ribavirin therapy (PEG-IFN/RBV). Furthermore, co-infection with EBV did not affect the response to IFN-based therapy in HCV-infected patients.

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Introduction

Hepatitis C virus (HCV) is a major factor of liver disease and one of the most important health issues worldwide [1]. The prevalence of HCV in Egypt is over 14.7% of adult population, with Egypt having the highest prevalence of HCV in the world. Consequently, Egyptians also have a high frequency of hepatocellular carcinoma (HCC) [2].

HCV persistence is evident in approximately two-thirds of the infected subjects [3]. The past two decades has seen interferon (IFN) as the cornerstone of chronic HCV treatment. Pegylated-IFN-α (PEG-IFN) and ribavirin (RBV) have resulted in sustained virological response (SVR) of up to 40–50% for genotypes 1 and 4 and up to 70–80% in genotypes 2 and 3 [4].

Many viral and host factors could affect treatment response; observed variation in response to IFN-based therapy for chronic HCV might be related to a combination of them [5]. In addition,

there are several immunological factors involved in determining the disease outcome in HCV-infected patients including the adaptive immune response and the innate immune system [6]. A series of studies on single-nucleotide polymorphisms (SNPs) in the *IL-28B* gene region, which encodes IFN-λ3, were conducted to assist in understanding the contribution of the innate immune system to the disease outcome in HCV-infected patients. In addition, SNPs predict spontaneous and type-1 IFN-induced clearance of HCV infections [7]. One of these SNPs, the rs12979860 C/T SNP, resides at 3 kb upstream of the *IL-28B* gene, and variations at this position are associated with approximately two- to threefold differences in spontaneous clearance and response to treatment of HCV genotypes 1 and 4. The C/C genotype is associated with a higher rate of SVR, while it is lower in the T/T genotype [8].

In addition, co-infection with other pathogens may be an interfering factor against host genotype-based prediction [9]. For example, co-infection with human immunodeficiency virus (HIV) can modify the outcomes of HBV and HCV infection with a higher likelihood of chronic liver disease and a greater risk of rapid evolution to liver fibrosis [10].

EBV is a member of the herpes virus family responsible for infectious mononucleosis (IM) [11]. The virus infects >90% of the adult population around the world [12]. EBV infects human B lymphocytes, resulting in latent infection, in which the entire EBV genome is retained as an episome, and restricted numbers of EBV genes are expressed [13]. Latent cells are normally removed by cytotoxic T lymphocytes in immunocompetent patients, whereas the transformed cells can proliferate and cause various lymphoproliferative disorders in immunosuppressed patients [14].

Previous studies reported contradictory results concerning the effect of EBV coinfection in chronic HCV patients. The results of Medina et al. [15], suggested that viral co-infection with EBV could accelerate the course of chronic HCV infection, might lead to a more severe liver damage histologically, and might facilitate disease progression to fibrosis, cirrhosis, and HCC. However, Challine et al. [16], showed that markers of EBV infection did not influence HCV replication and anti-HCV antibodies or markers of HCV replication did not influence markers of EBV infection. Therefore, this study was conducted to explore whether co-infection with EBV could significantly affect the treatment response to IFN in patients with chronic HCV infection and therefore could be considered as a significant predictor to anti-viral therapy in association with Interleukin-28B polymorphism.

Patients and methods

In this study 105 adult Egyptian patients of both genders with chronic HCV infection (genotype 4) were recruited. They were diagnosed by anti-HCV antibodies and HCV–RNA in addition to the histological evidence of chronic HCV infection. Patients with positive HBsAg and other causes of liver disease were excluded. Between 2007 and 2010, all patients had been treated with Peg-IFN-α2a or -2b plus weight-based RBV at Cairo-Fatemic Hospital, Ministry of Health and Population (MOHP) Cairo, Egypt. The study was approved by the institution ethics committee, and an informed consent was obtained from all subjects. All data (demographic, laboratory, and histopathological) in addition to viral response were obtained from the patients' medical records. A total of 38 patients achieved a SVR, and the remaining 67 patients were NRs who failed to achieve an SVR.

Serum samples were collected before the start and after 12 weeks of therapy. Collected sera were used for detection of EBV antibodies and were subjected to purification of viral nucleic acid for molecular detection of EBV, as well as genomic DNA was purified from whole blood samples for IL-28B SNP analysis.

EBV detection

Serological testing EBV immunoglobulin G, M, and EBNA-1 IgG

Anti-VCA IgG, anti-VCA IgM, and EBNA-1 IgG antibodies were detected with commercial enzyme immunoassays according to the manufacturer's instructions (ETI-VCA-G™, ETI-EBV-M-reverse™, and ETI-EBNAG™, Techno Genetics, Italy). Samples were considered positive for EBV if the titre of anti-VCA IgM was >10 AU/ML [17].

Molecular detection of EBV
Extraction of viral DNA. According to the manufacturer's instructions, viral DNA was purified from the serum using QIAamp®MinElute®Virus Spin extraction kit (Qiagen, Germany).

Amplification of EBV DNA. DNA samples (200 ng) were subjected to polymerase chain reaction (PCR) using Taq DNA polymerase (HotStart-IT® Taq DNA Polymerase, Affymetrix Inc., USA) for EBER-2 (5′PRIME, Stratagene Inc., USA) and forBNLF1 and BZLF1, specific oligonucleotides listed in Table 1, and a DNA Thermocycler (biometra T Gradient, Germany). The expected products were 108 bp for EBER-2, 994 bp for BZLF1, and 337 or 307 bp according to BNLF1 polymorphism as previously described [18]. The standard cycle procedure was a 5-min denaturation at 95 °C, then 40 cycles of 1-min denaturation at 95 °C, 1 min of annealing at 57 °C for BZLF1, 56 °C for BNLF1, 55 °C for EBER-2, and a 2-min extension at 72 °C. Cycling was followed by a 10-min extension at 72 °C. BZLF1–PCR product at 1 μL was taken for a second round of PCR with the use of internal primers (Table 1). The PCR products were analysed by electrophoresis through 2% agarose gel. According to Wen et al. [13], and Hafez et al. [19], samples were considered positive for EBV if there was an amplification of two/three genes.

Detection of IL-28B rs12979860 polymorphism

According to the manufacturer's instructions, genomic DNA was purified from whole-blood samples using QIAamp (Promega, USA). Genotyping for the IL-28B rs12979860 C/T polymorphism was performed by a PCR-based restriction fragment length polymorphism assay as previously described (see Ref. [20]). A 242-base-pair product was obtained using the forward primer 5′-GCTTATCGCA TACGGCTAGG-3′, and the reverse primer 5′AGGCTCAGGGTCAATC ACAG-3′ in a total volume of 20 μL containing: 10 ng of genomic DNA, 10 mM of Tris–HCl (pH 8.3), 50 mM of KCl, 0.01% Tween-20, 0.2 mM of deoxyribonucleotides, 24 pmol of each primer, 2.0 mM of MgCl₂, and 0.5 U of Hot-Start Taq DNA polymerase. The PCR cycles were as follows: 40 cycles of denaturation at 95 °C for 30 s, annealing at 62 °C for 30 s, and elongation at 72 °C for 30 s using a Techne TC-412 thermal cycler. Ten microlitres of the amplicons were digested with 1 U of the BstU-I restriction endonuclease at 60 °C overnight. The digested fragments were 135, 82, and 25 bp for the C-allele and 160 and 82 bp for the T-

Table 1
Primers used in polymerase chain reaction amplifications for EBV genes.

Primer name		Primer sequence
BZLF1	ZES2 (first PCR)	AGGGGAGATGTTAGACAGGT
	ZAS2 (first PCR)	AGTATGCCAGGAGTAGAACA
	ZES (second PCR)	GCCACCTTTGCTATCTTTGC
	ZEAS (second PCR)	AGGCGTGGTTTCAATAACGG
BNLF1	LMP2CS	CTAGCGACTCTGCTGGAAAT
	LMP2CAS	GAGTGTGTGCCAGTTAAGGT
EBER-2	EBER-2S	CCCTAGTGGTTTCGGACACA
	EBER-2AS1	ACTTGCAAATGCTCTAGGCG

Table 2
Baseline demographic and laboratory findings of chronic HCV patients in relation to IFN response.

	Treatment response		*p* value
	SVR $N = 38$	NR $N = 67$	
Gender n (%)			
Female	7 (18.4%)	23 (34.3%)	0.08
Male	31 (81.6%)	44 (65.7%)	
Peg-IFN n (%)			
Peg-IFNα2a	18 (47.4%)	36 (53.7%)	0.53
Peg-IFNα2b	20 (52.6%)	31 (46.3%)	
Age (years)	43.44 ± 8.450	42.89 ± 8.902	0.76
BMI (kg/m^2)	28.208 ± 3.6496	29.217 ± 4.5428	0.25
Glucose (70–120 mg/dl)	98.30 ± 26.76	98.51 ± 29.69	0.97
Creatinine (0.5–1.5 mg/dl)	0.86 ± 0.17	0.88 ± 0.21	0.60
Albumin (3.5–5.5 g/dl)	4.23 ± 0.50	4.07 ± 0.47	0.11
ALP (30–120 U/L)	76.04 ± 52.39	98.82 ± 68.40	0.08
Total bilirubin (0.5–1.5 mg/dl)	0.78 ± 0.28	0.79 ± 0.27	0.89
Indirect bilirubin (0–0.2 mg/dl)	0.58 ± 0.26	0.56 ± 0.21	0.65
WBC (4–11 × 10^3/mm^3)	6.37 ± 1.67	6.97 ± 1.99	0.12
ANC × 10^3/mm^3	3.30 ± 1.14	3.62 ± 1.55	0.28
Haemoglobin (12–18 g/dl)	13.98 ± 1.32	13.93 ± 1.39	0.86
PLT count (150–450 × 10^3/mm^3)	211.71 ± 57.48	216.48 ± 64.07	0.71
TSH (0.2–6 mIU/L)	1.64 ± 0.99	1.66 ± 1.08	0.90
AST (0–40 IU/L)	59.67 ± 51.42	57.28 ± 32.22	0.78
ALT (0–40 IU/L)	78.18 ± 64.67	63.22 ± 44.29	0.17
AFP[**] (up to 10 IU/ml)	3.32/4.27	6.39/7.30	0.01[*]
HCV RNA log IU/ml	4.74 ± 1.30	4.52 ± 1.47	0.44

AFP: α-fetoprotein; ALP: alkaline phosphatase; ALT: alanine aminotransferase; AST: aspartate aminotransferase; BMI: body mass index; HCV: hepatitis C virus; IFN: interferon; PLT: platelet count; TSH: Thyroid-stimulating hormone; SVR: sustained virological responder; NR: nonresponder.
All values are represented by mean ± SD.
 [*] *p*-Value < 0.05 was considered statistically significant.
 [**] Values are represented by median/IQR.

Table 3
EBV markers among chronic HCV patients at baseline and FUP in relation to IFN response.

	SVR ($N = 38$)	NR ($N = 67$)	*p*-Value
At baseline			
Positive EBV[**]	21 (55%)	42 (63%)	0.4
Anti-VCA IgM	0.89 (3.36)	3.02 (10.01)	0.01[*]
Anti-VCA IgG	117.99 (102.35)	122.6 (104.92)	0.97
EBNA-1 IgG	102.40 (106.7)	108.20 (73.2)	0.83
At FUP (week 12)			
Positive EBV[**]	20 (53%)	38 (57%)	0.69
Anti-VCA IgM	4.79 (9.07)	6.58 (9.12)	0.53
Anti-VCA IgG	123.07 (168.94)	98.80 (90.74)	0.13
EBNA-1 IgG	34.00 (107.95)	114.00 (113.10)	0.02[*]

EBV, Epstein–Barr virus; EBNA, Epstein–Barr nuclear antigen.
Cutoff value for the detection of anti-VCA IgM, anti-VCA IgG, and EBNA-1 IgG was 10 AU/ML.
All values are represented by median/IQR.
 [*] *p*-Value < 0.05 was considered statistically significant.
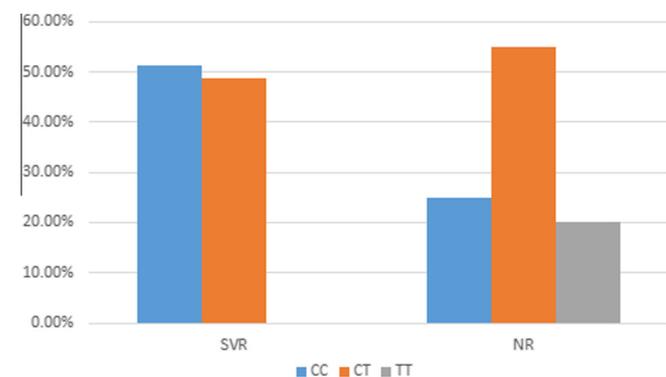 [**] Values are represented by N (%).

Table 4
Interleukin-28 genotype polymorphism among chronic HCV patients in relation to IFN response.

IL-28 Polymorphism		Treatment response		*p*[*] value
		SVR ($N = 38$)	NR ($N = 67$)	
Genotype	CC ($N = 33$)	18 (51.4%)	15 (25%)	
	CT ($N = 50$)	17 (48.6%)	33 (55%)	0.003[*]
	TT ($N = 12$)	0 (0%)	12 (20%)	
Allele	C ($N = 112$)	53 (45.7%)	63 (54.3%)	<0.01[*]
	T ($N = 74$)	17 (23%)	57 (77%)	

10 samples are missed in the IL-28B polymorphism detection.
 [*] *p* < 0.01 was considered highly statistically significant.

allele variants. The fragments were resolved by electrophoresis in an ethidium-bromide-stained 3.5% agarose gel.

Quantitative variables were presented by mean and standard deviation (SD) for normally distributed data, and analysed by Student's *t*-test. They were presented by median and Inter-quartile range (IQR) if not normally distributed and analysed by the Mann–Whitney *U* test. Qualitative variables were presented by number and percent and compared by the chi-square test. Univariate regression analysis was performed, in which failure of response to IFN treatment is the dependent factor. In all tests, $p < 0.05$ was considered significant.

Results

Baseline demographic and laboratory data in relation to patients' outcome are shown in Table 2. Low median (3.32 ng/mL) baseline serum α-fetoprotein (AFP) levels were significantly related to the achievement of SVR, while higher median (6.39 ng/mL) baseline serum AFP levels were associated with NR HCV patients ($p = 0.01$).

Fig. 1. IL-28 rs12979860 C/T genotype distribution among SVR and NR patients. SVR, sustained virological responder; NR, nonresponder.

Table 5
Baseline different EBV genes in relation to IFN response.

			Treatment response			
			SVR $N = 38$	NR $N = 67$	Total	p-Value
EBER 2 PCR	−ve	N (%)	19 (50.0%)	45 (67.2%)	64 (61.0%)	0.08
	+ve	N (%)	19 (50.0%)	22 (32.8%)	41 (39.0%)	
BNLF1 PCR	−ve	N (%)	27 (71.1%)	41 (61.2%)	68 (64.8%)	0.31
	+ve	N (%)	11 (28.9%)	26 (38.8%)	37 (35.2%)	
BZLF1 PCR	−ve	N (%)	25 (96.2%)	36 (90.0%)	61 (92.4%)	0.64
	+ve	N (%)	1 (3.8%)	4 (10.0%)	5 (7.6%)	

EBER, Epstein–Barr virus-encoded small RNAs; BNLF1, BamHI N Leftward Frame 1; BZLF1, BamHI Z Leftward Frame 1.
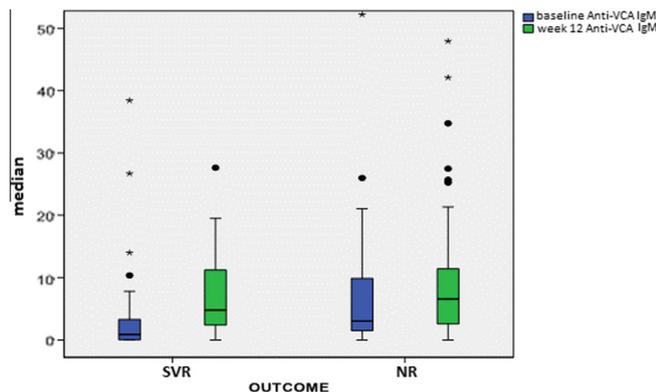

Fig. 2. Changes in baseline and FUP (week-12) patterns of anti-VCA IgM in both groups (SVR and NR). SVR, sustained virological responder; NR, nonresponder; VCA, viral capsid antigen; FUP, follow-up.

IL-28 rs12979860 C/T polymorphism in SVR patients and NRs is shown in Table 3 and Fig. 1. The results showed that CC genotype was significantly associated with SVR patients, while TT genotype was significantly associated with lower SVR rates ($p = 0.003$). The polymorphism genotype frequencies in patients with SVR and NR were represented as follows: C/C (51.4% vs. 25%), C/T (48.6% vs. 55%), and T/T (90% vs. 20%), respectively, as shown in Table 3 and Fig. 1. The comparison of allele frequency between SVR patients and NRs reported a significant association between C alleles and SVR, while the T allele was found to be more frequent in NRs ($p < 0.01$). The C- and T-allele frequencies in patients with SVR and NRs were represented as follows: C alleles (45.7% vs. 54.3%) and T alleles (23% vs. 77%), as shown in Table 3.

At baseline assessment, interpretation of EBV infection in all HCV patients revealed that active EBV infection was present in 61% at baseline (55% in SVR patients and 63% among NRs) and dropped to 55% after 12 weeks of IFN therapy (53% in SVR patients and 57% among NRs) with no significant difference between groups at baseline or FUP. However, latent infection as diagnosed by anti-VCA IgG was positive among all HCV patients before IFN therapy. Baseline serological tests for EBV antibodies revealed that anti-VCA IgM levels were significantly higher in NRs in comparison to responders; $p = 0.01$, as shown in Table 4; however, the expression of different EBV genes did not show any statistically significant treatment outcome, as shown in Table 5.

After 12 weeks of IFN therapy, a significant decline of EBNA-1 IgG was evident within the SVR group ($p = 0.02$). However, other serological markers for EBV did not show such significance at baseline or FUP for the SVR patients and NRs. Figs. 2–4 show the pattern of serological markers' changes at baseline and FUP.

Relation between EBV infection and IL-28B polymorphism

After comparing CC with CT and TT genotypes, it was evident that EBV infection was not associated with IL-28B polymorphism
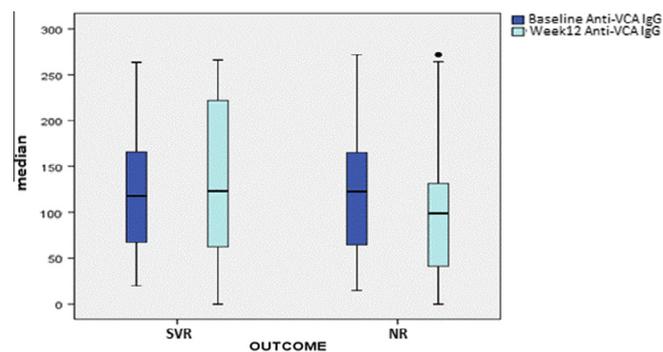

Fig. 3. Changes in baseline and FUP (week-12) patterns of anti-VCA IgG in both groups (SVR and NR). SVR, sustained virological responder; NR, nonresponder; VCA, viral capsid antigen; FUP, follow-up.
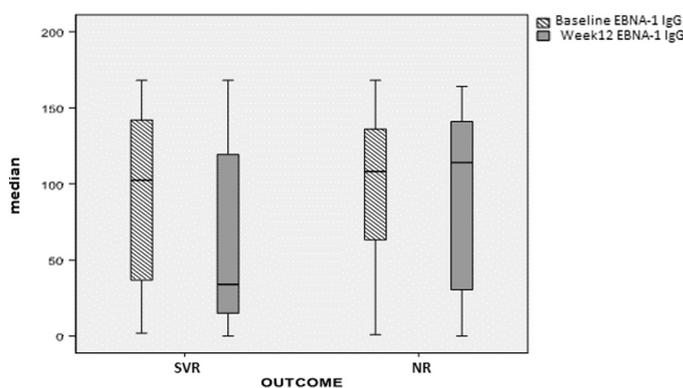

Fig. 4. Changes in baseline and FUP (week-12) patterns of EBNA-1 IgG in both groups (SVR and NR). SVR, sustained virological responder; NR, nonresponder; FUP, follow-up.

Table 6
Relation between EBV infection and IL-28B polymorphism among chronic HCV patients.

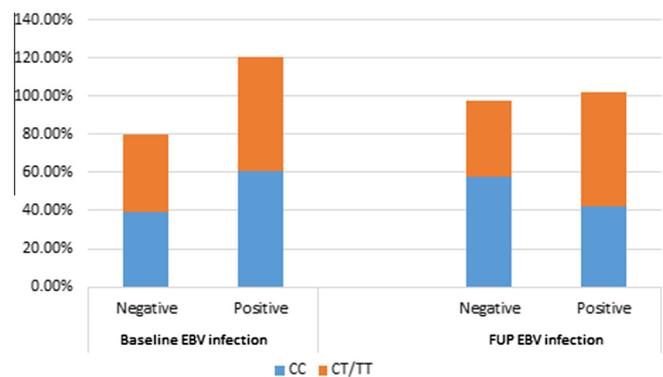| Positive EBV	CC		CT and TT		p-Value
At baseline					
Negative	13	39.4%	25	40.3%	0.93
Positive	20	60.6%	37	59.7%	
FUP at week 12					
Negative	19	57.6%	25	40.3%	0.11
Positive	14	42.4%	37	59.7%	

Fig. 5. Correlation between EBV positivity and IL-28 rs12979860 C/T polymorphism.

Table 7
Relation between quantitative EBV antibodies and IL-28 polymorphism among chronic HCV patients.

Baseline and FUP viral markers of EBV infection	IL-28 polymorphism		p-Value
	CC	CT and TT	
Baseline			
Anti-VCA IgM	2.22/5.0	2.53/8.4	0.54
Anti-VCA IgG**	120.78 ± 63.91	115.47 ± 61.61	0.69
EBNA-1 IgG**	95.55 ± 49.21	95.59 ± 52.92	1.0
FUP at week 12			
Anti-VCA IgM	6.3/6.8	5.3/10.7	0.86
Anti-VCA IgG	128.4/188.4	86.7/102.6	0.003*
EBNA-1 IgG	90.1/112.2	97.4/122.0	0.83

Values are expressed as median/IQR.
* p-Value < 0.01 was considered highly statistically significant.
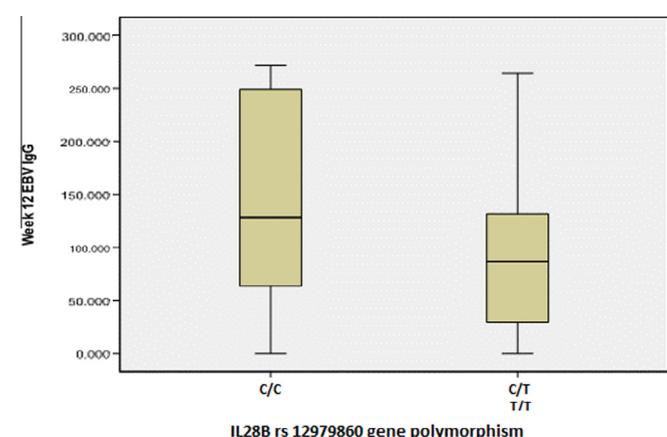** Values are expressed in mean ± SD.

Fig. 6. EBV IgG titre in relation to IL-28 rs12979860 C/T polymorphism.

at baseline or FUP (p = 0.93 and 0.11), respectively, as shown in Table 6 and Fig. 5. In addition, on comparing quantitative EBV antibodies with CC versus CT and TT genotypes, it was found that anti-VCA IgG titre was significantly elevated at FUP among patients with HCV infection with CC genotype (p = 0.003). No other statistical significance was observed at baseline or FUP (Table 7 and Fig. 6).

Univariate and multivariate regression analysis for failure of SVR among patients with HCV infection showed that IL-28 polymorphism was the only parameter with statistical significance (p = 0.01), whereas other baseline parameters such as AFP, HCV, viremia, and fibrosis did not show such significance (Tables 8 and 9).

Discussion

HCV infection is one of the leading threats to public health worldwide and is still a growing global health problem. There are a series of viral, host, and treatment characteristics that influence the likelihood of HCV treatment success establishing them as useful predictors of treatment response when assessing the benefits and risks of therapy [5]. To our knowledge, this is the first study that explores the possible role of EBV infection in addition to IL-28 polymorphism as a predictor of IFN treatment response among patients with chronic HCV infection. Baseline biochemical, virological, and pathological parameters, which are well known factors associated with SVR, were analysed.

In this study, we were able to demonstrate that low median (3.32 ng/mL) baseline serum AFP levels were significantly related to the achievement of SVR, while higher median (6.39 ng/mL) baseline serum AFP levels were associated with NR patients with HCV infection. This finding was in accordance with previous studies conducted by Dellgren et al. [21], and Males et al. [22], who highlighted the same findings on HCV genotype 4. Abdoul et al. [23], illustrated the association between serum AFP level and SVR in 93 patients with chronic HCV infection. He found that the SVR rate was much higher among patients with serum AFP levels below rather than above a median value of 5.7 ng/mL, denoting that serum AFP should be added to the list of predictive factors of treatment response in patients with chronic HCV infection.

The gene expression and the predictive power of IL-28B rs12979860 in response to treatment of HCV genotype 4 were recently studied with CC genotype of higher response rate [24,25].

In our study, a significant difference (p = 0.003) was observed in IL-28B rs12979860 genotype frequencies between SVR patients and NR patients: C/C (51.4% vs. 25%), C/T (48.6% vs. 55%), and T/T (0% vs. 20%), respectively. The C/C genotype was more frequent in SVR patients, while the T/T genotype was associated with NR patients with HCV infection, suggesting a protective role of the CC genotype. This finding could be related to the presence of the C alleles in the former which was significantly associated with better outcome (p < 0.01). Matching with our results, El Awady et al. [9], reported that the CC genotype was significantly associated with higher SVR rate (p = 0.025). Both baseline AFP levels and IL-28B polymorphism were significant predictors of SVR.

Co-infection with other pathogens was, in some instances, an interfering factor against host genotype-based prediction [9]. The EBV has an important and multifaceted role in liver pathology [26]. Reactivation may be due to a HCV co-infection which may lead to downregulation of the immune system [27].

In this study, EBV infection reduced from 64 (61%) cases at baseline to 58 (55%) cases after 12 weeks of therapy with no significant difference between SVR and NR patients. The high incidence of EBV infection in patients with HCV infection could be due to similar pathobiology of EBV and HCV infection. It was possible that HCV infection triggered reactivation of EBV, and that the two viruses could induce host lymphoproliferation cooperatively, as well as prolongation of survival and transformation. These findings agreed with those of the study conducted by Ghanem et al. [12], who observed the common EBV infection in patients with HCV infection and reported that the EBV DNA was detected in 29% of chronic HCV-infected patients compared with 7.7% of the control group cases.

Table 8
Regression table of the selected biomarkers.

	Univariate		Multivariate	
	OR (95% CI)	p-Value	OR (95% CI)	p-Value
Active CMV infection	1.4 (0.66–2.82)	0.4		
Active EBV infection	1.03 (0.42–2.51)	0.94		
IL-28B polymorphism	3.0 (1.23–7.50)	0.02*	3.2 (1.31–7.69)	0.01*

CMV, cytomegalovirus; EBV, Epstein–Barr virus; IL, interleukin.
*p < 0.05 was considered statistically significant.

Table 9
Regression table of laboratory data.

	OR (95% CI)	p-Value
Female gender	2.1 (0.6–7.3)	0.25
Age > 50 (years)	0.51 (0.12–2.2)	0.36
BMI > 30 (kg/m^2)	0.93 (0.28–3.1)	0.90
AST > 40 (IU/L)	0.43 (0.09–2.01)	0.29
ALT > 40 (IU/L)	0.53 (0.12–2.28)	0.40
AFP > 10 (IU/ml)	4.2 (0.81–21.94)	0.09
HCV viremia > 600,000 (IU/ml)	1.0 (0.21–5.1)	0.97
HAI > A1	0.55 (0.15–2.1)	0.38
Fibrosis > 0.21	3.6 (0.77–17.1)	0.1

BMI, body mass index; AFP, α-fetoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; IFN, interferon; TSH, thyroid-stimulating hormone.

In the current study, the role of EBV antibodies on the treatment outcome of patients with HCV infection revealed that higher baseline levels of anti-VCA IgM were associated with NR patients ($p = 0.01$); a comparable increase was noted in anti-VCA IgM among both SVR and NR patients after 12 weeks of therapy. In fact, these results could not be accounted as the level of anti-VCA IgM did not exceed the cutoff value either at baseline or at FUP. Although we were not able to detect a consistent relation between EBV infection and the response to IFN therapy, it was found that there was a trend of increasing the titre of EBNA-1 IgG in NR patients at FUP. This result has been previously attributed and addressed to the possible role of HCV infection in B cells, which may induce dysfunction of Ig, so an increase of polyclonal IgG is commonly observed. These Igs include both HCV-specific and non-specific antibodies [28]. In conclusion, co-infection with EBV did not affect the response of HCV to IFN-based therapy, but it could result in HCV persistence and progression of chronic liver damage. This was consistent with the study conducted by Medina et al. [15], who highlighted that EBV co-infection in chronic HCV patients has been proven to accelerate the course of chronic HCV infection.

The relation between *IL-28B* gene polymorphism and EBV infection revealed that the FUP anti-VCA IgG median was elevated among HCV patients with CC genotype (128.4 IU/ML) compared to the CT and TT genotype (86.7 IU/ML) with *p*-value 0.003. The explanation of this observation was not clearly understood up till now. Otherwise, there was no correlation between qualitative or quantitative values of EBV markers with IL-28B polymorphism at baseline or after 12 weeks of therapy. Martin et al. [29], has shown that there was no correlation found between IL-28B polymorphisms and outcome in chronic viral infections such as hepatitis B or HIV [29].

Our data showed that, IL-28B polymorphism is a strong predictor to PEG-IFN/RBV therapy. In addition, co-infection with EBV did not affect the response of HCV to IFN-based therapy. There was no correlation between IL-28B polymorphism and co-infection with EBV in patients with chronic HCV infection.

Conflict of interest

The authors declare that they have no conflict of interests.

Acknowledgment

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