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RESEARCH ARTICLE

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Impact of *IFNL4* rs12979860 and rs8099917 polymorphisms on response to Peg-Interferon-α and Ribavirin in patients with congenital bleeding disorder and chronic hepatitis C

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Background: The aim of this study was to determine whether two polymorphisms of the human interferon lambda 4 (*IFNL4*) gene (rs12979860 and rs8099917) can predict sustained virologic response (SVR) following antiviral therapy in patients with inherited bleeding disorder and chronic hepatitis C (CHC).

Methods: This retrospective study was conducted on 294 patients with congenital bleeding disorder and CHC who were treated with Peg-Interferon-α (PegIFN) and Ribavirin (RBV). Baseline patient and viral parameters were measured and analyzed statistically to assess their combined and individual contributions to SVR prediction.

Results: The most prevalent variants of rs12979860 and rs8099917 identified among the study patients were CT (45.9%) and TT (57.6%), respectively. Overall, SVR was achieved in 69% of the study patients. The rate of SVR was lower in patients with HCV genotype-1 than in those with HCV genotype-3 (62% vs 88%; *P*<.001; OR=0.23). Multivariate analysis of SVR predictors in patients with HCV genotype-1 infection included age (<24 years), BMI (<25), absence of cirrhosis, HCV RNA level (<400 000 IU/mL), rs8099917 TT and rs12979860 CC, all of which were associated with a higher SVR rate. In HCV genotype-3 infection, only rs12979860 CC was significantly associated with SVR.

Conclusion: These results demonstrate that polymorphisms of the *IFNL4* gene are highly associated with SVR to PegIFN and RBV combination therapy in patients with a congenital bleeding disorder and CHC. Assessment of rs12979860 and rs8099917 genotypes can guide physicians in choosing an optimal treatment regimen, including less expensive therapies that may only be available in many geographic locales.

KEYWORDS

chronic hepatitis C, hemophilia, Pegylated-Interferon, rs12979860, rs8099917, sustained virologic response

1 | INTRODUCTION

Despite new highly efficacious treatment options, hepatitis C infection and chronicity persist as major health problems worldwide. According to a recent world health organization (WHO) report, 130–150 million people have been chronically infected with the hepatitis C virus (HCV) globally. A significant number of patients with chronic hepatitis C (CHC) will develop cirrhosis or liver cancer. Approximately, 500 000 people die each year from hepatitis C-related liver diseases.[1] Before 1985, multi-transfused patients, including those with congenital

bleeding disorders, were particularly vulnerable to HCV exposure from non-screened blood and plasma-derived clotting factor concentrates. Most of these patients ultimately developed CHC. Treated or recombinant clotting factors are now utilized routinely, and new cases of HCV infection in patients with congenital bleeding disorders are uncommon.[2] The prevalence of hepatitis C among hemophilic patients varies from 24% to 95% throughout the world.[3] Prior to the introduction of efficacious HCV direct-acting antiviral agents (DAAs) in 2011, A 24-48 week course of Pegylated-Interferon-α-2a (PegIFN-α-2a) or PegIFN-α-2b combined with Ribavirin (RBV; PegIFN/RBV) was the recommended treatment for CHC.[4] In countries in which DAAs are not available, PegIFN/RBV remains an effective treatment option. HCV medications can lead to a sustained virologic response (SVR), which in turn improves patients' survivability and prevents disease progression and development of hepatocellular carcinoma (HCC). Thus, treating CHC patients with available antiviral agents is highly recommended.[5,6] However, certain factors, including host and viral parameters, can affect the treatment response to antiviral therapy and must be considered prior to starting any antiviral regimens.[7] Key viral determinants of response are HCV genotype-1 and HCV RNA level (high baseline viremia), both of which have negative effects on-treatment response.[8] In 2009, three genome-wide association studies (GWAS) found that single nucleotide polymorphisms (SNPs) in the interferon-λ (*IFNL*) genomic region, located on human chromosome 19, are associated with spontaneous and treatment-induced HCV clearance.[9–11] The most studied SNPs in this genomic region with regard to treatment response are rs12979860 and rs8099917, which were shown to be the strongest baseline predictors of SVR in patients with HCV genotype-1 infection.[12–14] In addition, the rs12979860 SNP was shown to be significantly associated with spontaneous clearance of HCV infection.[15,16] Historically, the rs12979860 and rs8099917 SNPs have been referred to as *IFNL3* (*IL28B*) polymorphisms, however, the discovery of the *IFNL4* gene by Prokunina-Olsson et al.[17] localized the rs12979860 SNP on *IFNL4*.

Patients with congenital bleeding disorder and HCV comprise a special treatment group, with little data to support treatment recommendations. We conducted this study to evaluate the impact of host and viral factors on-treatment response to PegIFN/RBV in patients with congenital bleeding disorder and CHC to provide treatment guidance.

2 | MATERIALS AND METHODS

2.1 | Study population

In this retrospective study (2010–2014), 294 HCV-infected patients with congenital bleeding disorders were enrolled and treated at the Tehran Hepatitis Clinic—a subsidiary of the Baqiyatallah Research Center for Gastroenterology and Liver Diseases (BRCGL). All patients had HCV RNA (>50 IU/mL) in their sera for longer than 6 months and had no previous history of antiviral therapy for CHC. The patient exclusion criteria were: coinfection with hepatitis B or human immunodeficiency viruses, acute hepatitis C, HCC, liver transplantation, low (<50 mL/min) creatinine clearance, poorly controlled psychiatric disease, poorly controlled diabetes, malignant or neoplastic disease, severe cardiac or chronic pulmonary disease, active substance abuse, immunologically mediated disease, retinopathy and clinical or laboratory evidence of liver decompensation. The Ethics Committee of the Baqiyatallah Research Center for Gastroenterology and Liver Diseases approved the study design. All study participants provided informed consent. The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008.

2.2 | Treatment regimen and treatment response definition

The treatment regimen consisted of subcutaneous, once-weekly injections of 180 μg of PegIFN-α-2a (Pegasys®, Roche, Basel, Switzerland) or 1.5 μg/kg of PegIFN-α-2b (PegIntron®, Schering-Plough, Las Piedras, Puerto Rico, USA), and oral Ribavirin (Copegus®, Roche or Rebetol®, Schering-Plough) administered at a weight-adjusted dose of 1000–1200 mg/day as a combination therapy for CHC. The treatment duration was 24 weeks for patients with HCV genotype-3 infection or 48 weeks for patients with HCV genotype-1 infection. The treatment duration could be extended depending on the patient's response and compliance. In patients with HCV genotype-1 infection who achieved partial early virologic response (EVR), the treatment course was extended to 72 weeks. All patients with mixed HCV genotype infections had HCV genotype-1 as a component of their HCV pool, and thus were treated the same as patients with HCV genotype-1 infection.

The serum HCV RNA levels were measured at weeks 4, 12, 24, 48 and 72 during treatment, and 24 weeks after treatment cessation. Undetectable HCV RNA (<10 IU/mL) at the end of a 4-week treatment course was considered to be a rapid virologic response (RVR). Early virologic response was defined as undetectable HCV RNA at the end of week 12 (complete EVR, cEVR) or quantified as a ≥2-log decrease in HCV RNA at the end of week 12 compared to the baseline HCV RNA level (partial EVR, pEVR). Undetectable HCV RNA 6 months after treatment cessation was considered to be SVR, which indicated treatment success. Treatment non-response was defined as a <2-log decrease in the HCV RNA level at week 12 compared to the baseline, or detectable HCV RNA at week 24. If an undetectable level of HCV RNA was achieved at the end of treatment and the HCV RNA became detectable again 6 months later, the case was considered to be a relapse.

2.3 | Laboratory and liver fibrosis assessments

HCV RNA levels were quantitated using COBAS® TaqMan® HCV Test v2.0 (Roche Diagnostics) according to the manufacturer's instructions. The detailed protocol of the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method for genotyping the rs12979860 and rs8099917 SNPs has been described previously.[18] The study patients did not undergo liver biopsy because determining the histological severity is not required before starting

HCV treatment. Furthermore, the liver biopsy procedure is life threatening and costly in patients with congenital bleeding disorders. Liver elastography (Fibroscan) was performed on a proportion of patients and the results were defined as F0-F4; patients with results of >F3 or >12.5 Kpa were considered as severe fibrotic or cirrhotic. For instances in which Fibroscan was not accessible, evidence of cirrhosis included small liver size or irregular liver margins, dilated portal vein and/or splenomegaly on abdominal sonography, and endoscopic findings that included esophageal varices or hypertensive gastropathy.

2.4 | Statistical analysis

Categorical variables were expressed as frequencies and percentages. Continuous variables with normal distributions were expressed as mean ± standard deviation (SD); continuous variables that deviated from normal distributions were expressed as the median (interquartile range). The Fisher's exact test was used to analyze the categorical variables, *t* tests were used to analyze continuous variables with normal distributions, and the Mann-Whitney *U*-test was utilized to analyze continuous variables with non-normal distributions. The Hardy-Weinberg Equilibrium (HWE) was assessed for the rs12979860 and rs8099917 SNPs, and the Linkage Disequilibrium (LD) between these SNPs was calculated. All baseline variables with P <.2 in the univariate analyses were entered to logistic regression models. Two regression models for multivariate analyses were used with the inclusion of one of the two analyzed SNPs in each model to avoid multicollinearity.[19] Odds ratios (OR) were calculated for the dichotomous variables and corresponding 95% confidence intervals were produced. P values <.05 were considered to be statistically significant. Statistical analyses were performed using SPSS version 20.0 (IBM SPSS, Chicago, IL, USA). Statistical graphs were generated using GraphPad Prism version 6.0 (GraphPad, San Diego, CA, USA).

3 | RESULTS

3.1 | Patients' baseline characteristics

Patients' demographics, clinical and laboratory characteristics are presented in Table 1. The majority of the study population was comprised of male hemophilic patients. Their mean±SD age was 28.79±10.17 years. HCV genotype-1a was the most prevalent, followed by HCV genotype-3a. The cirrhosis criteria were observed in 31 (10.5%) patients. The CT variant of the rs12979860 SNP and the TT variant of the rs8099917 SNP were the most prevalent in the study group (45.9% and 57.6%, respectively; Table 1). The distributions of both rs12979860 and rs8099917 genotypes were in HWE (P=.86 and P=.99, respectively) and the two SNPs were in moderate LD (D'=1.0, r^2=.55).

3.2 | Hepatitis C treatment response

In this study, 271 (92.2%) patients received PegIFN-α-2a and 23 (7.8%) received PegIFN-α-2b. In the 18 (8.5%) genotype-1 patients who achieved pEVR, treatment was prolonged for 72 weeks. Treatment was withdrawn in 30 (14.1%) patients with HCV genotype-1 infection

TABLE 1 Baseline characteristics of patients

	All patients (n=294)
Sex, n (%)	
Male	271 (92.2)
Female	23 (7.8)
Age (years)	
Mean±SD	28.79±10.17
Range (min–max)	12–75
BMI,	
Median (IQR)	22.9 (6.1)
Range (min–max)	13.0–38.7
Bleeding disorder type, n (%)	
Hemophilia A	202 (68.9)
Hemophilia B	52 (17.6)
VWD	24 (8.1)
Others	16 (5.4)
Bleeding severity, n (%)	
Mild	55 (18.9)
Moderate	57 (19.3)
Severe	180 (61.1)
Unknown	2 (0.7)
Serum ALT (IU/L)	
Median (IQR)	41 (36)
Range (min–max)	8–293
Serum AST (IU/L)	
Median (IQR)	34 (22)
Range (min–max)	12–188
Cirrhosis	
Yes	31 (10.5)
No	263 (89.5)
HCV RNA[a] (Log IU/mL)	
Median (IQR)	5.97 (6.12)
Range (min–max)	3.37–7.62
HCV genotype, n (%)	
1a	170 (57.8)
1b	27 (9.2)
3a	81 (27.6)
Mixed genotypes	16 (5.4)
rs12979860, n (%)	
CC	119 (40.5)
CT	135 (45.9)
TT	40 (13.6)
C	373 (63.4)
T	215 (36.6)
rs8099917[a], n (%)	
TT	159 (57.6)
GT	101 (36.6)
GG	16 (5.8)
T	419 (75.9)
G	133 (24.1)

n, number; SD, standard deviation; IQR, interquartile range; ALT, alanine transaminase; AST, aspartate transaminase; VWD, Von Willebrand disease.
[a]The data were missed in <10% of patients.

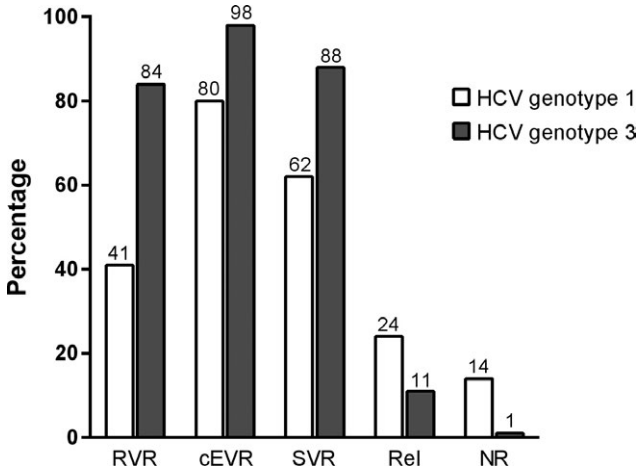

FIGURE 1 Rate of virologic responses, relapse and non-response in patients with congenital bleeding disorder and chronic hepatitis C. The data for assessment of cEVR, SVR, relapse, and non-response were available in all 294 patients. The data for assessment of RVR were available in 151 patients. RVR, rapid virologic response; cEVR, complete early virologic response; SVR, sustained virologic response; Rel, relapse; NR, non-response

and for one (1.2%) patient with HCV genotype-3 infection due to viral breakthrough or non-response.

Within the cohort of 294 patients, 203 (69%) attained SVR, 31 (10.5%) exhibited no response or breakthrough and 60 (20.4%) relapsed. The rate of SVR was higher in patients treated with PegIFN-α-2a compared to that in patients treated with PegIFN-α-2b; however, the difference was not statistically significant ($P \geq .05$). Among the 18 patients with pEVR who underwent 72 weeks of prolonged treatment, 8 (44.4%) achieved SVR. The rates of RVR, cEVR, SVR, relapse, and non-response in relation to HCV genotype are shown in Fig. 1. The rate of virologic responses was significantly lower in patients with HCV genotype-1 infection than that in patients with HCV genotype-3 infection, including RVR ($P < .001$; OR=0.13; 95% CI=0.06–0.30), cEVR ($P < .001$; OR=0.10; 95% CI=0.02–0.44) and SVR ($P < .001$; OR=0.23; 95% CI=0.11–0.47). The rates of relapse and non-response were lower in HCV genotype-3 patients than in HCV genotype-1 patients ($P = .015$; OR=0.4; 95% CI=0.19–0.85) and ($P = .001$; OR=0.08; 95% CI=0.01–0.57), respectively.

3.3 | Factors associated with treatment response among patients with HCV genotype-1 infection

Among the baseline parameters measured in the HCV genotype-1 patients, age <24 years, BMI <25, absence of cirrhosis, HCV RNA level<400 000 IU/mL, rs12979860 CC and rs8099917 TT genotypes were significantly correlated with SVR, as determined by univariate analyses (Table 2). Moreover, achieving RVR and cEVR during the treatment course was also associated with SVR among HCV genotype-1-infected patients (Table 2). In the multivariate analyses, all of the aforementioned baseline parameters remained statistically significant in their combined ability to predict SVR ($P \leq .05$; Table 3). In logistic regression model 1 (with inclusion of rs12979860), the rs12979860 CC genotype was the strongest predictor of SVR, while in logistic regression model 2 (with inclusion of rs8099917), an absence of cirrhosis was the strongest predictor of SVR (Table 3).

3.4 | Factors associated with treatment response in HCV genotype-3 infection

Among the baseline parameters in HCV genotype-3 infection, only the rs12979860 SNP was significantly associated with SVR ($P = .048$; Table 4). In addition, cEVR was associated with attainment of SVR ($P = .014$). Moreover, RVR did not predict SVR in patients with HCV genotype-3 infection. The data for RVR were not collected in 37% of patients owing to the routine management of the study population (Table 4).

3.5 | Sustained virologic response, virologic non-response, relapse, and rs12979860 in patients with HCV infection

HCV genotype-1-infected patients with the rs12979860 CC genotype exhibited the highest SVR rate, whereas patients with the CT and TT genotypes showed significantly decreased SVR ($P < .05$). In addition, the rs12979860 CC genotype patients exhibited the lowest rate of relapse and virologic non-response, which increased significantly in those with the CT and TT genotypes ($P < .001$; linear-by-linear association, Fig. 2a). In the HCV genotype-3 infections, approximately 95% of patients with the rs12979860 CC and CT genotypes achieved SVR, whereas 25% of patients with the rs12979860 TT genotype achieved SVR ($P < .001$; linear-by-linear association, Fig. 2b).

4 | DISCUSSION

This study demonstrated that among HCV-infected patients with inherited bleeding disorders, SVR was achieved in 62% of HCV genotype-1 patients and 88% of HCV genotype-3 patients—findings that are higher than those determined in similar studies.[20,21] In patients with HCV genotype-1 infection, several variables were identified as key predictors of HCV treatment response: young age (<24), BMI <25, absence of cirrhosis, optimal low HCV RNA level (<400 000 IU/mL), and rs12979860 CC and rs8099917 TT SNPs. Mancuso *et al*[22] found 42% and 85% SVR, respectively, among HCV genotype-1- and genotype-3-infected hemophilic patients. A study by Maor *et al*[23] showed a response rate of 37% SVR in 51 HCV-infected hemophilic cases. Another study conducted on 367 Iranian hemophilic patients who underwent treatment with PegIFN and RBV showed 61% SVR. In the latter study, Age <24, HCV genotype-non-1, BMI <25 and baseline HCV RNA<600 000 IU/mL were independent predictors of SVR.[24] A recent study on hemophilic children and adults demonstrated 83.9% and 62.9% SVR rates, respectively.[25] Alavi-moghaddam *et al*[26] reported an excellent success rate of 95.6% in 45 non-cirrhotic hemophiliac patients (mean age 30.4±12.6 years), of which 55.6% were infected with HCV genotype-1 and 42.2% with HCV genotype-3. Similarly, different studies showed that high pretreatment HCV RNA

TABLE 2 The impact of baseline parameters and on-treatment response on achievement of SVR in patients with HCV genotype-1 infection

	SVR (n=132)	NVR (n=81)	OR (95% CI)	*P* value[a]
Sex, n (%)				
Female	8 (6.1)	10 (12.3)	Ref.	.131
Male	124 (93.9)	71 (87.7)	2.18 (0.82–5.78)	
Age, n (%)				
>24	78 (59.1)	64 (79.0)	Ref.	.003
<24	54 (40.9)	17 (21.0)	2.61 (1.38–4.93)	
BMI, n (%)				
>25	30 (22.7)	39 (48.1)	Ref.	<.001
<25	102 (77.3)	42 (51.9)	3.16 (1.74–5.73)	
Cirrhosis, n (%)				
Yes	7 (5.3)	16 (19.8)	Ref.	.001
No	125 (94.7)	65 (80.2)	4.40 (1.72–11.22)	
HCV RNA (IU/mL), n (%)				
>400 000	98 (74.2)	73 (90.1)	Ref.	.005
<400 000	34 (25.8)	8 (9.9)	3.17 (1.38–7.24)	
rs12979860, n (%)				
CT+TT	71 (53.8)	64 (79.0)	Ref.	<.001
CC	61 (46.2)	17 (21.0)	3.23 (1.71–6.10)	
rs8099917[b], n (%)				
GT+GG	39 (33.1)	48 (60.8)	Ref.	<.001
TT	79 (66.9)	31 (39.2)	3.14 (1.73–5.67)	
RVR[c], n (%)				
No	18 (32.7)	41 (91.1)	Ref.	<.001
Yes	37 (67.3)	4 (8.9)	21.07 (6.53–67.96)	
cEVR, n (%)				
No	8 (6.1)	34 (42.0)	Ref.	<.001
Yes	124 (93.9)	47 (58.0)	11.2 (4.84–25.98)	

SVR, sustained virologic response; NVR, non-virologic response; OR, odds ratio; Ref, reference; BMI, body mass index; RVR, rapid virologic response; cEVR, complete early virologic response.

[a]Fisher's-exact test.

[b]The data were missed in less than 10% of patients.

[c]The data were missed in more than 10% of patients.

levels, HCV genotype-1 infection, presence of cirrhosis, high BMI, and older age at the time of infection were parameters associated with a reduced chance of achieving SVR.[27] This study determined that SVR was strongly associated with RVR (OR=21) and cEVR (OR=11) in patients with HCV genotype-1 infection, which confirms the same observation determined previously among non-hemophilic HCV-infected individuals and indicates the importance of a rapid drop in HCV RNA level on HCV eradication.[28] Andriulli *et al*[29] found that achieving RVR was the main on-treatment predictor for achieving SVR. The current study demonstrated that attaining RVR played more of a key role in attaining SVR than either of the polymorphisms of the *IFNL4* gene. The theory of viral kinetics maintains that RVR is a stronger predictor of SVR than baseline predictors.[7] Previously, it was assumed that polymorphisms of *IFNL4* were alternative SVR predictors to RVR. However, these data are indicating instead that RVR is a reflection of known and unknown predictors of treatment outcome and cannot be replaced by polymorphisms of *IFNL4*.[7] The current study found a higher SVR rate in patients with HCV genotype-1 infection carrying rs12979860 CC and rs8099917 TT host genotypes than those cases with rs12979860 non-CC and rs8099917 non-TT genotypes. A recent study from Iran found that the rs12979860 CC genotype was significantly associated with achieving SVR.[13] Thompson *et al*[30] found that polymorphism(s) of *IFNL4* was the best predictor of anti-HCV treatment response, above all other known baseline predictors. This study revealed that the HCV genotype-1 patients with rs12979860 CC or rs8099917 TT achieved 78% and 53% SVR, respectively. A recent study by Maor *et al*[23] showed that SVR was attained in 70% and 50% of hemophilic patients who carried rs12979860 CC and rs8099917 TT genotypes, respectively.

Two meta-analyses reported that favorable rs12979860 genotypes are associated with SVR in HCV genotype-1 and -4 patients, but not in HCV genotype-3 patients.[31,32] However, the results of this and other studies associating polymorphisms of *IFNL4* and HCV

TABLE 3 Multivariate analysis of baseline predictors of SVR in patients with HCV genotype-1 infection

	Logistic regression model 1		Logistic regression model 2	
	Adjusted OR (95% CI)	Adjusted *P* value	Adjusted OR (95% CI)	Adjusted *P* value
Age <24	2.50 (1.23–5.08)	.011	2.40 (1.13–5.08)	.023
Male sex	1.74 (0.56–5.43)	.337	1.27 (0.37–4.36)	.707
BMI <25	2.76 (1.41–5.41)	.003	3.03 (1.50–6.12)	.002
Absence of cirrhosis	2.81 (1.01–7.93)	.048	5.48 (1.74–17.20)	.004
HCV RNA <400 000 IU/mL	2.95 (1.20–7.24)	.018	3.19 (1.23–8.28)	.017
rs12979860 CC genotype	3.90 (1.93–7.87)	<.001	–	–
rs8099917 TT genotype	–	–	4.68 (2.30–9.49)	<.001

BMI, body mass index.

TABLE 4 The impact of baseline parameters and on-treatment response on achievement of SVR in patients with HCV genotype-3 infection

	SVR (n=71)	NVR (n=10)	OR (95% CI)	*P* value[a]
Sex, n (%)				
Female	4 (5.6)	1 (10.0)	Ref.	.492
Male	67 (94.4)	9 (90.0)	1.86 (0.19–18.55)	
Age, n (%)				
>24	46 (64.8)	7 (70.0)	Ref.	>.999
<24	25 (35.2)	3 (30.0)	1.27 (0.30–5.34)	
BMI, n (%)				
>25	22 (31.0)	5 (50.0)	Ref.	.290
<25	49 (69.0)	5 (50.0)	2.22 (0.59–8.49)	
HCV RNA[b] (IU/mL), n (%)				
>400 000	55 (78.6)	6 (60.0)	Ref.	.240
<400 000	15 (21.4)	4 (40.0)	0.41 (0.10–1.64)	
rs12979860, n (%)				
CT+TT	32 (45.1)	8 (80.0)	Ref.	.048
CC	39 (54.9)	2 (20.0)	4.88 (1.01–24.59)	
rs8099917[b], n (%)				
GT+GG	25 (35.7)	5 (55.6)	Ref.	.290
TT	45 (64.3)	4 (44.4)	2.25 (0.55–9.15)	
RVR[c], n (%)				
No	6 (13.0)	2 (40.0)	Ref.	.170
Yes	40 (87.0)	3 (60.0)	4.44 (0.61–32.33)	
cEVR, n (%)				
No	0 (0)	2 (20.0)	Ref.	.014
Yes	71 (100)	8 (80.0)	35.5 (1.47–857.1)	

SVR, sustained virologic response; NVR, non-virologic response; OR, odds ratio; Ref, reference; BMI, body mass index; RVR, rapid virologic response; cEVR, complete early virologic response.
[a]Fisher's-exact test.
[b]The data were missed in less than 10% of patients.
[c]The data were missed in more than 10% of patients.

genotype-2 and -3 patient responses to PegIFN/RBV treatment are controversial.[33] Sarrazin *et al*[34] reported an association between the rs12979860 CC genotype and SVR in a subgroup of HCV genotype-3 patients who attained RVR, but not in HCV genotype-3 patients who did not attain RVR. It has been shown that the intrahepatic expression profile of interferon-stimulated genes (ISGs) varies according to different rs12979860 and rs8099917 genotypes. Patients carrying favorable genotypes of polymorphisms of *IFNL4* exhibit decreased expression levels of genes shown to promote an antiviral state.[35] The expression of hepatic ISGs is strongly associated with treatment

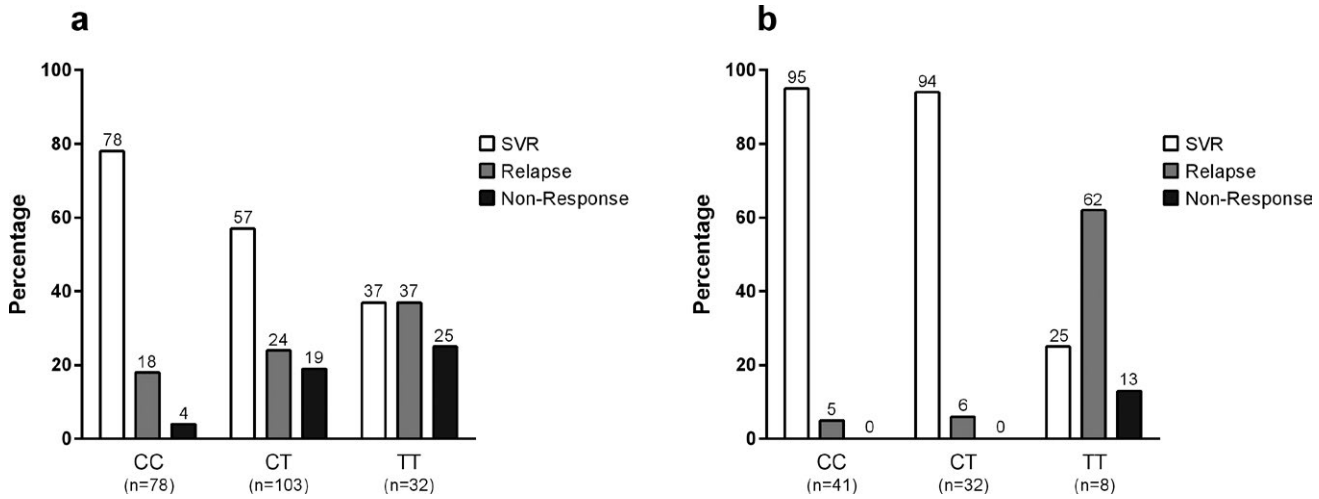

FIGURE 2 The impact of rs12979860 genotypes on the rate of sustained virologic response (SVR), relapse and non-response in patients with congenital bleeding disorder and chronic hepatitis C. (a) The rate of SVR, relapse and non-response in patients with congenital bleeding disorder and HCV genotype-1 infection in relation to rs12979860 genotypes, (b) The rate of SVR, relapse and non-response in patients with congenital bleeding disorder and HCV genotype-3 infection in relation to rs12979860 genotypes

response, in which low ISGs pretreatment levels have been associated with successful IFN-based therapies, whereas patients having high ISGs levels respond poorly to interferon.[7,36] Recently, the dinucleotide variant rs368234815 (TT/ΔG), which is located in exon 1 of the *IFNL4* gene and upstream of the *IFNL3* gene, was found to be in strong LD with rs12979860 in Caucasians. In patients of African ancestry, the LD between rs12979860 and rs368234815 is moderate, and the *IFNL4* rs368234815 genotype predicts viral decline, and the rate of SVR to PegIFN-α/RBV better than rs12979860 among the African ancestry patient group.[17,36] In a similar study, our group determined a perfect correlation between two genetic variants of rs368234815 and rs12979860 in Iranian patients with HCV infection.[37]

Recently, several new DAAs including NS3, NS5A, and NS5B inhibitors have been approved for treatment of HCV infection.[38,39] These compounds have potent antiviral activities, broad genotypic coverages and are administered orally once daily in treatment-naïve and treatment-experienced patients, which can lead to a 95% SVR rate.[40] A recent study evaluated the efficacy of a Ledipasvir/Sofosbuvir fixed-dose DAA combination plus RBV in 14 patients with inherited bleeding disorders and HCV genotype-1 infection.[41] In the latter study, all cases achieved SVR 12 weeks after completing antiviral therapy.[41] Despite their ability to resolve HCV with a high SVR rate, these new DAAs are costly and thus may not be affordable in low-income communities. The limitation of the present study was its necessarily retrospective design; in our opinion, a prospective study design would produce more reliable results. Moreover, the assessment of liver cirrhosis herein was performed via ultrasonography and physical exam findings in a proportion of patients.

In conclusion, given that hemophilic patients comprise a special patient group and the majority of these patients is infected with difficult-to-treat HCV genotypes, we believe that a decision-making policy for treating them is required. The present study showed a negative impact of different factors including the presence of cirrhosis, older age and high BMI on achieving SVR, thus, we recommend treating these patients as soon as possible. As approximately 80% of patients with HCV genotype-1 and the rs12979860 CC genotype did achieve SVR, and that the statistical correlation of these two factors with a favorable outcome is strong, we recommend conducting an evaluation of polymorphisms of *IFNL4* before making a treatment decision. If the treatment-naïve patients harbor a favorable CC genotype, treatment with PegIFN and RBV can still be considered. Given that a high proportion (95%) of patients with HCV genotype-3 infection and rs12979860 CC and CT genotypes achieved SVR, a treatment course with PegIFN and RBV for this patient group is highly recommended. Conversely, the patients with HCV genotype-3 infection and the rs12979860 TT genotype had a low (25%) SVR rate, which warrants consideration of IFN-free regimens for these patients.

AUTHOR CONTRIBUTIONS

Maryam Keshvari, Seyed Moayed Alavian, Bita Behnava, Ali Pouryasin and Heidar Sharafi designed the study, Maryam Keshvari, Seyed Moayed Alavian, and Bita Behnava contributed to the sample collection, Ali Pouryasin and Heidar Sharafi performed the study, Maryam Keshvari and Heidar Sharafi analyzed the data, and Maryam Keshvari, Johanna C. Craig and Heidar Sharafi drafted the paper.

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