Risk factors for recurrences in patients with hepatitis C virus after achieving a sustained virological response: a multicentre study from Turkey

Ferhat Arslan[1], Bahadir Ceylan[2], Ahmet Riza Sahin[3], Özgür Günal[4], Bircan Kayaaslan[5], Kenan Uğurlu[6], Alpaslan Tanoğlu[7],Gülsen Iskender[8], Selma Tosun[9], Aynur Atilla[4], Fatma Sargın[10], Ayse Batirel[11], Ergenekon Karagöz[12], Abdullah Sonsuz[13], Ali Mert[2]

[1]Department of Infectious Diseases and Clinical Microbiology, Faculty of Medicine, Istanbul Medeniyet University, Istanbul, Turkey;
[2]Department of Internal Medicine, Faculty of Medicine, Istanbul Medipol University, Istanbul, Turkey;
[3]Department of Infectious Diseases and Clinical Microbiology, Sutcu Imam University Medical Faculty, Kahramanmaras, Turkey;
[4]Department of Infectious Diseases and Clinical Microbiology, Samsun Education and Training Hospital, Samsun, Turkey;
[5]Department of Infectious Diseases and Clinical Microbiology, Faculty of Medicine, Yildirim Bayazit University, Istanbul, Turkey;
[6]Department of Infectious Diseases and Clinical Microbiology, 25 Aralik State Hospital, Gaziantep, Turkey;
[7]Department of Gastroenterology, GATA Haydarpasa Education and Training Hospital, Istanbul, Turkey;
[8]Department of Infectious Diseases and Clinical Microbiology, Dr. Abdurrahman Yurtaslan Oncology Education and Training Hospital, Ankara, Turkey;
[9]Department of Infectious Disease and Clinical Microbiology, Bozyaka Education and Training Hospital, Izmir, Turkey;
[10]Department of Infectious Diseases and Clinical Microbiology, Goztepe Education and Training Hospital, Istanbul, Turkey;
[11]Department of Infectious Diseases and Clinical Microbiology, Dr. LutfiKirdar Education and Training Hospital, Istanbul, Turkey;
[12]Department of Infectious Diseases and Clinical Microbiology, Istanbul Sancaktepe Prof Dr Ilhan Varank Education and Training Hospital, Istanbul, Turkey;
[13]Department of Gastroenterology and Hepatology, Cerrahpasa Faculty of Medicine, Istanbul University, Istanbul, Turkey

SUMMARY

In this study, we aimed to determine the late relapse rate in hepatitis C patients with sustained virological response after interferon-based regimens, and evaluated the predictors of late relapse while comparing the real-life data of our country with that of others. A multicenter retrospective study was performed to investigate the data of patients infected with HCV who obtained sustained virological response after classical or pegylated interferon alpha (PegIFNα) and ribavirin (RBV) for 48 weeks. Sustained virological response was based on negative HCV RNA level by PCR at the end of six months after the therapy. The information of patients enrolled in the study was retrieved from the hospital computer operating system and outpatient follow-up archives. We evaluated the age, gender, HCV RNA levels, HCV genotype, six-month and further follow-up of patients with sustained virologic response, presence of cirrhosis, steatosis and relapse. In all, 606 out of 629 chronic hepatitis C patients (mean age was 53±12 years; 57.6 % of them were female) with sustained virological response were evaluated. We excluded 23 patients who relapsed within six months after the end of treatment (EOT). The mean follow-up period of the patients was 71 months (range: 6-136) after therapy. Late relapse rate was 1.8% (n=11) in all patients. Univariate Cox proportional hazard regression models identified that cirrhosis and steatosis were associated with the late relapse [(p=0.027; Hazard Ratio (HR) 2.328; 95% confidence interval (CI): 1.309-80.418), (p=0.021; HR 1.446; 95% CI: 1.243-14.510, respectively]. In multivariable Cox regression analysis, steatosis was the only independent risk factor for late relapse (p=0.03; HR 3.953; 95% CI: 1.146-13.635). Although the late relapse rate was approximately 2% in our study, clinicians should consider that pretreatment steatosis may be an important risk factor for late relapse.

Keywords: hepatitis C, HCV, relapse, steatosis.

INTRODUCTION

A great deal of progress and success have been achieved in treatment of chronic hepatitis C (CHC) infection with newly introduced direct-acting antiviral agents [1]. Besides, on a global perspective, all these favorable achievements still remain as a distant hope for patients living in countries with limited resources [2]. The rate of long-term relapse (developing after 24 weeks) in patients with CHC infection has been reported to be 0-17% [3, 7]. We could not find any study including quite a number of cases genotype 1 patients and focused their late relapse in medical literature. In another aspect, we are of the opinion that this late relapse rates may reflect the new therapies long term results.
In this study, we aimed to determine the late relapse rate in a total of 629 patients with sustained virological response and the risk factors of late relapse.

PATIENTS AND METHODS

Study patients and design
In this study, the records of adult CHC patients who were admitted to 15 center of chronic hepatitis outpatient clinics of university and training and research hospitals after presenting sustained virological response (SVR) following therapy with recombinant or pegylated interferon alpha (PegIFNα) + ribavirin (RIB) and were followed up between 2000-2015 were evaluated retrospectively. SVR was based on negative HCV RNA level by PCR at the end of six months after EOT. Late relapse was defined HCV RNA detectability after have achieving an SVR.
Patients who were not treated adequately in terms of therapy duration (12 months for genotype 1 or 4 patients, and 6 months for genotype 2 or 3 patients) or due to drug-related side effects and those who were lost to follow-up were excluded. The patients with HBV/HCV, HIV/HCV co-infection, drug abuse, malignant disease, or autoimmune hepatitis and those who were pregnant were also excluded. Patients who relapsed within six months after EOT were not included in the study.
The data of the patients in terms of age, gender, serum HCV RNA level by PCR, HCV genotype, treatment regimens, presence of cirrhosis and Child Pugh scores, presence of steatosis were obtained from patients' data files.

Treatment regimens
Patients who received interferon-alpha 2a or 2b or pegylated INF alpha 2b 1.5 mg/kg/w or 2a 180 mg/kg/w; subcutaneously + RIB (1200 mg/day or 1000 mg/day for those with body weight of >75 kg and <75 kg, respectively; per oral) combination were included. The treatment regimen was continued for 12 months in patients with HCV genotype 1 or 4 and 6 months in those with genotype 2 or 3.

HCV RNA and HCV genotype assays
HCV RNA was assessed using bDNA (branched DNA) signal amplifier (Versant HCV RNA 3.0 Assay, Bayer Corporation Diagnostics, USA [detection range 615-7690000 U/ml]) or RT-PCR (real time polymerase chain reaction, Cobas TaqMan HCV test v 2.0 [detection range 25-391000000 U/ml]) in 70% of patients. The patients were grouped according to their pre-treatment HCV RNA levels. That is, one group comprised the patients with pre-treatment HCV RNA levels of ≥600000 IU/L and the other group included patients with pre-treatment RNA levels of <600000 IU/mL.
HCV genotype was assessed by using Innolipa HCV II commercial kit (Bayer Diagnostics, USA). The patients were grouped according to their HCV genotypes. That is, the patients having genotype 1 and those with genotype 2, 3 or 4 were analyzed as separate groups.

Evaluation of cirrhosis and liver steatosis
Liver biopsy findings were evaluated using the modified Knodell (Ishak) scores. A fibrosis score of 5/6 was accepted as cirrhosis. Child Pugh score was calculated if a patient had been diagnosed as cirrhosis. Cirrhosis was also accepted according to radiological (ultrasonography, magnetic resonance imaging) appearances (liver contour lobulation, caudate/left lobe hypertrophy splenomegaly and thrombocytopenia). All patients were evaluated with ultrasonography for the diagnosis of hepatic steatosis.

Corresponding author
Ferhat Arslan
E-mail: ferhatarslandr@hotmail.com

Statistical analysis

The data were expressed as mean ± SD or %. Late relapsed and non-relapsed HCV patients were presented by age, genders, quantitative HCV RNA, follow up time and the proportion of cirrhosis and steatosis in Table 1.

The Chi-square test or Fisher's exact test (when Chi-square test assumptions do not hold due to low expected cell counts), where appropriate, was used to compare proportions in late relapsed and non-relapsed patient groups. A *p*-value lower than 0.05 was considered as statistically significant.

Associations between possible risk factors and late relapse were evaluated using the Multivariable Cox proportional hazard regression models based on variables with a *p*-value of ≤0.05. The following variables were considered in the univariate analysis for late relapse: age (<60 years, ≥60 years), gender, HCV RNA level (<6x10^5 IU/mL, ≥6x10^5 IU/mL), HCV genotype, treatment regimens (standard IFN or pegylated IFN), presence of cirrhosis and steatosis. Variables that were found significant in univariate analysis were evaluated with multivariable analysis. All *p*-values were based on two-sided hypothesis tests, and α were set at 0.05. Analysis was performed with SPSS Statistics version 16.0 (IBM, Chicago, IL, USA).

RESULTS

Investigation of the hospital records of a total of 606 patients with sustained virological response after treatment with pegylated INF α2b + RIB (n=298), pegylated INF α2a + RIB (n=285), INF α2b + RIB (n=32), or INF α2a + RIB (n=14) combinations revealed that they completed the therapy period and showed virological response at the end of therapy. The mean follow-up period of the patients was 71 months (range, 6-136) after the therapy.

The mean age of the patients was 53±12 and 58% of them were female. Genotype 1 was detected in 98% of the patients. Late relapse was recorded in

Table 1 - Clinical and laboratory features of study patients.

	Non-relapsed patients (n = 595)	*Late relapsed patients (n =11)*	*P value*
Variables			
Age	53±12 years	58±8.5 years	0.133
Gender (Female)	349/595 (58.6)	5/11 (45.5)	1
HCV RNA	850000 (IQR, 284946- 2835000 IU/ml)	2107950 (IQR, 594500- 3130000 IU/ml)	0.699
Cirrhosis	9/585 (1.5%)	1/11 (9.1 %)	0.169
Steatosis	164/589 (27%)	7/11 (63.6)	0.016
Follow up duration	43±27 months	42±30 months	0.0001
Relapse duration	-	17±5.6 months	-

Table 2 - Results of univariate and multivariate Cox regressions model analysis for the development of late relapse.

Variable	*N (%)*	*Hazard Ratio*	*(%95 Confidence Interval)*	*P value (univariate)*	*P value (multivariate)*
Gender (Female)	349 (58.6)	1.134	0.346-3.716	0.835	
Age >60 years	379 (62.7)	2.342		0.276	
Liver steatosis	171 (28.2)	1.446 3.953	1.243-14.510 1.146-13.635	0.021	0.03
Cirrhosis	10 (1.7)	2.328	1.309-80.418	0.027	0.063
Pre-treatment HCV RNA level (>2x10^{6}IU/mL)	361 (59.7)	1.945	0.516-7.73	0.326	
Standard INF+RIB	48 (8.1)	0.966	0.124-7.553	0.974	

11 patients (1.8%). The mean late relapse time was 16.9±5.3 months. Ten patients were diagnosed as cirrhosis before the treatment. All cirrhotic patients had a Child Pugh classification of A. The clinical and laboratory features of late relapsed vs. non-relapsed patients are summarized in Table 1. Gender, age, genotypes, HCV RNA load at the beginning of therapy, classical IFN- based treatment were not found as predictive factors to predict late relapse rate. Univariable Cox proportional hazard regression models identified that cirrhosis and steatosis were independently associated with the late relapse [p=0.027; Odds Ratio (OR) 2.328; 95% confidence interval (CI): 1.309-80.418, and p=0.021; OR 1.446; 95% CI: 1.243-14.510, respectively]. Multivariable Cox proportional hazard regression models identified that steatosis is only independent factor for late relapse (p=0.03; HR 3.953; 95% CI: 1.146-13.635) (Table 2).

DISCUSSION

Chronic hepatitis C virus (HCV) infection, which is a global health problem, affects approximately 170 million people all over the world [2]. Additionally, long-term carriage may lead to the development of cirrhosis, liver decompensation, hepatocellular carcinoma and even death [6]. Although the treatment regimens for hepatitis C virus (HCV) infection has moved beyond interferons and toward direct-acting antiviral agents (DAAs) such as protease inhibitors and NS5A and NS4B inhibitors, payers and the governments even in high income countries have been limiting the coverage of these therapies because of their high costs. This situation makes it difficult to access DAAs in some regions of the world. For this reason, in some countries, interferon based regimens are still in use.

It is well-known that SVR is a good marker of response to CHC treatment [8-10]. Moreover, late HCV recurrence (relapse or re-infection) still remains as a problem. In high-risk group patients (such as HIV-positive subjects, patients on hemodialysis, intravenous drug users), increased recurrence rates support the higher possibility of re-infection [11]. In this study, particularly patients who have low risk of re-infection were investigated and compared with medical literature in Medline (Table 3).

The phenomenon of late relapse and re-infection

Table 3 - Chronic HCV infection studies with late relapse rates in patiens with low risk for reinfection.

Study/publicationyear	*Total number of patients with SVR*	*Follow Up Time*	*Late Relapse, n (%)*	*Risk factors for relapse*
Veldt et al, 2004	286	5 years	12 (4.7)	None
E. Formann, 2006	187	29 months	(0)	NA
Desmond et al, 2006	147	Mean 2.3 years (range 0.3-10.3)	(0)	NA
daCostaFerreira et al, 2010	174	Median duration 47 months (12-156)	(0)	NA
Swain et al, 2010	1077	Annually (for 5 years)	(0)	NA
Sood et al, 2010	100	6 months to 8 years	8 (8)	Cirrhosis
Giannini et al, 2010	231	Median duration 41 months weeks	2 (1)	NA
Li et al, 2012	146	33.45 +/- 16.41 months (range: 12-85)	8 (8.9)	Older Age
Rutter et al, 2013	103	21 months (range: 7-64)	2 (1)	One patient was cirrhotic, both carried the genotype 1b
Uyanikoglu et al, 2013	196	33.5 months (range, 6 to 112)	2 (0)	NA
Papastergiou et al, 2013	145	68.8 ± 35 months	2 (1)	NA
M. P. Manns 2013	1002	5 years	(0)	NA
Giordanino, Chiara, 2014	115	9.2 years	(0)	NA

in patients with CHC infection may be confused with each other. Even though the term "late relapse" is used, it shouldn't be overlooked that some of the patients with high relapse prevalence constitute those with high risk of re-infection [7, 12]. In a meta-analysis, the rate of late relapse in low-risk patients for re-infection has been reported as less than 2% [11]. In a study conducted with 196 CHC patients in our country, relapse after SVR has been detected in 1.02% in a period of 33 months in average and those cases had low levels of HCV viremia [13].

Most of the studies stated/emphasized the predictive factors for relapse developing after SVR as age, HCV genotype, IL-28B genotype/polymorphism, presence of steatosis, cirrhosis, viruses in peripheral blood mononuclear cells, ribavirin concentration at the end of treatment [5, 14, 20].

In addition to difficult achievement of SVR in cases with advanced liver injury (especially in patients infected with genotype 1 HCV), there are some studies which reveal that risk of development of early relapse is higher in those patients [21, 23]. There are few studies investigating the relationship between late relapse and advanced liver injury (except for transplanted patients) other than this study [24, 25]. Late relapse was detected in five out of 28 cirrhotic patients and this result was statistically significant compared to non-cirrhotic patients in the study conducted by Sood et al. [24, 25]. Rahman et al reported significantly higher rates of late relapse in treatment experienced cirrhotic patients (three out of six) who achieved SVR compared to non-cirrhotic patients during a 5-year prospective study [26].

Hepatosteatosis develops as a result of deposition of fat droplets in hepatocytes [27]. HCV (especially genotype 3) is a risk factor for hepatosteatosis in addition to environmental factors triggering it [27]. It is known that presence of steatosis causes progressive fibrosis and decreases SVR rates [28]. Furthermore, it is under debate whether hepatosteatosis is an independent risk factor for recurrence in patients with SVR or not.

In a study evaluating patients infected with Genotype 2 and 3, it has been stated that presence of steatosis had positive correlation with early relapse and the relapse rate was around 36.4% in patients with steatosis and high viral load [15]. This situation may be interpreted as being irrelevant to genotype 3. In studies focusing on genotype 1 HCV patients similar to our study, it has been reported that hepatosteatosis had a negative effect on SVR [34]. In another study evaluating liver transplant patients, the sensitivity of presence of steatosis in forecasting of virologic relapses in post-transplant liver biopsies was around 89% [30]. To the best of our knowledge, we could not come across any studies focusing on relation between late relapse and steatosis in non-transplanted HCV patients in medical literature. We believe that our study is unique in this aspect.

Major limitations of our study are its retrospective design, inability to perform genetic studies in patients with relapse, and detection of steatosis only by ultrasound. However, inclusion of large number of patients and long-term follow-up are its strong aspects.

In conclusion, the rate of late relapse after SVR in patients with CHC infection in our study is consistent with previous medical literature. Although SVR has been achieved in patients with cirrhosis and steatosis, the possibility of late relapse should be kept in mind.

Conflict of interest

There is no conflict of interest.

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