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The effect of direct-acting antivirals (DAA) on confirmed noninvasive fibrous parameters in chronic hepatitis C patients

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ABSTRACT

Aims: Chronic hepatitis C (CHC) is an important public health problem in terms of the number of people it affects worldwide and the diseases it causes. The high sustained virological response (SVR) rates achieved by the use of direct-acting antiviral (DAA) drugs in the recent period have shown that a new era has begun in this disease. It was aimed to evaluate the effect of DAAs on confirmed noninvasive fibrous parameters together with their effectiveness.

Methods: 75 patients who were started on DAA treatment for CHC were included in the study. In addition, laboratory parameters values at the beginning of the treatment, 12 and 24 weeks after the end of the treatment, hepatitis C virus ribonucleic acid (HCV RNA) results and Aminotransferase-to-Platelet Ratio Index (APRI), Fibrosis-4 (FIB-4) scores were compared.

Results: The most common comorbidity in patients is hypertension (HT), and the most common source of transmission is surgical operations. Genotype 1b was the dominant genotype. The SVR rates of all patients 12 and 24 weeks after the end of treatment were 100%. The APRI and FIB-4 scores of the patients decreased significantly at the 12th and 24th weeks at the end of the treatment compared to the beginning of the treatment.

Conclusion: The confirmed noninvasive fibrous parameters used in the treatment of CHC are useful in evaluating the results of the treatments applied.

Keywords: Chronic hepatitis C, direct-acting antiviral agents, sustained virological response, fibrosis-4, aminotransferase-to-platelet ratio index

INTRODUCTION

Hepatitis C virus (HCV) infections are a major public health problem affecting estimated 80 million people worldwide and can result in hepatocellular carcinoma and cirrhosis.[1,2] Chronic infection due to HCV induces the progression of fibrosis in the liver. Elimination of HCV with antiviral treatment, which is defined as a permanent virological response (SVR), prevents the progression of chronic hepatitis and its related complications.[2] Direct-acting antivirals targeting HCV have reformed the treatment of CHC infection by significantly increasing the rates of permanent virological response (SVR) and diminishing the adverse effects of treatment.[3] In Turkey, it has been reported that the rates of SVR increased above 90% with the direct-acting antivirals (DAA) included in the reimbursement scope as of June 2016.[4]

Since liver histology rather than plasma viremia is the most important prognostic factor in patients with CHC infection, it is necessary to determine how virologic clearance is related to histologic recovery.[3] The technique defined as noninvasive fibrosis measurements is the calculation of hepatic fibrosis using non-invasive techniques. The indices called Aminotransferase-to-Platelet Ratio Index (APRI) and Fibrosis-4 (FIB-4) provide identification of the liver parenchyma, especially in patients at high risk of disease complications, without exposing them to invasive diagnostic methods such as liver biopsy. The use of these methods is also very useful and important in the treatment and post-treatment follow-up of CHC patients without an additional invasive procedure.[5]

The main aim of this study is to evaluate the demographic characteristics and treatment response of CHC patients using DAA followed in our center, and also to reveal the change of proven noninvasive fibrosis parameters with treatment. Thus, it is aimed to bring a modern

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approach to the management of patients with chronic HCV infection by combining the management of HCV infection, which has undergone a great change with the emergence of DAA agents, with proven noninvasive fibrous measurements.

METHODS

The study was carried out with the permission of Van Training and Research Hospital Ethics Committee (Date: 18.02.2021, Decision No: 2021/04). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

75 CHC patients who were followed-up and treated at the infectious diseases outpatient department of a training and research hospital between 1 January 2018 and 31 December 2020 were included in this retrospective cross-sectional analysis.

Patient Selection Criteria

- 18 years or older,
- Having met the criteria for CHC and received DAA treatment,
- Completing DAA treatment and following up regularly in our center (Patients who discontinued the treatment or were out of follow-up were not included in the study).

Definition of Sustained Virological Response and Non-Invasive Tests Used for Liver Fibrosis

- **SVR-12:** Invisible HCV RNA in plasma - 12 weeks following the end of DAA treatment, utilizing a high - sensitivity PCR assay
- **SVR-24:** Invisible HCV RNA in plasma - 24 weeks following the end of DAA treatment, utilizing a high - sensitivity PCR assay

The treatment to be used by the patients was decided by considering the genotype, cirrhosis status, treatment experience, comorbidities and the drugs they used within the framework of the health practice communiqué rules determined by the Republic of Turkiye Ministry of Health.

The treatment methods, genotypes, risk factors for HCV infection, demographic characteristics and laboratory values were all recorded. For these procedures, patient files, forms created for patient follow-up, hospital system and anamnesis taken from patients were used.

The laboratory values of the patients were recorded at the beginning of the treatment, 12 weeks after the end of the treatment, and 24 weeks after the end of the treatment.

APRI score (AST-platelet ratio index): It is a non-invasive serological marker showing the degree of liver fibrosis and was confirmed and suggested by Wai et al.[6] in 2003.

Formula: [AST (IU/L)/AST upper limit of normal (IU/L)]/platelet count (10^9/L)×100.[5]

FIB-4 index: Another confirmed noninvasive serological measurement.

Formula: Age (years)×AST (IU/L)/platelet count (10^9/L)×√ALT (IU/L).[5]

These two scores have been able to reliably identify liver fibrosis in many studies.[5]

Statistical Analysis

The data in the study were evaluated using the SPSS 22.0 statistics package program. The compatibility of the data to normal distribution was evaluated by Kolmogorov-Smirnov analysis. It was seen that the data were suitable for normal distribution. Numerical variables that fit the normal distribution are presented with mean (SD), and categorical variables are presented with percentage (%). In comparison of the differences between groups, the comparison of independent groups proportions of categorical variables was performed by chi-square and FisherExact tests, and comparison of dependent group proportions was performed by McNemar test. In comparison of the means of two groups independent of numerical variables, those that fit the normal distribution were evaluated using Student's t-test. One-way analysis of variance (OneWay: ANOVA) was used to compare more than two independent sample means.

A P value less than 0.05 P was considered statistically significant, and values with p=0.000 were given as P<0.001.

RESULTS

The mean age of 75 patients who received DAA was 51.4 (15.8) years, and 51 (68%) of these patients were male. The most common comorbidity in patients was hypertension (HT) with 27 patients (36%). It was followed by chronic renal failure (22.6%), diabetes mellitus (14.6%), coronary artery disease (10.6%), renal transplantation (6.6%), chronic obstructive pulmonary disease (6.6%), liver cirrhosis (5.3%), HCC (4%), non-HCC malignancies (4%), and chronic hepatitis B (CHB) infection (2.6%).

There was no additional disease in 29 (38.6%) patients. The most common reason among the sources of HCV transmission in the patients was the history of surgical operation. It was detected in 20 (26.7%) patients. The second risk factor was dialysis with 17 (22.7%) patients. Intravenous (IV) drug addiction in 16 (21.3%) patients, dental treatment in 7 (9.3%) patients, being

imprisoned in 4 (5.3%) patients, blood transfusion in 3 (4%) patients, 2 (2.7%) patients tattoo/piercing has been identified other risk factors. No risk factor could be identified in 6 (8%) patients.

Eight (10.7%) patients were genotype 1a, 50 (66.7%) genotype 1b, and 17 (22.7%) genotype 3. Twenty-three of the patients (30.7%) had at least one prior non-DAA treatment experience. Treatment regimens were applied as follows; Paritaprevir + Ritonavir + Ombitasvir + Dasabuvir (ProD) in 30 patients, Ledipasvir/Sofosbuvir (LED/SOF) in 23 patients, Glecaprevir + Pibrentasvir (GLE/PIB) in 16 patients, and Sofosbuvir + Ribavirin (SOF/RIB) in 6 patients. (**Table 1**).

The laboratory median values of the patients at the beginning of the treatment, 12 weeks after the end of the treatment and 24 weeks after the end of the treatment are shown in **Table 2**. Accordingly, the changes in AST, ALT, total bilirubin, ALP, GGT, LDH, AFP, albumin, thrombocyte, white blood cell, INR, PTT values were found to be statistically significantly different when compared to the values 12 and 24 weeks after the end of the treatment, according to the beginning of the treatment. The SVR rates of the patients at 12 and 24 weeks were 100%, and HCV-RNA was not detected in these months. When the patients' APRI and FIB-4 scores at the beginning of the treatment were compared with the values 12 and 24 weeks after the end of the treatment, there was a statistically significant decrease (**Table 3**).

Table 1. Demographic and clinical data of the patients

	n (%)
Age [Mean (SD)]	51.4(15.8)
Gender	
Male	51 (68%)
Female	24 (32%)
Comorbidity	
Metabolic syndrome (HT, DM,CAD)	46 (61.3%)
COPD	5 (6.7%)
Renal comorbidities (CRF, RT)	22 (29.3%)
Hepatic comorbidities (cirrhosis, HCC, CHB)	9 (12%)
Malignite (not HCC)	3 (4%)
None	29 (38.7%)
Treatment Received	
LED/SOF	23 (30.7%)
PrOD	30 (40%)
SOF+RIB	6 (8%)
GLE/PIB	16 (21.3%)
Risk Factor	
Operation	20 (26.7%)
Dialysis	17 (22.7%)
Iv Drugs	16 (21.3%)
Dental treatment	7 (9.3%)
Tattoo/piercing	2 (2.7%)
Prisoner	4 (5.3%)
Blood transfusion	3 (4%)
Unknown	6 (8%)
Genotype	
1a	8 (10.7%)
1b	50 (66.7%)
3	17 (22.6%)
Previous Treatment	
Naive	52 (69.3 %)
Experienced	23 (30.7%)

HT: Hypertension, DM: Diabetes mellitus, CAD: Coronary artery disease, COPD: Chronic obstructive pulmonary disease, CRF: Chronic renal failure, RT:Renal Transplantation, HCC: Hepatocellular carcinoma, CHB: Chronic hepatitis b, LED/SOF: Ledipasvir/Sofosbuvir, ProD: Paritaprevir + Ritonavir + Ombitasvir + Dasabuvir, SOF+RIB:Sofosbuvir +Ribavirin, GLE/PIB: Glecaprevir +Pibrentasvir, IV: Intravenous.

Table 2. Changes of laboratory tests at baseline and SVR12, and SVR24

	Baseline Median (min-max)	At 3th month Median (min-max)	At 6th month Median (min-max)	p
ALT (U/L)	45 (13.4-171)	20 (7-130)	15 (2-83)	<.001
AST (U/L)	31 (11.8-110)	20 (7-106)	16 (5-85)	
T. bil (mg/dl)	0.6 (0.2-4)	0.47 (0.15-4.2)	0.4 (0.18-5)	<.001
ALP (U/L)	96 (41-240)	86 (42-380)	82 (38-355)	<.001
GGT (U/L)	42 (6-580)	30 (6-400)	25 (6-350)	<.001
LDH (U/L)	110 (10-325)	110 (20-375)	130 (23-345)	
AFP (U/ml)	3.7 (0.5-76.91)	2.8 (0.5-72)	2.5 (0.3-65)	<.001
Albumin (g/dl)	4.1 (2.9-5)	4.2 (3.2-5.2)	4.3 (3.2-5.3)	<.001
Creatine (mg/dl)	0.85 (0.3-8.5)	0.8 (0.4-7.5)	0.82 (0.45-9.2)	.092
Platelets ($\times10^3$/ml)	185(35-368)	220 (45-400)	235 (75-485)	<.001
Leukocyte ($\times10^3$/ml)	7.1 (2.27-10.7)	7.2 (3.35-11.4)	6.91 (3.6-12.4)	<.001
Hemoglobin (g/dl)	14.3 (8.5-17.4)	14.1 (8-17.1)	14.2 (8.2-17)	.638
INR	1.08 (0.84-1.54)	1.01 (0.8-1.24)	0.95 (0.75-1.12)	<.001
PTT (second)	33.1 (22.9-51.5)	30.8 (26.2-41.4)	29.7 (26.2-41.4)	<.001
HCV-RNA level ($\times10^3$ IU/ml)	359.181(3.38-26502)	0	0	<.001

AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, T.Bil: Total bilirubin, ALP: Alkaline phosphatase, GGT: Gamma glutamyl transferase, LDH: Lactate dehydrogenase, AFP: Alpha fetoprotein, INR: "international normalized ratio", PTT: partial thromboplastin time, HCV-RNA: Hepatitis C virus ribonucleic acid.

Table 3. Changes in liver fibrosis scores following anti-viral treatment regimens

	Baseline Median (min-max)	At 3th month Median (min-max)	At 6th month Median (min-max)	p
APRI	0.00045 (0.00014-0.00529)	0.000245665 (0.0000875-0.002722222)	0.00017 (0.00011-0.00152)	<.001
FIB-4	0.00126 (0.00021-0.01442)	0.001043 (0.000219-0.00878)	0.000841(0.000212-0.004732)	<.001

APRI: "aspartate aminotransferase to platelet ratio index", FIB-4: "Fibrosis-4"

DISCUSSION

The primary goal in the treatment of CHC is to avert the development of fibrosis in the liver and to reverse the hepatic and extrahepatic effects of HCV.[7] Unlike previous treatments, direct-acting antivirals have higher efficacy and better safety profile. Similar studies with different genotypes and different regimens have reported a sustained virological response (SVR) of 90% to 100% about direct-acting antivirals. In this study, SVR12 and SVR24 were detected as 100% and were higher than many reported rates.[8,9]

HCV genotype determination is very important in terms of determining the antiviral treatment option, adjusting the treatment dose, determining the treatment time and treatment answer. The predominant genotype in our study was genotype 1b. Also; genotype 3 and genotype 1a were followed up. The findings were similar to other studies conducted in our country.[10,11]

The transmission route of HCV differs according to the development level of the countries. While IV drug use is the most important mode of transmission in developed countries, unsafe injections and medical procedures are the most important transmission route of HCV infection in developing countries.[12] In the study conducted by Karaca et al.[13] with 320 HCV patients, it was reported that 98% of the patients had a history of surgical operation. In this study, it was seen that the commonly risk factor in patients was the past of surgical operation with a rate of 26.6%.

Transmission via blood transfusion was the best defined route in HCV patients and was the most common cause of transmission in previous years. Transfusion-associated HCV infection has decreased due to routine testing of HCV antibodies in blood banks since 1990, and it has become one of the least common transmission routes today.[12] As revealed in our study, one of the least common modes of HCV transmission is a history of transfusion, and it was seen only in 3 patients.

The prevalence of HCV in patients with failured renal function is advanced than the general population (9.5%).[6] HCV can directly lead to diseases that cause kidney damage such as membranoproliferative glomerulonephritis and cryoglobulinemia.[14] In addition, procedures such as dialysis and blood transfusion increase the risk of HCV transmission in patients with CRF.[14] In our study, we attribute CRF being the most common comorbidity after HT to these reasons. In addition, 5 of our patients had renal transplantation.

Histologic changes in the liver affect the prognosis in CHC infection and the relationship between virologic clearance and histologic improvement needs to be investigated.[3] Due to the invasive procedure of liver biopsy, many noninvasive fibrosis markers have been enhanced to assess the stage of liver fibrosis.[15] APRI and FIB-4 are widely used among these and are known to be safe in predicting fibrosis in CHC patients before treatment.[16,17]

Non-invasive measures to assess fibrosis have been widely implemented in CHC patients after successful treatment with DAA, and many studies have shown reduced liver fibrosis and inflammation after treatment with DAA.[18,19] Huang et al.[20] stated that APRI and FIB-4 indices could be used to determine fibrous regression in the liver after DAA. In a study of 392 HCV patients receiving DAA, the median LSM (liver stiffness measure=liver stiffness measure) decreased from 12.7 kPa to 8.6 ($P<0.001$) after SVR, while a significant decrease was also observed in APRI and FIB-4 values.[19] In another study, it was shown that the APRI and FIB-4 indices showed a rapid and continuous decrease at the end of the treatment and at the 12th week after the treatment.[21] Similarly, in this study, there was a significant reduction in APRI and FIB-4 values at 12- and 24-weeks post-treatment in all treatment groups. Upon completion of the therapeutic regimen, it was concluded that the diminution in parameters could be attributed to the accelerated decline in AST and ALT concentrations as a result of amelioration in necrotic inflammation.

In individuals suffering from chronic hepatitis C, platelet count is often influenced by factors such as liver fibrosis, necrotic inflammation of the liver, and insufficiency of thrombopoietin.[22] As platelets move through the liver that has been damaged, they come into contact with the endothelium of the hepatic sinusoids and gather cells and proteins that are involved in the healing process.[23] This interaction leads to a cycle of platelet and white blood cell buildup, which ultimately causes harm to the liver cells.[24] In previous studies, hepatic necroinflammatory activity has been shown to be associated with low platelet counts in CHC patients.[25] This study revealed that the decline in hepatic necroinflammation was associated with increased platelet count at 12- and 24-weeks post-treatment. This result is consistent with some studies reporting an increase in platelet count 12 and 24 weeks after DAA treatment.[26,27] Giannini et al.[28] revealed that the advancing decrease in liver functions in patients with CHC was associated with decreased thrombopoietin. The rise in thrombopoietin production and the subsequent decrease in necrotic inflammation may be the reason for the increased platelet count seen in our study.

In a study comparing biopsies of 40 patients before and 24 weeks after SVR, fibrosis healing was noticed in 38% of patients. However, prominent reductions in LSM, APRI, and FIB-4 were also seen in patients without fibrosis. In 83% of the patients, regression in inflammation was detected.[20] These findings support the conclusion that the rapid decrease in noninvasive measurements observed as early as 24 weeks in our study is mainly due to the improvement in necroinflammation. It has also been confirmed in many other studies that the early decrease in fibrous indices results from the regression in necroinflammation.[21]

In another study comparing long-term liver biopsies, liver biopsies performed 5 years after SVR were compared with APRI and FIB-4 scores. Compared with the first biopsy, significantly lower histological fibrosis was detected from the second biopsy performed 5 years later ($p<0.0001$), confirming the ability of noninvasive indices such as APRI and FIB-4 to predict liver fibrosis.[5] However, this study was conducted with other antiviral treatments used before DAA, and the number of studies showing long-term results of DAAs is extremely limited. The present study leads us to the conclusion that noninvasive fibrous indices calculated in the long term have a stronger correlation with fibrosis and better reflect fibrosis. These results are important in terms of knowing what noninvasive tests reflect at what stage. Because the availability of noninvasive fibrosis measurements together with the high SVR rates developed by the use of DAAs has changed the opinion in the management of CHC infection. In the near future, there will be a dramatic increase in the number of patients reaching SVR, and the need for continuous and non-invasive testing of liver fibrosis status will emerge. Because many studies have reported that the risk of HCC development continues even after HCV eradication and that an important risk factor for HCC development is residual liver fibrosis.[29,30]

Limitations

There are some limitations in the study. Firstly, no study examining the relationship between temporary changes in non-invasive fibrous indicators during and after DAA treatment has been conducted using histological methods. In addition, due to the invasive structure of liver biopsy, there are limitations such as sampling failure, intra-observer and interobserver histological examination diversities, and risk of various complications (pain, bleeding…). Second, this study was managed in only-one center and the number of patients was limited. Our third limitation was that the design of our study was retrospective. Our last limitation was the collection of patient data up to SVR24. Additional research is needed to see the long-period effects of DAAs.

CONCLUSION

In the study, 100% SVR was achieved in patients 12 and 24 weeks after the end of DAA treatment. This rapid response was consistent with the regression of confirmed fibrous scores APRI and FIB-4. The sudden decline in APRI and FIB-4 values may primarily be caused by the decrease in necrotic inflammation. In patients with CHC identified after SVR, more research is required to clarify the link between noninvasive index and fibrosis stage.

In order to see the long-term impacts of DAAs and long-term alterations in liver histology, prospective studies with larger patient numbers are needed many years after SVR. In addition, large-scale and long-term follow-up studies are required to determine the prognosis and management strategies of patients with CHC infection after achieving by effective treatment viral eradication.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Van Training and Research Hospital Ethics Committee (Date: 18.02.2021, Decision No: 2021/04).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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