ORIGINAL ARTICLE

DYNAMICS OF FIBROTIC CHANGES IN THE LIVER AFTER THE SUCCESSFUL ERADICATION OF HEPATITIS C VIRUS IN PATIENTS WITH NAFLD

DOI: 10.36740/WLek202210113

Mariya A. Derbak[1], Nataliya V. Lizanets[1], Oksana T. Hanych[1], Olesya M. Horlenko[1], Hanna Y. Mashura[1], Serhii O. Boiko[1], Nad'a Rozumyková[2]

[1]UZHHOROD NATIONAL UNIVERSITY, UZHHOROD, UKRAINE

[2]SLOVAK MEDICAL UNIVERSITY IN BRATISLAVA, BRATISLAVA, SLOVAKIA

ABSTRACT

The aim: To assess the dynamics of serum levels of angiopoietin-2 and transforming growth factor-β1 in patients with chronic hepatitis C (CHC) with concomitant nonalcoholic fatty liver disease (NAFLD) after successful DAAs.

Materials and methods: 82 patients with CHC were examined, of which 56 were diagnosed with NAFLD and increased body weight. Ang-2, TGF-β1, leptin, adiponectin, and the degree of liver fibrosis were determined for all participants. The patients were divided into groups: 1 gr. (n=23) – CHC + increased body weight + hepatic steatosis, 2 gr. (n=33) – CHC + increased body weight + nonalcoholic steatohepatitis, 3rd gr. (n=26) – CHC. All patients received DAAs for 12 weeks.

Results: From 82 patients F_{3-4} had 31 people, F_{1-2} – 25, F_{0-1} – 11, F_0 -15 patients. F_{3-4} and steatosis S2-3 ($p<0.05$) was more common in patients of 2 gr.. Serum Ang-2 levels were higher ($p<0.05$) in patients of 2 gr. with F_{3-4}, than in patients with F_{0-2}. Fibrosis regression occurred more often in patients with 1 and 3 gr. with F_{1-2} than in patients 2 gr. and F_{3-4} and was accompanied by a decrease in Ang-2 and TGF-β1 levels.

Conclusions: High levels of Ang-2 and TGF-β1 are registered in patients with CHC+NAFLD, which correlate with the degree of liver fibrosis and significantly decrease after successful DAAs in patients with low initial stages of liver fibrosis and normal body weight.

KEY WORDS: chronic hepatitis C, NAFLD, increased body weight, fibrosis, regression, angiopoietin-2, transforming growth factor-β1

Wiad Lek. 2022;75(10):2392-2396

INTRODUCTION

In recent years, the treatment of chronic hepatitis C (HCV) has undergone significant progress [1]. Direct-acting antiviral agents (DAAs) have made a breakthrough in the treatment of CHC with the possibility of sustained virological response (SVR) in more than 95% of patients [2,3]. Most patients who achieve SVR have a reduction in the degree of liver fibrosis and have a lower risk of developing hepatocellular carcinoma (HCC). However, some patients may experience progression of liver fibrosis and/or development of HCC after successful HSV eradication with DAAs. [4]. Long-term persistence of HCV in the liver tissue, followed by inflammation, leads to angiogenesis, fibrosis, cirrhosis and HCC [5]. The rate of progression of fibrosis has been linked to various factors, in particular other liver diseases, including alcoholic and nonalcoholic fatty liver disease (NAFLD), nonalcoholic steatohepatitis (NASH), obesity, hepatitis B virus infection, and HIV infection. The process of fibrogenesis in CHC is an appropriate reaction of the body to liver damage, which is initiated and maintained by a chronic inflammatory process [6, 7]. Cytokines are involved in the regulation of the development of the inflammatory reaction of liver tissue, apoptosis and necrosis of liver cells, the development of cholestasis and fibrosis [8]. The progression of liver fibrosis is accompanied by angiogenesis, regardless of the etiology of the liver disease [9,10]. One of the key factors in the development of fibrogenesis is transforming growth factor β (TGF-β1), which is the main profibrogenic cytokine that promotes the activation of liver myofibroblasts [11]. TGF-β also has proangiogenic ability and regulates the differentiation, proliferation and migration of pericytes [12]. Angiogenesis in liver damage is associated with increased levels of vascular endothelial growth factor (VEGF) and angiopoietins [13]. Angiopoietins are a group of vascular growth factors, and the most well-studied are angiopoietin-1 (Ang-1) and angiopoietin-2 (Ang-2) [14]. Ang-1 helps maintain the vasculature by acting as an endothelial preservation factor. Ang-2 is a biological antagonist of Ang-1, which is highly expressed at sites of vascular remodeling. It reduces vascular stability and makes VEGF more accessible to endothelial cells [15]. Prediction of fibrotic changes in the liver after HCV treatment is a crucial clinical problem [16].

Since the issue of NAFLD progression in CHC patients who have completed treatment with DAAs has not been definitively studied, and the data of scientific studies are

contradictory, this determined the relevance of our research.

THE AIM

To assess the dynamics of serum levels of angiopoietin-2 (Ang-2) and transforming growth factor-β1 (TGF-β1) in patients with chronic hepatitis C (CHC) with concomitant nonalcoholic fatty liver disease (NAFLD) after successful DAAs.

MATERIALS AND METHODS

The study was conducted at the Department of Faculty Therapy with the consent of the patients, and the methodology was in accordance with the Declaration of Helsinki of 1975 and its revision of 1983. The study was approved by the local ethics committee (protocol No. 6/2 dated 09/07/2021), and the participants read and signed the consent form , the structure of which corresponded to the officially accepted one.

Criteria for inclusion in the study: patients with a verified diagnosis of CHC genotype 1b with and without NAFLD, who agreed to follow-up.

Criteria for the exclusion of patients from the study: the presence of markers of infection with other hepatitis viruses (A, B, D), markers of autoimmune hepatitis/cross syndrome (anti-LKM-1, anti-SLA and anti-LC-1) and HIV infection, use of corticosteroids, nonsteroidal anti-inflammatory and immunosuppressive drugs, and the patient's decision to stop participating in the study.

Taking into account the specified criteria, only 82 patients with a verified diagnosis of CHC were included in the study, of which 56 had CHC combined with NAFLD and 26 patients with CHC without NAFLD. There were 53.7% (44) men, 46.3% (38) women. The average age of patients is 58.5±1.5 years. All patients received specific antiviral therapy: sofosbuvir 400 mg + daclatasvir 60 mg once a day for 12 weeks. The control group (n=25, average age 33.2±1.5 years) consisted of practically healthy individuals.

The diagnosis of HCV was established according to the International Classification of Diseases of the 10th revision and confirmed by the detection of RNA-HCV in the blood of patients by the method of polymerase chain reaction (PCR) in real time (RT-PCR) with determination of viral load and genotyping. The degree of activity of the pathological process was determined by the level of increased activity of ALT, according to the international classification of liver diseases (Los Angeles, 1994). The diagnosis of NAFLD was established according to the unified clinical protocol "Non-alcoholic steatohepatitis" (2014) and the adapted evidence-based clinical guideline "Non-alcoholic fatty liver disease" (2012), according to the recommendations of the European Association for the Study of the Liver (EASL).

In the work, Enzyme-Immuno-Sorbent-Assay (ELISA) was used to determine the levels of serum Angiopoietin-2 (Ang-2), transforming growth factor – β1 (TGF-β1), and leptin and adiponectin, according to the instructions attached to the kits Diagnostics Biochem Canada and DRG (USA) reagents. Indicators of biochemical blood analysis – total bilirubin, total protein, activity of serum cytolytic enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST), activity of cholestatic enzymes alkaline phosphatase (ALP) and γ-glutamyl transpeptidase (GGT) were carried out in certified laboratories ("Dila" and "Synevo").

The degree of fibrosis and steatosis of the liver was determined by a non-invasive diagnostic method – FibroMax, which includes: FibroTest, ActiTest, SteatoTest, AshTest, NashTest and is carried out by BioPredictive (Paris, France) in commercial laboratories "Dila" and "Synevo" (BioPredictive, Paris). All participants underwent abdominal ultrasound and the amount of HCV RNA, angiopoietin-2, TGF-β1, leptin, and adiponectin was determined. The degree of liver fibrosis was assessed before and 24 and 48 weeks after the end of treatment. Body mass index (BMI) was determined for all patients. A BMI of 18.5-24.9 kg/m2 was considered normal body weight. A BMI > 24.9 kg/m2 was considered overweight.

The analysis and processing of the results of the examination of patients was carried out with the help of the Statistics for Windows v.7.0 computer program (StatSoft Inc, USA) using parametric and non-parametric methods for evaluating the obtained results. The difference was considered to be significant at $p<0.05$.

RESULTS

82 patients with CHC were examined, of which 56 (68,3%) were diagnosed with various degrees of NAFLD and increased body weight. According to the set tasks, patients were divided into groups: 1 gr. (n=23) – CHC + increased body weight + hepatic steatosis, 2 gr. (n=33) – CHC + increased body weight + nonalcoholic steatohepatitis, 3rd gr. (n=26) – CHC + normal body weight. The groups were representative by age and gender. All patients received DAAs for 12 weeks.

Of the 82 patients, 31 (37.8%) had progressive liver fibrosis (F_{3-4}), 25 (30.5%) had moderate fibrosis (F1-2), 11 (13.4%) had minimal fibrosis (F_{0-1}), and 15 people (18.3%) did not have fibrosis (F_0). Comparing groups of patients, it should be noted that progressive liver fibrosis F_{3-4} is more often registered in patients with CHC combined with NASH. It was also established that patients of group 2 (CHC+NASH) significantly more often than patients of groups 1 and 3 had pronounced steatosis of the liver S 2-3 (45.5% versus 30.4% and 3.9% of patients; $p< 0.05$) (Table I).

During the study of serum level of TGF-β1 in patients with CHC, an elevated level was found in 60 (73.2%) patients, a decreased level in 8 (9.8%) and a normal level in 14 (17.0%) patients. Serum Ang-2 levels were significantly higher in patients with CHC + NASH + increased body weight than in patients with CHC + steatosis + increased body weight and CHC without steatosis (by 1.8 and 2.3 times, respectively; $p<0$,05). It should be noted that se-

Table I. Data of the non-invasive FIBROMAX method before treatment

Indicator		Groups of patients		
		1	2	3
		CHC + increased body weight + hepatic steatosis (n=23) abs/%	CHC + increased body weight + NASH (n=33) abs/%	CHC + normal body weight (n=26) abs/%
Fibrosis degrees FibroTest	F_0 (n=15)	3/13.0	5/15.2	7/26.9
	F_{0-1} (n=11)	3/13.0	3/9.1	5/19.2
	F_2 (n=25)	10/43.5	9/27.2	6/23.1
	F_{3-4} (n=31)	7/30.4	16/48.5*	8/30.8
Activity of the necro-inflammatory process ActiTest	A_0 (n=19)	6/26,1	5/15.2	8/30.8
	A_1 (n=20)	7/30.4	7/21.2	6/23.1
	A_2 (n=23)	6/26.1	10/30.3	7/26.9
	A_3 (n=20)	4/17.4	11/33.3*	5/19.2
Steatosis degrees SteatoTest	S_{0-1} (n=37)	10/34.8	7/21.2	20/76.9
	$S_{>1}$ (n=22)	6/26.1	11/33.3	5/19.2
	$S_{>2}$ (n=23)	7/30.4	15/45.5*	1/3.9
Inflammation in metabolic disorders NashTest	N_0	0	0	0
	N_1 (n=17)	0	17/51.5	0
	N_2 (n=16)	0	16/48.5	0

Notes: * – significant difference in degrees of fibrosis, steatosis, and inflammation between groups (p<0,05)

Table II. Levels of fibrogenesis and angiogenesis cytokines in the examined patients

Group	Indicator			
	TGF-β1, pg/ml	Ang-2, pg/ml	Leptin (ng/ml)	Adiponectin (mcg/ml)
1 (n=23)	224,3±36,3*	325,4±22,5*	19,4±1,7*‥	52,4±12,3*‥
2 (n=33)	457,2±28,7*‥	573,2±45,8*‥	27,5±2,2*‥	30,5±2,7*‥
3 (n=26)	176,2±15,3*	254,6±27,5*	12,3±1,5	68,5±7,2
Control (n=20)	134,0±14,5	128,5,0±5,2	6,5±0,7	77,4±9,5

Note. Significance of the difference: * – with the control group; •• – with group 3 (the indicator was calculated according to the Mann-Whitney test, p<0.05).

rum levels of Ang-2 were significantly higher (p<0.05) in patients with CHC + NASH with BMI > 24.9 kg/m2 and progressive degrees of fibrosis (F_{3-4}), than in patients with a degree of fibrosis to F_2 and normal body weight.

The average values of Ang-2 and TGF-β1 were increased in all CHC patients with the highest values in group 2, which was significantly (p<0.01) different from the corresponding values of patients in groups 1 and 3. IAs blood leptin levels increased, the degree of hepatic steatosis increased, corresponding to higher levels of Ang-2. Serum levels of leptin and adiponectin were significantly higher in patients with CHC + NAFLD than in patients with CHC. The concentration of leptin in patients with CHC + NASH was increased by 4.2 times, and in patients with CHC with simple steatosis – by 2.9 times, compared to the control group. At the same time, the serum level of adiponectin in patients of groups 2 and 1 was reduced by 2.5 times and 1.4 times, respectively, in comparison with patients of group 3 (Table II).

Based on the results of Fibromax, 24 weeks after the end of successful antiviral therapy (DAAs), regression of liver fibrosis stages was registered in 65.6% (54/82) of patients. Liver fibrosis regression was defined as a decrease in fibrosis by more than one stage for patients with data from F_2 to F_4, and for patients with liver fibrosis F_{0-1}, if the stage of liver fibrosis did not worsen.

DISCUSSION

12 weeks after the successful elimination of the hepatitis C virus, 97.6% (80/82) of patients with NAFLD had normalization of markers of cytolysis (ALT, AST) and cholestasis (bilirubin, ALP, GGT). Similar data were obtained by a group of scientists led by Van der Meer AJ [17], who found that ALT, AST and alkaline phosphatase significantly decreased 12 weeks after successful treatment with DAAs.

24 weeks after HCV eradication, a significant decrease in the activity of the necroinflammatory process was estab-

lished in 96.3% (79/82) of patients. It was found that the regression of liver fibrosis was significantly more frequent ($p<0.001$) in patients with CHC without steatosis and with lower stages of fibrosis before treatment than in patients with CHC combined with steatosis or NASH.

The study of the dynamics of the Ang-2 level in patients with CHC after treatment with DAAs showed a significant decrease (value $p<0.001$) in persons who underwent regression of liver fibrosis, and a slight decrease in patients without regression of fibrosis (value $p=0.072$). These data overlap with the data obtained by Makhlouf MM. etc. [18]. In patients with CHC + NASH + increased body weight who had higher initial stages of liver fibrosis, serum Ang-2 levels did not decrease significantly after treatment with DAAs, and no regression of liver fibrosis was registered. Our results are consistent with the data of Osawa et al., who found a significant decrease in Ang-2 among F_{0-3} patients ($p <0.001$), and a slight decrease (p 0.136) in F_4 patients [19]. Similar data were obtained by Lefere et al. [20] who found that serum Ang-2 levels are significantly higher in patients with NASH than in patients with simple hepatic steatosis but without CHC.

A positive correlation of leptin and Ang-2 values ($r=0.49$; $p<0.05$) with the degree of fatty infiltration of the liver was revealed, while a similar relationship between the adiponectin/leptin ratio was negative ($r=-0.34$; $p <0.05$). As blood leptin levels increased and the adiponectin/leptin ratio decreased, the degree of hepatic steatosis increased, corresponding to higher Ang-2 levels. It is obvious that angiogenesis is influenced by cytokines involved in the formation of NAFLD. Leptin is an adipokine that regulates satiety, has a key role in obesity and stimulates angiogenesis [21].

In CHC patients with low stages of fibrosis ($\leq F_2$) after treatment, a significant decrease in TGF-β1 levels was registered against the background of regression of fibrosis and a decrease in the necroinflammatory activity of the process according to Fibromax data. However, in 10 out of 16 (62.5%) patients with CHC+NASH with fibrosis stage F_{3-4} after successful DAAs, regression of liver fibrosis was not registered, and TGF-β1 levels remained high, which confirms the involvement of TGF-β1 in fibrogenesis. A high level of TGF-β1 in patients with CHC is associated with the risk of developing liver cirrhosis and hepatocellular carcinoma, as reported by Radwan M.I. et al. [22].

CONCLUSIONS

1. High serum levels of angiopoietin-2 and transforming growth factor – β1 are registered in patients with CHC+NAFLD, which correlate positively with the stage of fatty infiltration of the liver and the degree of liver fibrosis.
2. In patients with CHC + NAFLD, a decrease in the levels of angiopoietin-2 and transforming growth factor – β1 after successful eradication of HCV and low stages of liver fibrosis before treatment are predictors of regression of liver fibrosis.
3. Increased body weight, high leptin level, higher initial degree of liver fibrosis before treatment can be negative predictors of regression of liver fibrosis in patients with CHC + NAFLD after successful DAAs, which must be taken into account during further observation of such patients.
4. High serum levels of angiopoietin-2 and transforming growth factor-β1 may be an important biomarker of liver angiogenesis and fibrogenesis in patients with CHC on the background of NAFLD.

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ORCID and contributionship:
Mariya A. Derbak: 0000-0003-4791-4080 [A,E,F]
Nataliya V. Lizanets:0000-0003-3560-195X [B,D,F]
Oksana T. Hanych: 0000-0001-8213-1829 [B]
Olesya M. Horlenko: 0000-0002-2210-5503 [E,F]
Hanna Y. Mashura: 0000-0002-0299-0349 [C]
Serhii O. Boiko: 0000-0002-8073-3030 [E,F]
Nad'a Rozumyková: 0000-0003-1420-5712 [E,F]

Conflict of interest:
The Authors declare no conflict of interest.

CORRESPONDING AUTHOR
Mariya A. Derbak
Uzhhorod National University
20 Hryboiedova St., 88000 Uzhhorod, Ukraine
tel: +380506275075
e-mail: morika1415@gmail.com

Received: 15.04.2022
Accepted: 03.09.2022

A – Work concept and design, **B** – Data collection and analysis, **C** – Responsibility for statistical analysis, **D** – Writing the article, **E** – Critical review, **F** – Final approval of the article