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Dynamics of TGF-1β and MMP-9 content in the serum of patients with chronic hepatitis C infected with HCV GT1 on the background of antiviral treatment depending on the severity of liver fibrosis

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A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of the article

The aim: to analyse the dynamics of TGF-1β and MMP-9 in the blood serum of patients with chronic hepatitis C (HCV) infected with HCV GT1 on the background of antiviral treatment depending on the severity of liver fibrosis.

Materials and methods. The study included 92 patients with GT1 HCV infection treated with antiviral therapy (AVT) – OBV / PTV / r+DSV±RBV. The severity of liver fibrosis was determined by elastometry. The content of TGF-1β (Elabscience, USA) and MMP-9 (Elabscience, USA) in the blood serum was determined by ELISA.

Results. In patients with CHC GT1 before the start of AVT, profibrogenic potential prevailed: increased TGF-1β ($p < 0.05$), decreased MMP-9 ($p < 0.05$) and a higher TGF-1β / MMP-9 ratio ($p < 0.05$). AVT (OBV / PTV / r+DSV±RBV) slows down fibrogenesis and activates antifibrotic processes, which is confirmed by an increase in MMP-9 ($p < 0.05$) and a decrease in TGF-1β / MMP-9 ($p < 0.05$) compared to the pre-treatment values. Prior to AVT, patients with GT1 HCV with liver fibrosis F 0–2 had lower MMP-9 levels ($p < 0.05$) than healthy subjects, with no changes in TGF-1β and TGF-1β / MMP-9 ratio ($p > 0.05$). In patients with F 3–4 liver fibrosis, serum TGF-1β and the TGF-1β / MMP-9 ratio were higher, and MMP-9 was lower, both compared with healthy subjects ($p < 0.05$) and compared with patients with F 0–2 ($p < 0.05$). In patients with F 0–2, at the time of completion of AVT, the studied parameters do not differ from healthy individuals ($p > 0.05$). In patients with F 3–4 at the time of completion of AVT, the TGF-1β / MMP-9 ratio remains higher both in comparison with healthy individuals ($p < 0.05$) and in comparison with patients with CHC GT1 with liver fibrosis F 0–2 ($p < 0.05$).

Conclusions. We demonstrated more significant dynamics of antifibrotic changes during direct-acting antiviral agents based AVT in patients with GT1 CHC in the presence of F 0–2 liver fibrosis compared to patients with F 3–4.

Keywords: chronic hepatitis C, viral infection, diagnosis, liver fibrosis, cytokines, antiviral therapy, treatment.

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Динаміка вмісту TGF-1β і MMP-9 у сироватці крові пацієнтів із хронічним гепатитом С, інфікованих HCV GT1, на фоні противірусного лікування залежно від ступеня вираженості фіброзу печінки

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Мета роботи – проаналізувати динаміку вмісту TGF-1β і MMP-9 у сироватці крові пацієнтів із хронічним гепатитом С (ХГС), інфікованих HCV GT1, на фоні противірусного лікування залежно від ступеня вираженості фіброзу печінки.

Матеріали і методи. Обстежили 92 хворих на ХГС GT1, які отримували противірусну терапію (ПВТ) – OBV / PTV / r+DSV±RBV). Ступінь вираженості фіброзу печінки визначено методом еластометрії. Методом ІФА визначено вміст TGF-1β (Elabscience, США) та MMP-9 (Elabscience, США) у сироватці крові.

Результати. У хворих на ХГС GT1 до початку ПВТ визначено превалювання профіброгенного потенціалу: підвищення TGF-1β ($p < 0,05$), зниження MMP-9 ($p < 0,05$) і вищий коефіцієнт TGF-1β / MMP-9 ($p < 0,05$). ПВТ (OBV / PTV / r+DSV±RBV) сприяє уповільненню процесів фіброгенезу й активації антифібротичних процесів, що підтверджує підвищення вмісту MMP-9 ($p < 0,05$) та зниження TGF-1β / MMP-9 ($p < 0,05$) порівняно з показниками до початку лікування. До початку ПВТ у хворих на ХГС GT1 із фіброзом печінки F 0–2 вміст MMP-9 нижчий ($p < 0,05$), ніж у здорових осіб, без змін вмісту TGF-1β і коефіцієнта TGF-1β / MMP-9 ($p > 0,05$). У хворих із фіброзом печінки F 3–4 вміст TGF-1β у сироватці крові та коефіцієнт TGF-1β / MMP-9 вищий, а вміст MMP-9 нижчий порівняно і зі здоровими ($p < 0,05$), і з хворими з F 0–2 ($p < 0,05$). У хворих із F 0–2 на час завершення ПВТ досліджені показники не відрізнялися від даних здорових осіб ($p > 0,05$). У хворих із F 3–4 на час завершення ПВТ коефіцієнт TGF-1β / MMP-9 залишається вищим порівняно і зі здоровими ($p < 0,05$), і з хворими на ХГС GT1 з фіброзом печінки F 0–2 ($p < 0,05$).

Висновки. Виявлено більш виражену динаміку антифібротичних змін внаслідок застосування ПВТ, що включала противірусні препарати прямої дії, у хворих на ХГС GT1, які мали фіброз печінки F 0–2, порівняно з пацієнтами з F 3–4.

Ключові слова: хронічний гепатит С, вірусна інфекція, діагностика, фіброз печінки, цитокіни, противірусна терапія, лікування.

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Chronic hepatitis C (CHC) remains a significant healthcare burden in many countries around the world [1]. Prolonged exposure to hepatitis C virus (HCV) provokes the development and progression of liver fibrosis, which leads to the formation of liver cirrhosis and/or hepatocellular carcinoma, which are the main causes of death in these patients [2,3,4]. The highest priority for the treatment of patients with HCV is etiotropic treatment with direct-acting antiviral agents (DAAs), which allows achieving a sustained virological response (SVR) in the majority of treated patients [3]. The possibility of HCV eradication reduces the likelihood of developing severe disease complications [3,5,6].

Currently, there are many antiviral therapy (AVT) regimens using DAAs that have proven to be highly effective in eradicating HCV [3,4,6,7,8]. While the high efficacy of DAAs in eradicating HCV has been proven [3,4,5,6,9], the question of whether fibrotic changes in the liver can regress after DAAs remains open. It is known that AVT with interferon-containing regimens had, in addition to antiviral effect, a certain impact on the regression of liver fibrosis due to immunomodulatory effect [10,11].

For example, study [10] compared histological changes in the liver before and after combined treatment with peg-interferon and ribavirin in patients with CHC. It has been shown that patients with liver fibrosis severity F ≥2 who achieved SVR had a reduced risk of progression of fibrotic changes in the liver, but did not experience a complete regression of existing changes in liver structure [10]. It is assumed that since the formation of liver fibrosis involves both extracellular matrix deposition and its degradation, it may be subject to some regression [11,12]. However, the issue of the antifibrotic effect of AVT is quite controversial and there are currently no convincing data on the regression of liver fibrosis after AVT with DAAs. In the current literature, there are few reports on the assessment of liver fibrosis regression both in experimental models and among patients with liver disease [11,13,14]. At the same time, the accumulation of clinical data on this issue is still ongoing.

It is known that fibrosis is a dynamic process, which, on the one hand, is regulated by the effects of profibrogenic and proinflammatory cytokines, the main of which is transforming growth factor 1β (TGF-1β) [11,12,15,16], and on the other hand, by the effects of antifibrotic cytokines, due to various subpopulations of macrophages that induce the expression of matrix metalloproteinases (MMPs), which ensures the breakdown of the formed collagen [16]. Pro-fibrogenic mediators are produced in response to prolonged damage to epithelial and endothelial cells, inflammatory reactions, and signs of oxidative stress [15].

In the case of prolonged inflammatory factors, as is the case in patients with CHC, activated myofibroblasts deposit an abnormally high amount of proteins in the extracellular space, which leads to fibrotic changes in the liver [15,16,17]. TGF-1β is a regulator of cell differentiation, migration, and proliferation, i. e., it is involved in both the reparative response and fibrosis formation [18]. Overexpression of this cytokine contributes to the formation of fibrotic changes, and prolonged induction of TGF-1β is usually associated with the severity of fibrotic changes, which may predict the degree of fibrosis progression [17,18,19]. At the same time, TGF-1β is released not only through the synthesis of new molecules, but also by activating existing latent forms of the cytokine [18].

Therefore, understanding the balance between the leading mediators of profibrogenic and antifibrotic mechanisms is especially important for assessing the intensity of fibrosis, which is of significant prognostic importance for assessing not only the natural course of CHC, but also for assessing the possible regression of fibrotic changes in the liver after virus eradication.

Aim

To analyse the dynamics of TGF-1β and MMP-9 in the blood serum of patients with chronic hepatitis C infected with HCV GT1 on the background of antiviral treatment depending on the severity of liver fibrosis.

Materials and methods

The study included 92 patients with CHC infected with HCV GT1, all of whom had subtype b. Gender composition: men – 40, women – 52. The patients' age ranged from 27 to 72 years, with a median age of 54.5 [44.5; 61.5] years.

Patients were examined at the Municipal Non-Profit Enterprise "Regional Infectious Disease Clinical Hospital" of the Zaporizhzhia Regional Council. All patients received DAAs under the State Programme in accordance with the Unified Clinical Protocol for Primary, Secondary (Specialised) and Tertiary (Highly Specialised) Medical Care "Viral Hepatitis C in Adults" (Order of the Ministry of Health of Ukraine No. 729 of 18.07.2016). The diagnosis of hepatitis C was etiologically confirmed by the detection of anti-HCV in the blood by enzyme-linked immunosorbent assay and RNA-HCV with virus genotyping by polymerase chain reaction. Patients enrolled in the study received a 12-week 3D regimen (OBV / PTV / r+DSV±RBV) consisting of ombitasvir (OBV), an NS5A protein inhibitor; pariteprevir (PTV), an NS3/4A protease inhibitor; ritonavir (r), which enhances the effects of ATV and dasabuvir (DSV), which is a non-nucleoside inhibitor of HCV RNA-dependent RNA polymerase; and in the presence of severe liver fibrosis, ribavirin (RBV) was added to the regimen [4,6]. According to the Protocol, the response to AVT was assessed by biochemical (ALT activity) and virological (detection of RNA-HCV in the blood) parameters at the time of AVT completion and 12 weeks after AVT.

The severity of liver fibrosis was determined in all patients with CHC GT1 by shear wave elastometry. Depending on the severity of liver fibrosis, patients were divided into groups: 48 patients with liver fibrosis F 0–2 and 44 patients with F 3–4. All patients were examined in the dynamics before the start of AVT, after completion of AVT and 12 weeks after completion of AVT to assess SVR.

On the basis of the Training Medical and Laboratory Center of Zaporizhzhia State Medical and Pharmaceutical University the content of transforming growth factor 1β (TGF-1β) (Elabscience, USA) and metallomatrix proteinase 9 (MMP-9) (Elabscience, USA) in the serum of patients with GT1 CHC and 20 healthy control subjects was studied by enzyme-linked immunosorbent assay. The

Table 1. Dynamics of TGF-1β and MMP-9 content in blood serum and TGF-1β / MMP-9 ratio in patients with CHC GT1 in the dynamics of AVT, Me [Q25; Q75]

Indicator, units of measurement	Healthy people, n = 30	Patients with CHC, n = 92	
		before AVT	at the time of AVT completing
TGF-1β, pg/ml	6.20 [4.90; 7.00]	12.30 [7.03; 15.16] [1]	8.16 [5.16; 12.81][1]
MMP-9, pg/ml	1269.43 [1088.70; 1331.50]	923.69 [627.13; 1117.96] [1]	1259.9 [909.72; 1520.90][2]
TGF-1β / MMP-9	0.005 [0.004; 0.006]	0.013 [0.006; 0.023][1]	0.008 [0.005; 0.013][1,2]

1: difference is significant compared to healthy subjects ($p < 0.05$); **2:** compared to the pre-AVT levels ($p < 0.05$).

TGF-1β / MMP-9 ratio was calculated for each patient with GT1 CHC and healthy subjects.

Statistical processing was performed using Statistica 13 (StatSoft Inc., No. JPZ804I382130ARCN10-J). The results of quantitative parameters are presented as median and interquartile ranges Me [Q25; Q75]. To assess the reliability of differences in quantitative indicators, the Mann–Whitney test was used between independent samples and the Wilcoxon test between dependent samples. The χ^2 test was used to assess differences between qualitative parameters. Correlation analysis (Spearman, gamma) was conducted. Differences at $p < 0.05$ were considered significant.

Results

According to the results of our study, it was found that in patients with CHC GT1 before the start of AVT with the OBV / PTV / r+DSV±RBV regimen, the median content of the profibrogenic cytokine TGF-1β in the patients' serum was higher (2-fold, $p < 0.05$), and the content of the fibrinolytic cytokine MMP-9 in the serum was lower (1.4-fold, $p < 0.05$) compared with healthy subjects.

Before the start of the AVT, the median TGF-1β / MMP-9 ratio, which reflects the processes of fibrogenesis / fibrinolysis, was statistically significantly higher (2.6 times, $p < 0.05$) than in healthy subjects, which confirmed the prevalence of profibrogenic potential. Assessment of the serum levels of these cytokines at the end of AVT showed that TGF-1β had only a downward trend ($p > 0.05$), and MMP-9 levels statistically significantly increased ($p < 0.05$) compared with the corresponding values before the start of therapy. It should be noted that at this stage of observation, a decrease in the median TGF-1β/MMP-9 ratio (1.6 times, $p < 0.05$) was recorded compared with the corresponding value before treatment, but it remained higher ($p < 0.05$) than in healthy subjects.

The positive dynamics in the recovery of indicators reflecting the processes of fibrogenesis / fibrinolysis suggests a slowdown in fibrogenesis and activation of antifibrotic processes *(Table 1)*.

When evaluating the efficacy of AVT in patients with GT1 CHC infection using the OBV / PTV / r+DSV±RBV regimen, it was found that the frequency of achieving SVR12 did not depend ($p > 0.05$) on the severity of liver fibrosis and was high in both study groups. SVR12 was achieved in 45 (93.8 %) patients with liver fibrosis F 0–2 and in 42 (95.5 %) patients with liver fibrosis F 3–4. Accordingly, the absence of virological response to treatment was recorded in 3 (6.3 %) patients with F 0–2 liver fibrosis and 2 (5.4 %) patients with severe F 3–4 liver fibrosis, as evidenced by the detection of HCV-RNA in the blood after completion of the AVT *(Fig. 1)*.

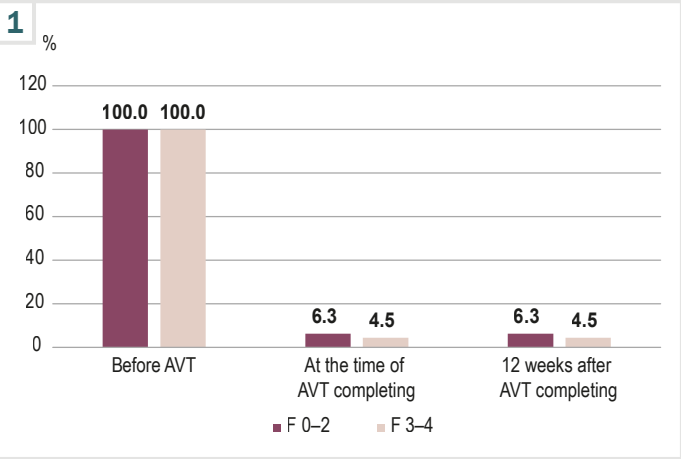

Fig. 1. Frequency of HCV-RNA detection in the blood of patients with CHC GT1 on the background of AVT according to the OBV / PTV / r+DSV±RBV regimen depending on the severity of liver fibrosis.

Evaluation of the biochemical response by alanine aminotransferase (ALT) activity in the blood serum in patients with GT1 HCV during AVT with the OBV / PTV / r+DSV±RBV regimen showed that before the start of AVT, most patients had cytolytic syndrome, but the frequency of detection of elevated serum ALT levels was higher in the group of patients with liver fibrosis F 3–4 compared with patients with F 0–2: 36 (81.8 %) vs. 25 (52.1 %) patients ($\chi^2 = 9.08$, $p < 0.01$). In the dynamics after AVT in patients with CHC GT1, a significant decrease in the proportion of patients with elevated serum ALT activity was noted in both study groups compared with the baseline.

Thus, among patients with F 0–2 liver fibrosis at the time of AVT completion, the number of patients with elevated ALT decreased from 25 (52.1 %) to 7 (14.6 %) patients ($\chi^2 = 15.9$, $p < 0.0001$) at the time of completion of AVT therapy and to 4 (9.1 %) at the time of SVR12 assessment ($\chi^2 = 21.79$, $p < 0.0001$) compared with the pre-AVT. And in the group of patients with F 3–4 liver fibrosis, the number of patients with elevated ALT decreased from 36 (81.8 %) to 6 (13.6 %) patients ($\chi^2 = 40.99$, $p < 0.0001$) at the time of AVT completion and to 4 (8.3 %) at the time of SVR12 assessment ($\chi^2 = 46.93$, $p < 0.0001$) compared with the pre-AVT. At the same time, a comparison of the frequency of elevated serum ALT activity in patients with F 0–2 and F 3–4 liver fibrosis did not reveal a statistically significant difference ($p > 0.05$) both at the time of AVT completion and 12 weeks after completion of AVT *(Fig. 2)*.

Table 2. Dynamics of TGF-1β, MMP-9 content in blood serum and TGF-1β / MMP-9 ratio in CHC patients with GT1 in the dynamics of AVT depending on the stage of liver fibrosis, Me [Q25; Q75]

Indicator, units of measurement	Healthy people, n = 30	CHC patients, n = 92			
		F 0–2, n = 48		F 3–4, n = 44	
		before AVT	at the time of AVT completing	before AVT	at the time of AVT completing
TGF-1β, pg/ml	6.2 [4.9; 7.0]	7.0 [4.5; 8.5]	6.9 [4.1; 10.5]	15.2 [13.4; 18.4][1,3]	9.8 [7.0; 14.8][1,2]
MMP-9, pg/ml	1269.43 [1088.70; 1331.50]	994.03 [753.41; 1151.13][1]	1348.10 [1041.80; 1654.90][2]	725.12 [488.74; 994.71][1,3]	1070.00 [638.89; 1313.80][2,3]
TGF-1β / MMP-9	0.005 [0.004; 0.006]	0.006 [0.004; 0.010]	0.006 [0.004; 0.009]	0.020 [0.013; 0.035][1,3]	0.012 [0.007; 0.023][1,3]

1: the difference is significant compared to healthy people ($p < 0.05$); **2:** compared to patients in the corresponding group before the start of AVT ($p < 0.05$); **3:** compared to patients with liver fibrosis F 0–2 during the corresponding observation period ($p < 0.05$).

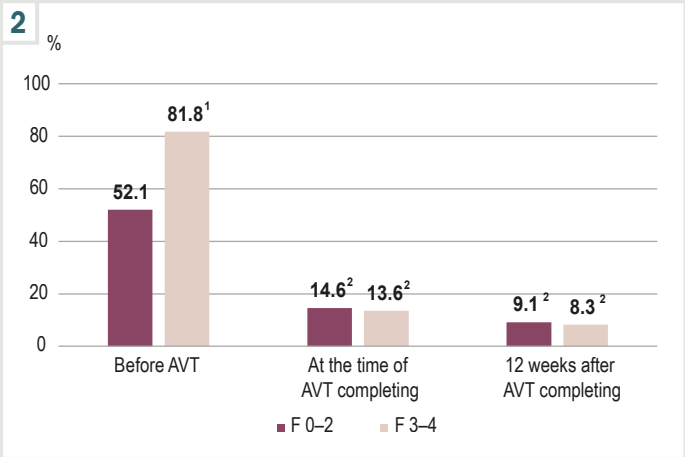

Fig. 2. The frequency of increased serum ALT activity in patients with CHC GT1 depending on the degree of liver fibrosis in the dynamics of AVT. **1:** the difference is significant compared to F 0–2 ($p < 0.05$); **2:** compared to patients in the corresponding group before AVT ($p < 0.001$).

Comparison of the severity of cytolytic syndrome in CHC patients with GT1 with different severity of liver fibrosis during AVT with the OBV / PTV / r+DSV±RBV regimen showed that before AVT, the median serum ALT activity in patients with liver fibrosis F 3–4 was higher compared with patients with liver fibrosis F 0–2 and was 1.47 [1.01; 2.37] mmol/hour.l versus 0.86 [0.48; 1.33] mmol/hour.l ($p < 0.05$). Subsequently, after AVT, both at the time of AVT completion and 12 weeks after AVT completion, serum ALT activity in patients, regardless of the severity of liver fibrosis, was lower ($p < 0.05$) than before AVT. It should be noted that after AVT, the median serum ALT activity in patients of both study groups remained within the reference values.

According to the results of our study, it was found that before the start of AVT in CHC patients with GT1 with liver fibrosis F 0–2, the median TGF-1β content was not statistically different from that of healthy individuals ($p > 0.05$). However, the content of MMP-9 in the blood serum was lower (1.3 times, $p < 0.05$) than in healthy subjects. At the same time, the median TGF-1β / MMP-9 ratio before AVT was not statistically different from the corresponding ratio in healthy subjects ($p > 0.05$).

In the group of patients with liver fibrosis severity F 3–4, the content of TGF-1β in the blood serum was higher both in comparison with healthy individuals (2.5 times, $p < 0.05$) and in comparison with patients with liver fibrosis F 0–2 (2.2 times, $p < 0.05$). The median content of MMP-9 in the blood serum of patients with liver fibrosis F 3–4 was significantly lower both in comparison with healthy individuals (1.8 times, $p < 0.05$) and in comparison with patients with liver fibrosis F 0–2 (1.4 times, $p < 0.05$). At the same time, the TGF-1β / MMP-9 ratio in patients with CHC with severe stages of liver fibrosis F 3–4 was 4 times higher ($p < 0.05$) compared with healthy subjects, and 3.3 times higher ($p < 0.05$) than in patients with stages of liver fibrosis F 0–2, which confirms the greater prevalence of fibrogenesis over fibrinolysis *(Table 2)*.

At the time of AVT completion with the OBV / PTV / r+DSV±RBV regimen, a statistically significant increase in serum MMP-9 levels was observed in CHC patients with GT1 with F 0-2 liver fibrosis compared with the corresponding indicator before AVT ($p > 0.05$). In patients with liver fibrosis F 0–2, at the time of AVT completion, the serum levels of TGF-1β and MMP-9, as well as the TGF-1β / MMP-9 ratio, did not statistically differ from the corresponding values in healthy subjects ($p > 0.05$). In patients with F 3–4 liver fibrosis, at the time of AVT completion, a statistically significant decrease in the content of TGF-1β in the blood serum was noted compared with the corresponding indicator before AVT (1.6 times, $p < 0.05$), but remained statistically significantly higher compared with healthy subjects (2.5 times, $p < 0.05$).

When assessing the content of MMP-9 in the blood serum at the time of AVT completion, an increase (1.5 times, $p < 0.05$) was noted compared with the corresponding indicator before the start of AVT. However, the serum levels of MMP-9 in patients with F 3–4 liver fibrosis during this observation period remained lower than in patients with F 0–2 liver fibrosis (1.3 times, $p < 0.05$). However, despite the above-mentioned dynamics of TGF-1β and MMP-9 in the serum of CHC patients with GT1 with liver fibrosis F 3–4, the TGF-1β / MMP-9 ratio at the time of AVT completion remained higher both in comparison with healthy individuals (2.4-fold, $p < 0.05$) and in comparison with CHC patients with GT1 with liver fibrosis F 0–2 (2-fold, $p < 0.05$). The indicated dynamics of the studied parameters suggests a slowdown in fibrosis formation and activation of fibrinolysis after AVT completion *(Table 2)*.

The Spearman correlation analysis allowed to demonstrate the interconnections of the studied cytokine levels with the liver stiffness index according to shear wave elastometry and laboratory parameters reflecting the functional state of the liver and hepatitis progression. Thus, direct correlations were established between the content of TGF-1β in the blood serum and the index of liver stiffness expressed in kPa ($r = +0.85$, $p = 0.0001$)

and with the activity of γ-glutamyl transpeptidase in the blood serum (r = +0.70, p = 0.035). There was also an inverse correlation between serum MMP-9 and liver stiffness according to elastography (r = -0.36, p = 0.005) and a direct correlation between MMP-9 and platelet count (r = +0.27, p = 0.02).

Clinical and pathogenetic significance of the studied cytokines in the progression of liver fibrosis and possible regression of fibrotic changes was confirmed by significant gamma correlations between the presence of severe liver fibrosis F 3–4 and the level of TGF-1β in the blood serum (gamma +0.96, p = 0.0001) and the level of MMP-9 in the blood serum (gamma -0.28, p = 0.01).

Discussion

In the current literature, many studies from around the world have confirmed the high efficacy of DAAs in eradicating HCV, in particular, the OBV / PTV / r+DSV±RBV regimen [7,8,9,20].

For example, study [9] showed that the presence or absence of previous experience of AVT in patients with compensated cirrhosis with CHC does not affect the effectiveness of 3D mode therapy and allows achieving SVR in 100 % of patients. High efficacy of 3D regimen, namely achievement of SVR in 92.6 %, was also demonstrated in special groups of patients with CHC GT1 and CHC GT4, in particular those who required programmed haemodialysis and had CHC / HIV co-infection [8].

The results of our study showed a high efficacy of AVT according to the OBV / PTV / r+DSV±RBV regimen, regardless of the stage of liver fibrosis. Namely, the frequency of achieving SVR was recorded: in 93.8 % of patients with liver fibrosis F 0–2 and in 95.5 % of patients with liver fibrosis F 3–4 (p < 0.05). In addition, the results of our study demonstrated that the frequency of achieving a biochemical response after AVT also did not depend on the severity of liver fibrosis.

The evolution of DAAs and the emergence of new regimens with DAAs have significantly increased the frequency of virological response, but the issue of the antifibrotic effect of AVT is currently being actively studied. It is known that fibrosis formation in the liver is constantly progressing without effective HCV eradication [11]. Therefore, the study of possible regression of liver fibrotic changes is carried out after successful AVT with both interferon-containing [10,11,21,22] and interferon-free [15,20,23,24] treatment regimens.

Literature data [10,11,15,20,21,22] support a possible regression of fibrotic changes in liver tissue after AVT completion and the formation of SVR in some patients. At the same time, patients with cirrhotic changes in the liver show significantly less regression than patients with mild or moderate fibrotic changes according to the METAVIR scale [10,11,15,20,21,22].

However, the issue of the antifibrotic effect in the setting of different AVT regimens is still under discussion [11]. A review article [11] analysed studies that investigated the possible regression of fibrotic changes in different degrees of liver fibrosis and liver cirrhosis in patients with CHC on different AVT regimens. This review demonstrates that the data from different studies have conflicting findings, which can be partly explained by the use of different methods for verifying liver fibrosis (morphological, serum markers, different calculation tests, etc.) at different stages of follow-up [11]. The results of non-invasive methods for assessing the progression of liver fibrosis are closely related to the presence of necroinflammatory activity in the liver, so the difference may be more significant when assessing the possible regression of liver fibrosis, as necroinflammatory activity decreases after AVT [11].

The vast majority of literature data on the AVT antifibrotic effect relates to observations of patients treated with interferon-containing regimens [10,11,21,22]. For example, study [21] demonstrated a tendency to regression of fibrotic changes in the liver in CHC patients after interferon treatment, which was confirmed by an 89 % reduction in collagen content based on the results of paired biopsies before and after AVT 61 months after reaching SVR. Another study [22] demonstrated that 10 years after interferon-containing AVT in CHC patients with F 4 liver fibrosis, regression of fibrotic changes was observed in 24 of 43 patients who achieved SVR, as confirmed by the FibroTest or transient elastometry [22]. However, study [10], based on the study of the dynamics of histological changes in paired liver biopsy results after peg-interferon therapy in combination with ribavirin in CHC patients, demonstrated that for patients with F 0–3 liver fibrosis who achieved SVR, the risk of progression of fibrotic changes in the liver is reduced, but there is no complete regression of existing changes.

Recently, in connection with the use of DAAs alone in clinical practice in AVT, an urgent issue has arisen to determine the possible regression of liver fibrosis with interferon-free regimens. A few studies [15,23,24,25] have demonstrated a decrease in fibrotic changes in the liver after AVT completion with DAAs and the achievement of SVR based on non-invasive methods for determining the severity of liver fibrosis (elastometry, calculated FIB-4 and APRI indices). Thus, according to the results of a study [23], in CHC patients after interferon-free AVT and the formation of SVR, a decrease in liver stiffness was found according to shear wave elastometry, but the coefficient of controlled attenuation increased, indicating the progression of liver steatosis. A study [24] demonstrated a decrease in FIB-4 and APRI in CHC patients and established liver cirrhosis after DAAs treatment in 6 months – in 21 %, and in one year – in 40 % of treated patients (p < 0.005).

At the same time, in the presence of signs of decompensation of liver cirrhosis among patients who initially had compensated liver cirrhosis, alcohol abuse during the observation period was significantly less likely to improve FIB-4 and APRI based on the statistical odds ratio [24]. The study of liver stiffness in the dynamics was the basis of a study conducted in South Korea, which compared the degree of regression of liver fibrosis among patients treated with peg-interferon and DAAs. It was proven that liver stiffness was significantly lower in the group of patients treated with DAAs compared to patients treated with peg-interferon both after 48 weeks (29 % vs. 9 %) and 96 weeks (39 % vs. 17 %). The results of this study concluded that HCV eradication with DAAs treatment may lead to an improvement in liver stiffness over time [25].

However, the results achieved in terms of HCV eradication and reduction in liver stiffness by elastography did not affect the subsequent risk of developing hepatocellular carcinoma at 144 weeks of follow-up [25].

In our study, we evaluated the possible antifibrotic effect of AVT with the OBV / PTV / r+DSV±RBV regimen based on the dynamics of serum markers that reflect the activity and ratio of fibrotic and fibrinolytic processes. According to the results of our study, it was found that AVT with the OBV / PTV / r+DSV±RBV regimen slows down fibrogenesis and activates antifibrotic processes, which is confirmed by the dynamics of the relevant parameters: an increase in the content of MMP-9 in the blood serum ($p < 0.05$) and a decrease in the TGF-1β / MMP-9 ratio (1.6 times, $p < 0.05$), compared with the corresponding indicators before treatment. Our results are in line with the current literature on the antifibrotic effect after successful antiviral therapy with DAAs [23,24,25].

In addition, in our study, we analysed the peculiarities of the dynamics of serum markers of fibrosis / fibrinolysis depending on the severity of liver fibrosis in the setting of AVT with the OBV / PTV / r+DSV±RBV regimen. The results of our study have shown that in the presence of severe liver fibrosis F 3–4, AVT with the OBV / PTV / r+DSV±RBV regimen, which is highly effective in eradicating HCV, decreases TGF-1β ($p < 0.05$), but remains higher than in healthy individuals at the end of AVT ($p < 0.05$). The serum level of MMP-9 increases with AVT ($p < 0.05$), but remains lower than in patients with F 0–2 liver fibrosis ($p < 0.05$). In patients with F 3–4 liver fibrosis, at the time of AVT completion, the TGF-1β / MMP-9 ratio remains higher both in comparison with healthy individuals ($p < 0.05$) and in comparison with CHC patients GT1 with F 0–2 liver fibrosis ($p < 0.05$). At the same time, in patients with liver fibrosis F 0–2, the content of MMP-9 in the blood serum increases ($p < 0.05$) and at the time of AVT completion, all the studied parameters do not differ from those of healthy individuals ($p > 0.05$).

To date, the current literature already contains a few publications that demonstrate the results of the assessment of antifibrotic changes in the liver in the context of the 3D treatment regimen. For example, study [20], which is a phase 3b clinical trial of TOPAZ-I and TOPAZ-II, evaluated the efficacy, safety, and clinical outcomes achieved within 3 years after the use of AVT with the OBV / PTV / r+DSV±RBV regimen in CHC patients GT1. It was demonstrated that the FIB-4 score improved during treatment, and patients with liver cirrhosis also had an improvement in the Child–Pugh score. Within 3 years after AVT completion, less than 1 % of patients developed decompensation of liver cirrhosis, and only 1.4 % of patients were diagnosed with hepatocellular carcinoma in the setting of existing liver cirrhosis [20].

In our opinion, studies of the possible antifibrotic effect after AVT using various non-invasive tests, including understanding the informative value of serum markers, deserve special attention. For example, study [15] investigated the relationship between the content of angiopoietin 2 (Ang-2) in the serum of CHC patients after successful eradication of HCV after AVT using DAAs as a possible marker of fibrotic and antifibrotic processes. It was found that the initial levels of Ang-2 correlated with the liver stiffness index determined by elastometry, splenic index and the stage of liver fibrosis. At the same time, 75 % of patients showed regression of liver fibrosis stage after DAAs. At the same time, a significant association of Ang-2 content and fibrosis stage progression with the ability to regress the fibrosis stage after treatment was found. Thus, in the ROC analysis, the level of Ang-2 ≥354 pg/ml (sensitivity – 88 %, specificity – 73 %, AUC-0.855) was determined, at which the absence of regression of liver fibrosis after treatment with DAAs is predicted [15].

In our study, using correlation analysis, we demonstrated the relationship between the levels of the profibrogenic cytokine TGF-1β and antifibrogenic MMP-9 in the blood serum and the liver stiffness index according to elastography. Namely, a direct correlation of TGF-1β in the blood serum with the index of liver stiffness expressed in kPa ($r = +0.85$, $p = 0.0001$) and an inverse correlation of MMP-9 in the blood serum with the index of liver stiffness ($r = -0.36$, $p = 0.005$) was proved. Therefore, the obtained results of changes in the content of these cytokines in the blood serum during AVT with the OBV / PTV / r+DSV±RBV regimen suggest a slowdown in fibrogenesis and activation of antifibrotic processes, which is confirmed by the dynamics of the relevant parameters: an increase in the content of MMP-9 in the blood serum ($p < 0.05$) and a decrease in the TGF-1β / MMP-9 ratio (1.6 times, $p < 0.05$), compared with the corresponding values before treatment. In our previous study, we proved the relationship of the studied cytokines in the progression of liver fibrosis in patients with chronic hepatitis C GT1 [26].

Conclusions

1. In CHC patients GT1 before AVT, the prevalence of profibrogenic potential is confirmed by an increase in TGF-1β (2-fold, $p < 0.05$), a decrease in MMP-9 (1.4-fold, $p < 0.05$) and a higher TGF-1β / MMP-9 ratio (2.6-fold, $p < 0.05$) than in healthy subjects. AVT according to the OBV / PTV / r+DSV±RBV regimen slows down fibrogenesis and activates antifibrotic processes, which is confirmed by an increase in MMP-9 content ($p < 0.05$) and a decrease in the TGF-1β / MMP-9 ratio (1.6 times, $p < 0.05$) compared to the corresponding values before treatment.

2. The effectiveness of AVT in CHC patients GT1 according to the OBV / PTV / r+DSV±RBV regimen in terms of virological and biochemical responses is high and independent ($p > 0.05$) of the severity of liver fibrosis, which is confirmed by the achievement of SVR12 in 93.8 % of patients with liver fibrosis F 0–2 and 95.5 % of patients with F 3–4, as well as the biochemical response regardless of the severity of liver fibrosis ($p > 0.05$).

3. Before AVT starting, in CHC patients GT1 with liver fibrosis F 0–2, the serum MMP-9 content is lower ($p < 0.05$) than in healthy subjects, with no changes in TGF-1β and the TGF-1β / MMP-9 ratio ($p > 0.05$). In patients with F 3–4 liver fibrosis, serum TGF-1β and TGF-1β / MMP-9 ratio were higher, and MMP-9 was lower both in comparison with healthy subjects ($p < 0.05$) and in comparison with patients with F 0–2 liver fibrosis ($p < 0.05$).

4. On the background of AVT (OBV / PTV / r+DSV±RBV) in CHC patients GT1 with liver fibrosis F 0–2, the content

of MMP-9 in the blood serum increases ($p < 0.05$) and at the time of AVT completion all the studied parameters do not differ from healthy subjects ($p > 0.05$). In patients with F 3–4, the content of TGF-1β decreases ($p < 0.05$), but at the time of AVT completion remains higher than in healthy individuals ($p < 0.05$). The content of MMP-9 in increases with AVT ($p < 0.05$), but remains lower than in patients with F 0–2 ($p < 0.05$). In patients with F 3–4, at the time of AVT completion, the TGF-1β / MMP-9 ratio remains higher both in comparison with healthy individuals ($p < 0.05$) and with CHC patients GT1 with F 0–2 ($p < 0.05$).

Prospects for further research in this area, in our opinion, are to evaluate the antifibrotic effect of pathogenetic treatment of CHC patients after HCV eradication.

Ethical approval

The Bioethics Committee of Zaporizhzhia State Medical and Pharmaceutical University has reviewed the materials presented in the article and found no violations of ethical standards as outlined in applicable regulatory documents, including the Declaration of Helsinki, the Council of Europe's Convention on Human Rights and Biomedicine (Oviedo Convention), and other relevant legal instruments. The study complies with the current legislation of Ukraine. The Committee's conclusion is documented in the minutes of the meeting (Extract from Protocol No. 7 dated May 22, 2025).

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