Journal of Advanced Zoology

ISSN: 0253-7214

*Volume **44** Issue **S-5 Year 2023** Page **1831-1837***

Immunohistochemical Study of Stellate Cells in Patients with Chronic Viral Heptitis C Histopathological study

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Article History

Received: 11 June 2023
Revised: 12 Sept 2023
Accepted: 03 Nov 2023

Abstract

Background: *Chronic hepatitis is defined as liver inflammation that lasts for at least 6 months. The Hepatitis C virus is responsible for 60 to 70% of chronic hepatitis cases; the virus causes continued inflammation that slowly damages the liver, eventually leading to cirrhosis, liver failure, and, in rare cases, liver cancer.* ***Aim of the work:*** *To evaluate the changes in distribution and percentage of alpha-smooth muscle actin-positive hepatic stellate cells and the correlation with the degree of the fibrosis in cirrhotic livers, in patients with HCV chronic hepatitis.* ***Material and methods:*** *50 hepatic core biopsies selected randomly were received from Histopathology Department at National Hepatology and Tropical Medicine Research Institute and examined for histopathological features using (hematoxylin and eosin), stage of hepatic fibrosis using stellate cell Masson s' trichrome stain, and examined for stellate cell activity using alpha smooth muscle actin (ASMA) immunostaining.* ***Results****: The relation between degree of ASMA expression by stellate cells and stage of fibrosis was highly significant with a p value <0.001, also the relation between degree of necroinflammation and degree of ASMA expression by stellate cells was highly significant with a p value <0.001. The relation between degree of necroinflammation and stage of fibrosis was highly significant with a p value <0.001. The relation between age of patient and stage of fibrosis also was statistically significant with a p value =0.012. The relation between age of patient and degree of necroinflammation was statistically significant with a p value =0.017.* ***Conclusion:*** *To summarize from hepatic core biopsies of patients suffering from chronic HCV, the number of active stellate cells was found to be positively associated with stage of hepatic fibrosis.*

Key words: *Chronic HCV, Stellate cells, Alpha smooth muscle actin.*

1. Introduction

Chronic liver injury manifests itself in the form of hepatic fibrosis. It causes hepatic impairment and plays a key role in the development of cirrhosis and hepatocellular cancer. The activation of hepatic stellate cells (HSC) is a key factor in the development of liver fibrogenesis in a variety of diseases[1] In the early stages of HCV-induced severe liver disease, hepatic fibrosis develops. Early identification and monitoring of liver fibrosis allows for prompt intervention to improve anti-HCV treatment outcomes 2 Hepatitis C virus (HCV) infection can result in liver damage ranging from minor to severe, such as fibrosis and cirrhosis. The activation of hepatic stellate cells (HSCs) is a critical step in HCV-induced liver fibrosis. HSCs express many HCV co-receptors that bind with HCV proteins and promote fibrogenesis in the liver. HSCs can also ingest apoptotic bodies of hepatocytes produced by HCV, resulting in a profibrogenic reaction 3,4 Stellate cells make up about 5-8 percent of total cells in a healthy liver. They're found in Disse 's perisinusoidal space, between the fenestrated endothelium of sinusoids and hepatocytes, with a higher frequency in periportal locations than in centrilobular places. The importance of stellate cells in the development of liver fibrosis and its progression to cirrhosis has been extensively documented 5,6 Stellate cells are triggered to myofibroblasts, which are exceptionally high fibrogenous cells, in response to stress and trauma. Because HSCs can express ASMA when activated, it was thought that ASMA expression by HSCs was a signal of their activation to

myofibroblast-like cells7 ASMA is a key marker of activated stellate cells that can predict the onset of fibrosis. Its presence in stellate cells in chronic hepatitis and cirrhosis reflects their role in extracellular matrix remodeling and, as a result, in fibrosis8 Our study aimed to evaluate the changes in distribution and percentage of alpha-smooth muscle actin-positive hepatic stellate cells and the correlation with the degree of the fibrosis in cirrhotic livers, in patients with HCV chronic hepatitis.

2. Materials and Methods

This study included archival paraffin blocks of 50 patients with HCV cirrhosis received from Histopathology unit of National Hepatology and Tropical medicine Research Institute from January to December 2020 , they were examined for classification of stage of fibrosis and also degree of necroinflammation. The relevant clinical information and demographic data was obtained from the medical records. Patients of both sexes and average age 19-69 years old were included in the study.

Selection criteria: Cases were selected from adults with HCV hepatitis attending the National Hepatology and Tropical Medicine Research Institute, Cairo, Egypt, and were included if they had positive HCV infection detected by PCR. Data were obtained from the medical records of the selected patients including age and gender.

Exclusion criteria: Patients who had any evidence of infection with other causes of hepatitis as autoimmune hepatitis, Drug induced hepatitis, HBV infection etc. all were excluded on the basis of analytical and clinical data.

Histopathologic Evaluation:

Core needle liver biopsies were performed for all the patients . Biopsy samples were fixed in 10% neutral formalin and Serial sections (5 micrometers [µm] thick) from formalin-fixed, paraffin-embedded blocks were processed and stained with:

1) Hematoxlyin and eosin (H&E) to evaluate the degree of inflammatory activity and degree of steatosis.
2) Masson's trichrome stain to detect degree of fibrosis using Metavir score.
3) Alfa smooth muscle actin (ASMA) immunostaining as a marker for hepatic stellate cells.

Assessment of Steatosis:

Macrovesicular steatosis was rated from 0 to 3 based on the percentage of hepatocytes found in the biopsy (0 = none; 1 = up to 33%; 2 = 34–66%; 3 = more than 66%).

ASMA Immunostaining:

It is widely known that ASMA is a good indicator of active HSCs and capillarized endothelial cells in perisinusoidal fibrosis, while it is negative in quiescent HSCs. We utilised a subsequent semiquantitative score to evaluate ASMA staining (0, absent; 1, focal; 2, diffuse).

Masson stain:

Trichrome stains expose the extracellular matrix, which is generally seen in portal tracts and the walls of major hepatic vein branches, allowing the quantity and distribution of fibrosis to be easily identified.

3.Results and Discussion

Table (1): Age distribution of the presented study.

Age groups (years)	Frequency	Percentage
<20	1	2%
20-30	2	4%
>30-40	5	10%
>40-50	23	46%
>50-60	17	34%
>60	2	4%
Total	50	100%

Most of our cases are in the age group > 40- 50 years constituting 46%.

Table (2): Distribution of studied cases according to ASMA score

ASMA score	Frequency	Percentage
0	7	14%
1	22	44%
2	21	42%
Total	50	100%

Most of our cases showed Grade 2 ASMA immunostaining, constituting 42% of all cases.

Table (3): Correlation between age and NI grade

	NI grade	
Age	0.337	Correlation coefficient
	0.017	P value
	50	Number

The relation between age and NI grade was statistically significant positive with p value = 0.017 and correlation coefficient= 0.337

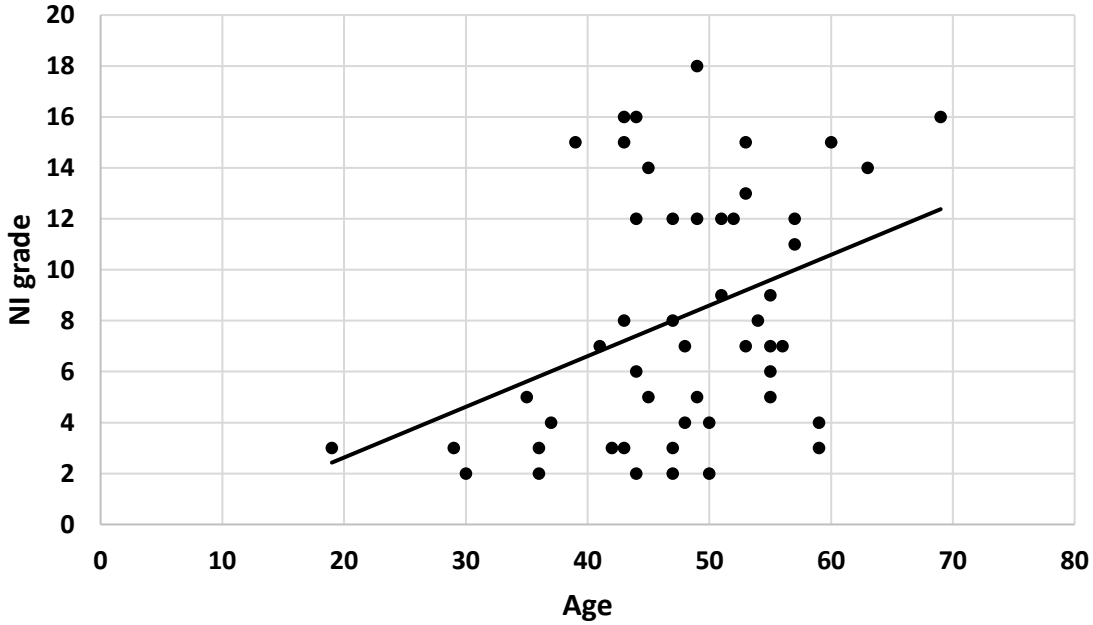

Fig. (1): Correlation between age and NI grade in the study sample

Table (4): Correlation between age and stage of fibrosis

	Stage of fibrosis	
Age	0.351	Correlation coefficient
	0.012	P value
	50	Number

The relation between age and stage of fibrosis was statistically significant positive with p value = 0.012 and correlation coefficient= 0.351.

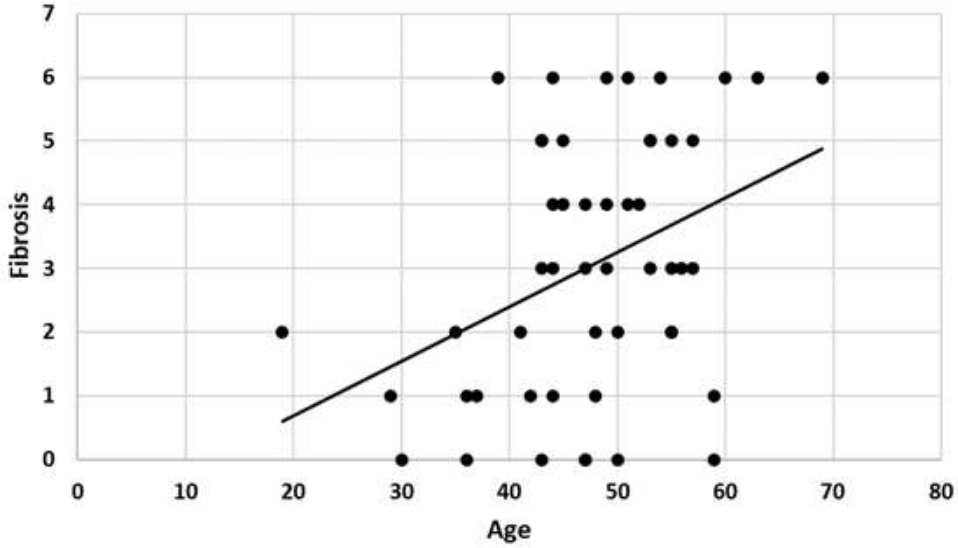

Fig. (2): Correlation between age and fibrosis score in the study sample

Table (5): Correlation between age and degree of steatosis

Age	Degree of steatosis	
	0.066	Correlation coefficient
	0.650	P value
	50	Number

The relation between age and stage of fibrosis was statistically unsignificant with p value = 0.650 and correlation coefficient= 0.066.

Table (6): Correlation between age and ASMA score

Age	ASMA score	
	0.255	Correlation coefficient
	0.074	P value
	50	Number

The relation between age and ASMA score was statistically unsignificant with p value = 0.074 and correlation coefficient= 0.255.

Table (7): Correlation between NI grade and Stage of fibrosis

NI grade	Stage of fibrosis	
	0.896	Correlation coefficient
	<0.001	P value
	50	Number

The relation between NI grade and stage of fibrosis was highly significant with a p value <0.001 and correlation coffecient =0.896.

Table (8): Correlation between Stage of Fibrosis and Degree of Steatosis

Stage of Fibrosis	Degree of Steatosis	
	-0.014	Correlation coefficient
	0.924	P value
	50	Number

The relation between Stage of Fibrosis and degree of steatosis was statistically insignificant with a p value =0.924 and correlation coffecient =-0.014.

Table (9): Correlation between Stage of Fibrosis and ASMA score

Stage of Fibrosis	ASMA score	
	0.925	Correlation coefficient
	<0.001	P value
	50	Number

The relation between Stage of Fibrosis and ASMA score was statistically highly significant with a p value <0.001 and correlation coffecient =-0.925.

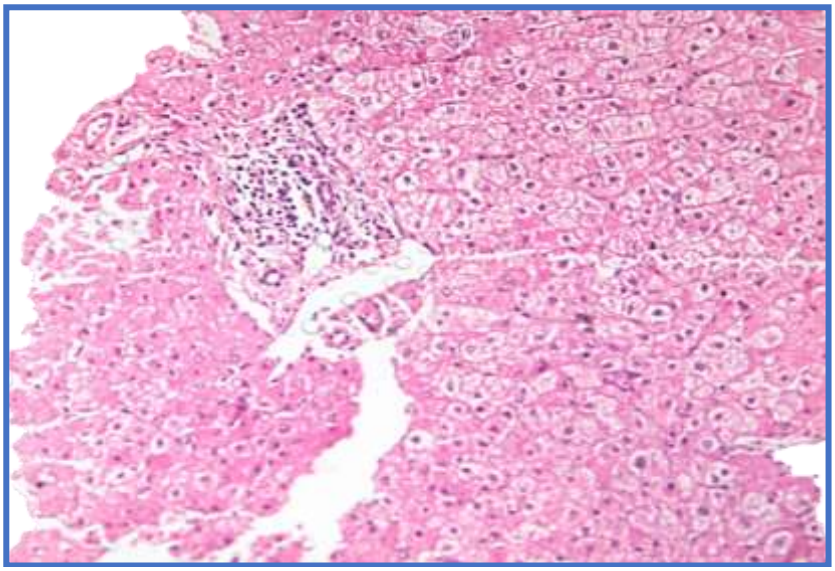

Fig. (3): Minimal portal inflammation, NI grade 1\18, H&E. (x200)

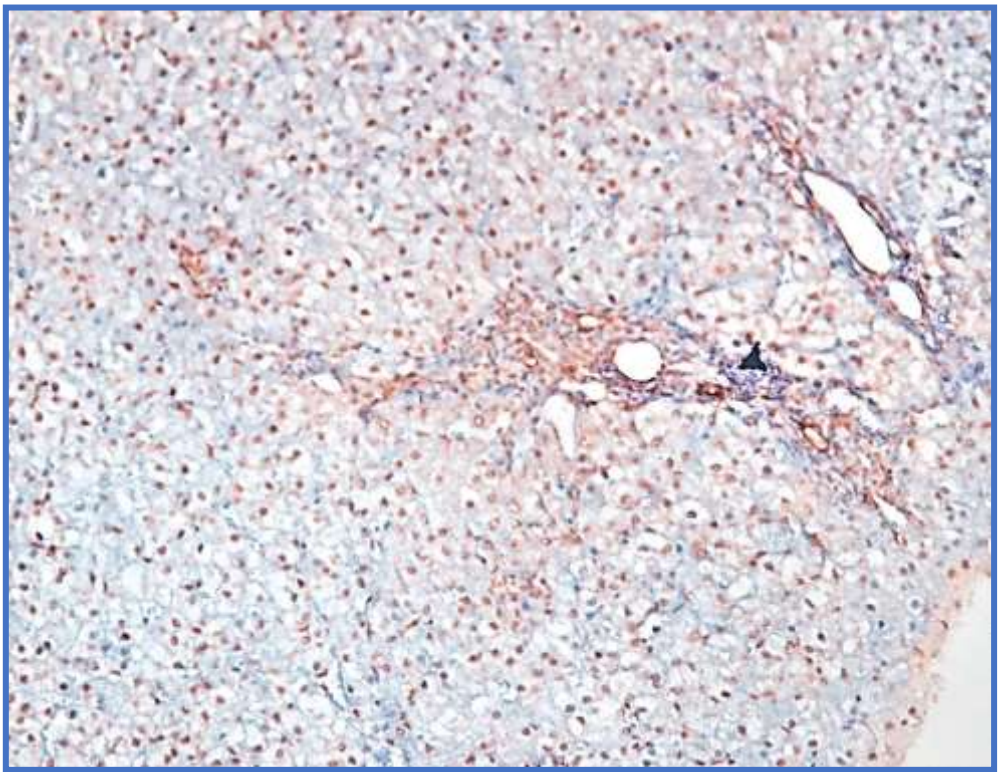

Fig. (4): ASMA immunostaining grade 1, Focal. (x200)

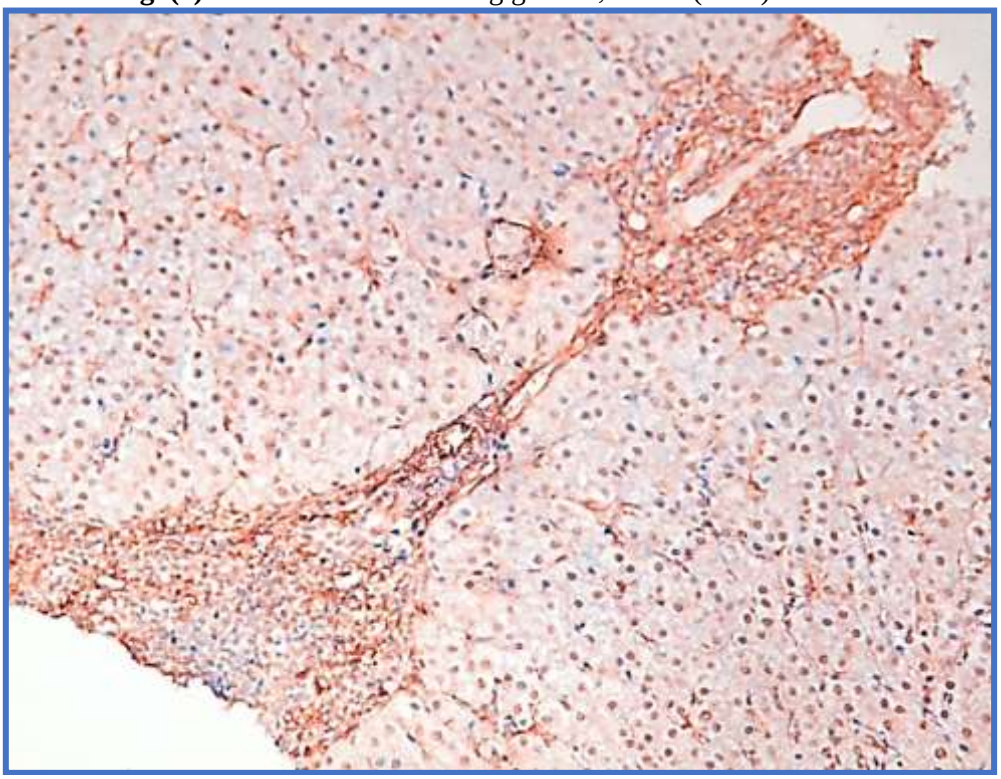

Fig. (5): ASMA immunostaining grade 1, focal. (x200).

Our study found a significant relation between age of patient and stage of fibrosis with p value of 0.012. This finding coincides with Schuppan D., et al.[9], who found that the age of onset of infection has consistently been proven to be a primary factor impacting the pace of fibrosis advancement in hepatitis C, with the speed of fibrosis progression being directly connected with the age of onset of infection in fibrosis progression analyses. Immune factors, increased fibrogenesis, or decreased fibrolysis may be involved in the influence of age on fibrosis progression, although the precise mechanisms are unknown[10] Also Bhattacharya P. and Mukherjee S. 11stated that regular heavy alcohol consumption; age (>50-55 years); female gender; hispanic or white racial background; human leukocyte antigen DRB; HIV and other immune suppressive conditions; degree of steatosis; severity of inflammation; necrosis and injury; and a high iron load are all factors that enhance fibrosis and disease progression.

The relation between age of patient and degree of steatosis was statistically insignificant in our study with p value = 0.650 and this goes with AdinolfiL. et al.,[12] who also found that neither patient age nor the prevalence or severity of steatosis had any statistically meaningful relationship to each other.

But this was in contrast to Lay et al., [13] who found that NAFLD prevalence rises with age, from 1 to 3 percent in toddlers, to 5 percent in teenagers, to 18 percent between 20 and 40 years, to 39 percent between 40 and 50 years, to over 40 percent in those over 70 years.

As for the relation between the age of patient and degree of stellate cell activity manifested by ASMA immunostaining, our study found an unsignificant relation between them with p value=0.074 and this coincides with Săndulescu L., et al.14who stated that there was also no correlation between age and the level of ASMA expression in stellate cells.

Concerning the relation between degree of steatosis and stage of fibrosis, our study found an unsignificant relation between them with p value=0.924, this finding was in contrast to Leandro et al., 15who stated that liver steatosis has been found to be strongly and independently associated with fibrosis in CHC patients, and inflammation of the liver in these patients may be a mediator of fibrogenesis.

In our study we found a statistically significant relation between degree of necroinflammation and degree of ASMA immunostaining (stellate cell activity) with p value of <0.001 and this coincides with TomanovicN. et al., [16] who also found that an increased number of activated HSCs and a higher level of necro inflammatory activity are positively correlated, also mentioned that with HCV, the immune system first tries to eradicate the virus, but in the context of persistent infection, it probably promotes hepatocyte destruction and fibrosis by direct cellular toxicity and the release of inflammatory cytokines.

As for the relation between stage of fibrosis and degree of ASMA expression, our study found a statistically significant relation between them with a p value < 0.001 and this coincides with Venturi C. et al., 17who stated that ASMA overexpression in the early stages of liver fibrosis may have both short- and long-term effects on the disease's progression.

This also coincides with Săndulescu L., et al., [14] who found that according to the expression of ASMA, patients with chronic HCV have increased activity in stellate cells, and several studies have identified a link between stellate cell activity and stage of fibrosis.

4. Conclusion

In chronic viral hepatitis C, the HSC activation index correlated significantly and independently with degree of necroinflammation and stage of fibrosis. Alpha-smooth muscle actin expression is a valid marker of activation of hepatic stellate cells, which precedes the deposition of fibrous tissue. Identification of the activated stellate cell opens new options in early diagnosis of liver fibrosis and future anti fibrogenic therapy.

References:

1. Adinolfi LE, Gambardella M, Andreana A, Tripodi MF, Utili R, Ruggiero G. Steatosis accelerates the progression of liver damage of chronic hepatitis C patients and correlates with specific HCV genotype and visceral obesity. Hepatology. 2001 Jun 1;33(6):1358-64.
2. Andreichenko IN, Tsitrina AA, Fokin AV, Gabdulkhakova AI, Maltsev DI, Perelman GS, Bulgakova EV, Kulikov AM, Mikaelyan AS, Kotelevtsev YV. 4-methylumbelliferone prevents liver fibrosis by affecting hyaluronan deposition, FSTL1 expression and cell localization. International journal of molecular sciences. 2019 Dec 13;20(24):6301.
3. Bhattacharya PK, Mukherjee S. Natural history of liver fibrosis progression in patients confected with hepatitis c virus and human immunodeficiency virus type 1. Revista da SociedadeBrasileira de Medicina Tropical. 2016 Nov;49:803-5.
4. Ertle J, Dechêne A, Sowa JP, Penndorf V, Herzer K, Kaiser G, Schlaak JF, Gerken G, Syn WK, Canbay A. Non-alcoholic fatty liver disease progresses to hepatocellular carcinoma in the absence of apparent cirrhosis. International journal of cancer. 2011 May 15;128(10):2436-43.
5. Gan L, Chitturi S, Farrell GC. Mechanisms and implications of age-related changes in the liver: nonalcoholic fatty liver disease in the elderly. Current gerontology and geriatrics research. 2011 Oct;2011.
6. Hickman IJ, Powell EE, Prins JB, Clouston AD, Ash S, Purdie DM, Jonsson JR. In overweight patients with chronic hepatitis C, circulating insulin is associated with hepatic fibrosis: implications for therapy. Journal of hepatology. 2003 Dec 1;39(6):1042-8.
7. Khatun M, Ray RB. Mechanisms underlying hepatitis C virus-associated hepatic fibrosis. Cells. 2019 Oct 14;8(10):1249.
8. Leandro G, Mangia A, Hui J, Fabris P, Rubbia–Brandt L, Colloredo G, Adinolfi LE, Asselah T, Jonsson JR, Smedile A, Terrault N. Relationship between steatosis, inflammation, and fibrosis in chronic hepatitis C: a meta-analysis of individual patient data. Gastroenterology. 2006 May 1;130(6):1636-42.

9. Liu B, Tian Y, He J, Gu Q, Jin B, Shen H, Li W, Shi L, Yu H, Shan G, Cai X. The potential of mecciRNA in hepatic stellate cell to regulate progression of nonalcoholic hepatitis. Journal of Translational Medicine. 2022 Sep 4;20(1):393.
10. Marcellin P, Asselah T, Boyer N. Fibrosis and disease progression in hepatitis C. Hepatology. 2002 Nov;36(S1):S47-56.
11. Otto J, Verwaayen A, Penners C, Hundertmark J, Lin C, Kallen C, Paffen D, Otto T, Berger H, Tacke F, Weiskirchen R. Expression of Cyclin E1 in hepatic stellate cells is critical for the induction and progression of liver fibrosis and hepatocellular carcinoma in mice. Cell Death & Disease. 2023 Aug 24;14(8):549.
12. Săndulescu LA, Rogoveanu I, Ciurea T, Comănescu MV, Streba CT, Ionescu AG, Oproaica A, Ene M. Immunohistochemical study of stellate cells in patients with chronic viral hepatitis C genotype 1. Rom J MorpholEmbryol. 2011 Jan 1;52(1):137-43.
13. Schuppan D, Ruehl M, Somasundaram R, Hahn EG. Matrix as a modulator of hepatic fibrogenesis. InSeminars in liver disease 2001 (Vol. 21, No. 03, pp. 351-372). Copyright© 2001 by Thieme Medical Publishers, Inc., 333 Seventh Avenue, New York, NY 10001, USA. Tel.:+ 1 (212) 584-4662.
14. Seitz T, Hellerbrand C. Role of fibroblast growth factor signalling in hepatic fibrosis. Liver International. 2021 Jun;41(6):1201-15.
15. Tomanovic NR, Boricic IV, Brasanac DC, Stojsic ZM, Delic DS, Brmbolic BJ. Activated liver stellate cells in chronic viral C hepatitis: histopathological and immunohistochemical study. J Gastrointestin Liver Dis. 2009 Jun 1;18(2):163-7.
16. Venturi C, Reding R, Quinones JA, Sokal E, Rahier J, Bueno J, Sempoux C. Relevance of activated hepatic stellate cells in predicting the development of pediatric liver allograft fibrosis. Liver Transplantation. 2016 Jun;22(6):822-9.
17. Villesen IF, Daniels SJ, Leeming DJ, Karsdal MA, Nielsen MJ. thesignalling and functional role of the extracellular matrix in the development of liver fibrosis. Alimentary Pharmacology & Therapeutics. 2020 Jul;52(1):85-97.