Egypt. J. Chem. **Vol. 65,** No. 9 pp. 93 - 98 (2022)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Cartilage Oligomeric Matrix Protein as A Serological Biomarker for the Assessment of Liver Fibrosis Before and After Treatment of HCV Infection

Abdallah A. Ragab[a], Sanaa O. Abdallah[a], Gamal E. Shiha[b], Hisham Ismail[c], Mohamed S. Albannan[d], Mohamed A. El-Desouky[a]*

[a]Faculty of Science, Cairo University, Giza 12613, Egypt

[b]Faculty of Medicine, Mansoura University, Mansoura 35516, Egypt

[c]Faculty of Science, Minia University, Minia 61519, Egypt

[d]Biotechnology Research Center, New Dameitta 34517, Egypt

Abstract

The goal of this study was to determine cartilage oligomeric matrix protein (COMP) levels as a biochemical marker in various phases of hepatic fibrosis and to estimate its effectiveness for hepatic fibrosis monitoring. Eighty-eight patients with various stages of hepatic fibrosis were enrolled in this trial, all of whom had a sustained virological response after using direct-acting antivirals. Patients were followed-up after one year of treatment. FibroScan and COMP were determined before and after one year from the end of treatment. Liver enzymes, albumin, total bilirubin, creatinine, and glucose were measured.COMP levels increased with the progression of liver fibrosis, according to our findings. COMP enabled the correct identification of F2-F4, F3-F4 and F4 with areas under the curve of 0.765, 0.788 and 0.790, respectively. Our findings showed that 68.4%, 87.1%, and 98.6% of F4, F3-F4, and F2-F4, respectively, were positive for COMP. The COMP levels after treatment were not significantly changed (P >0.05), especially in patients with F3 and F4 because more than 55% of the liver fibrosis had not changed and remained stationary. In conclusion, this work provides a promising marker that might be used as a potential serologic biomarker for liver fibrosis staging and monitoring liver fibrosis after eradication of HCV infection.

Key words: Liver fibrosis; Cirrhosis; COMP; Liver enzymes, Diagnosis

1. Introduction

Hepatitis C virus (HCV) infection is a leading cause of chronic liver disease (cirrhosis and hepatocellular cancer) globally (1). The evaluation of liver fibrosis advancement is useful not only for diagnostic and treatment supervision decisions, but also for disease monitoring (2)(3). Indirect and direct biomarkers are two types of non-invasive biochemical markers. The former assesses the components released into the bloodstream as a result of hepatic inflammation, subsequently, indicates changes in liver function (4, 5). These markers have the benefit of being very inexpensive and simple to use, but they lack diagnostic accuracy for detecting hepatic fibrosis (6). Direct indicators, on the other hand, include measurements of hepatic metabolic activity, collagen production, and (ECM) remodelling proteins, which are pathophysiologically generated from extracellular matrix (ECM) turnover and/or changes in fibrogenic cell types in the liver throughout the fibrosis process (5, 7). Collagens, in fact, need a varied range of chaperones that are required for effective collagen folding and the formation of a hard three-dimensional structure. One of these proteins is COMP, which binds five separate chains and ensures that they are folded correctly (8). COMP is a glycoprotein that is present mostly in the ECM of cartilage, synovium, ligaments, and tendons (9). It is made up of five identical subunits that are connected by disulfide bonds to produce a big protein with a molecular weight of 524 kDa. COMP's C-terminal domain may bind to collagen I, II, and IX, as well as other ECM components such fibronectin, matrilins, proteoglycans, and heparin, with great affinity (10, 11). As a result, this research focuses on identifying the expression of COMP in various phases of hepatic fibrosis before and after therapy, as well as measuring its performance as a surrogate marker for liver fibrosis diagnosis.

Subjects and Methods

2.1 Patients

This is a prospective cohort study. In the pre-treatment stage, we started with a number of 228 Egyptian individuals chronically infected with HCV

*Corresponding author e-mail: ma.des38@yahoo.com

Receive Date: 05 December 2021, Revise Date: 19 January 2022, Accept Date: 25 January 2022

DOI: 10.21608/EJCHEM.2022.109105.4994

genotype 4, but for several reasons, including the death of a number of participants, the failure of others to complete the treatment, and the delay of some Egyptian villagers on the date of follow-up after treatment, the number decreased to 88 individuals, including 53 males and 35 females, with a mean age (±SD) of 56.89 (±11.60) years. The Research and Ethical Committee of the Faculty of Medicine, Mansoura University, Mansoura, Egypt, accepted the study protocol. All individuals gave their informed permission after being fully informed about the diagnostic techniques and the nature of the condition. The study protocol followed the 1975 Helsinki Declaration's ethical requirements. These individuals were examined prospectively and performed all of the necessary laboratory tests to determine if they were eligible for antiviral medication. Abdominal ultrasonography was also performed using a Toshiba Aplio XG ultrasound machine (Toshiba, Minato, Tokyo, Japan), as well as a Triphasic multi-slice spiral computed tomography (MSCT) utilising a Philips Brilliance CT 16 slice where necessary (Philips, Amsterdam, Netherlands).

2.2 Laboratory tests

The Egyptian Liver Research Institute and hospital laboratories determined all regular laboratory values. An automated haematology analyzer was used to conduct a complete blood count (Sysmex Corporation, Kobe, Japan). Cobas Integra 400 plus was used to measure liver transaminases (ALT and AST), creatinine, albumin, alkaline phosphatase, total bilirubin, and glucose (Roche, Basel, Switzerland).

All patients were tested negative for HBsAg (Dia.Pro, Milan, Italy).The diagnosis of HCV was based on positive test for anti-HCV antibodies (Axiom Diagnostics, Worms, Germany).

The presence of HCV-RNA was subsequently validated using a real-time HCV-RNA polymerase chain reaction (PCR) (Cobas Ampliprep, Cobas Taqman 48; Roche, Rotkreuz, Switzerland) as directed by the manufacturer. Using the ELISA method, COMP levels in patients' serum were measured before and after one year of therapy (USA & Canada, R&D Systems, Inc; 614 McKinley Place NE, Minneapolis, MN 55413, USA). A standardised medical history was taken, as well as a physical examination.

Patients were observed every 4 weeks after starting antiviral therapy until the termination of the therapeutic course and 12 weeks after that (follow-up 12) to determine persistent virological response (SVR). After a year of therapy, a liver stiffness measurement (LSM) was performed.

2.3 Exclusion criteria

Patients with hepatic focal lesions that were not malignant (dysplastic nodules, cirrhotic nodules, and haemangiomas) were excluded. Patients who had co-infection with either the hepatitis B virus (HBV), or the human immunodeficiency virus (HIV) were excluded. Patients having a history of prior interferon (IFN) therapy, decompensated cirrhosis (Child Pugh C and B scores of more than 7) or ascites, liver transplantation, renal impairment, or other malignancies were also excluded.

2.4 FibroScan

A FibroScan was used to detect the stage of liver fibrosis before and after one year of therapy. The following cut-off values were used to categorise distinct stages of liver fibrosis: F0–F1 ≤ 7 kPa; F2 = >7 kPa; F3 = 10.2 kPa; F4 = 16.3 kPa (12). When the following requirements were satisfied, transient elastography was considered as reliable: (a) ten successful measurements; (b) a success rate of more than 60%; and (c) an interquartile range (IQR) that is less than 30% of the median value (13). The median of all valid values was determined to be liver stiffness. Two skilled operators performed an examination using the XL probe on individuals with a high BMI (30 kg/m^2). FibroScan 502 was used to do transient elastography (Echosens, Paris, France).

In this paper, the term reversal of cirrhosis refers to the complete restoration of normal architecture following the onset of cirrhosis, whereas regression of fibrosis or cirrhosis simply implies that the fibrosis content is lower than before, without indicating that the histology has reverted to normal (14).

Cirrhosis reversal is defined as the reduction of fibrosis stage from F4 to ≤F2 (≤10.2 kPa) or from F3 to ≤F1 (≤7 kPa). Fibrosis regression is defined as a one-stage reduction in fibrosis, such as from F4 to ≤F3 (≤16.3 kPa) or from F3 to ≤F2 (≤10.2 kPa). Stationary fibrosis occurs when the fibrosis stage remains constant, such as F4 to F4 (>16.3 kPa) or F3 to F3 (>10.2 kPa and ≤16.3 kPa). Fibrosis progression is defined as an increase in the fibrosis stage from F3 to F4 (>16.3 kPa).

2.5 Anti-viral treatment

In accordance with Egyptian national therapy, all individuals got a 12- or 24-week course of one of several DAA regimens (15) and 2014 WHO (16) Treatment recommendations for CHC infection caused by genotype 4.

2.6 Statistical analysis

Version 24 of the SPSS (Statistical Package for Social Sciences) was used to conduct statistical analyses (IBM Corp., USA). The median value (IQR) for continuous variables was used. The frequency of categorical variables was provided as (%). The area under the curve (AUC) was calculated using ROC curves (receiver operator characteristic) (AUROC). Two groups of patients were established (below and above the cut-off values). Based on the closest point to the top left point in the ROC curve, the optimal cut-off values for the independent

variables were chosen. Significant was defined as a *P* value of <0.05 or more.

2. RESULTS

A quantitative PCR method was used to screen all eligible patients for HCV-RNA. All patients were tested positive for the presence of HCV-RNA and the mean value (±SD) of HCV-PCR (Log_{10}) was 5.71 (±0.59) at baseline time. According to the FibroScan distribution for fibrosis score categorization, 13.5 % (n=12) of the sample had no fibrosis (F0), 8% (n=7) had mild fibrosis (F1), 8% (n=7) had moderate fibrosis (F2), 27.3 % (n=24) had advanced fibrosis (F3), and 43.2 % (n=38) had cirrhosis (F4).

Table 1 shows a comparison of the characteristics studied in patients before and after therapy. Surprisingly, the treated patients had significantly lower levels of ALT, AST, ALP, and total bilirubin than the control group (P<0.05). Treated patients, on the other hand, had significantly greater levels of albumin and platelet count than those who did not get treatment (P<0.05). Table 2 shows changes in liver fibrosis stages across patients before and after therapy. Patients with F4 demonstrated reversal of hepatic fibrosis to F2 or below in two patients (5.3 %), 11 patients (28.9%) showed just one stage improvement to F3 (fibrosis regression), and 25 patients (65.8%) remained at F4 with no change in fibrosis stage (stationary). 4 patients (16.7 %) had their fibrosis reversed to F0 or F1, 6 patients (25 %) had their fibrosis regress to F2, 12 patients (50 %) stayed stable at F3, and 2 patients (8.3%) developed to F4. For patients with F2, no patients showed reversal fibrosis. Five patients (71.4%) showed fibrosis regression to F1, one patient (14.3%) remained stationary at F2 while one patient (14.3%) progressed to F3. For patients with F1, no patients showed reversal fibrosis, 2 patients (28.6%) showed fibrosis regression to F0, 4 patients (57.1%) remained stationary at F1 while 1 patient (14.3%) progressed to F2. For patients with F0, no patients showed reversal and regression fibrosis. Seven patients (58.3%) remained stationary at F0 while five patients (41.7%) progressed to F1.

COMP levels in relation to various fibrosis stages before therapy, on the other hand, were determined and displayed in Table 3. As a consequence, patients with significant fibrosis (F2-F4) had a greater level of COMP than those with non-significant fibrosis (F0-F1), with a median (IQR) of 2.93 (1.86-4.99), and the difference was statistically significant (*P* <0.001). As demonstrated in Table 3, patients with advanced fibrosis and cirrhosis had greater COMP concentrations than those with non-advanced fibrosis and non-cirrhosis, respectively, with a statistically significant difference (*P* <0.001).

Before and after a year of therapy, different levels of COMP were measured, and the findings are summarised in Table 4. ROC analysis was used to establish the diagnostic accuracy of COMP. COMP allows for the accurate diagnosis of advanced fibrosis (F3-F4) and cirrhosis (F4), with AUCs of 0.788 and 0.790, respectively. Our data revealed that 68.4 % (26/38) of cirrhotic patients tested positive for COMP, but only 14 % (7/50) of non-cirrhotic patients tested positive at a cut-off point of 2.99. The vast majority of patients who received a positive COMP test (26/33, or 78.8%) had well-documented cirrhosis. In the case of advanced fibrosis, 87.1 % (54/62) tested positive for COMP. Notably, the vast majority of patients with a positive COMP test (54/67, or 80.6 %), at a cut-off point of 1.56, had extensive fibrosis that had been well-documented. In the case of significant fibrosis, 98.6% (68/69) of the patients tested positive for COMP. The vast majority of patients with a positive COMP test (26/33, or 78.8%) had severe fibrosis that had been reported.

3. DISCUSSION

Hepatitis C has a wide range of long-term effects, from minor alterations to severe fibrosis (17). Patients with advanced fibrosis have a higher risk of developing cirrhosis and hepatocellular cancer. Antiviral medication is required to prevent disease development and consequences (18). The evaluation of fibrosis gives a wealth of information that is valuable not only for disease diagnosis and prognosis, but also for therapeutic decisions and monitoring of the natural history or treatment progression (7). Indeed, there is a growing interest in developing non-invasive approaches for diagnosing the prevalence and severity of liver fibrosis. While some non-invasive models focus on tests that aren't widely available and come at a premium cost (so-called "direct indicators"), others use normal clinical and laboratory data but don't directly represent extracellular matrix metabolism (so-called "indirect markers") (7, 19). Serum levels of proteins directly associated to the hepatic fibrogenic process might be employed as surrogate indicators of liver fibrosis, it's worth emphasising (20, 21).

Table 1: Changes of biochemical and hematological tests among patients before and after treatment

Variables	Baseline	Follow up	*P* value
ALT (IU/L)	42.45 (29.25-79.75)	20.00 (14.00-25.50)	<0.001
AST (IU/L)	46.50 (28.00-83.00)	24.00 (20.25-31.50)	<0.001
ALP (IU/L)	93.23±33.72	91.35±40.71	0.666
Albumin (g/dL)	3.58±0.56	4.23±0.54	<0.001
T. bilirubin (mg/dL)	0.82±0.46	0.77±0.48	0.140

Platelet count (10^9/L)	152.28±81.43	174.60±86.67	0.006
INR	1.21 (1.16-1.38)	1.06 (1.04-1.10)	<0.001

Note: Data are presented as mean ± SD (if normally distributed) or median (IQR) if not normally distributed (ALT, AST and INR).
Abbreviations: ALT: alanine aminotransferase; AST: aspartate aminotransferase; ALP: alkaline phosphatase; T. bilirubin: total bilirubin; INR: international normalization ratio.

Table 2: Changes in liver fibrosis stages among patients before and after treatment

Variables	F0	F1	F2	F3	F4	Total
Reversal	0	0	0	4	2	6
	(0.0 %)	(0.0 %)	(0.0 %)	(16.7 %)	(5.3 %)	(6.8 %)
Regression	0	2	5	6	11	24
	(0.0 %)	(28.6 %)	(71.4 %)	(25.0 %)	(28.9 %)	(27.3 %)
Stationary	7	4	1	12	25	49
	(58.3 %)	(57.1 %)	(14.3 %)	(50.0 %)	(65.8 %)	(55.7 %)
Progressive	5	1	1	2	0	9
	(41.7 %)	(14.3 %)	(14.3%)	(8.3 %)	(0.0 %)	(10.2 %)
Total	12	7	7	24	38	88

Table 3: Levels of COMP in relation to different fibrosis stage before treatment in comparison to FibroScan

Fibrosis stage	Number	Median (IQR)	*P* value
F0	12	1.71 (1.14-2.68)	<0.001
F1	7	2.07 (1.42-2.40)	
F2	7	2.25 (1.62-2.80)	
F3	24	2.31 (1.48-2.54)	
F4	38	3.34 (2.57-5.11)	
non-significant fibrosis (F0-F1) vs. significant fibrosis (F2-F4)			
F0-F1	19	1.48 (1.05-2.41)	<0.001
F2-F4	69	2.93 (1.86-4.99)	
non-advanced (F0-F2) vs. advanced fibrosis (F3-F4)			
F0-F2	26	1.60 (1.24-2.38)	0.001
F3-F4	62	3.02 (2.03-5.31)	
non-cirrhosis (F0-F3) vs. cirrhosis (F4)			
F0-F3	50	2.14 (1.49-2.86)	<0.001
F4	38	3.34 (2.57-5.11)	

Table 4: Levels of COMP before and after one year of treatment in relation to fibrosis change in comparison to FibroScan

Change of liver fibrosis	Number	Before	After
Reversal	6	1.62 (1.28-2.54)	2.00 (1.27-3.74)
Regression	24	2.34 (1.34-3.28)	2.42 (1.91-2.77)
Stationary	49	2.63 (1.81-5.19)	2.96 (2.12-4.79)
Progressive	9	2.77 (1.88-2.95)	2.27 (1.63-2.97)
P value		0.318	0.095

Note: Data are presented as median (IQR).

Table 5: Correlations of COMP to biochemical tests, hematological tests and LSM by FibroScan before and after one year of treatment

Variables	Before		After	
	r	*P*	r	*P*
ALT (IU/L)	0.384	<0.001	0.073	0.501
AST (IU/L)	0.552	<0.001	0.330	<0.001
Alkaline phosphatase (IU/L)	0.234	0.028	0.312	0.003
T. bilirubin (mg/dL)	0.414	<0.001	0.456	<0.001
Albumin (g/dL)	-0.652	<0.001	-0.154	<0.151
Platelet count (109/L)	-0.434	<0.001	-0.498	<0.001
LSM by FibroScan	0.330	0.002	0.499	0.001

They represent increased ECM deposition in the liver, either as a result of enhanced production by activated stellate cells or sluggish clearance by Kupffer and endothelial sinusoidal cells (22). Hepatic

metabolic activity, extracellular matrix remodelling proteins, collagen production, and matrix breakdown are among the studies performed (21). Indeed, COMP is one of these proteins. COMP is a glycoprotein found mostly in the extracellular matrix (ECM) of cartilage, synovium, ligaments, and tendons (9). It is made up of five identical subunits that are joined together by disulfide connections to produce a high molecular weight protein of 524 kDa. COMP's C-terminal domain may bind to collagen I, II, and IX, as well as other ECM components such fibronectin, matrilins, proteoglycans, and heparin, with great affinity (10). This study describes the difference in liver fibrosis in CHC patients after SVR following DAAs over a long period of time, as well as the diagnostic performance of COMP for recognising distinct phases of liver fibrosis.

COMP is an ECM component that has been found to be expressed in patients with fibrosis and cirrhosis. The COMP assay detects peptides produced during cartilage breakdown (23, 24). COMP has mostly been utilised in clinical settings to measure cartilage damage in patients with rheumatoid arthritis (RA) and osteoarthritis (OA) (25). COMP fragments could be found in patients' sera during liver remodelling, confirming our theory, and the amount of COMP presumably signifies the intensity of fibrogenic activity. Patients with chronic liver diseases had a significantly higher rate of COMP positive. Indeed, regardless of the cause of liver disease, individuals with cirrhosis had a significant rise in COMP. Our AUC was virtually identical to that reported by Andreasson et al., (26) who found that COMP had potential as a discriminator of severe fibrosis with an AUC of 0.79.

According to our data, the baseline individuals had lower amounts of serum albumin and blood platelets. Because albumin is entirely synthesised in the liver, these findings may be explained by the fact that albumin levels diminish in chronic hepatitis C patients as the liver's synthetic function degrades with growing hepatic fibrosis (27, 28). Furthermore, liver fibrosis may result in thrombocytopenia due to decreased thrombopoietin production and/or platelet sequestration in an enlarged spleen (29, 30) .

Reversal of cirrhosis, on the other hand, refers to the complete restoration of normal architecture after the onset of cirrhosis, whereas regression of fibrosis or cirrhosis simply means that the fibrosis content is lower than before, without specifying the extent of regression or indicating that the histology has returned to normal (14, 16). Our findings revealed that 5.3 % of CHC patients with F4 had their liver cirrhosis reversed, and 28.9% had their hepatic fibrosis regression, while the remaining 65.8% remained stable at F4 with no change in liver fibrosis stage. Patients with severe hepatic fibrosis stage F3 had a 16.7% reversal of the fibrosis stage to F0-1 and a 25% regression of the fibrosis stage to F2, while 50% remained stable in this stage. Despite viral clearance, 8.3 % of F3 patients progressed to F4 infection.

4. CONCLUSION

This research represents an advance in biomedical science because it provides a promising non-invasive marker that might be used as a potential serologic biomarker for liver fibrosis staging and may facilitate definitive therapy as well as a complementary diagnostic tool after eradication of HCV infection. To validate the effectiveness of COMP in clinical practise, more prospective trials with a larger number of patients are needed.

5. Conflict of Interest

There was no conflict of interest stated by the authors.

6. Acknowledgements

The authors would like to thank everyone at the Egyptian Liver Research Institute and Hospital (ELRIAH) in Mansoura, Egypt, for their kind help during the study.

7. References

1. Huang R, Rao H, Yang M, Gao Y, Wang J, Jin Q, et al. Noninvasive measurements predict liver fibrosis well in hepatitis C virus patients after direct-acting antiviral therapy. Digestive diseases and sciences. 2020;65(5):1491-500.
2. Elmdams MR, Gad SAB, Gawish MSF. A Novel Noninvasive Index for Assessment of Liver Fibrosis and Cirrhosis in Patients with Chronic Hepatitis C Virus Infection. The Egyptian Journal of Hospital Medicine. 2021;82(1):156-63.
3. Zarski J-P, David-Tchouda S, Trocme C, Margier J, Vilotitch A, Hilleret M-N, et al. Non-invasive fibrosis tests to predict complications in compensated post-hepatitis C cirrhosis. Clinics and Research in Hepatology and Gastroenterology. 2020;44(4):524-31.
4. Baranova A, Lal P, Birerdinc A, Younossi ZM. Non-invasive markers for hepatic fibrosis. BMC gastroenterology. 2011;11(1):1-15.
5. Pinzani M, Rombouts K, Colagrande S. Fibrosis in chronic liver diseases: diagnosis and management. Journal of hepatology. 2005;42(1):S22-S36.
6. Morling JR, Guha IN. Biomarkers of liver fibrosis. Clinical liver disease. 2016;7(6):139.
7. Grigorescu M. Noninvasive biochemical markers of liver fibrosis. Journal of gastrointestinal and liver diseases: JGLD. 2006;15(2):149-59.
8. Meurer SK, Karsdal MA, Weiskirchen R. Advances in the clinical use of collagen as biomarker of liver fibrosis. Expert Review of Molecular Diagnostics. 2020;20(9):947-69.

9. Hedbom E, Antonsson P, Hjerpe A, Aeschlimann D, Paulsson M, Rosa-Pimentel E, et al. Cartilage matrix proteins. An acidic oligomeric protein (COMP) detected only in cartilage. Journal of biological chemistry. 1992;267(9):6132-6.
10. Magdaleno F, Arriazu E, de Galarreta MR, Chen Y, Ge X, de la Rosa LC, et al. Cartilage oligomeric matrix protein participates in the pathogenesis of liver fibrosis. Journal of hepatology. 2016;65(5):963-71.
11. Acharya C, Yik JH, Kishore A, Van Dinh V, Di Cesare PE, Haudenschild DR. Cartilage oligomeric matrix protein and its binding partners in the cartilage extracellular matrix: interaction, regulation and role in chondrogenesis. Matrix Biology. 2014;37:102-11.
12. Shiha G, Seif S, Maher M, Etreby S, Samir W, Zalata K. Comparison between transient elastography (Fibroscan) and liver biopsy for the diagnosis of hepatic fibrosis in chronic hepatitis C genotype 4. Egyptian Liver Journal. 2014;4(4):106-11.
13. Poynard T, Ingiliz P, Elkrief L, Munteanu M, Lebray P, Morra R, et al. Concordance in a world without a gold standard: a new non-invasive methodology for improving accuracy of fibrosis markers. PloS one. 2008;3(12):e3857.
14. Friedman SL, Bansal MB. Reversal of hepatic fibrosis—fact or fantasy? Hepatology. 2006;43(S1):S82-S8.
15. Chung R, Davis G, Jensen D. AASLD/IDSA HCV Guidance Panel. Hepatitis C guidance: AASLD-IDSA recommendations for testing, managing, and treating adults infected with hepatitis C virus. Hepatology. 2015;62(3):932-54.
16. Shiha G, Soliman R, Mikhail N, Ibrahim A, Serwah AH, Khattab M. Changes in hepatic fibrosis stages after achieving SVR following direct-acting anti-viral treatment: a prospective study. GastroHep. 2020;2(1):39-48.
17. Maasoumy B, Wedemeyer H. Natural history of acute and chronic hepatitis C. Best practice & research Clinical gastroenterology. 2012;26(4):401-12.
18. Westbrook RH, Dusheiko G. Natural history of hepatitis C. Journal of hepatology. 2014;61(1):S58-S68.
19. Sebastiani G, Alberti A. How far is noninvasive assessment of liver fibrosis from replacing liver biopsy in hepatitis C? Journal of viral hepatitis. 2012;19:18-32.
20. Fontana RJ, Lok AS. Noninvasive monitoring of patients with chronic hepatitis C. Hepatology. 2002;36(S1):S57-S64.
21. Attallah AM, El-Far M, Malak CAA, Omran MM, Farid K, Hussien MA, et al. Fibro-check: a combination of direct and indirect markers for liver fibrosis staging in chronic hepatitis C patients. Annals of hepatology. 2015;14(2):225-33.
22. Ladero JM. Noninvasive evaluation of liver fibrosis in patients with chronic hepatitis C. Hepatitis monthly. 2011;11(9):698.
23. Halász K, Kassner A, Mörgelin M, Heinegård D. COMP acts as a catalyst in collagen fibrillogenesis. Journal of biological chemistry. 2007;282(43):31166-73.
24. Mann HH, Özbek S, Engel J, Paulsson M, Wagener R. Interactions between the cartilage oligomeric matrix protein and matrilins: implications for matrix assembly and the pathogenesis of chondrodysplasias. Journal of Biological Chemistry. 2004;279(24):25294-8.
25. Norman GL, Gatselis NK, Shums Z, Liaskos C, Bogdanos DP, Koukoulis GK, et al. Cartilage oligomeric matrix protein: A novel non-invasive marker for assessing cirrhosis and risk of hepatocellular carcinoma. World journal of hepatology. 2015;7(14):1875.
26. Andréasson K, Hesselstrand R, Saxne T, Holmberg A, Norrgren H, Jönsson G. Cartilage oligomeric matrix protein: a new promising biomarker of liver fibrosis in chronic hepatitis C. Infectious Diseases. 2015;47(12):915-8.
27. Fahim FA, Esmat AY, Hassan GK, Abdel-Bary A. Biochemical changes in patients with combined chronic schistosomiasis and viral hepatitis C infections. Disease markers. 2000;16(3, 4):111-8.
28. Attallah AM, Omran MM, Farid K, El-Bendary M, Emran TM, Albannan MS, et al. Development of a novel score for liver fibrosis staging and comparison with eight simple laboratory scores in large numbers of HCV-monoinfected patients. Clinica Chimica Acta. 2012;413(21-22):1725-30.
29. Rios R, Sangro B, Herrero I, Quiroga J, Prieto J. The role of thrombopoietin in the thrombocytopenia of patients with liver cirrhosis. American Journal of Gastroenterology. 2005;100(6):1311-6.
30. Scharf RE. Thrombocytopenia and Hemostatic Changes in Acute and Chronic Liver Disease: Pathophysiology, Clinical and Laboratory Features, and Management. Journal of Clinical Medicine. 2021;10(7):1530.