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The Difference of Hypoxic Inducible Factor-1α, Vascular Endothelial Growth Factor, and Transforming Growth Factor-β1 Based on Liver Fibrosis Severity in Patients with Chronic Hepatitis B

Libya Husen, Gontar Alamsyah Siregar*, Masrul Lubis

Division of Gastroenterology and Hepatology, Department of Internal Medicine, Faculty of Medicine, Universitas Sumatera Utara, Medan, Indonesia

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***Correspondence:** Gontar Alamsyah Siregar, Department of Internal Medicine, Faculty of Medicine, Universitas Sumatera Utara, Dr. Mansyur 5 Medan, Indonesia. E-mail: gontarsir@gmail.com
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Abstract

BACKGROUND: Hepatitis B is a global health problem. The disease damages hepatocytes and creates tissue hypoxic condition. Hypoxia triggers production of several mediators such as hypoxic inducible factor (HIF)-1α, vascular endothelial growth factor (VEGF), and transforming growth factor (TGF)-β1. The mediators act in liver fibrosis and cirrhosis, and hepatocellular carcinoma.

AIM: The objective of the study was to determine the difference in serum HIF-1α, VEGF, and TGF-β1 levels based on liver fibrosis severity in patients with chronic hepatitis B.

MATERIALS AND METHODS: This cross-sectional study was performed in Haji Adam Malik Hospital Medan, Indonesia, from January to July 2020. Subjects were chronic hepatitis B patients aged 18 years or older. Exclusion criteria were other chronic diseases, malignancies, or pregnancy. Liver fibrosis was determined using shear wave elastography and categorized as follow: F1, F2, F3, and F4. Serum HIF-1α, VEGF, and TGF-β1 levels were measured using enzyme-linked immunosorbent assay. Specimens were obtained from venous blood.

RESULTS: A total of 63 patients were enrolled in this study with mean age of 40.3 (SD 11.69) years. Subjects were dominated by males (58.7%). There were no differences in serum HIF-1α, VEGF, and TGF-β1 levels based on liver fibrosis grading and also based on hepatitis B envelope antigen (HBeAg) status and gender. Associations between liver fibrosis grading, HBeAg, and gender were absent. There was a positive correlation between liver fibrosis severity and age (r = 0.311, p = 0.013).

CONCLUSION: Serum HIF-1α, VEGF, and TGF-β1 levels were not different among chronic hepatitis B patients based on liver fibrosis severity.

Introduction

Hepatitis B infection is a serious global health problem. Approximately 240 million of world's population is infected with hepatitis B virus (HBV). Hepatitis B infection increases the risk of liver cirrhosis, liver decompensation, and hepatocellular carcinoma [1], [2]. The prevalence of hepatitis B infection is the highest in Asia-Pacific region [3]. In Indonesia, the prevalence of hepatitis B patients ranges from 4.0% to 20.3% [4]. HBV is transmitted vertically and horizontally. Around 90% of vertically transmitted infection in perinatal period will become chronic while only 5% of adult cases grow chronic [5].

Liver fibrosis results from chronic infection, including HBV infection, which causes hepatocyte death and its replacement with fibrous tissue. This process triggers hypoxia condition that activates hypoxic inducible factor (HIF). HIF itself functions as regulator of vascular endothelial growth factor (VEGF) and transforming growth factor (TGF)-β1 [6], [7], [8], [9]. The expression of HIF, VEGF, and TGF-β1 is important for angiogenesis and hepatocyte repair. However, in chronic infection, all of the mediators act as fibrogenesis agents and cause a wide spectrum of liver damage from fibrosis to cirrhosis [10].

Liver cirrhosis is an advanced condition of liver fibrosis and also known as F4 grade. Before reaching the stage, one should pass mild (F1), moderate (F2), and severe (F3) grades of fibrosis. The progression is influenced by the amount of HBV deoxyribonucleic acid (DNA), genotype, onset of infection, and comorbidity disease [11]. Gold standard for evaluating fibrosis grades is histopathology examination. However, this examination is invasive and possesses high probability of sampling error [12], [13]. Other accurate and non-invasive examination is liver elastography. This method utilizes ultrasound wave to measure liver stiffness in kilopascal (kPa) unit. Unfortunately, this device is not widely available particularly in remote areas [14], [15].

In this study, we aimed to determine whether serum HIF-1α, VEGF, and TGF-β1 levels were different among chronic hepatitis B patients based on liver

fibrosis severity from liver elastography examination. We hoped that serum HIF-1α, VEGF, and TGF-β1 may play important role in predicting liver fibrosis severity in resource-limited health centers.

Materials and Methods

This cross-sectional study was performed in outpatient clinic of Haji Adam Malik Hospital Medan, Indonesia. The study period ranged between January and July 2020. Subjects were chronic hepatitis B patients aged 18 years or older. Subjects with other chronic diseases, malignancies, or who were pregnant were excluded from this study. Written informed consent was obtained from each subject. Baseline characteristics were gathered from direct interview. Liver fibrosis was determined using shear wave elastography. Numerical results were expressed in kPa while categorical grades were determined as follow: F1 (≤7.1 kPa), F2 (>7.1–9.3 kPa), F3 (>9.3–14.5 kPa), and F4 (>14.5 kPa). Serum HIF-1α, VEGF, and TGF-β1 levels were measured using enzyme-linked immunosorbent assay. Specimens were obtained from venous blood. Serum HIF-1α level was expressed in ng/mL while serum VEGF and TGF-β1 levels in ng/mL. Data analysis was done with statistical software and the results will be presented in tables. This study was approved by the Health Research Ethical Committee, Medical School, Universitas Sumatera Utara.

Results

A total of 63 patients were enrolled in this study. Mean age of subjects was 40.3 (standard deviation [SD] 11.69) years. Male subjects were dominant in this study (58.7%). Subject's distribution based on fibrosis grading was roughly equal. Of all subjects, 20 were naïve patients and 27 were hepatitis B envelope antigen (HBeAg) reactive. Mean fibrosis severity of subjects in this study was 7.9 kPa. Median serum HIF-1α, VEGF, and TGF-β1 levels were 0.09 ng/mL, 561.9 ng/L, and 37.0 ng/L, respectively (Table 1).

Based on statistical analysis, we did not observe any difference in serum HIF-1α, VEGF, and TGF-β1 levels based on liver fibrosis grading. In this study, the highest serum HIF-1α level was observed in F1 group. In contrast, serum VEGF and TGF-β1 levels were highest in F3 group (Table 2).

We further evaluate the associations between liver fibrosis staging and HBeAg status and gender. There was no statistically significant association between liver fibrosis staging and HBeAg status (p = 0.335). Similar result was observed in the association between liver fibrosis staging and gender (p = 0.459) (Table 3).

Table 1: Baseline characteristics of subjects

Characteristics	n = 63
Mean age, years (SD)	40.3 (11.69)
Gender, n (%)	
Male	37 (58.7)
Female	26 (41.3)
Live fibrosis grade, n (%)	
F1	22 (34.9)
F2	21 (33.3)
F3	20 (31.7)
HBeAg status, n (%)	
Nonreactive	36 (57.1)
Reactive	27 (42.9)
Therapy, n (%)	
Naïve	20 (31.7)
Tenofovir	16 (25.4)
Pegasys	2 (3.2)
Sebio	21 (33.3)
Combination	4 (6.3)
Mean severity of liver fibrosis, kPa (SD)	7.9 (2.46)
Median serum HIF-1α level, ng/mL (median)	0.09 (0.00–12.70)
Median serum VEGF level, ng/L (median)	561.9 (11.5–4688.0)
Median serum TGF-β1 level, ng/L (median)	37.0 (3.5–4997.0)

SD: Standard deviation; HBeAg: Hepatitis B envelope antigen; HIF-1α: Hypoxic inducible factor-1α; VEGF: Vascular endothelial growth factor; TGF-β1: Transforming growth factor-β1.

Table 2: The difference in serum HIF-1α, VEGF, and TGF-β1 levels based on liver fibrosis grading

Mediators	F1	F2	F3	p*	95% CI
Median serum HIF-1α level, ng/mL (min-max)	0.24 (0.03–1.92)	0.06 (0.00–2.09)	0.09 (0.02–2.58)	0.086	0.034–0.189
Median serum VEGF level, ng/L (min-max)	569.0 (11.5–4437.8)	449.4 (106.4–2280.2)	810.7 (220.8–4688.0)	0.180	0.146–0.361
Median serum TGF-β1 level, ng/L (min-max)	38.4 (8.4–3589.5)	24.1 (3.5–859.4)	51.5 (3.6–4997.0)	0.347	0.188–0.415

*Kruskal–Wallis test; CI: Confidence interval; HIF-1α: Hypoxic inducible factor-1α; VEGF: Vascular endothelial growth factor; TGF-β1: Transforming growth factor-β1.

Table 3: The associations between liver fibrosis staging and HBeAg status and gender

Stages	HBeAg status, n (%)		p*	Gender		p*
	Nonreactive	Reactive		Male	Female	
F1	12 (19.0)	10 (15.9)	0.335	12 (19.0)	10 (15.9)	0.459
F2	10 (15.9)	11 (17.5)		11 (17.5)	10 (15.9)	
F3	14 (22.2)	6 (9.5)		14 (22.2)	6 (9.5)	

*Chi-square test; HbeAg: Hepatitis B envelope antigen.

The severity of liver fibrosis was also measured in numerical scale. Based on HBeAg status and gender, there was no statistically significant difference in severity of liver fibrosis with p values of 0.335 and 0.327, respectively (Table 4).

Table 4: The difference in liver fibrosis severity based on HBeAg status and gender

Mean liver fibrosis severity, kPa (SD)		
HBeAg status		p*
Nonreactive	2.0 (0.86)	0.335
Reactive	1.8 (0.76)	
Gender		
Male	2.0 (0.84)	0.327
Female	1.8 (0.78)	

*T independent test; SD: Standard deviation; HbeAg: Hepatitis B envelope antigen.

We also analyzed the correlation between liver fibrosis severity and age of subjects. We found a positive significant correlation between both variables (r = 0.311, p = 0.013). The higher the age of subjects, the more severe the liver fibrosis becomes (Table 5).

Table 5: The correlation between liver fibrosis severity and age

Variables	Mean	SD	r	p*
Liver fibrosis severity, kPa	7.9	2.46	0.311	0.035
Age, years	40.3	11.69		

*Pearson's correlation test; SD: Standard deviation.

Discussion

Chronic hepatitis B is initiated by unresolved acute hepatitis B infection, marked by the presence of HBsAg and HBV DNA. HBeAg, in the other hand, is a marker of high viral load and could be remained positive in months to years after the onset of infection [16]. As time goes by, liver damage will occur. At first, hepatocytes experience damage and apoptosis. This will impair hepatic blood circulation and induce hypoxic condition. Those situations induce secretion of fibrogenic mediators which activate hepatic stellate cells (HSCs) to produce fibrous connective tissues [17], [18].

HIF is one of mediators secreted in hypoxic condition. It consists of HIF-1α and HIF-1β heterodimers [19], [20], [21]. HIF plays important role in maintaining tissue oxygenation by promoting cell metabolism, proliferation, and migration [21], [22]. Most organs follow the same rule, including liver. HIF, in the other hand, is also related to VEGF and TGF-β1 which promotes fibrogenesis through HSC activation [23], [24].

In acute liver injury, HIF-1α has protective effect but not in chronic liver disease [18], [25], [26]. HIF-1α increases the activities of fibrogenesis and angiogenesis in chronic liver disease [21]. Chronic liver damage results in extracellular matrix accumulation, including fibrocytes, replacing damaged liver tissues [18]. In an animal study, administration of HIF-1α inhibitor resulted in alleviation of liver damage and abortion of liver failure. This was suspected from decreased reactive oxygen species amount, inhibited necrosis process, and decreased hepatocyte mitochondria permeability due to inactivation of HIF-1α [26]. Liver fibrosis was also less progressive in HIF-1α knockout mice even after receiving similar treatment with normal mice. This finding supported the theory that HIF-1α plays important role in fibrogenesis in liver damage [27]. Another study in mice showed positive association between HIF-1α expression and liver fibrosis severity. In addition, HIF-1α expression was also correlated with angiogenesis and VEGF expression. In this study, HIF-1α expression was measured histopathologically [28]. Those studies were not in line with our result. We did not observe a difference in serum HIF-1α level based on liver fibrosis grading. Furthermore, median serum HIF-1α level was higher in subjects with mild grade liver fibrosis.

Even though the highest serum VEGF level in this study was observed in F3 grade, the difference was no statistically significant compared to F1 and F2 grades. There are still controversies regarding this finding. The expression of VEGF was increased in hypoxia condition and related to angiogenesis. Its expressions were different among liver diseases and the highest was in acute hepatitis. In chronic hepatitis, the expression of VEGF was not increased compared to healthy subjects as control. Serum VEGF level was also not in line with inflammation score in chronic hepatitis and liver cirrhosis [29]. Our result is in contrast with a study by El-Assal *et al*. [30]. They reported that tissue VEGF expression was higher in liver cirrhosis patients compared to subjects without liver cirrhosis. However, they also did not find any association between tissue VEGF expression and histopathology findings in patients with hepatocellular carcinoma. This might be caused by decreased VEGF role after the tumor reached a significant size [31]. In the other hand, serum VEGF level per thrombocyte count was associated with the severity of liver cirrhosis based on a study from Korea. The higher the serum VEGF level per thrombocyte count, the lower the therapeutic response of patients and this was obviously related to poor disease outcome [7]. Another study from Indonesia reported that serum VEGF level was associated with disease progressivity of liver cirrhosis. Serum VEGF level is in concordance with liver cirrhosis severity based on Child Pugh scoring system [8].

TGF-β is involved in the pathogenesis of disease from liver fibrosis to hepatocellular carcinoma [18], [31]. A study by Yang *et al*. showed that TGF-β expression was increased in the hepatic tissue of mice suffering from liver fibrosis and hepatocellular carcinoma. The elevation of TGF-β expression was suspected to increase fibrogenesis, anti-apoptotic, pro-oncogenic, and angiogenic activities in hepatocytes [31]. An *in vitro* study proved that the addition of TGF-β significantly increased collagen I and fibronectin levels which led to liver fibrosis. Administration of TGF-β inhibitor (ferulic acid) resulted in fibrogenesis inhibition [32]. The expression of TGF-β in hepatic tissues of mice suffering from fibrosis was increased according to a study by Fan *et al*. The increment was followed by increased activity of HSC, one of important cells that promote fibrogenesis activity. They also found that extracellular matrix protein 1 secreted by hepatocytes might inhibit TGF-β activity [33]. Our result was different with several literatures above. Serum TGF-β1 level was not significantly different based on fibrosis grading but we found that subjects with severe grade fibrosis had the highest serum TGF-β1 level.

In this study, male subjects were dominant. Males were also dominant in each grade of fibrosis group. The severity of liver fibrosis was higher in males compared to females even though the difference was not statistically significant. Male predilection in liver fibrosis could be caused by the difference in the lifestyle. Males were prone to high risk lifestyles compared to females such as alcohol consumption, smoking, and unhealthy diet [18], [31], [34]. According to Yang *et al*. males had higher risk for liver fibrosis due to the absence of estrogen. This was supported by the fact that gender predisposition began to disappear by age [35].

Gao *et al*. found that hepatitis B infection markers which associated with liver fibrosis severity were not HBeAg, but HBsAg and HBV DNA. Uniquely, HBsAg and HBV DNA levels in patients with moderate

and severe grades fibrosis were lower compared to those with mild grade [36]. Analysis result by Chen *et al*. showed that qualitative HBeAg level was positively associated with liver fibrosis severity [37]. In this study, HBeAg was measured qualitatively and most subjects were not reactive. Those might affect the result of our study, where there was no relationship between HBeAg status and fibrosis grading in this study. The severity of liver fibrosis was also milder in subjects with reactive HBeAg but the difference was not statistically significant.

Petta *et al*. conducted a study in 2017. They reported that age was a factor influencing the severity of liver fibrosis. The relationship was mediated by lipid profile. The older the subject, the more severe liver fibrosis became [38]. Similar result was reported by Klisic *et al*. They stated that advanced age was an independent risk factor for the severity of liver fibrosis. In their study, the severity of liver fibrosis was assessed using BARD score [39]. Another study confirming those findings were reported by van Santen *et al*. Advanced age was associated with more severe fibrosis. In their study, liver fibrosis severity was measured using similar method with our study [40]. Our result was consistent with previous studies results. We found a positive correlation between age and liver fibrosis severity.

There were some differences in our result with previous studies. This might be caused by our study's limitations. We examined the level of mediators from serum sample while most previous studies used histopathological method from tissue specimen. Besides, there was a difference in fibrosis grading evaluation. Regardless of that, according to authors' knowledge this was the first study determining the difference in serum HIF-1α, VEGF, and TGF-β1 levels based on liver fibrosis severity in general population, not study animal, in Indonesia.

As the conclusion, there was no difference in serum HIF-1α, VEGF, and TGF-β1 levels based on severity of liver fibrosis in this study. Similar result was reported based on HBeAg status and gender. There was no association between liver fibrosis grading and HBeAg status and gender. Positive correlation was observed between severity of liver fibrosis and age.

References

1. Ott JJ, Stevens GA, Groeger J, Wiersma ST. Global epidemiology of hepatitis B virus infection: New estimates of age-specific HBsAg seroprevalence and endemicity. Vaccine. 2012;30(12):2212-9. https://doi.org/10.1016/j.vaccine.2011.12.116
   PMid:22273662
2. Schweitzer A, Horn J, Mikolajczyk RT, Krause G, Ott JJ. Estimations of worldwide prevalence of chronic hepatitis B virus infection: A systematic review of data published between 1965-2013. Lancet. 2015;386(10003):1546-55. https://doi.org/10.1016/s0140-6736(15)61412-x
   PMid:26231459
3. Liaw Y. Antiviral therapy of chronic hepatitis B: Opportunities and challenges in Asia. J Hepatol. 2009;51(2):403-10.
   PMid:19467727
4. Kementerian Kesehatan Republik Indonesia. Badan Penelitian dan Pengembangan Kesehatan Kementerian Kesehatan Republik Indonesia, Riset Kesehatan Dasar 2013. Jakarta: Kementerian Kesehatan Republik Indonesia; 2013. Available from: https://www.kemkes.go.id/resources/download/general/hasil%20riskesdas%202013.pdf. [Last accessed on 2020 Dec 05]. https://doi.org/10.6066/jtip.2013.24.2.121
5. Lesmana CR, Lesmana LA. Perjalanan Penyakit Hepatitis B: Hepatitis B di Indonesia Dari Molekul Sampai Terapi. Jakarta: Digestive Disease and GI Oncology Centre Mediastra Hospital; 2015.
6. Makhlouf MM, Awad A, Zakhari MM, Fouad M, Saleh WA. Vascular endothelial growth factor level in chronic liver diseases. J Egypt Soc Parasitol. 2002;32(3):907-21.
   PMid:12512823
7. Kim SJ, Choi IK, Park KH, Yoon SY, Oh SC, Seo JH, *et al*. Serum vascular endothelial growth factor per platelet count in hepatocellular carcinoma: Correlations with clinical parameters and survival. Jpn J Clin Oncol. 2004;34(4):184-90. https://doi.org/10.1093/jjco/hyh039
   PMid:15121753
8. Nova F, Siregar GA, Sungkar T. Comparison of serum vascular endothelial growth factor (VEGF) in degrees Child Pugh cirrhosis patients. Indian J Appl Res. 2019;9:37-9.
9. Abdelmoaty MA, Bogdady AM, Attia MM, Zaky NA. Circulating vascular endothelial growth factor and nitric oxide in patients with liver cirrhosis: A possible association with liver function impairment. Indian J Clin Biochem. 2009;24(4):398-403. https://doi.org/10.1007/s12291-009-0071-5
   PMid:23105867
10. Assy N, Paizi M, Gaitini D, Baruch Y, Spira G. Clinical implication of VEGF serum levels in cirrhotic patients with or without portal hypertension. World J Gastroenterol. 1999;5(4):296-300. https://doi.org/10.3748/wjg.v5.i4.296
    PMid:11819451
11. Bravo AA, Sheth SG, Chopra S. Liver biopsy. N Engl J Med. 2001;344(7):495-500.
    PMid:11172192
12. Rousselet MC, Michalak S, Dupré F, Croué A, Bedossa P, Saint-André JP, *et al*. Sources of variability in histological scoring of chronic viral hepatitis. Hepatology. 2005;41(2):257-64. https://doi.org/10.1002/hep.20535
    PMid:15660389
13. Mulherin SA, Miller WC. Spectrum bias or spectrum effect? Subgroup variation in diagnostic test evaluation. Ann Intern Med. 2002;137:598-602. https://doi.org/10.7326/0003-4819-137-7-200210010-00011
    PMid:12353947
14. Shiha G, Ibrahim A, Helmy A, Sarin SK, Omata M, Kumar A, *et al*. Asian-pacific association for the study of the liver (APASL) consensus guidelines on invasive and non-invasive assessment of hepatic fibrosis: A 2016 update. Hepatol Int. 2017;11(1):1-30. https://doi.org/10.1007/s12072-016-9760-3
    PMid:27714681
15. Fraquelli M, Rigamonti C, Casazza G, Conte D, Donato MF, Ronchi G, *et al*. Reproducibility of transient elastography in the evaluation of liver fibrosis in patients with chronic liver disease. Gut. 2007;56(7):968-73. https://doi.org/10.1136/gut.2006.111302

PMid:17255218

16. McMahon BJ. The natural history of chronic hepatitis B virus infection. Hepatology. 2009;49 Suppl 5:S45-55.
PMid:19399792
17. Sun M, Kisseleva T. Reversibility of liver fibrosis. Clin Res Hepatol Gastroenterol. 2015;39 Suppl 10:S60-3.
PMid:26206574
18. Bataller R, Brenner DA. Liver fibrosis. J Clin Invest. 2005;115(2):209-18.
PMid:15690074
19. Matak P, Heinis M, Mathieu JR, Corriden R, Cuvellier S, Delga S, *et al*. Myeloid HIF-1 is protective in *Helicobacter pylori*-mediated gastritis. J Immunol. 2015;194(7):3259-66. https://doi.org/10.4049/jimmunol.1401260
PMid:25710915
20. Koyasu S, Kobayashi M, Goto Y, Hiraoka M, Harada H. Regulatory mechanisms of hypoxia-inducible factor 1 activity: Two decades of knowledge. Cancer Sci. 2018;109(3):560-71. https://doi.org/10.1111/cas.13483
PMid:29285833
21. Corrado C, Fontana S. Hypoxia and HIF signaling: One axis with divergent effects. Int J Mol Sci. 2020;21(16):5611. https://doi.org/10.3390/ijms21165611
PMid:32764403
22. Verrecchia F, Mauviel A. Transforming growth factor-beta and fibrosis. World J Gastroenterol. 2007;13(22):3056-62. https://doi.org/10.3748/wjg.v13.i22.3056
PMid:17589920
23. Latasa MU, Boukaba A, García-Trevijano ER, Torres L, Rodríguez JL, Caballería J, *et al*. Hepatocyte growth factor induces MAT2A expression and histone acetylation in rat hepatocytes: Role in liver regeneration. FASEB J. 2001;15(7):1248-50. https://doi.org/10.1096/fj.00-0556fjev1
PMid:11344103
24. Roth KJ, Copple BL. Role of hypoxia-inducible factors in the development of liver fibrosis. Cell Mol Gastroenterol Hepatol. 2015;1(6):589-97.
PMid:28210703
25. Kron P, Linecker M, Limani P, Schlegel A, Kambakamba P, Lehn JM, *et al*. Hypoxia-driven Hif2a coordinates mouse liver regeneration by coupling parenchymal growth to vascular expansion. Hepatology. 2016;64(6):2198-209. https://doi.org/10.1002/hep.28809
PMid:27628483
26. Xie F, Dong J, Zhu Y, Wang K, Liu X, Chen D, *et al*. HIF1a Inhibitor rescues acute-on-chronic liver failure. Ann Hepatol. 2019;18(5):757-64. https://doi.org/10.1016/j.aohep.2019.03.007
PMid:31402229
27. Mesarwi OA, Shin MK, Bevans-Fonti S, Schlesinger C, Shaw J, Polotsky VY. Hepatocyte hypoxia inducible factor-1 mediates the development of liver fibrosis in a mouse model of nonalcoholic fatty liver disease. PLoS One. 2016;11(12):e0168572. https://doi.org/10.1371/journal.pone.0168572
PMid:28030556
28. Bozova S, Elpek GO. Hypoxia-inducible factor-1alpha expression in experimental cirrhosis: Correlation with vascular endothelial growth factor expression and angiogenesis. APMIS. 2007;115(7):795-801. https://doi.org/10.1111/j.1600-0463.2007.apm_610.x
PMid:17614845
29. Akiyoshi F, Sata M, Suzuki H, Uchimura Y, Mitsuyama K, Matsuo K, *et al*. Serum vascular endothelial growth factor levels in various liver diseases. Dig Dis Sci. 1998;43(1):41-5. https://doi.org/10.1023/a:1018863718430
PMid:9508533
30. El-Assal ON, Yamanoi A, Soda Y, Yamaguchi M, Igarashi M, Yamamoto A, *et al*. Clinical significance of microvessel density and vascular endothelial growth factor expression in hepatocellular carcinoma and surrounding liver: Possible involvement of vascular endothelial growth factor in the angiogenesis of cirrhotic liver. Hepatology. 1998;27(6):1554-62. https://doi.org/10.1002/hep.510270613
PMid:9620326
31. Yang L, Inokuchi S, Roh YS, Song J, Loomba R, Park EJ, *et al*. Transforming growth factor-β signaling in hepatocytes promotes hepatic fibrosis and carcinogenesis in mice with hepatocyte-specific deletion of TAK1. Gastroenterology. 2013;144(5):1042-54. https://doi.org/10.1053/j.gastro.2013.01.056
PMid:23391818
32. Mu M, Zuo S, Wu R, Deng K, Lu S, Zhu J, *et al*. Ferulic acid attenuates liver fibrosis and hepatic stellate cell activation via inhibition of TGF-β/Smad signalling pathway. Drug Des Devel Ther. 2018;12:4107-15. https://doi.org/10.2147/dddt.s186726
PMid:30584275
33. Fan W, Liu T, Chen W, Hammad S, Longerich T, Hausser I, *et al*. ECM1 prevents activation of transforming growth factor β, hepatic stellate cells, and fibrogenesis in mice. Gastroenterology. 2019;157(5):1352-67. https://doi.org/10.1053/j.gastro.2019.07.036
PMid:31362006
34. Mantaka A, Koulentaki M, Samonakis D, Sifaki-Pistolla D, Voumvouraki A, Tzardi M, *et al*. Association of smoking with liver fibrosis and mortality in primary biliary cholangitis. Eur J Gastroenterol Hepatol. 2018;30(12):1461-9. https://doi.org/10.1097/meg.0000000000001234
PMid:30106760
35. Yang JD, Abdelmalek MF, Pang H, Guy CD, Smith AD, Diehl AM, *et al*. Gender and menopause impact severity of fibrosis among patients with nonalcoholic steatohepatitis. Hepatology. 2014;59(4):1406-14. https://doi.org/10.1002/hep.26761
PMid:24123276
36. Gao Y, Zou G, Ye J, Pan G, Rao J, Li F, *et al*. Relationship between hepatitis B surface antigen, HBV DNA quantity and liver fibrosis severity. Zhonghua Gan Zang Bing Za Zhi. 2015;23(4):254-7.
PMid:26133815
37. Chen Y, Li Y, Li N, Fan X, Li C, Zhang P, *et al*. A noninvasive score to predict liver fibrosis in HBeAg-positive hepatitis B patients with normal or minimally elevated alanine aminotransferase levels. Dis Markers. 2018;2018:3924732. https://doi.org/10.1155/2018/3924732
PMid:30405859
38. Petta S, Eslam M, Valenti L, Bugianesi E, Barbara M, Cammà C, *et al*. Metabolic syndrome and severity of fibrosis in nonalcoholic fatty liver disease: An age-dependent risk profiling study. Liver Int. 2017;37(9):1389-96. https://doi.org/10.1111/liv.13397
PMid:28235154
39. Klisic A, Abenavoli L, Fagoonee S, Kavaric N, Kocic G, Ninić A. Older age and HDL-cholesterol as independent predictors of liver fibrosis assessed by BARD score. Minerva Med. 2019;110(3):191-8. https://doi.org/10.23736/s0026-4806.19.05978-0
PMid:30784251
40. van Santen DK, van der Loeff MF, van Dissel JC, Martens JP, van der Valk M, Prins M. High proportions of liver fibrosis and cirrhosis in an ageing population of people who use drugs in Amsterdam, the Netherlands. Eur J Gastroenterol Hepatol. 2018;30(10):1168-76. https://doi.org/10.1097/meg.0000000000001213
PMid:30028776