RESEARCH ARTICLE

Increased Levels of TNF-α, IL-6, and IL-10 are Associated with The Degree of Liver Fibrosis in Chronic Hepatitis B Patients with NUC Therapy

Ummi Maimunah[1,2*], Ulfa Kholili[1,2], Rheza Rahmadika Putra[2], Dio Brimantyo[2], Hendy Wirantara[2]

[1]Gastroenterology and Hepatology Division, Department of Internal Medicine, Faculty of Medicine, Universitas Airlangga/Dr. Soetomo General Hospital, Jl. Mayjend. Prof. Dr. Moestopo No.6-8, Surabaya 60286, Indonesia
[2]Department of Internal Medicine, Faculty of Medicine, Universitas Airlangga/Dr. Soetomo General Hospital, Jl. Mayjend. Prof. Dr. Moestopo No.6-8, Surabaya 60286, Indonesia

*Corresponding author. Email: ummima@gmail.com

Received date: Dec 15, 2023; Revised date: Jan 17, 2024; Accepted date: Jan 19, 2024

Abstract

BACKGROUND: Liver fibrosis in chronic hepatitis B (CHB) involves the host immune responses mainly T-lymphocyte regulatory cells and cytokines production. Tumor necrosis factor (TNF)-α, interleukin (IL)-6, and IL-10 have been reported to play a crucial role in the development of liver fibrosis. However, their association with liver fibrosis in treated CHB patients remains unclear. Therefore, this study was conducted to investigate the association between TNF-α, IL-6, and IL-10 with the degree of liver fibrosis in treated CHB patients.

METHODS: This was a cross-sectional prospective study including 101 treated chronic hepatitis B subjects. TNF-α, IL-6, and IL-10 serum levels were measured with quantitative sandwich enzyme-linked immunosorbent assay (ELISA) kit. Transient elastography result was classified according to METAVIR score. Data was analyzed by the Spearman correlation test with a $p<0.05$ was considered statistically significant.

RESULTS: From 101 subjects, there were significant differences were seen in TNF-α, IL-6, and IL-10 between patients with mild, significant and advance fibrosis. TNF-α (r=0.292; $p<0.05$), IL-6 (r=0.221; $p<0.05$), and IL-10 (r=0.208; $p<0.05$) were significantly correlated with the degree of fibrosis. After multivariate analysis, TNF-α was the only one cytokine parameter which significantly correlated with the degree of fibrosis.

CONCLUSION: Levels of TNF-α, IL-6 and IL-10 are associated with the degree of liver fibrosis. These parameters may potentially be used to evaluate the development of liver fibrosis in treated CHB patients.

KEYWORDS: chronic hepatitis B, liver fibrosis, cytokines, transient elastography

Indones Biomed J. 2024; 16(1): 72-8

Introduction

Chronic hepatitis B (CHB) remains an important global health problem leading to significant morbidity and mortality, despite highly effective preventive vaccines and oral antivirals.(1) According to the World Health Organization (WHO), HBV chronically infects more than 350 million people with 15-40% of those people having chronic liver disease, such as liver fibrosis, liver cirrhosis, and hepatocellular carcinoma (HCC).(2,3) Besides that, Indonesia is one of the intermediate-to-high endemic areas of HBV infection, in which 7.1% of the population is infected.(2,4) Several studies reported that liver fibrosis is reversible when the causative agents, including HBV infection and alcohol, are removed.(5,6) Therefore, patients with HBV infection should be screened for liver fibrosis earlier to prevent disease progression.

Nowadays, liver biopsy is the gold standard examination to determine the degree of liver fibrosis, the

liver histology and evaluate the treatment outcomes in CHB.(7) Due to the limitations of operators, post-procedure risks, and limited use in developing countries, liver biopsy cannot be widely used.(8) Moreover, the primary issues with liver biopsy are sampling error and significant variability in fibrosis assessment.(9) Transient elastography, one-dimensional ultrasound-based using Fibroscan, is a tool to measure liver stiffness by assessing the speed of low frequency elastic shear waves (50Hz) transmitted and reflected by liver parenchyma. Fibroscan is a non-invasive, painless, good objectivity, sensitivity, and specificity examination.(10) However, the Fibroscan device and its maintenance are expensive, requiring reliable operators and decreased accuracy in obesity, pregnancy, and narrow intercostal space making it unsuitable for routine monitoring of liver fibrosis/cirrhosis in every patient and area.(11) In order to accurately evaluate liver fibrosis, non-invasive and easy-to-use tests are needed due to the limitations of liver biopsy and Fibroscan.

Outcomes of the HBV infection are influenced by viral characteristics, history of treatment, and the host immune system. Nucleos(t)ide analogues (NUC) treatment in CHB patients still causes the liver fibrosis development through the immune system, in addition to HBV DNA and HBeAg. Both innate and adaptive immune systems, including T-lymphocyte immune regulatory (T-reg) and cytokines, are involved in host defense.(12,13) Cytokines in liver cells play a fundamental role by producing pro-inflammatory mediators through Th1 cells such as tumor necrosis factor (TNF)-α and interleukin (IL)-6 or anti-inflammatory mediators through T-reg cells such as IL-10.(14)

Several studies have shown that biomarkers and radiological examination can be used to assess the degree of liver fibrosis. There has been little research on how cytokines, such as TNF-α, IL-6, and IL-10, are related to the development of liver fibrosis in Indonesia. Therefore, we conducted a study to investigate the relationship between those cytokines and the degree of liver fibrosis in patients who were undergoing NUC treatment for CHB.

Methods

Study Design and Participants

A cross-sectional prospective study was conducted on chronic HBV patients who were evaluated for treatment at Dr. Soetomo Hospital, Surabaya, from April 2023 to October 2023. The objective of the study was explained to the patients, and subsequently, written consent was obtained. The study protocol has been approved by the Human Research Ethics Committee at Dr Soetomo Hospital (No. 632/KEPK/III/2023). One hundred-one patients with previous antiviral treatment for chronic hepatitis B were recruited. Clinical, demographic, and laboratory data, including aspartate aminotransferase (AST), alanine transaminase (ALT), hemoglobin (Hb), white blood cell (WBC), platelet (PLT), albumin, partial thromboplastin time (APTT), activated PTT (APTT), total bilirubin (TBil), direct bilirubin (DBil), hepatitis B e antigen (HBeAg), as well as HBV DNA were collected from medical records. The inclusion criteria were age over 18 years, patients with serologically confirmed chronic hepatitis B who had received antiviral therapy for at least one year. Patients diagnosed with alcoholic liver disease, decompensated chronic liver disease, autoimmune disease, diabetes mellitus, pregnancy, morbid obesity, sepsis, malignancy, and evidence of HCV or HIV coinfection, were excluded from the study.

TNF-α, IL-6 and IL-10 Measurement

Samples were collected from blood serum, and we allowed the serum to clot for 10-20 minutes at room temperature, the blood was then centrifuged at 2000-3000 rpm for 20 minutes. Then, we collected the supernatant without sediment. All specimens were kept at −80°C until tested. Circulating levels of cytokines TNF-α, IL-6, and IL-10 were measured using the ELISA method. Human TNF-α (Cat No. E0082Hu, BT-Lab, Shanghai, China), Human IL-6 (Cat No. E090Hu, BT-Lab), and Human IL-10 (Cat No. E0102Hu, BT-Lab) ELISA KIT utilizes an antibody specific for human TNF-α, IL-6, and IL-10 coated on a 96-well plate. Recombinant human TNF-α, IL-6, and IL-10 dilutions were used to produce the standard curves. The wavelength at which absorbance may be detected was 450 nm. The overall procedures were conducted in the Central Laboratory Installation, Dr. Soetomo Hospital.

Fibrosis Measurement

The fibrosis stage was measured with transient elastography using FibroScan 502 (Echosens, Paris, France) with Vibration Regulated Transient Elastography (VCTE) technology. Fibroscans were performed on CHB patients in the morning on an empty stomach or at least 2 hours after eating by three certified internists independently. We used 10 validated scores as the median value elastography. The median of the 10 validated values was considered the liver elastic modulus and was expressed in kilopascals (kPa). Liver fibrosis was graded on a 5-point scale (F0: no fibrosis, F1: minimal fibrosis, F2: fibrosis with few septa,

F3: numerous bridging fibers without cirrhosis), F4: liver cirrhosis or advanced severe fibrosis). As stated in the METAVIR score; F0-F1 was defined as mild fibrosis; F2–F3 as significant fibrosis; and F4 as advanced fibrosis.(15)

Statistical Analysis

Data were analyzed using SPSS version 26 (IBM Corporation, Armonk, NY, USA). Univariate analysis were performed to analyze mean, median, maximum and minimum value and SD. Spearman correlation was used when variables were not normally distributed. Mann-Whitney was used for post hoc analysis. A $p<0.05$ was considered statistically significant.

Results

Patient Clinical Profiles

There were total 101 subjects included in this study. The baseline clinical characteristics of enrolled subjects were described in Table 1. The average age of the enrolled patients was 45 years, with a majority of male subjects (63.4%). More than half of the subjects had mild fibrosis (51.5%), had negative HBeAg (69.3%), and had undetected HBV DNA (56.4%).

Compared with subjects in mild fibrosis, subjects with significant fibrosis had higher levels of ALT, AST, PPT, APTT, TBil and lower levels of albumin, hemoglobin, WBC counts, and platelet counts. Similarly, subjects with advanced fibrosis had higher levels of ALT, AST, TBil, DBil, PPT, APTT, and lower levels of albumin, hemoglobin, WBC counts, platelet counts. No significant differences were observed in gender, hemoglobin, and APTT between subjects with mild, significant and advanced fibrosis (Table 2).

Post-hoc analysis showed that in mild *vs* significant fibrosis, there were significant differences in AST, WBC, and PLT. In the mild *vs* advanced fibrosis stage, there were significant differences in all parameters. In the significant *vs* advanced fibrosis stage, there were significant differences in AST, PPT, TBil, and DBil (Table 3).

TNF-α, IL-6, IL-10 Serum Levels with the Degree of Fibrosis in Patients with CHB

TNF-α was significantly correlated with the degree of fibrosis (r=0.292; $p<0.05$), IL-6 also significantly correlated with the degree of fibrosis (r=0.221; $p<0.05$) and IL-10 was also significantly correlated with the degree of fibrosis (r=0.208; $p<0.05$) (Table 4).

After post hoc analysis in TNF-α, IL-6, and IL-10, there were significant differences in mild *vs* advanced fibrosis stage. There was no significant difference in the mild *vs* significant fibrosis stage. TNF-α was the only cytokine parameter that significant differences in the significant *vs* advanced fibrosis stage (Table 5).

After multivariate analysis of data including baseline characteristics, laboratory data, and other cytokines, TNF-α was the only cytokine parameter that significantly correlated with the degree of fibrosis ($p=0.022$). But ALT and PLT also showed some significance (Table 6).

Discussion

HBV infection indirectly harms liver cells by triggering the body's immune response. Imbalances in T lymphocyte subsets and their cytokines play a significant role in the onset and progression of chronic liver diseases.(16,17) Chronic HBV infection causes prolonged inflammation and liver fibrosis due to the imbalanced T helper (Th) immune responses and its cytokines especially TNF-α, IL-6, and IL-10 which have pro-inflammatory and anti-inflammatory properties, respectively, in the liver.(18) The majority of this study involved the use of NUC, particularly tenofovir and

Table 1. Clinical characteristics of research subjects (n=101).

Variable	n (%)	Mean±SD
Age (years)		45.36±12.32
Gender		
Male	64 (63.4%)	
Female	37 (36.6%)	
Liver Stiffness Data		
F0-F1 (Mild)	52 (51.5%)	
F2-F3 (Significant)	28 (27.7%)	
F4 (Advanced)	21 (20.8%)	
Therapy		
Tenofovir	90 (89.1%)	
Entecavir	10 (9.9%)	
Lamivudine	1 (1.0%)	
HBeAg		
Negative	70 (69.3%)	
Positive	31 (30.7%)	
HBV DNA		
Undetected	57 (56.4%)	
Detected	44 (43.6%)	
<2000 IU/mL	32 (31.7%)	
2000-20.000 IU/mL	2 (2.0%)	
>20.000 IU/mL	10 (9.9%)	

Table 2. Characteristics of research subjects based on the degree of liver fibrosis.

Variable	Fibrosis Stage			*p*- value
	Mild	Significant	Advanced	
Gender [c]				
Male	29 (55.8%)	21(75%)	14 (66.7%)	0.220
Female	23 (44.2%)	7 (25%)	7 (33.3%)	
Age (years) [b]	40.58±11.74	49.64±10.00	51.48±12.15	0.000*
AST (U/L) [a]	24(14-47)	25.5 (19-71)	34 (22-62)	0.000*
ALT (U/L) [a]	25(11-58)	29.5(14-117)	33(14-93)	0.049*
Hb (g/dL) [b]	14.35(10.6-17.4)	13.95(11-16.60)	12.90(7.50-17.9)	0.073
WBC (10^3/uL) [a]	7.51(3.94-44.20)	6.29(2.71-12.26)	5.19(2.09-10.38)	0.000*
PLT (10^3uL) [b]	281.48±71.74	212.50±82.87	192.90±62.77	0.000*
Albumin (g/dL) [b]	4.52(3.99-5.76)	4.45(3.88-4.99)	4.47(2.85-5.09)	0.022*
APTT [a]	28.13±4.18	28.74±3.39	30.15±4.33	0.061
PPT [a]	11.59±3.20	11.80±2.53	12.24±1.27	0.003*
TBil (mg/dL) [a]	0.69±0.67	2.31±8.96	1.22±1.11	0.006*
DBil (mg/dL) [a]	0.24±0.27	0.22±0.18	0.38±0.29	0.033*
HBeAg [c]				
Negative	34 (65.4%)	20 (71.4%)	16 (76.2%)	0.637
Positive	18 (34.6%)	8 (28.6%)	5 (23.8%)	
HBV DNA [c]				
Undetected	32 (61.5%)	14 (50%)	11 (52.4%)	0.686
Detected	20 (38.5%)	14 (50%)	10 (47.6%)	
<2000 IU/mL	13 (25%)	11 (39.3%)	8 (38.1%)	
2000-20.000 IU/mL	2 (3.8%)	0 (0%)	0 (0%)	
>20.000 IU/mL	5 (9.6%)	3 (10.7%)	2 (9.5%)	

[a]Kruskal–Wallis test, the value is presented in mean±SD value. [b]Anova test, the value is presented in median (min-max). [c]Chi-square test, value is presented in n (%). Significant if $p<0.05$.

entecavir, which have been shown to effectively suppress HBV replication and increase the level of inflammatory cytokines like TNF-α and IL-6 while concurrently reducing the level of anti-inflammatory cytokine IL-10.(19)

Hepatic fibrosis and liver tissue damage are associated with elevated blood TNF-α levels during HBV infection.(12,20) As per prior research, our investigation demonstrated a noteworthy association, which is shown in Table 4, between TNF-α and the advancement of liver fibrosis as determined by FibroScan (r=0.292; $p<0.05$). Macrophages and monocytes create TNF-α, which is one of the key cytokines for eliminating HBV. Through interacting with particular receptors, such as soluble TNF receptors (sTNFR), TNF-α plays a significant role in beginning fibrogenesis and participates in the initiation, angiogenesis, proliferation, and metastasis of many malignancies.(20) When TNF-α binds to its receptors, nuclear factor Kappa B (NF-κB) is produced more readily and subsequently induces the expression of pro-inflammatory cytokines and therefore promotes liver fibrosis. Increased soluble TNF-α and its receptors are seen in the patient's blood and liver in both acute and chronic cases of hepatitis B.(21) Even if the liver enzymes were normal in a previous investigation, the higher TNF-α levels in the blood of individuals with mild liver inflammation might be utilized to predict the degree of liver inflammation.(12)

TNF-α was shown to be the only cytokine parameter that significantly correlated with the degree of fibrosis when

Table 3. Post hoc analysis of subjects characteristics through the degree of fibrosis.

Variable	Fibrosis Stage		
	Mild *vs* Significant	Mild *vs* Advanced	Significant *vs* Advanced
AST [b]	0.017*	0.000*	0.034*
ALT [b]	0.071	0.033*	0.585
WBC [b]	0.008*	0.000*	0.130
PLT [a]	0.000*	0.000*	0.153
Albumin [a]	0.166	0.027*	0.664
PPT [b]	0.968	0.001*	0.012*
TBil [b]	0.716	0.003*	0.005*
DBil [b]	0.479	0.025*	0.017*

[a]Tukey test, [b]Mann-Whitney test. Significant if $p<0.05$.

Table 4. Association of TNF-α, IL-6, IL-10 serum levels with the degree of fibrosis.

Variable	Fibrosis Stage				
	Mild	Significant	Advanced	*p-value*[a]	r
TNF-α (pg/mL)	14.47±14.89	21.16±31.85	71.53±86.93	0.003	0.292
IL-6 (pg/mL)	9.08±9.94	13.85±15.92	26.41±31.09	0.026	0.221
IL-10 (pg/mL)	13.62±12.60	21.60±25.12	55.69±67.54	0.036	0.208

[a]Spearman correlation.

contrasted with other significant bivariate analysis in this research. Other studies have shown a correlation between more prominent inflammation and severe liver fibrosis when there is an elevated level of TNF-α in the serum. (22,23) The observed trend can be accounted for by the rise in stellate cell activity, which exhibits a high sensitivity to anti-inflammatory cytokines, and TNF-α's capacity to stop stellate cell apoptosis.(24-26)

An essential pro-inflammatory cytokine, IL-6 controls the production of numerous acute phase proteins and is linked to the immunological control of chronic liver disease. Normal human peripheral blood has very low concentrations of IL-6, which is also not expressed in the majority of normal resting cells. However, when infection or other stimuli are present, single macrophages and T lymphocytes become activated, which increases IL-6 synthesis and release, encourages the proliferation of activated T cells and the differentiation of immature thymocytes into mature CTL, which in turn kills the virus inside the cell but also exacerbates liver inflammation and damages more liver cells.(13) This study found that for CHB patients, IL-6 level in advanced fibrosis group significantly increased compared with mild fibrosis group (r=0.221; $p<0.05$), which in turn the level of serum IL-6 was closely related to the liver fibrosis (Table 4). According to prior studies that revealed serum IL-6 levels to be much higher than normal during the acute phase of cirrhosis and hepatitis, and to rise even more in the case of serious illness or death, this study's findings are consistent. Elevated IL-6 was connected with the severity of CHB. It was also positively correlated with the number of white blood cells and neutrophils, C-reactive protein (CRP), and TBIL levels, and negatively correlated with serum total protein level (27,28), which is not found in this study.

By preventing Th1 cells from proliferating and producing IL-2, interferon (IFN), leukotrienes, and other cytokines, IL-10 is a crucial anti-inflammatory cytokine that can also reduce immune cell function in patients, inhibit Th1-type immune response, and encourage Treg proliferation and activation. All of these effects can lead to a persistent hepatitis B virus, induce tolerance to foreign pathogens, and decrease immune cell function in patients.(29,30) In this study, serum IL-10 level in advanced fibrosis group increased significantly compared with mild fibrosis group (r=0.208; $p<0.05$), suggesting that the body can effectively remove virus-infected liver cells and Th2 cytokine IL-10 cannot inhibit Th1 cell response, causing IL-10 shows an upward trend (Table 4). The increase in IL-10 in this study is in line a study with 857 samples of chronic hepatitis B patients and 100 samples of normal patients, where the amount of IL-10 increased with chronicity and liver cell damage.(31) Another research with 390 samples showed an increase in IL-10 in peripheral blood examination. Shows a strong correlation for the progression of infection from inactive carrier to malignancy.(32)

Immunological changes have been noticed in CHB patients treated with antiviral therapy, reflecting the restoration of the host immunity against HBV. For instance, the frequency of Toll-like receptors (TLRs), programmed death-1 (PD-1) and an increase of active Th1 cytokines such as TNF-α, IL-12 induced, and high serum levels of IL-12 and IL-10, have been associated with HBeAg seroconversion in

Table 5. Post hoc analysis of TNF-α, IL-6, IL-10 through the degree of fibrosis.

Variable	*p-value*[a]	Fibrosis Stage		
		Mild *vs* Significant[b]	Mild *vs* Advanced[b]	Significant *vs* Advanced[b]
TNF-α	0.003*	0.600	0.001*	0.013*
IL-6	0.048*	0.359	0.022*	0.186
IL-10	0.021*	0.106	0.009*	0.207

[a]Kruskal-Wallis test, [b]Mann-Whitney test. Significant if $p<0.05$.

Table 6. Multivariate ordinal regression analysis through the degree of fibrosis.

Variable	Multivariate	
	Wald	*p-value**
TNF-α	5.220	0.022*
IL-6	2.596	0.107
IL-10	0.137	0.711
Age	1.018	0.313
AST	10.829	0.001*
ALT	0.353	0.552
Wbc	2.657	0.103
PLT	11.853	0.001*
Albumin	0.865	0.352
PPT	2.664	0.103
TBil	0.330	0.566
DBil	0.996	0.318

*Ordinal regression, significant if $p<0.05$.

HBeAg-positive CHB patients treated with α-IFN, and with early, spontaneous, HBeAg seroconversion.(33-35)

In our post hoc and multivariate analysis (Table 5 and 6), we discovered significant differences in all three of the cytokines we examined in this study between the groups with mild and advanced fibrosis; TNF-α was the only cytokine found to be significant in multivariate analysis. This was consistent with a study which found that NUC administration decreases the production of IL-10, which is followed by a reciprocal stimulation of TNF-α and IL-6.(19) Nevertheless, in contrast to another study with animal, in this study we were unable to identify the decline in serum IL-10 (19,36), which could be attributed to gene polymorphism. Post hoc analysis revealed that AST was the only parameter significant across all fibrosis stage comparisons (Table 3). However, it is important to emphasize that the degree of normal to mildly increased aminotransferase is a poor guide to the severity of the disease in patients with established chronic viral hepatitis.(37)

Although cytokines are essential for preserving a patient's immune system against HBV infection, they also contribute to hepatocellular damage, particularly in those with long-term infections.(12,38) Numerous investigations have revealed that genes with cytokine polymorphisms are both functionally linked to hepatocellular carcinoma and liver disorders, as well as increasing a person's susceptibility to cancer.(39) Serum cytokine levels can differ between people. Additionally, different signaling pathways, target cells, and physiological conditions including stress, fitness, and eating state all affect how cytokines are released and function.(40)

This research has limitation due to the cytokines that evaluated and the various types of drug that consumed by patients. Therefore, further study is needed to evaluate other cytokines such as TGF-β which is associated with HBx protein, MMP-2 which is associated with matrix degradation disruption and PDGF which is associated with chemotaxis and retinoid loss process due to liver fibrosis. Besides that, prospective cohort study is needed to evaluate and compare the role of cytokines in naïve and treated CHB patients.

Conclusion

There are increased levels of TNF-α, IL-6, and IL-10 in treated CHB patients are correlated with the severity of liver fibrosis. These parameters may be useful in assessing liver fibrosis in treated patients with CHB. While our findings suggest potential clinical implications, it is important to note that further investigation is required before considering these parameters as tools for daily clinical practices.

Acknowledgments

The authors would like to thank the team from Endoscopy Installation of Dr. Soetomo General Hospital, Surabaya for their help in collecting blood samples. The authors would also like to thank the Pathology Clinic Installation of Dr. Soetomo General Hospital, Surabaya for processing and examining in blood sampling of patients. This research was supported by Airlangga Research Fund Universitas Airlangga (9493/UN3.FK/PT.01.03/2023)

Authors Contribution

UM and UK designed and conceptualized the study. RRP, DB and HW collected and analyzed the data. RRP and DB drafted the manuscript, HW aided in interpreting results and discussion. All authors took parts in giving critical revision of the manuscript.

References

1. Elbrolosy AN, Elabd NS, ElGedawy GA, Abozeid M, Abdelkreem M, Montaser B, *et al.* Toll-like receptor 2 polymorphism and IL-6 profile in relation to disease progression in chronic HBV infection: A case control study in Egyptian patients. Clin Exp Med. 2023; 23(1): 117-29.
2. Muljono DH. Epidemiology of hepatitis B and C in Republic of Indonesia. Euroasian J Hepato-Gastroenterology. 2017; 7(1): 55-9.
3. Christian JE, Yuliawuri H, Suherman EA. CRISPR target-based

single-guide RNA (sgRNA) for diagnostic testing of hepatitis B virus. Mol Cell Biomed Sci. 2023; 7(2): 70-4.
4. Fatmawati, Nasrul E, Zubir N, Sandra F. Programmed cell death protein 1-overexpressed CD8+ T lymphocytes play a role in increasing chronic hepatitis B disease progression. Indones Biomed J. 2021; 13(3): 310-5.
5. Sun M, Kisseleva T. Reversibility of liver fibrosis. Clin Res Hepatol Gastroenterol. 2015; 39(1): S60-3.
6. Rockey DC. Liver fibrosis reversion after suppression of hepatitis B virus. Clin Liver Dis. 2016; 20(4): 667-79.
7. Kang J, Lee M. Noninvasive diagnostic and prognostic assessment tools for liver fibrosis and cirrhosis in patients with chronic liver disease. In: Tsoulfas G, editor. Liver Cirrhosis - Update and Current Challenges. London: IntechOpen; 2017. p.35-75.
8. Parikh P, Ryan JD, Tsochatzis EA. Fibrosis assessment in patients with chronic hepatitis B virus (HBV) infection. Ann Transl Med. 2017; 5(3): 40. doi: 10.21037/atm.2017.01.28.
9. Cheng JYK, Wong GLH. Advances in the diagnosis and treatment of liver fibrosis. Hepatoma Res. 2017; 3(8): 156-69.
10. Ferraioli G, Wong VWS, Castera L, Berzigotti A, Sporea I, Dietrich CF, *et al.* Liver ultrasound elastography: An update to the World Federation for Ultrasound in Medicine and Biology guidelines and recommendations. Ultrasound Med Biol. 2018; 44(12): 2419-40.
11. Chang PE, Goh GBB, Ngu JH, Tan HK, Tan CK. Clinical applications, limitations and future role of transient elastography in the management of liver disease. World J Gastrointest Pharmacol Ther. 2016; 7(1): 91-106.
12. Mourtzikou A, Alepaki M, Stamouli M, Pouliakis A, Skliris A, Karakitsos P. Evaluation of serum levels of IL-6, TNF-α, IL-10, IL-2 and IL-4 in patients with chronic hepatitis. Inmunologia. 2014; 33(2): 41-50.
13. Ming DS, Lin ZZ, Qiu XD. Analysis of IL-6, IL-10, IL-17 levels in the blood of patients with chronic hepatitis B. Int J Clin Exp Med. 2016; 9(6): 9437-42.
14. Lan T, Chang L, Wu L, Yuan YF. IL-6 plays a crucial role in HBV infection. J Clin Transl Hepatol. 2015; 3(4): 271-6.
15. de Lédinghen V, Vergniol J. Transient elastography (FibroScan). Gastroenterol Clin Biol. 2008; 32(6): 58-67.
16. Mondelli MU, Varchetta S, Oliviero B. Natural killer cells in viral hepatitis: Facts and controversies. Eur J Clin Invest. 2010; 40(9): 851-63.
17. Bertoletti A, Maini MK and Ferrari C. The host pathogen interaction during HBV infection: Immunological controversies. Antivir Ther 2010; 15(Suppl3): 15-24.
18. Chen Z, O'Shea JJ. Th 17 cells: A new fate for differentiating helper T cells. Immunol Res 2008; 41(2): 87-102.
19. Murata K, Tsukuda S, Suizu F, Kimura A, Sugiyama M, Watashi K, *et al.* Immunomodulatory mechanism of acyclic nucleoside phosphates in treatment of hepatitis B virus infection. Hepatology. 2020; 71(5): 1533-45.
20. Poovorawan K, Tangkijvanich P, Chirathaworn C, Wisedopas N, Treeprasertsuk S, Komolmit P, *et al.* Circulating cytokines and histological liver damage in chronic hepatitis B infection. Hepat Res Treat. 2013; 2013: 757246. doi: 10.1155/2013/757246.
21. Abramson A, Menter A, Perrillo R. Psoriasis, hepatitis B, and the tumor necrosis factor-alpha inhibitory agents: A review and recommendations for management. J Am Acad Dermatol. 2012; 67(6): 1349-61.
22. Yao QY, Feng YD, Han P, Yang F, Song GQ. Hepatic microenvironment underlies fibrosis in chronic hepatitis B patients. World J Gastroenterol. 2020; 26(27): 3917-28.
23. Deng YQ, Zhao H, Ma AL, Zhou JY, Xie SB, Zhang XQ, *et al.* Selected cytokines serve as potential biomarkers for predicting liver inflammation and fibrosis in chronic hepatitis B patients with normal to mildly elevated aminotransferases. Medicine. 2015; 94(45): e2003. doi: 10.1097/MD.0000000000002003.
24. Lee UE, Friedman SL. Mechanisms of hepatic fibrogenesis. Best Pract Res Clin Gastroenterol. 2011; 25(2): 195-206.
25. Liu C, Chen X, Yang L, Kisseleva T, Brenner DA, Seki E. Transcriptional repression of the transforming growth factor β (TGF-β) pseudoreceptor BMP and activin membrane-bound inhibitor (BAMBI) by nuclear Factor κB (NF-κB) p50 enhances TGF-β signaling in hepatic stellate cells. J Biol Chem. 2014; 289(10): 7082-91.
26. Tarrats N, Moles A, Morales A, García-Ruiz C, Fernández-Checa JC, Marí M. Critical role of tumor necrosis factor receptor 1, but not 2, in hepatic stellate cell proliferation, extracellular matrix remodeling, and liver fibrogenesis. Hepatology. 2011; 54(1): 319-27.
27. Ramakers BP, de Goeij M, van der Hoeven JG, Peters WH, Pickkers P. Inflammation-induced hepatotoxicity in humans. Shock. 2009; 31(2): 151-6.
28. Wen XY, Xiang CY, Yuan YR. Change of serum TNF-α, IL-6, IL-8, IL-12 and IL-18 in patients with hepatitis B. China J Modern Med. 2004; 14: 54-6.
29. Fickenscher H, Hör S, Küpers H, Knappe A, Wittmann S, Sticht H. The interleukin-10 family of cytokines. Trends Imunol. 2002; 23(2): 89-96.
30. Chan SL, Mo FK, Wong CS, Chan CM, Leung LK, Hui EP, *et al.* A study of circulating interleukin-10 in prognosticatication of unresectable hepatocellular carcinoma. Cancer. 2012; 118: 3984-92.
31. Rybicka M, Woziwodzka A, Sznarkowska A, Romanowski T, Stalke P, Dręczewski M, *et al.* Genetic variation in IL-10 influences the progression of hepatitis B infection. Int J Infect Dis. 2020; 96: 260-5.
32. Saxena R, Chawla YK, Verma I, Kaur J. Association of interleukin-10 with hepatitis B virus (HBV) mediated disease progression in Indian population. Indian J Med Res. 2014; 139(5): 737-45.
33. Wu JF, Wu TC, Chen CH, Ni YH, Chen HL, Hsu HY, *et al.* Serum levels of interleukin-10 and interleukin-12 predict early, spontaneous hepatitis B virus e antigen seroconversion. Gastroenterology. 2010; 138(1): 165-72.e1-3.
34. Lang T, Lo C, Skinner N, Locarnini S, Visvanathan K, Mansell A. The hepatitis B e antigen (HBeAg) targets and suppresses activation of the toll-like receptor signaling pathway. J Hepatol. 2011; 55(4): 762-9.
35. Rossol S, Marinos G, Carucci P, Singer MV, Williams R, Naoumov NV. Interleukin-12 induction of Th1 cytokines is important for viral clearance in chronic hepatitis B. J Clin Invest. 1997; 99(12): 3025-33.
36. Hsieh HG, Huang HC, Lee FY, Chan CY, Lee JY, Lee SD. Kinetics of cytokine expression in cirrhotic rats. J Chin Med Assoc. 2011; 74(9): 385-93.
37. Giannini EG, Testa R, Savarino V. Liver enzyme alteration: A guide for clinicians. CMAJ. 2005; 172(3): 367-79.
38. Tacke F, Hammerich L, Heymann F. Role of IL-17 and Th17 cells in liver diseases. Clin Dev Immunol. 2011; 2011: 345803. doi: 10.1155/2011/345803.
39. Trehanpati N, Vyas AK. Immune regulation by T regulatory cells in hepatitis B virus-related inflammation and cancer. Scand J Immunol 2017; 85(3): 175-81.
40. Monastero RN, Pentyala S. Cytokines as biomarkers and their respective clinical cutoff levels. Int J Inflam. 2017: 2017: 4309485. doi: 10.1155/2017/4309485.