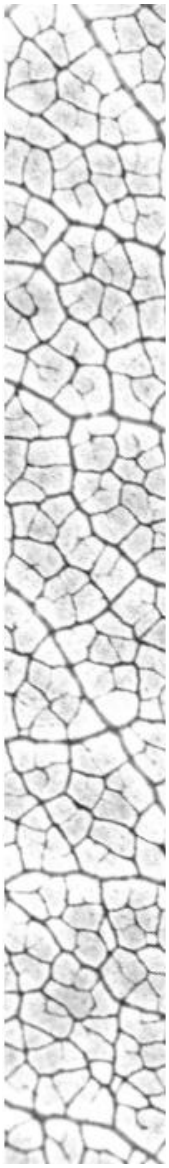

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Serum hyaluronic acid and its association with liver stiffness in chronic hepatitis B patients

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Abstract

Background: Serum hyaluronic acid has an important role in the pathogenesis of liver fibrosis and is one of the most studied extracellular liver matrices. The purpose of this study was to examine the relationship between serum hyaluronic acid level and liver stiffness in hepatitis B chronic patients.
Methods: This was an observational cross-sectional analytic study consisting of 43 samples of chronic hepatitis B patients. Serum hyaluronic acid was measured by the ELISA method. Liver stiffness describing fibrosis was examined using Fibro scan.
Results: Among forty-three subject was predominantly by females (65.1%). The average age of the study subjects was 44.023 years old. The median serum hyaluronic acid level of the study subjects was 40.318 ng/mL. The median liver stiffness in the study subjects was 5.8 kPa. There was a significant positive correlation between serum hyaluronic acid levels and liver stiffness in the study subjects with a moderate strength of r = 0.448 (P = 0.003).
Conclusion: Serum hyaluronic acid levels associated with liver stiffness.

Keywords: hepatitis B virus infection, hyaluronic acid, liver stiffness

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INTRODUCTION

Infection of hepatitis B virus (HBV) is one of the causes of liver fibrosis which still major worldwide health problems (Mastutik et al. 2015). Early-life HBV acquisition is generally asymptomatic, but associated with a certain risk of chronic infection (Darmawan et al. 2015). Transmission of HBV is parenteral, in contact with blood or other body fluids (Lubis et al. 2018, Tanadi et al. 2017). In addition, intra-familial transmission is a potential source of HBV-infected patients (Gunardi et al. 2017).

Understanding the pathogenesis of liver fibrosis has an important role for management, monitoring, and long-term prognosis (Kim et al. 2012). There are some tools to diagnose hepatitis B or cirrhosis and predicting the prognosis, such as aspartate aminotransferase to platelet ratio index (APRI) (Husni et al. 2019). Whereas, the assessment of the degree of fibrosis can be done through several methods, such as liver biopsy, measurement of serum fibrosis markers, and measurement of liver stiffness. Measurement of liver stiffness using fibro scan is an accurate examination of fibrosis in chronic hepatitis B that has been recommended and done routinely in various countries including Indonesia (Castera 2014, Harkisoen et al. 2014, Jia et al. 2015). Many components of the pathogenesis of liver fibrosis in chronic hepatitis B affecting liver stiffness include the extracellular liver matrix. Nonetheless, the extracellular matrix correlation of the heart with liver stiffness is not yet fully understood (Castera 2014).

Advanced liver fibrosis in chronic hepatitis B will develop into liver cirrhosis and liver cancer which increases morbidity and mortality. The world mortality rate due to hepatitis B reaches 650,000 patients per year. One of the causes of this high mortality is due to late diagnosis, which causes delay in intervention (Osakabe et al. 2011, World Health Organization 2015). Cirrhosis is one of the final stages of chronic liver disease, which is a global problem with 800,000 deaths per year worldwide (LiuT. et al. 2012). An understanding

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of the pathogenesis of liver fibrosis has an important role to improve prognosis (Harkisoen et al. 2014). Serum ferritin may predict the prognosis of patients with decompensated cirrhosis because it represents immune-mediated and infectious stimuli. The extent of liver disease and possible future complications may be demonstrated by ferritin (Siregar et al. 2018;).

In the pathogenesis of liver fibrosis by chronic hepatitis B, there is accumulation of various extracellular matrices. This process causes changes in structure and consistency of the liver to harden resulting in an increase in liver stiffness (LiuT. et al. 2012). Hyaluronic acid is one of the extracellular matrices whose serum levels will increase with liver fibrosis (Rostami et al. 2013). Several studies comparing several extracellular matrices (hyaluronic acid, laminin, and collagen type IV) with the degree of liver fibrosis in chronic viral hepatitis patients showed that their concentration correlated with liver fibrosis. One of the strongest correlations was hyaluronic acid (r = 0.849, P <0.001) (Parsian et al. 2010). However, extracellular matrix studies with liver stiffness in chronic hepatitis B are still limited. Different studies show that there is still controversy in the correlation of hyaluronic acid to liver stiffness. Study in China showed hyaluronic acid did not have a significant correlation with liver stiffness (r=0.099, P = 0.277) (LiuJ. et al. 2019). Whereas, other studies in China in 74 chronic hepatitis B patients showed a significant positive correlation between serum hyaluronic acid and liver stiffness (r=0.517, P = 0.048) (Gou et al. 2010). Another study in Japan in 212 chronic hepatitis B patients showed that serum hyaluronic acid levels were significantly positively correlated with liver stiffness (r = 0.578, P <0.0001) (Osakabe et al. 2011).

Hyaluronic acid examination is quite easy, objective, non-invasive and correlates with liver fibrosis which is also expected to have a correlation with liver stiffness (Rostami et al. 2013). This study aimed to observe the correlation between serum hyaluronic acid level and liver stiffness in chronic hepatitis B patients in the Gastroenterohepatology Outpatient Installation at Dr. Soetomo General Hospital Surabaya (Fallah et al, 2009).

MATERIALS AND METHODS

This was an observational analytic study with a cross sectional design, carried out in the Gastroenterohepatology Outpatient Installation of the Dr. Soetomo General Hospital, Surabaya, Indonesia. The population of this study was patients at the Gastroenterohepatology Installation of the Dr. Soetomo General Hospital, Surabaya who diagnosed chronic hepatitis B. The study sample was collected by consecutive sampling technique.

Inclusion criteria included age 18-65 years patients who were willing to take part in the study. Patients with clinical cirrhosis, hepatocellular carcinoma, obesity, pregnancy, ascites, narrowing of the intercostal space, increase in ALT, malignancy, history of alcohol consumption, hepatitis C virus infection, non-alcoholic fatty liver disease, SLE, rheumatoid arthritis, and scleroderma were excluded from the study. This study has obtained ethical feasibility from the ethics committee of the dr. Soetomo General Hospital, Surabaya, Indonesia (Elmiawati Latifah et al., 2019).

Sample Collection

Serum hyaluronic acid levels are the levels of hyaluronic acid circulating in blood serum. Taking serum blood examinations was carried out study subjects, measured using Hyaluronic Acid (Echelon, Salt Lake City, UT) Enzyme Linked Immune Assay (ELISA) kits by the Prodia Surabaya laboratory. The results of the examination are stated in ng/mL. For preparation of serum hyaluronic acid examination, patients were fasting for at least 8 hours.

Measurement of liver stiffness was done using fibro scan. The examination was carried out by one trained operator. Values were expressed in kPa. The value range was 2.5 - 75.0 kPa. The results of the examination are valid if a minimum of 10 valid examinations are obtained and the success rate of at least 60% and the interquartile range (IQR) is less than 33% median.

Statistical Analysis

Correlation of serum hyaluronic acid levels with liver stiffness was calculated using the Spearman non parametric test because data distribution was not normal. The correlation test output was expressed by the value p, the direction of correlation, and the correlation coefficient (r). The value of P <0.05 was stated to be statistically significant. The direction of positive correlation means that the higher the value of the independent variable, the higher the value of the dependent variable. The magnitude of the correlation was stated to be very weak (r = 0-0.2), weak (r = 0.2-0.4), moderate (r = 0.4-0.6), strong (r = 0.6-0.8), and very strong (r = 0.8-1.0)

RESULTS

The sample of this study was total of 43 people who met the inclusion and exclusion criteria. Mean age was 44.023±12.781 years with the youngest age range of 24 years and the oldest age of 64 years. The male was 15 subjects (34.9%) and the female was 28 subjects (65.1%). Most of the study subjects were patients who had not received therapy (55.8%). Clinical evaluation of research subjects is shown in **Table 1**.

All research subjects were examined for serum hyaluronic acid levels. The median serum hyaluronic acid in the study subjects was 40,318 ng/mL with the lowest levels of 7,693 ng/mL and the highest levels were 209,939 ng/mL. All study subjects were also examined for liver stiffness using fibro scan. The median value of

Table 1. Clinical Evaluation of Subjects

Variables	Frequency	Percentage (%)
Therapy status		
Naïve	24	55.8
On therapy	19	44.2
Entecavir	1	5.26
Lamivudine	2	10.53
Peg-Interferon	3	15.79
Tenofovir	6	31.58
Telbivudine	7	36.84
Degree of fibrosis		
F0/1	31	44.3
F2	4	5.7
F3	12	17.1
F4	23	32.9

Table 2. Results of Serum Hyaluronic Acid and Liver Stiffness

Variables	Median	Range (min-max)	Normality (p)
Hyaluronic Acid (ng/mL)	40,318	7,693-209,939	0.000
Liver Stiffness (kPa)	5.8	3.4-32.9	0.000

liver stiffness in the study subjects was 5.8 kPa with the lowest value of 3.4 kPa and the highest value was 32.9 kPa. Results of serum hyaluronic acid levels and liver stiffness are shown in **Table 2**.

Both variables were tested for normality and the two variables were not normally distributed. Correlation between serum hyaluronic acid and liver stiffness was performed using Spearman's nonparametric test. The results of the Spearman correlation test obtained P = 0.003 and coefficient correlation (r) of 0.448. There was a significant correlation between serum hyaluronic acid levels and liver stiffness with the strength of the moderate positive correlation. This means that the higher the serum hyaluronic acid, the higher the liver stiffness. The correlation scatter diagram of the two variables is shown in **Fig. 1**.

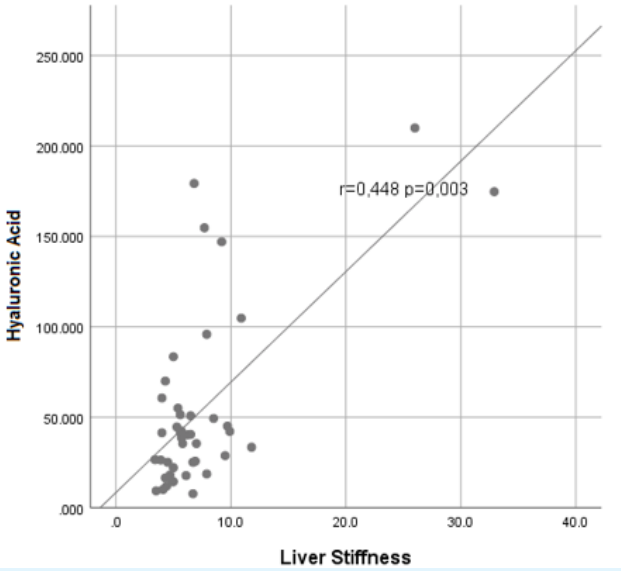

Fig. 1. Scatter diagram of correlation of serum hyaluronic acid levels with liver stiffness

DISCUSSION

In this study, there was a significant positive correlation between serum hyaluronic acid levels and liver stiffness in the study subjects with a moderate strength. This means that the higher the serum hyaluronic acid level, the liver stiffness will also increase.

Hepatitis B infection is a leading cause of chronic liver disease. The hepatitis B virus causes injury to hepatocytes (Castera 2014, Harkisoen et al. 2014, Jia et al. 2015). Hepatocyte injury will be followed by an inflammatory process with immune system activation, such as cytokine (Rey et al. 2018). Immune system causes the activation process of liver stellate cells (LiuT. et al. 2012). In addition, the hepatitis B virus has a regulator component HBx protein which also stimulates activation of liver stellate cells directly. Activated liver stellate cells become myofibroblast. Myofibroblast stimulates an increase in extracellular matrix synthesis. In addition myofibroblast also regulates the increase in tissue inhibitors of metalloproteinases (TIMP) and decreases in matrix metalloproteinase (MMP) which causes a decrease in degeneration of extracellular matrix. Continuous increase in synthesis and decrease in degeneration cause accumulation of extracellular matrix in the form of elastin, glycoprotein, proteins, glycogen, and hyaluronic acid. Excessive accumulation of extracellular matrix in the long term leads to liver fibrosis (LiuT. et al. 2012). This process causes the consistency of the liver to harden and an increase in liver stiffness. Hyaluronic acid correlates with liver fibrosis and is one of the most useful serum fibrosis markers to predict liver fibrosis in chronic hepatitis B patients (Parsian et al. 2010). Hyaluronic acid also correlates with liver stiffness and disease activity (Harkisoen et al. 2014).

Various studies have shown that the correlation between hyaluronic acid and liver stiffness is still controversial. Research in China showed hyaluronic acid was not found to have a significant correlation with liver stiffness (r = 0.099, P = 0.277). This difference in results could be caused by the method of examining liver stiffness using 2D-WSE instead of fibro scan as in other studies (LiuJ. et al. 2019). In another study in which liver stiffness examination methods used fibro scan, results were similar to this study. Research in China showed a significant positive correlation between serum hyaluronic acid and liver stiffness (r = 0.517, P = 0.048) (Gou et al. 2010). Another study in Japan showed that serum hyaluronic acid levels were significantly positively correlated with liver stiffness (r = 0.578, P <0.0001) (Osakabe et al. 2011). Both of these studies showed a positive correlation with a strong moderate relationship similar to the results of this study.

Moderate relationships in this study and in some similar studies are thought to show there are still other factors that affect liver stiffness in addition to serum hyaluronic acid. Some of the suspected influential factors are the varying stages of hepatitis B, the effects

of chronic hepatitis B therapy, and other extracellular matrix components that were not examined in this study. This strong correlation with moderate relationships suggests that serum hyaluronic acid levels as a single serum marker are not strong enough to show changes in liver stiffness in chronic hepatitis B. Unlike liver stiffness, serum hyaluronic acid levels cannot be recommended for routine clinical examination. Many other studies showed serum hyaluronic acid have a better clinical value when used as a supplement, where serum hyaluronic acid is used as data in an algorithm. The combination of hyaluronic acid with other fibrosis markers is expected to have a stronger correlation with liver stiffness (Jia et al. 2015).

The limitations of this study are not examining HbeAg, HBV DNA, quantitative HBsAg, and liver biopsy that show the patients' clinical stage, and this study did not examine the effect of therapy.

CONCLUSION

The results of this study indicate a significant positive correlation between serum hyaluronic acid levels and liver stiffness.

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