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β-Catenin Plasma Test: Liver Fibrosis Degree Assessment in Chronic Hepatitis B Patients

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ABSTRACT

Liver fibrosis caused by chronic hepatitis B infection leads to significant mortality and morbidity. Monitoring and evaluation of liver fibrosis progression depend on the ability to detect the fibrosis. Liver biopsy as a gold standard for liver fibrosis is an invasive technique, while Fibroscan ® with transient elastography as a non-invasive technique has a limitation. Therefore, a biomarker is needed to detect liver fibrosis. β-catenin is a multifunctional protein, which has a Wnt-regulated transcription factor and resides in hepatocytes. Hepatitis B virus infection activates Wnt/ β-catenin and affects the expression of target genes for liver fibrosis. This study aimed to analyze the diagnostic performance of plasma β-catenin levels using transient elastography as a standard reference to assess the degree of liver fibrosis in patients with chronic hepatitis B infection. This was an observational analytic study with a cross-sectional design. The analysis was performed on 70 chronic Hepatitis B patients between December 2020 and January 2021 at Dr. Moewardi Hospital, Surakarta. This ROC analysis was used to determine the cut-off point. The best AUC point was chosen using a 2x2 diagnostic test table. The cut-off point for plasma β-catenin was 73.132 pg/mL and AUC was 0.793 (CI 95%: 0.681-0.906; $p<0.001$), indicating that the results were statistically significant with $p<0.05$. Sensitivity of 74.3%; specificity of 71.4%; PPV of 72.2%; NPV of 73.5%; LR (+) 2.6; LR (-) 0.36 were obtained. The β-catenin level was <73.132 pg/mL. A total of 26 subjects were at risk for liver fibrosis with transient elastography ≥8 kPa. Plasma β-catenin levels had moderate performance as a liver fibrosis marker.

Keywords: Liver fibrosis, chronic hepatitis B, liver biopsy, transient elastography, β-catenin

INTRODUCTION

Hepatitis B is a liver infection caused by the Hepatitis B Virus (HBV) and has moderate-high endemicity in Indonesia (2.5%-10%), which can lead to chronic infection and a high risk of death from cirrhosis and hepatocellular carcinoma (HCC).[1] Research by Poynard *et al*. in 2010 reported chronic HBV prevalence of 5%; 2.8% and 0.3% developed into liver fibrosis and cirrhosis of the liver at the age of 40 years or above, respectively.[2] According to the medical record of Dr. Moewardi Hospital (RSDM) Surakarta, there were 4,535 cases in 2018 and 8,580 cases in 2019 with a total increase of 89.2%. In addition, there were 5,807 cases from 2020 to August.

Liver fibrosis with an incidence rate of about 2.8% is a healing response to liver inflammation characterized by excessive deposition of Extra Cellular Matrix (ECM) protein, and replacement of scar tissue with collagen due to repeated liver damage, which can result from chronic hepatitis B infection, chronic hepatitis C infection, alcoholic liver disease, and non-alcoholic steatohepatitis.[3-5] Liver fibrosis is one of the leading causes of morbidity with the 11th highest mortality rate, which indicates the development of chronic inflammation and contributes to 45% of all causes of mortality worldwide.[6,7]

Diagnosis and determination of the degree of hepatic fibrosis is important to predict the morbidity, mortality, and complications of portal hypertension. The liver fibrosis test method consists of liver biopsy as the gold standard liver biopsy, which has an overall complication rate of 6.0%. The most common complaints are pain and bleeding with an overall risk of death of 0.03%.[8] Transient Elastography (TE) with Fibroscan ® is a non-invasive method, which measures liver stiffness (LSM), observes liver stiffness quickly, and indicates liver fibrosis. Its diagnostic accuracy based on Stage I, stage 2, and Stage 3 is 5.55 kPa, 8.0 kPa, and 10.95 kPa, respectively. However, this test has several limitations such as over-estimation of TE readings due to obesity, ascites, Body Mass Index (BMI) > 30-35 kg/m^2, old age, and liver inflammation.[9] Therefore, other biomarkers are needed to detect liver fibrosis.

Many biomarkers are currently being developed to assess the degree of liver fibrosis, one of which is

β-catenin, a multifunctional protein that plays a role in physiological homeostasis and works as a coregulatory transcription factor regulated by Wnt and resides in hepatocytes. Previous research suggested that the mechanism of HBV infection and HBx protein influences and activates Wnt/β-catenin through the Canonical pathway and affects the expression of target genes for liver fibrosis. Wnt/β-catenin resides in hepatocytes (pericentral hepatocytes) and activates Hepatic Stellate Cells (HSC) in the space of Disse (the gap between hepatocytes and liver sinusoidal endothelium), which can cause liver fibrosis in the long-term if collagen deposition occurs. [10,11]

Previous research suggested that detected serum or plasma β-catenin in humans might be associated with several disease progressions. Receiver Operating Curve (ROC) analysis of β-catenin in chronic hepatitis B-cirrhosis patients yielded an Area Under Curve (AUC) of 0.75 (cut-off 42 pg/mL, 95% CI 0.67-0.83), sensitivity of 66.43%, specificity of 75.41% and accuracy of 70.92%. However, research on β-catenin–TE diagnostic tests for liver fibrosis remains limited. Recent studies have shown that aberrations in the Wnt/β-catenin pathway play a role in the development of fibrotic organs, thus enabling them to become new therapeutic targets in fibrotic disorders.[3,7,12,13] Based on the background above, the author would like to analyze the diagnostic performance of plasma β-catenin levels with TE to assess the degree of liver fibrosis in patients with chronic hepatitis B infection.

METHODS

This research was observational analytic research carried out from December 2020 until January 2021 at the Clinical Pathology Laboratory Installation, RSDM Surakarta with a cross-sectional approach and selected research by consecutive sampling. The research subjects were all patients with chronic hepatitis B (HBsAg was still detected for more than 6 months) who were diagnosed by clinicians at the Internal Medicine Outpatient Clinic in the Gastroenterohepatology (GEH) sub-section of RSDM Surakarta based on inclusion and exclusion criteria. The inclusion criteria were as follows: patients with chronic hepatitis B diagnosed by a clinician, males, and females aged 18-75 years, agreed to participate in the study by signing an informed consent. Exclusion criteria were as follows: had a history of or currently suffering from heart disease, (increased levels of Troponin I hs, EKG), had a history of or currently suffering from kidney disease (reference price of creatinine 0.7-1.3 mg/dL; male 0.9-0.3 mg/dL; female 0.6-1.1 mg/dL), patients with HCV, HCC, alcoholic liver disease, non-alcoholic steatohepatitis, patients with obesity, ascites patients with a diagnosis of inflammation or malignancy (based on data obtained from history or medical record), sample with inadequate volume, hemolyzed, lipemic and icteric sample.

Plasma β-catenin levels were measured using the sandwich Enzyme-Linked Immunosorbent Assay (ELISA) method with an ELISA microplate reader Rayto 2100 C. The laboratory test was preceded by a precision test and an analytic accuracy test. Data on the characteristic of research subjects were displayed as mean and standard deviation if the data were normally distributed and median (minimum-maximum) if the data were not normally distributed. Independent T-test (data with normal distribution) or Mann-Whitney (data with abnormal distribution) or Chi-Square test (nominal data) was used and $p<0.05$ was reported as statistically significant. The cut-off value for plasma β-catenin levels was calculated using the ROC curve; the widest AUC value with the maximum cut-off point was determined using a 2x2 diagnostic test table. Data were statistically analyzed using SPSS version 22 software.

This study has received approval from the Biomedical Research Ethics Commission of Dr. Moewardi General Hospital of Surakarta number 1.321/XII/HREC/2020.

RESULTS AND DISCUSSIONS

This study involved 70 chronic hepatitis B patients which consisted of 35 patients with significant fibrosis (TE ≥ 8 kPa) and 35 patients with non-significant fibrosis (TE <8 kPa). The basic characteristics of the research subjects are presented in Table 1.

Based on the characteristics of research subjects in Table 1, it can be seen that there were significant age differences ($p=0.007$), gender ($p=0.028$), SGOT ($p=0.019$), SGPT ($p=0.026$), and platelets ($p=0.007$) between significant fibrosis group and non-significant fibrosis with $p<0.05$.

The significant fibrosis group had an average age of 46.14±11.53 years and consisted of 19 (54.3%) male subjects and 16 female subjects (45.7%). This supported previous research, which found that the liver infection prevalence was also observed to be higher in males than in females (10.7% vs. 4.4%).[2,14] Hepatitis B virus infection is considered to be a sex hormone-responsive virus that is regulated differently

Table 1. Basic characteristics of research subjects

Variable	Transient Elastography Test		p
	Significant Fibrosis (n=35)	Non-Significant Fibrosis (n=35)	
Age	46.14±11.53	38.66±10.78	0.007[a]*
Gender			0.028[c]*
Male	19 (54.3%)	10 (28.6%)	
Female	16 (45.7%)	25 (71.4%)	
BMI (kg/m2)	24.20±1.92	23.06±2.92	0.059
SGOT (u/L)	25 (12–75)	23 (12–49)	0.019[b]*
SGPT (u/L)	24 (12–73)	21 (10 –73)	0·026[b]*
Creatinine (mg/dL)	0.70 (0.40–1.80)	0.70 (0.40–1.90)	0.425[b]
Hemoglobin (g/dL)	13.88±1.66	14.14±1.40	0.477[a]
Leukocyte (/uL)	7.26±2.10	8.06±1.96	0.103[a]
Platelet (10^3/uL)	230.77±80.67	285.89±84.55	0.007[a]*

Abbrev: BMI = Body Mass Index; SGOT = Serum Glutamic Oxaloacetic Transaminase; SGPT = Serum Glutamic Pyruvic Transaminase; u/L = micro per liter; g/dL = gram per deciliter; mg/dL = milligram per deciliter; pg/mL: pictogram per milliliter; a Independent T-test (normal distribution), mean±SD; bMann-Whitney test (abnormal distribution), median (min-max); c Chi-Square/Fisher Exact test (nominal data); *p<0.05 was significant

Table 2. Characteristics of research variable

Variable	Transient Elastography		p
	Significant Fibrosis (n=35)	Non-Significant Fibrosis (n=35)	
β-catenin (pg/mL)	62.90±20.54	120.70±63.65	<0.001*

by the hepatic androgen or hepatic-estrogen axis. Once stimulated, the androgen receptor actively binds to androgen-responsive elements in viral enhancer I. The high rate of liver fibrosis progression in males is caused by the estrogen receptor variant, which is more greatly expressed in male patients along with loss of estrogen responsiveness, resulting in decreased estradiol production and response.[2,15]

The median values of SGOT and SGPT serum levels in the significant fibrosis group in this study were 25 (12-75) u/L and 24 (12-73) u/L, respectively. This supported previous research, which suggested that liver changes are found as blunt, irregular liver edges and rough liver parenchyma with increased SGOT levels due to damage to the liver membrane together with the damage to the mitochondrial membrane. Significantly increased biochemical activity is related to chronic hepatocellular injury. There is an increase in SGPT levels and SGPT activity decreases as fibrosis is formed and liver elasticity is also reported.[16]

The mean value of platelets in the significant group in this study was 230.77±80.67 x10^3/uL. Decreased liver function in chronic liver disease corresponds to the severity of the liver fibrosis that occurs characterized by decreased platelet count. This supported previous research, which suggested a possibility of disrupted thrombopoietin production in chronic liver disease, which might lead to a decreased platelet count.[17]

There were no significant differences in the results of hemoglobin, leukocytes, creatinine, and BMI for the characteristics of the study subjects. This supported previous research, which found that there was no significant difference in hemoglobin (p=0.76), leukocytes (p=0.32), creatinine (p>0.005), and BMI (p=0.08).[18-20]

Table 2 shows the characteristics of the research variables in the mean±SD, followed by the independent T-test. The average plasma β-catenin levels in the significant fibrosis and non-significant fibrosis group was 62.90±20.54 pg/mL and 120.70±63.65 pg/mL, respectively with p=<0.001, which indicated a significant difference between significant fibrosis and non-significant fibrosis groups (p< 0.05). The significant fibrosis group had lower results compared to the non-significant fibrosis group.

A cut-off value of β-catenin was determined using ROC curve (Figure 1).

The AUC value was determined for the β-catenin cut-off and then the optimal cut-off point was selected. Based on the ROC curve, the cut-off value for β-catenin was 73.132 pg/mL, with an AUC of 0.793 (95% CI: 0.681-0.906). The graph of the intersection of the sensitivity and specificity values is shown in Figure 2.

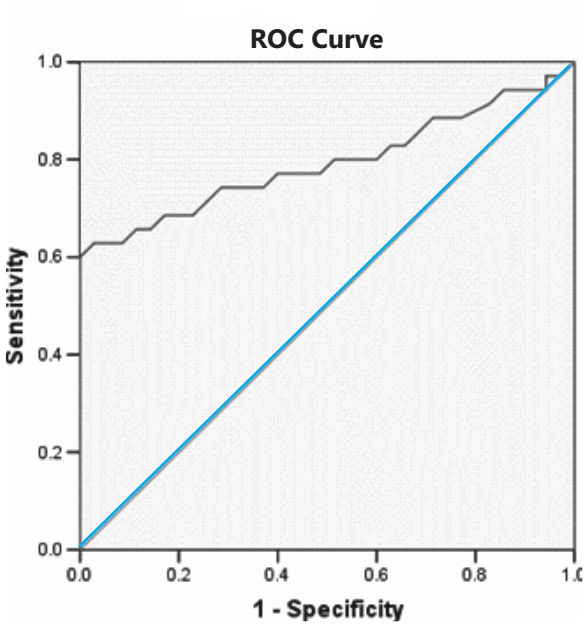

Figure 1. ROC curve of β-catenin to liver fibrosis in chronic B hepatitis

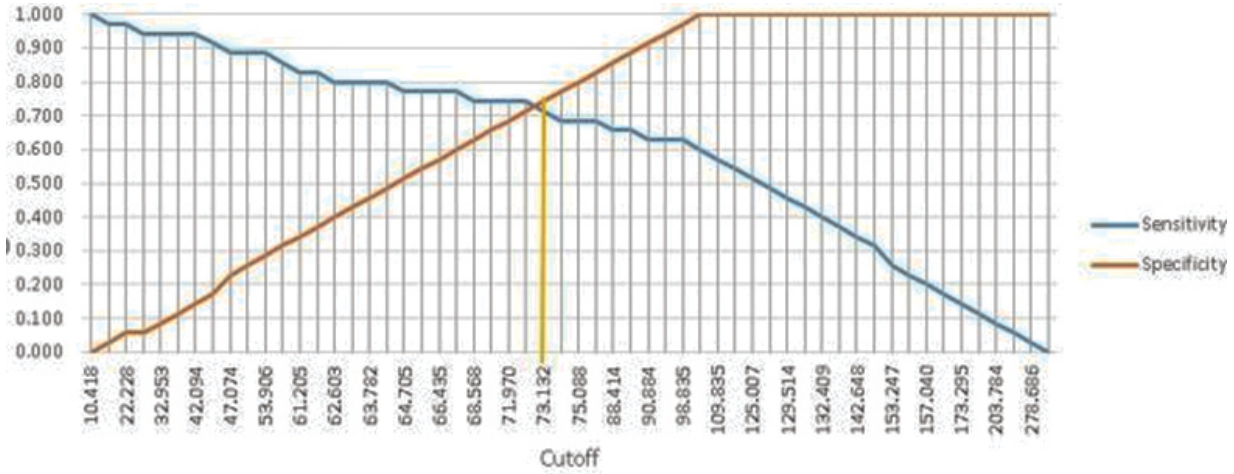

Figure 2. Graph of the intersection of cut-off of β-catenin with sensitivity and specificity value

Table 3. Cross-tabulation of β-catenin diagnostic test to transient elastography

β-catenin	Transient Elastography Test		Total
	Significant Fibrosis (≥8)	Non-Signicant Fibrosis (<8)	
< 73.132 pg/mL	26	10	36
≥73.132 pg/mL	9	25	34
Total	35	35	70

Based on the cut-off on the ROC curve, β-catenin was divided into 2 groups, a group with the β-catenin cut-off value >73.132 pg/mL and a group with the β-catenin cut-off value <73.132 pg/mL. The diagnostic test table is shown in Table 3.

Based on data in Table 3, it was known that with a β-catenin cut-off <73.132 pg/mL, 36 subjects were divided into 26 subjects with significant fibrosis and 10 subjects with non-significant fibrosis. A total of 34 subjects with β-catenin cut-off >73.132 pg/mL were divided into 9 subjects with significant fibrosis and 25 subjects with non-significant fibrosis. The β-catenin <73.132 pg/mL showed 26 subjects with a high risk of liver fibrosis and transient elastography ≥ 8. Based on the cross-tabulation above, a diagnostic test on β-catenin cut-off for liver fibrosis was carried out to determine the sensitivity, specificity, PPV, NPV, LR (+), and LR (-). The results of

the diagnostic test calculation using a cut-off of 73.132 pg/mL are presented in Table 4.

Table 4. Diagnostic test results of β-catenin cut-off of 73.132 pg/mL

Parameter	Formula	Results
Sensitivity (Sn)	$\frac{a}{a+c}$	74.3%
Spesificity (Sp)	$\frac{d}{d+b}$	71.4%
PPV	$\frac{a}{a+b}$	72.2%
NPV	$\frac{d}{c+d}$	73.5%
LR (+)	$\frac{Sn}{[1-Sp]}$	2.6
LR (-)	$\frac{[1-Sn]}{Sp}$	0.36

Abbrev: Sn: sensitivity, Sp: specificity, PPV: Positive Predictive Value, NPV: Negative Predictive Value, LR (+): Likelihood Ratio (+), LR (-): Likelihood Ratio (-)

Diagnostic test results of plasma β-catenin for the incidence of liver fibrosis in patients with chronic hepatitis B showed a sensitivity of 74.3%; specificity of 71.4%; PPV of 72.2%; NPV of 73.5%; LR (+) of 2.6 and LR (-) of 0.36 with AUC of 0.79 CI 95% (0.68-0.90).

The PPV of 72.2% indicated a probability of liver fibrosis of 72.2% with β-catenin levels <73.132 pg/mL. Meanwhile, the NPV value of 73.5%, indicated a probability of liver fibrosis not to occur of 73.5% if the β-catenin result is > 73.132 pg/mL. The LR+ of 2.6 indicated that the probability of patients with β-catenin <73.132 pg/mL having liver fibrosis is 2.6 times greater than patients with β-catenin > 73.132 pg/mL. The LR- of 0.36 indicated that the probability of patients with β-catenin > 73.132 pg/mL to have liver fibrosis is 0.36 times less than patients with β-catenin levels < 73.132 pg/mL.

The results of the diagnostic test showed that plasma β-catenin levels had a moderate performance as a marker of liver fibrosis in chronic hepatitis B. The AUC value in the results of this study (AUC 0.793) was classified as moderate accuracy, which ranges from 0.7 to 0.8.[21] Diagnostic test studies on β-catenin for liver fibrosis in chronic hepatitis B infection remains limited. Several previous studies suggested a 42 pg/mL β-catenin cut-off with an AUC of 0.75, a sensitivity of 66.43%, and a specificity of 75.41% for liver cirrhosis in chronic hepatitis B patients.[12] In addition to conditions caused by chronic hepatitis B infection, β-catenin was also suggested as a potential marker in detecting colorectal cancer with an AUC of 0.74, a sensitivity of 86.4%, a specificity of 51.56% with an average of 31.79±20.23 pg/mL.[22]

Based on these studies, it was found that the trend of decreased cut-off and average β-catenin levels in these disorders. A mean of 62.90±20.54 pg/mL was obtained in the significant fibrosis group in this study. Previous studies suggested that liver fibrosis occurs due to continuous liver injury, which can cause damage to hepatocyte microvilli and endothelial fenestrations resulting in liver dysfunction, a 3-8 fold increase in collagen, and regeneration of hepatocytes. During regeneration, there is an increase in scar tissue/fibrosis scar followed by the destruction of healthy liver cells. β-catenin resides in hepatocytes and is directly involved in the regeneration of hepatocytes.[23]

The state of continuous injury results in a continuous 2.5-fold increase in β-catenin in response to hepatocellular injury and contributes to the transcriptional activity of target genes that play a role in the development of fibrosis. Therefore, there is an observable increase in β-catenin levels at the start of liver injury. With the formation of fibrous scars and the destruction of healthy hepatocytes in liver fibrosis, β-catenin continues its activity to produce serine phosphorylation residues, which can eliminate inter-hepatocyte contact, which plays an important role in reconstruction to enable normal function of the liver. Therefore, as fibrosis develops, β-catenin levels become lower in damaged liver cells. A previous study by Duan *et al.* suggested that increased β-catenin levels were associated with necrosis of hepatocytes, which then releases β-catenin from hepatocytes to the extracellular environment during the chronic hepatitis B infection phase. However, β-catenin levels decrease progressively along with the severity of the liver injury, as shown by histological observation, which shows an even distribution of β-catenin in the hepatocytes membrane.[24,12]

CONCLUSIONS AND SUGGESTIONS

Measurement of plasma β-catenin levels at a cut-off of 73.132 pg/mL had moderate performance as a marker of liver fibrosis in chronic hepatitis B with a sensitivity of 74.3%; specificity of 71.4%; PPV of 72.2%; NPV of 73.5%; LR (+) 2.6; LR (-) 0.36 with an AUC of 0.793 and CI 95% (0.681-0.906). Measurement of β-catenin levels can be used as a

diagnostic test for liver fibrosis; however, further research was needed and it was recommended to perform a combination test, which can further improve performance to obtain a better diagnostic test value.

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