RESEARCH ARTICLE

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Potential of Apolipoprotein A1 (ApoA1) for Detecting Liver Cirrhosis and Hepatocellular Carcinoma

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Abstract

Objective: Liver cirrhosis and hepatocellular carcinoma (HCC) are chronic liver diseases that can cause serious health problems. Meanwhile, the methods used to detect liver cirrhosis and HCC are limited. Apolipoprotein A1 (ApoA1) is a protein that makes up high-density lipoprotein (HDL), which plays a role in liver cirrhosis and HCC, and can be used as a biomarker. This study aims to determine the ability of ApoA1 to detect and differentiate liver cirrhosis and hepatocellular carcinoma. **Methods:** This cross-sectional study was conducted on 47 patients with liver cirrhosis and HCC at Margono Soekarjo Regional General Hospital, Purwokerto, Indonesia. This study also involved 33 healthy participants from blood donors at the Blood Transfusion Unit, Indonesian Red Cross, Banyumas. Serum ApoA1 levels were analyzed by ELISA method. Receiver Operating Characteristics (ROC) were used to evaluate the diagnostic power of ApoA1 and differentiate between cirrhotic, HCC, and healthy patients. Multivariate binary logistic regression test to determine the most influential variables on the incidence of cirrhosis, HCC, and health. **Results:** ApoA1 was able to differentiate cirrhosis from HCC, cirrhosis from healthy and HCC from healthy, with sensitivity 56.7%, 86.7%, 70.6%, specificity 70.6%, 93.9%, 84.9%, respectively, and AUC 68.5%, 92.6%, 75.0%. AFP (p = 0.002, OR 1.004) and bilirubin (p = 0.021, OR 1.259) were variables that contributed to cirrhosis - HCC. Age (p = 0.011, OR 0.766) and AST (p = 0.003, OR 0.834) are variables that play a role in health - cirrhosis. ALT (p = 0.024, OR 0.965) and PT (p = 0.004, OR 0.253) are variables that play a role in healthy - HCC. **Conclusion:** ApoA1 was best for detecting healthy from cirrhosis, followed by healthy from HCC and cirrhosis from HCC. ApoA1 is not the primary variable determining the incidence of cirrhosis - HCC, healthy - HCC, and healthy – HCC.

Keywords: Apolipoprotein A1- biomarker- hepatic cirrhosis- hepatocellular carcinoma

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Introduction

Liver cirrhosis is a chronic liver disease caused by a liver injury that triggers necroinflammation and fibrogenesis. It involves nodular diffusive regeneration with dense connective tissues and loss of liver parenchyma (Zhou et al., 2014; Tsochatzis et al., 2014). This disease is responsible for 31 million deaths worldwide (Stasi et al., 2015) and is related to increased hepatocellular carcinoma (HCC) incidence. HCC is among the leading malignancy globally, the 5th most common cancer case in males and 7th in females (Bosetti et al., 2014). In Indonesia, the liver cancer ratio between 2005 and 2007 is 4.0 in 100,000 (3rd rank) for men and 1.4 in 100,000 (11th rank) for women (Yano et al., 2015).

The progression of liver cirrhosis to HCC results in an unfavorable prognosis (Forner et al., 2018). HCC diagnosis is often delayed due to the absence of clear signs or symptoms. The tumor may grow relatively too big until detection (El-Serag and Rudolph, 2007). Currently, there is no effective medication for patients diagnosed with HCC (Armengol et al., 2018); this drives decreased patient life expectancy. In addition, poor prognosis in HCC patients is also attributed to the high rates of relapse and metastasis incidence following surgery (Gani, 2017). As for liver cirrhosis, this disease can be progressive despite advancements in understanding pathogenesis, therapy, and complication, leading to difficulties in the initial diagnosis (Muir, 2015).

Currently, alpha-fetoprotein (AFP) is a commonly used biomarker to diagnose HCC, differentiating between liver cirrhosis and HCC. However, some problems are that not all HCC are AFP-positive HCC (HCC with positive AFP). While most cases are AFP-negative HCC (HCC

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with negative AFP), generally, they have a better cure rate than AFP-positive HCC. In addition, AFP-negative HCC is generally a low-grade HCC (TNM I or Okuda I), which is difficult to diagnose using imaging methods (Wang and Zhang, 2020). Therefore, it is necessary to develop biomarkers to identify the factors to predict cirrhosis and HCC disease development. Apolipoprotein A1 (ApoA1) is the primary high-density lipoprotein (HDL) that functions as an anti-atherogenic agent. ApoA1 inhibits tumor angiogenesis and induces the anti-tumor immune microenvironment that prevents tumor development (Ma et al., 2016). ApoA1 plays a role in the steatosis mechanism in liver cirrhosis (Naveau et al., 2009). ApoA1 is believed to have an essential role in the mechanism of liver cirrhosis and HCC, and it has the potential to become a biomarker (Ardakani et al., 2016). Thrombocytes, AST, haptoglobin, and ApoA1 could predict liver cirrhosis with the area under receiver operating characteristics (AUROC) of 0.924 and 95% confidence interval (CI) of 0.877 – 0.971 (Lee et al., 2010). The levels of ApoA1 increased in early fibrosis (F0 – F3) rather than in advanced fibrosis (F4). Therefore, this study aims to find out the diagnostic potential of ApoA1 to discriminate between liver cirrhosis, hepatocellular carcinoma, and healthy conditions.

Materials and Methods

Participants

This study used a prospective cross-sectional study conducted for six months in 2019 inpatients at the internal medicine department, Margono Soekarjo Regional General Hospital. The study involved 80 subjects divided into 30 patients with liver cirrhosis, 17 patients with HCC, and 33 healthy participants. The number of patients with cirrhosis hospitalized in the Internal Medicine Department of Margono Soekarjo Regional General Hospital in 2019 was 243 (data from Margono Soekarjo Regional General Hospital). The ratio of HCC to cirrhosis is 1 : 3 (Singal et al., 2020). Patients included in this study were selected based on inclusion and exclusion criteria, with consecutive sampling.

Inclusion criteria. Liver cirrhosis and HCC group comprised adult patients diagnosed with liver cirrhosis and HCC, respectively. A hepatogastroenterologist performed a diagnosis of liver cirrhosis and HCC. Liver cirrhosis diagnosis was based on clinical symptoms, laboratory, and liver imaging. HCC diagnosis was based on examining serum AFP and nodules in the liver through abdominal ultrasonography and CT scan (Ratnasari et al., 2017).

Exclusion criteria. Patients were excluded if they suffered from a malignant disease other than HCC, with complications or accompaniment of other severe ailments.

Healthy subjects were blood donors at the Blood Transfusion Unit (BTU) of the Banyumas Regency Indonesian Red Cross. All healthy subjects should be free from either liver cirrhosis or cancer and Hepatitis B surface antigen (HBsAg) and HCV antibodies (anti-HCV) negative.

Clinical data and ApoA1 measurements

Clinical data and medical history were obtained from interviews and medical records. Peripheral venous blood samples (10 mL) were collected from all participants and divided into three tubes. Three milliliters of blood were collected in a tube containing EDTA for blood count examination. Three milliliters were collected in a tube containing sodium citrate to examine prothrombin time (PT). The rest was centrifuged with the serum separated for ApoA1 and blood biochemical examination. The serum level of ApoA1 was examined at the Research Laboratory of the Faculty of Medicine, Universitas Jenderal Soedirman using the ELISA method (Wuhan Fine Biotech, Co., Ltd) at the sensitivity of 0.188 ng/ml and assay range of 0.313 – 20 mg/ml.

Platelet count, prothrombin time (PT), alpha-fetoprotein (AFP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), albumin, bilirubin was observed. The examination was carried out at the Clinical Pathology Laboratory at Margono Soekarjo Regional General Hospital. These data were used to compare with ApoA1 to determine whether ApoA1 was the most influential variable on cirrhosis and HCC.

Statistical analyses

Statistical analysis using STATA 15. The analysis results presented are the mean and standard deviation, percentage, median, and minimum - maximum. ROC (The Receiver Operating Characteristic) curve analysis was used to determine the sensitivity, specificity, and cut-off point based on the Youden Index, with 95% confidence interval.

Laboratory results other than ApoA1 were also analyzed to determine whether ApoA1 was the most influential variable on cirrhosis, HCC, and health conditions. The test used is a multivariate stepwise logistic regression method. In univariate analysis, the included numerical variables are linear.

Results

Clinical characteristics of the three groups study

Eighty (80) respondents participated in this study—30 patients with liver cirrhosis, 17 patients with HCC, and 33 healthy participants. The mean and median values of gender, age, and biological parameters of the study groups are presented in Table 1.

Based on Table 1, cirrhosis and HCC were more common in males. The mean age of cirrhosis, HCC, and healthy was 56.2, 53.18, and 38.96 years. Most of the etiology was hepatitis B. We combined hepatitis C with other etiologies, considering there were only 3 cases of hepatitis C. Most patients with cirrhosis and HCC have no history of diabetes mellitus and do not consume alcohol. Based on the stage of CTP, most patients with cirrhosis and HCC are in stages B and C.

Diagnostic performance of ApoA1

Receiver Operating Characteristics (ROC) curves were used to analyze the diagnostic performance of ApoA1 in distinguishing between liver cirrhosis, HCC, and healthy. The results showed that ApoA1 could differentiate cirrhosis from HCC with a sensitivity of 56.67%,

Table 1. Characteristics and Clinical Conditions in the Study Group

Variable	Cirrhosis (n = 30)			HCC (n = 17)			Healthy (n = 33)		
	n (%)	Mean (SD)	Median (Min-Max)	n (%)	Mean (SD)	Median (Min-Max)	n (%)	Mean (SD)	Median (Min-Max)
Gender									
Male	21 (26)			10 (13)			30 (38)		
Female	9 (11)			7 (9)			3 (4)		
Age		56.2 ± 8.32	55 (36 - 70)		53.18 ± 13.75	55 (28 - 79)		38.06 ± 11.37	40 (19 - 56)
Etiology									
Hepatitis B	15 (32)			8 (17)			NA		
Hepatitis C and others	15 (32)			9 (19)			NA		
Diabetes mellitus									
Yes	5 (11)			0 (0)			NA		
No	25 (53)			17 (36)			NA		
Alcohol									
Yes	3 (6)			4 (9)			NA		
No	27 (57)			13 (28)			NA		
ApoA1 (ng/mL)		7.96 ± 8.07	5.31 (1.24 - 41.33)		4.14 ± 2.73	4.24 (0.26 - 9.62)		1.88 ± 1.11	1.71 (0.43 - 5.84)
AFP (ng/mL)		63.30 ± 216.96	4.25 (0.5 - 1000)		475.56 ± 463.77	239.5 (2.8 - 1000)		2.81 ± 2.48	2.2 (0.5 - 14.2)
AST (U/L)		100.7 ± 143.72	54 (18 - 723)		334.35 ± 320.91	230 (59 - 1378)		20.48 ± 8.34	19 (9 - 47)
ALT (U/L)		72.50 ± 78.65	48 (6 - 384)		121.12 ± 100.14	93 (14 - 411)		38.39 ± 23.56	30 (16 - 131)
Albumin (g/dL)		2.34 ± 0.64	2.25 (1.14 - 3.65)		2.28 ± 0.55	2.13 (1.56 - 3.8)		3.95 ± 0.26	3.92 (3.52 - 4.42)
Bilirubin (mg/dL)		2.35 ± 2.32	1.35 (0.19 - 9.75)		8.62 ± 10.02	2.03 (0.66 - 29.91)		0.47 ± 0.27	0.42 (0.19 - 1.25)
Platelet (/mL)		181633.3 ± 159108.4	101000 (37000 - 622000)		235235.3 ± 160398.1	200000 (32000 - 735000)		272181.8 ± 53443.58	263000 (163000 - 390000)
PT (second)		13.13 ± 2.86	12.4 (10.2 - 21.5)		14.74 ± 3.09	14.2 (10.4 - 20.6)		10.55 ± 0.87	10.3 (9.3 - 14.2)
CTP									
A	5 (11)			2 (4)			NA		
B	19 (40)			9 (19)			NA		
C	6 (13)			6 (13)			NA		

ApoA1, apolipoprotein A1; AFP, alpha-fetoprotein; AST, aspartate aminotransferase; ALT, alanine aminotransferase; PT, prothrombin time; CTP, Child Turcotte Pugh

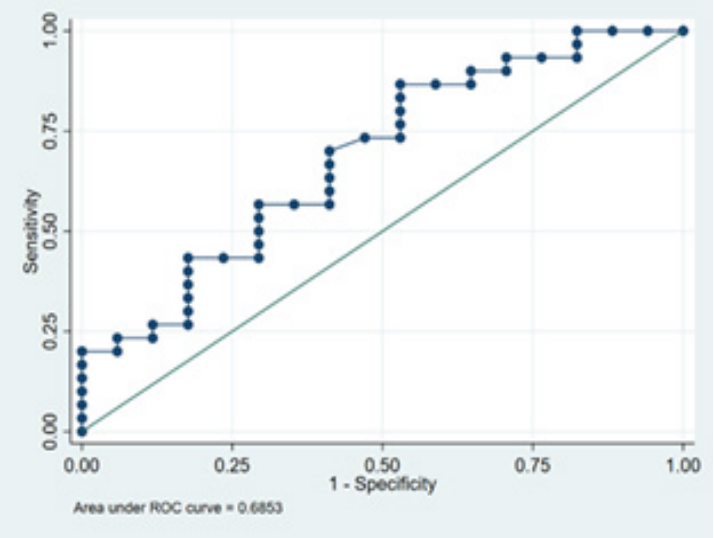

Figure 1. ROC ApoA1 in Discriminating Cirrhosis from HCC.

Table 2. Diagnostic Performance of ApoA1 Distinguish between Cirrhosis, HCC, and Healthy

	Cut-off	Sensitivity	Specificity	AUC (95% CI)
Cirrhosis vs HCC				
ApoA1	≥ 4.89	56.70%	70.60%	68.5% (52.3% – 84.8%)
Cirrhosis vs healthy				
ApoA1	≥ 3.5	86.70%	93.90%	92.6% (85.6% – 99.7%)
HCC vs healthy				
ApoA1	≥ 2.6	70.60%	84.90%	75.0% (57.8% – 92.3%)

AUC, area under the curve

specificity of 70.6%, and area under the curve (AUC) of 68.5%; the cut-off value was 4.89. ApoA1 differentiated cirrhosis from healthy with 86.7% sensitivity, 93.9% specificity, and 92.6% AUC; the cut-off value was 3.5. ApoA1 also differentiated HCC from healthy with a sensitivity of 70.6%, specificity of 84.9%, and AUC of 75.0%. The cut-off value was 2.6 (Table 2) (Figures 1, 2, and 3).

ApoA1 and laboratory indicators for detecting liver cirrhosis and HCC

In multivariate analysis, ApoA1 was not the main factor contributing to the incidence of cirrhosis – HCC, but AFP and bilirubin were the most involved (Table 3).

Table 4 shows that ApoA1 is not the primary variable contributing to the incidence of healthy – cirrhosis. The most influential are age and AST.

Table 5 shows that ApoA1 is not the primary variable

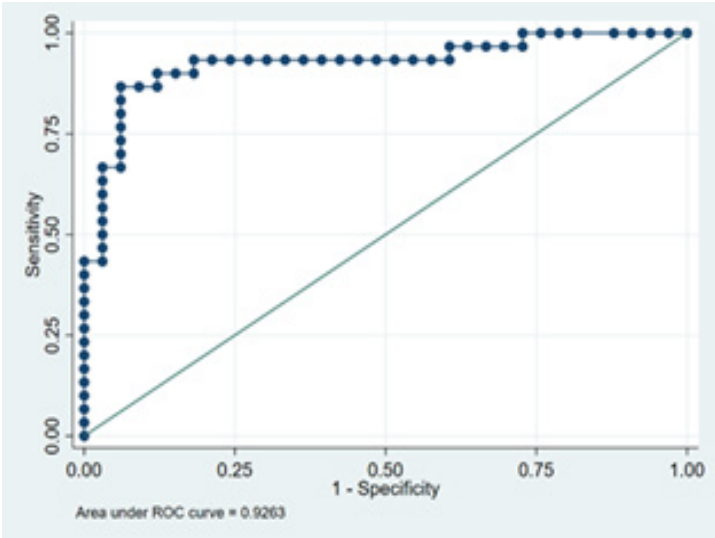

Figure 2. ROC ApoA1 in Discriminating Cirrhosis from Healthy.

Table 3. Univariate and Multivariate Binary Logistic Regression Analysis in Cirrhosis and HCC

Variable	Univariate analysis		Multivariate analysis	
	p-value	OR (min - max)	p-value	OR (min - max)
Gender (male, female)	0.439	1.633 (0.472 - 5.656)		
Age (years)	0.346	0.973 (0.918 - 1.030)		
Etiology (hepatitis B; hepatitis C + others)	0.846	1.125 (0.342 - 3.703)		
Alcohol (yes; no)	0.223	2.769 (0.539 - 14.228)		
ApoA1 (ng/mL)	0.064	0.795 (0.623 - 1.014)		
AFP (ng/mL)	0.003	1.003 (1.001 - 1.006)	0.002*	1.004 (1.001 - 1.006)
AST (U/L)	0.009	1.007 (1.002 - 1.011)		
ALT (U/L)	0.094	1.006 (0.990 - 1.014)		
Albumin (g/dL)	0.751	0.849 (0.308 - 2.337)		
Bilirubin (mg/dL)	0.021	1.208 (1.029 - 1.419)	0.021*	1.259 (1.036 - 1.531)
Platelet (/ml)	0.274	1.000 (0.999 - 1.000)		
PT (seconds)	0.087	1.198 (0.974 - 1.474)		
CTP (C)	0.367	2.500 (0.341 - 18.332)		

*, < 0.05

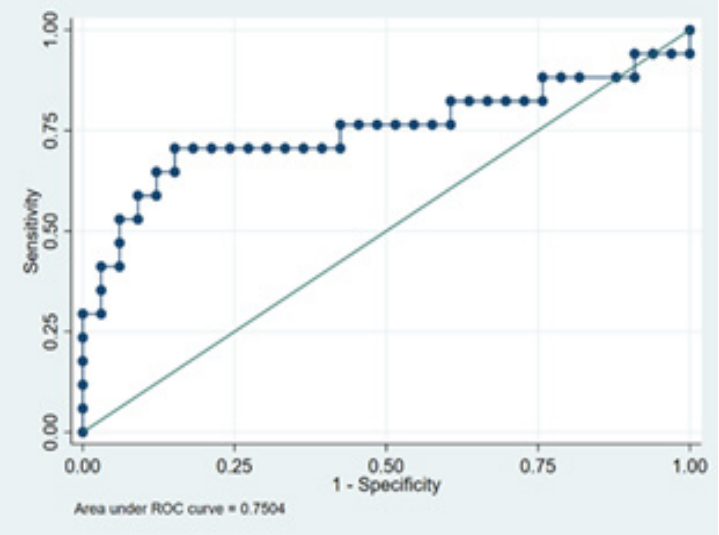

Figure 3. ROC ApoA1 in Discriminating HCC from Healthy

affecting healthy – HCC. The most important are ALT and PT.

Discussion

In this study, most patients with liver cirrhosis and HCC were caused by hepatitis B, aged over 50, and

Table 4. Univariate and Multivariate Binary Logistic Regression Analysis in Healthy and Cirrhosis

Variable	Univariate analysis		Multivariate analysis	
	p-value	OR (min - max)	p-value	OR (min - max)
Gender (male, female)	0.045	0.233 (0.056 - 0.966)		
Age (years)	0.000	0.805 (0.717 - 0.903)	0.011*	0.766 (0.622 - 0.942)
ApoA1 (ng/mL)	0.000	0.310 (0.176 - 0.546)		
AFP (ng/mL)	0.114	0.850 (0.695 - 1.040)		
AST (U/L)	0.000	0.866 (0.802 - 0.935)	0.003*	0.834 (0.741 - 0.938)
ALT (U/L)	0.047	0.981 (0.963 - 0.999)		
Bilirubin (mg/dL)	0.001	0.042 (0.007 - 0.260)		
Platelet (/ml)	0.006	1.000 (1.000 - 1.000)		
PT (seconds)	0.001	0.297 (0.146 - 0.606)		

*, < 0.05

Table 5. Univariate and Multivariate Analysis of Binary Logistic Regression in Healthy and HCC

Variable	Univariate analysis		Multivariate analysis	
	p-value	OR (min - max)	p-value	OR (min - max)
Gender (male, female)	0.013	0.143 (0.031 - 0.660)		
Age (years)	0.002	0.902 (0.846 - 0.963)		
ApoA1 (ng/mL)	0.003	0.526 (0.345 - 0.801)		
AFP (ng/mL)	0.126	0.846 (0.683 - 1.048)		
ALT (U/L)	0.002	0.966 (0.944 - 0.988)	0.024*	0.965 (0.936 - 0.995)
Bilirubin (mg/dL)	0.002	0.005 (0.000 - 0.141z0		
Platelet (/ml)	0.234	1.000 (0.999 - 1.000)		
PT (seconds)	0.001	0.278 (0.134 - 0.577)	0.004*	0.253 (0.099 - 0.646)

*, < 0.05

male. Previous studies stated that in Asia-Pacific, most cirrhosis and HCC were over 40 years old, male (Zhu et al., 2016), and caused by hepatitis B (Sarin et al., 2020). Meanwhile, the low level of alcohol is due to the low level of alcohol consumption in Indonesia (Sornpaisarn et al., 2020). Based on the stage of CTP, the majority of cirrhosis and HCC are in stages B and C, which indicate a decompensated condition. It may be related to the delay in the early detection of disease (Table 1).

Based on the descriptive data in Table 1, the highest levels of AFP, AST, ALT, bilirubin, and PT were in the HCC group, followed by cirrhosis, and the lowest was healthy. Increased AFP in HCC is associated with the pro-oncogenic properties of AFP (Sauzay et al., 2016). Elevated AST and ALT are associated with hepatic cell necrosis and cell membrane blistering (McGill, 2016). An increase in bilirubin indicates a more severe liver tissue damage and reflects changes in liver function (Nallagangula et al., 2018). Prothrombin time is prolonged in cirrhosis and HCC due to decreased synthesis of procoagulant proteins II, VII, IX, and X, factors V and XI (Northup and Caldwell, 2013). Meanwhile, albumin levels and platelet counts decreased in HCC, especially in cirrhosis. Decreased albumin levels are related to impaired albumin synthesis by the liver (Spinella et al., 2016). While the decreased platelet count is associated with several factors, including decreased platelet production, increased platelet uptake in the spleen, and increased platelet breakdown (Mitchel et al., 2016).

Meanwhile, ApoA1 levels were highest in cirrhosis and lowest in healthy individuals (Table 1). This result is different from that of previous research. Ni et al., (2020) showed that ApoA1 levels in HCC were higher than those in cirrhosis, and ApoA1 expression was similar to the high-density lipoprotein (HDL) expression pattern. The study by Ahn et al., (2009) stated that an increase in circulating HDL was associated with a reduced risk of liver malignancy. The mechanism of HDL and ApoA1 with cancer is associated with antioxidants, anti-inflammatory, anti-apoptotic, and immune-modulatory activities (Ganjali et al., 2019). Meanwhile, we did not examine the relationship between ApoA1 and HDL in this study. Therefore, further research is needed.

Regardless of other variables, ApoA1 has the potential to discriminate against cirrhosis from healthy (AUC 92.6%, cut-off 3.5). The ability of ApoA1 to discriminate against HCC from healthy individuals AUC 75.0%, cut-off 2.6. Meanwhile, the ability of ApoA1 to discriminate against cirrhosis from HCC was only 68.6% at a cut-off of 4.89 (Table 3, Figures 1 – 3). Lee et al., (2010) stated that ApoA1 increased in early fibrosis (F0 – F3) and decreased in late fibrosis. Another study reported higher ApoA1 in patients with greater levels of steatosis (Naveau et al., 2009). ApoA1 and liver cirrhosis are related to lipid metabolism and liver fibrosis. Meanwhile, in HCC, the results of previous studies showed different results. Previous research by Pleguezuelo et al., (2010) showed an increase in ApoA1 levels in HCC compared to cirrhosis. Another study of ApoA1 in HCC patients associated with relapse and survival time showed that decreased serum ApoA1 was associated with higher relapse and shorter survival times (Ma et al., 2016).

ApoA and ApoC genes play a role in steroid metabolism through peroxisome proliferator-activated receptors (PPAR) signaling pathways and steroid metabolism (Wang et al., 2019). ApoA1 is a central protein involved in the mechanism of cirrhosis and the PPAR signaling pathway. Elevated ApoA1 levels are associated with liver injury (Safaei et al., 2016). Ardakani et al., (2016) demonstrated that ApoA1 is a central protein for cirrhosis and HCC. ApoA1 is a component of HDL (high-density lipoprotein) involved in forming plasma cholesterol esters. In HCC, ApoA1 has the potential to suppress tumor growth and metastasis. ApoA1 is involved in reverse cholesterol transport and the humoral immune response.

However, in this study, a multivariate analysis combining ApoA1 with other variables showed that ApoA1 was not a major factor in developing cirrhosis – HCC, cirrhosis – healthy, or HCC – healthy. AFP (p = 0.002, OR 1.004) and bilirubin (p = 0.021, OR 1.259) were the main variables determining the incidence of cirrhosis – HCC (Table 3). In cirrhosis – healthy, the most influential variables were age (p = 0.011, OR 0.766) and AST (p = 0.003, OR 0.834) (Table 4). Meanwhile, in the incidence of cirrhosis – HCC, the most influential variables were ALT (p = 0.024, OR 0.965) and PT (p = 0.004, OR 0.253) (Table 5). These results may be due to the biological and clinical characteristics of the respondents in this study.

In this study, we analyzed the potential of ApoA1 as a tool to discriminate between patients who had cirrhosis

and HCC and who were healthy. Without observing other variables, the results show that ApoA1 has the best potential to discriminate patients with cirrhosis from healthy participants, followed by HCC from healthy and then cirrhosis from HCC. Multivariate test to determine whether ApoA1 is the most influential factor on the incidence of cirrhosis – HCC, healthy – cirrhosis, and HCC– healthy. The results of multivariate analysis showed that ApoA1 had not been able to become the main factor. The weakness of this study is that HDL levels, the patient's metabolic status, or other factors that might influence ApoA1 levels were not examined. In addition, the limited time of sampling and relatively low number of samples may also affect the result of this study. We suggest that future studies involve more participants and be conducted within a more extended period. Future studies should pay attention to factors that may affect ApoA1 levels.

In conclusion, ApoA1 levels were highest in liver cirrhosis, followed by HCC and healthy. ApoA1 has the best performance in detecting healthy cirrhosis, followed by healthy HCC and cirrhosis from HCC. ApoA1 was not the main factor determining the incidence of cirrhosis – HCC, healthy – cirrhosis, and healthy – HCC.

Author Contribution Statement

NSA Gumilas and D Novrial: designed the study, NSA Gumilas, IM Harini, DA Ernawati, and V Indriani: conducted the experiments, data analysis, interpretation of the data, and wrote the manuscript, NSA Gumilas, D Novrial and DW Kurniawan: finalized the manuscript.

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Approval

This study was approved by the Medical Research Ethics Committee of the Faculty of Medicine at Universitas Jenderal Soedirman (Ref.1913/KEPK/IV/2019). All respondents involved in this study have voluntarily given written consent.

Conflict of Interest

The authors declare that they have no conflict of interest.

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