RELATIONSHIP BETWEEN *ABSOLUTE LYMPHOCYTE COUNT* AND ASCITES IN PATIENTS WITH LIVER CIRRHOSIS

Nathasya Rizkyana Riyadi, Austin Bertilova Carmelita**, Lia Sasmithae***, Donna Novina Kahanjak**, Ravenalla Al Hakim Sampurna Putra S*****

*Faculty of Medicine, University of Palangka Raya, Palangka Raya
** Department of Physiology, Faculty of Medicine, University of Palangka Raya, Palangka Raya
***Department of Internal Medicine, Faculty of Medicine, University of Palangka Raya, Palangka Raya
**** Department of Family and Community Medicine,
Faculty of Medicine, University of Palangka Raya, Palangka Raya

Corresponding author :
Nathasya Rizkyana Riyadi. Faculty of Medicine, University of Palangka Raya, Palangka Raya. Phone: +62-82250466865. E-mail : nathasyariyadi@gmail.com

ABSTRACT

Background: *Cirrhosis is the final stage of liver disease characterized by fibrosis and irreversible nodule formation due to chronic inflammation. The most common liver cirrhosis complication is ascites, where pathological fluid accumulates in the peritoneal cavity. In cirrhosis, lymphocytes infiltrate the liver and contribute to stellate cell activation, differentiation, and the fibrogenic response. Many recent studies have not studied a relationship between the absolute lymphocyte count and ascites in liver cirrhosis. Therefore, it is important to investigate the relationship between lymphocytes and ascites in liver cirrhosis.*

Method: *This is an observational study with a cross-sectional design study. Held in August–October 2022 at Dr. Doris Sylvanus Hospital's Medical Record Installation. 64 samples were selected based on inclusion and exclusion criteria. Meanwhile, absolute lymphocyte count were measured from the patient's blood tests or by calculation. Ascites was diagnosed by physical examination and radiology. The relationship between absolute lymphocyte count and ascites was analyzed using the chi-square test, with the results considered statistically significant when the p-value < 0.05.*

Result: *The medical records from April 2019 to April 2022, there were 64 samples, of which 15 and 49 were without and with ascites. Based on the chi-square test results, there was a significant relationship between absolute lymphocyte count and the incidence of ascites, with p = 0.02.*

Conclusion: *This study concluded that there is a relationship between absolute lymphocyte count and ascites in patients with liver cirrhosis, where absolute lymphocyte count tends to decrease.*

Keywords: *absolute lymphocyte count, ascites, liver cirrhosis*

ABSTRAK

Latar belakang: *Sirosis hati adalah tahap akhir dari penyakit hati dengan karakteristik fibrosis dan terbentuknya nodul pada organ hati yang bersifat ireversibel akibat peradangan kronis. Salah satu dari komplikasi sirosis hati yang paling sering terjadi adalah asites. Asites merupakan keadaan di mana terdapat akumulasi cairan patologis di rongga peritoneum. Pada sirosis hati, limfosit menginfiltrasi organ hati dan berkontribusi pada aktivasi dan diferensiasi sel stelata serta respons fibrogenik. Namun belum ditemukannya banyak penelitian*

terbaru yang mempelajari hubungan antara absolute lymphocyte count dengan asites pada sirosis hati. Atas dasar itu, perlu dilakukan penelitian untuk mengetahui apakah terdapat hubungan dari absolute lymphocyte count dengan asites pada sirosis hati

Metode: *Penelitian ini merupakan penelitian observasional dengan desain studi cross-sectional. 64 Sampel dipilih berdasarkan kriteria inklusi dan eksklusi. Absolute lymphocyte count diukur dari hasil pemeriksaan darah atau melalui perhitungan. Asites didiagnosis dari pemeriksaan fisik dan radiologi. Analisis hubungan antara dengan asites pada pasien sirosis hati dilakukan dengan uji chi-square dengan hasil penelitian dianggap signifikan secara statistik jika nilai p < 0,05*

Hasil: *Dari data rekam medis April 2019–April 2022, terdapat 64 sampel, 15 tidak asites dan 49 asites. Berdasarkan hasil uji chi-square, terdapat hubungan yang signifikan antara absolute lymphocyte count dengan kejadian asites, dengan p = 0,02.*

Simpulan: *Kesimpulan penelitian ini adalah terdapat hubungan antara absolute lymphocyte count dengan asites pada pasien sirosis hati, dimana absolute lymphocyte count akan cenderung menurun pada pasien sirosis hati dengan asites.*

Kata kunci*: absolute lymphocyte count, asites, sirosis hati*

INTRODUCTION

Ascites is a condition with an accumulation of pathological fluid in the peritoneal cavity. It is one of the most common complications of the liver, resulting in a critical developmental stage of cirrhosis, marked by a decompensated phase. Patients affected with ascites are projected to have a 2 and 5 years survival rate of 38% and 78%, where about 75% are due to liver cirrhosis.[1–3] Furthermore, cirrhosis is the last stage of liver disease, characterized by fibrosis and the creation of permanent nodules due to persistent inflammation, which is the 11th world's leading cause of death, compared to 2000.[4]

The liver comprises two main components, parenchymal and non-parenchymal cells. When chronic inflammation damages hepatocytes and forms fibrous tissue, which activates stellate cells, and lymphocytes infiltrating the liver. The continuation of this complication can cause damage, resulting in organ malfunction.[5,6] Additionally, fibrous and nodular tissue can increase intrahepatic resistance to portal blood flow, which is the primary cause of ascites.[7]

Research conducted by Li et al involved 174 patients with liver cirrhosis, with the most common complication ascites in 114 patients (65.5%). The study found that patients who did not survive showed higher neutrophil counts and lower lymphocyte counts than the surviving group. Lymphocyte levels in the blood of cirrhotic liver patients with ascites have also been studied by Romaneli et al, which is the first study to describe differences between lymphocyte subsets in ascitic fluid of patients with either hepatitis C virus (HCV) or alcoholic liver disease (ALD)-related cirrhosis. This study also examined the subset of lymphocytes present in blood and found no significant differences in ascitic fluid and peripheral blood samples.[8,9] Researchers have yet to find many recent studies examining the relationship between absolute lymphocyte count in blood and ascites in liver cirrhosis. On that basis, the researcher wanted to know whether there is a relationship between lymphocytes and the incidence of ascites in liver cirrhosis so that it can be considered as a parameter in assisting the diagnosis of ascites in patients with liver cirrhosis.

METHOD

This study was conducted from August to October 2022 at the Dr. Doris Sylvanus Hospital's Medical Record Installation using analytic observational with a cross-sectional design study. All medical records of patients with liver cirrhosis and with or without ascites complications treated as outpatients or inpatients between April 2019 and 2022 were included in the population. Patients with liver cirrhosis, medical records, complete laboratory examination data, and liver cirrhosis of any etiology were included in the sampling, while patients with incomplete medical records were excluded.

Based on the operational definition, ascites is a complication of liver cirrhosis diagnosed on physical and radiological examinations from medical records. Meanwhile, absolute lymphocyte count is the value of the cell in blood circulation, based on the tests or calculations with the formula absolute lymphocyte count (ALC) = white blood cell count x relative lymphocyte count.

In statistical analysis, the chi-square test was used and Fisher's exact test was used when it failed to meet the parameters. Data analysis was performed using Windows's statistical package for social sciences (SPSS) software.

RESULT

In this research, 64 samples of record fulfilled the inclusion criteria. The majority of respondents came from the age group of 56–65 years, with 20 patients at 31.3% and 49 male patients at 76.6%, as well as abnormal absolute lymphocyte count of 33 at 51.6 %, and a history of hepatitis B with 53 patients at 82.8%.

Table 1. Sample characteristics

Sample characteristics	Without ascites (%)	With ascites (%)	n (%)
Age			
17–25 years	1 (1.6%)	2 (3.1%)	3 (4.7%)
26–35 years	0 (0%)	4 (6.3%)	4 (6.3%)
36–45 years	2 (3.1%)	6 (9.4%)	8 (12.5%)
46–55 years	5 (7.8%)	14 (21.9%)	19 (29.7%)
56–65 years	5 (7.8%)	15 (23.4%)	20 (31.3%)
> 65 years	2 (3.1%)	8 (12.5%)	10 (15.6%)
Gender			
Male	11 (17.2%)	38 (59.4%)	49 (76.6%)
Female	4 (6.3%)	11 (17.2%)	15 (23.4%)
Absolute lymphocyte count			
Abnormal (< 1.0 x 10^3/µL)	4 (12.1%)	29 (45.3%)	33 (51.6%)
Normal (1.0–4.8 x 10^3/µL)	11 (17.2%)	20 (31.3%)	31 (48.4%)
History of hepatitis			
No hepatitis	3 (4.7%)	6 (9.4%)	9 (14.1%)
Hepatitis B	11 (17.2%)	42 (65.6%)	53 (82.8%)
Hepatitis C	1 (1.6%)	1 (1.6%)	2 (3.1%)

Table 2. Distribution of absolute lymphocyte count values

Variable	Absolute lymphocyte count		
	Mean	Minimum	Maximum
Without ascites	1.38 ± 0.72	0.51	2.66
With ascites	1.3 ± 0.72	0.28	3.1

From Table 3, there were 31 patients with normal absolute lymphocyte count values (1.0–4.8 x 10^3/µL), consisting of 11 and 20 at 35.5% and 64.5% without and with ascites. In contrast, those with abnormal absolute lymphocyte count values (< 1.0 x 10^3/µL) were 33, consisting of 4 and 9 at 12.1% and 87.9% without and with ascites. The relationship between the absolute lymphocyte count and the incidence of ascites in individuals with liver cirrhosis was found to be $p = 0.02$ ($p < 0.05$). As a result, there is a relationship between the absolute lymphocyte count and the incidence of ascites in patients with liver cirrhosis.

The absolute lymphocyte count in patients without and with ascites had a mean value of 1.38 ± 0.72 and 1.3 ± 0.72 and a median of 1.17 (0.51–2.66) and 1.05 (0.28–3.1), respectively.

Table 3. Bivariate analysis of absolute lymphocyte count

Absolute lymphocyte count	Without ascites		With ascites		Total	P
	n	%	n	%	n	
Abnormal (< 1.0 x 10^3/µL)	4	12.1%	29	87.9%	33	$p = 0.02$
Normal (1.0–4.8 x 10^3/µL)	11	35.5%	20	64.5%	31	
Total	15	23.40%	49	76.60%	64	

DISCUSSION

In this research, most liver cirrhosis patients were 56–65 years old, and the highest frequency of ascites was found. This is in line with study by Vaz et al, involving 598 patients in Halland, Sweden, with the highest incidence in the 60–69 years range.[10] Similarly, Sepanlou et al found that the number of compensated cases peaks at 45–49, 40–44, and 50–54 years in females, males, and both genders.[11] Referring to the research by Wang et al, hepatitis B has the highest prevalence of 40–49 years and can develop liver cirrhosis in 10–20 years. Therefore, in the 50–69 age group, there will be many hepatitis patients with liver cirrhosis complications. In this research, 56–65 years has the highest frequency for liver cirrhosis patients.[12,13] In older patients, the incidence decreases because victims have a lower life expectancy due to higher complications, resulting in liver changes that lead to death.[14] Any sources have not explained the incidence of ascites in this age group. However, ascites is the most common complication due to portal hypertension. The transition from compensated to decompensated liver cirrhosis is affected by many predisposing factors, where 33% have transitioned within 4 years.[15–18]

Based on gender, male patients were higher than females, with 49 patients at 76.6%, and had the highest frequency of ascites, with 38 at 59.4%. This result is in line with the study conducted by Nababan et al, who analyzed 241 victims of decompensated cirrhosis, with a male predominance of 74.3%.[19] Rubin et al further examined the effect of gender on the clinical description of hospitalized cirrhotic patients and found that ascites complications were commonly found in males.[20] This research found that males dominate many cases of liver cirrhosis patients with a history of hepatitis, one of the common causes associated with etiology. It has a higher prevalence due to sex hormones on inflammatory cytokine release in the opposite ways because estrogen induces pro-inflammatory cytokines while androgens suppress the responses. Another research stated that after prophylactic vaccination against hepatitis B virus (HBV), males have lower antibodies than females, hence hepatitis tends to be higher. Liver cirrhosis is also higher in this gender due to higher-risk behaviors such as alcohol consumption.[11]

The result of absolute lymphocyte count value reported 29 patients with ascites at 45.3%. There was no research on the lymphocyte value in liver cirrhosis patients with ascites. Meanwhile, some research used a variable ratio of neutrophil lymphocytes.[17,21] Chronic liver injury causes the generation of pro-inflammatory mediators and leukocyte infiltration, especially lymphocytes, in the subendothelial region. Interactions with endothelial cells stimulate lymphocyte recruitment from circulation, controlled by various chemokines. Through surface integrins, lymphocytes can interact with extracellular matrix components and endothelial cells during pathogenesis, contributing to cell activation, differentiation, and fibrogenic responses. Chemoattractant molecules attract lymphocytes to the injury site after moving through the endothelium via a complicated method. Myofibroblasts release lymphocyte migration-promoting cytokines such as IL-6, hepatocyte growth factor, and TGF-β. As a result, the value in the plasma may drop during fibrogenesis owing to liver infiltration.[6]

Concerning the history of hepatitis, 53, 2, and 9 patients at 82.8%, 3.1%, and 14.1% had a history of hepatitis B, C, and no history, respectively. Pravitasari et al found that out of 78 samples, 54 and 15 patients, 62.4% and 19.23% have a history of hepatitis B and C.[22] The high history is because this disease is the leading cause of cirrhosis. Hepatitis B was found to be higher because hepatitis B virus (HBV) is highly contagious and can be transmitted in more ways than other viruses.[23] The hepatitis virus that replicates in hepatocytes induces hepatocyte cell death and inflammation in the liver. Chronic inflammation by HBV further causes hepatic stellate cell activation and differentiates into myofibroblasts, the main extracellular matrix that produces cells in the liver. Furthermore, increased extracellular matrix production results in hepatic fibrosis formation. Even though this fibrogenesis maintains tissue integrity, chronic fibrosis that persists can progress to liver cirrhosis.[24]

Absolute lymphocyte count and the incidence of ascites in patients with liver cirrhosis were significantly correlated, with $p = 0.02$ ($p < 0.05$). As a result, there is a relationship between the occurrence of ascites in patients and the absolute lymphocyte count. The immune response and the hepatic fibrotic process are closely related to cirrhosis. Hepatic fibrosis has been linked to T lymphocytes, including Th1, Th2, Th17, regulatory T cells, and mucosa-associated invariant T cells. These immune cells may be profibrotic or antifibrotic during fibrosis.[25,26]

Weiss et al discovered that patients with acute chronic liver failure (ACLF) have dysregulation in systemic immune cells, including leukocytosis generated by neutrophils and monocyte subsets and decreased count associated with memory lymphocyte, CD8 T cell, and natural killer cell depletion. ACLF patients also developed decompensated cirrhosis.[27] Low lymphocyte counts in the blood can also be produced by those in the liver, which plays a part in necroinflammatory processes. They get localized due to contact with endothelial cells, where numerous chemokines regulate the processes. Surface integrins allow lymphocytes to interact with extracellular matrix components and endothelial cells, promoting differentiation and the fibrogenic response.[6,28]

A robust innate immune response develops during the shift from compensated to decompensated states, boosting circulating neutrophils. Furthermore, neutrophils have a mechanism that inhibits lymphocyte production via arginase, nitric oxide synthase (NOS), and reactive oxygen species (ROS). It has a high arginase concentration in azurophilic granules, whereas T-cell activation depends on arginine availability in the body. The release of neutrophil arginase by degranulation or death reduces body arginine, which inhibits T-cell activation.[21] A decrease in lymphocytes can be caused by spontaneous bacterial peritonitis in patients with ascites. Popoiag et al found a relationship between the neutrophil-lymphocyte ratio (NLR) and the incidence of painful bladder syndrome (PBS),

where neutrophils and lymphocytes decreased, causing NLR to increase during PBS.[29]

CONCLUSION

According to this study, there is a significant relationship between absolute lymphocyte count and the incidence of ascites in liver cirrhosis patients at Dr. Doris Sylvanus General Regional Hospital, with a value of $p = 0.02$ ($p < 0.05$). The absolute lymphocyte count tends to decrease in liver cirrhosis patients with ascites.

Further research is necessary to analyze the relationship between absolute lymphocyte count and liver diseases such as cirrhosis with larger samples and broader locations. Other variables, such as neutrophils and monocytes in blood and ascites fluid, are also recommended.

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