CD4+ and CD8+ Counts in Liver and Their Correlation with Necroinflammatory and Fibrosis Grades in Chronic Hepatitis C

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ABSTRACT

Background: *Studies on the characteristics CD4+ and CD8+ in hepatitis C and their correlation with the severity of the disease have been rarely conducted. This study was aimed to obtain the mean difference between CD4+ and CD8+ count in liver to evaluate their correlation with fibrosis and necroinflammatory grades in chronic hepatitis C.*

Method: *A cross-sectional study was conducted between March and July 2010 with 30 liver biopsies obtained from patients with non-B and non-HIV chronic hepatitis C who visited the Outpatient Clinic of Hepatology Unit at Cipto Mangunkusumo Hospital in January 2008–February 2010. Fibrosis and necroinflammatory grades were determined using METAVIR methods on liver biopsies. The mean values of CD4+ and CD8+ in portal tracts and hepatic lobules in liver biopsy specimens were evaluated. Statistical analysis was performed by using independent T-test and Spearman test.*

Results: *There was a difference in mean CD4+ counts between portal tracts and the lobules (95% CI = 4.3-17.9; p = 0.002) and also differences in mean CD8+ counts in portal tracts and hepatic lobules (95% CI = 15.4-35.6; p < 0.001). There was no correlation between CD4+ and CD8+ counts, either in portal tracts or the lobules, and inflammatory grades as well as the liver fibrosis.*

Conclusion: *CD4+ and CD8+ counts are greater in portal area compared to the hepatic lobules, with greater CD8+ counts than CD4+. However, both CD4+ and CD8+ counts are not correlated to the severity of liver damage.*

Keywords*: CD4+, CD8+, intrahepatic, chronic hepatitis C, METAVIR*

ABSTRAK

Latar belakang: *Penelitian mengenai karakteristik sistem imun seluler terutama CD4+ dan CD8+ pada hepatitis C serta korelasi terhadap beratnya penyakit hepatitis C belum banyak dilakukan. Penelitian ini bertujuan untuk mendapatkan beda rerata antara hitung CD4+ dan CD8+ di portal dan lobulus hati serta untuk mengetahui korelasinya dengan derajat fibrosis dan nekroinflamasi hati pada hepatitis C kronik.*

Metode: *Penelitian ini merupakan studi potong lintang yang dilakukan antara bulan Maret-Juli 2010 pada 30 sampel biopsi hati pasien dengan hepatitis C kronik non-B dan non-HIV yang berobat ke Poliklinik Hepatologi Rumah Sakit Cipto Mangunkusumo antara bulan Januari 2008-Februari 2010. Derajat fibrosis dan nekroinflamasi ditentukan dengan metode METAVIR dari biopsi hati. Rerata hitung CD4+ dan CD8+ di lima portal dan lima lobulus dinilai dari sediaan biopsi hati. Analisis statistik dilakukan dengan uji-T independen dan tes Spearman.*

Hasil: *Terdapat perbedaan rerata hitung CD4+ di portal dan lobulus (CI 95 % = 4,3-17,9; p = 0,002) dan perbedaan rerata hitung CD8+ di portal dan lobulus (CI 95% = 15,4-35,6; p < 0,001). Tidak ada korelasi antara hitung CD4+ dan CD8+ di portal maupun lobulus dengan derajat nekroinflamasi dan fibrosis hati.*

Simpulan: *Hitung CD4+ dan CD8+ lebih banyak berada di portal daripada lobulus hati dengan jumlah hitung CD8+ lebih banyak daripada CD4+, namun baik pada CD4+ dan CD8+ tidak terdapat korelasi dengan derajat kerusakan hati. Hitung limfosit T CD8+ dan CD4+ di portal dan lobulus hati tidak dapat dijadikan prediktor kerusakan hati.*

Kata kunci*: CD4+, CD8+, intrahepatik, hepatitis C kronik, METAVIR*

INTRODUCTION

World Health Organization (WHO) has regarded hepatitis C virus (HCV) as one of global public health problems and estimates that about 3% of world's population, i.e. 170 million people have been infected with HCV.[1] The prevalence of hepatitis C ranges from 0.1-5% and varries associated with its geographical distribution. The lowest seroprevalence of hepatitis C is found in Europe and the prevalence is about 1% and the highest seropravelence is found in Africa, about 5.3%.[1]

In the United States, HCV accounts for 40-60% chronic hepatitis and one-third of those cases will develop into progressive fibrosis and cirrhosis and hepatitis C becomes the main cause of liver transplants.[2,3] In Japan, hepatitis C now has become the leading cause of hepatocellular carcinoma replacing hepatitis B and according to data in Centers for Disease Control and Prevention (CDC), there are approximately 8,000 – 10,000 of deaths each year in the United States attributed to cirrhosis and malignancy associated with HCV. In Southeast Asia, the prevalence is about 2.2% or 32.3 million cases.[3] The prevalence of HCV in Indonesia varies geographically as the country has extensive geographical diversity. A preliminary study of HCV in blood donors of various regions in Indonesia showed that the prevalence is about 3.1-4%.[3] New infections of HCV globally will occur in 3-4 million people each year with predominant HCV genotype 1, which is followed by genotype 2 and 3. Other genotypes such as genotype 4, 5 and 6 have specific geographical distribution. Most of acute HCV infections are asymptomatic and about 70-80% cases will develop into chronic infection and progressive liver disease leading to liver cirrhosis and hepatocellular carcinoma.[4]

The roles of immune system in chronic and acute hepatitis C are different and there are some hypothetical mechanisms that have tried to explain that generally, CD4+ and CD8+ are the important cellular immune system against the viral infection; however, they are not optimal for eradication of hepatitis C virus which may lead to persistent and chronic infection.[5] The HCV-specific CD4+ and CD8+ T cells are predominantly found in the liver.[6] Until now, few studies have been done that provide direct correlation between intrahepatic CD4+ and CD8+ and the process of liver damage in chronic hepatitis C, which have been shown as histopathological manifestation of hepatitis C. The results of those studies are still controversial.

This study was aimed to evaluate the most dominant cellular immunity against the pathology of liver cell disorder. The study was also aimed to obtain the correlation between CD4+ and CD8+, either in hepatic lobules or portal tracts, and the severity of liver damage in patients with chronic hepatitis C. The study examined cellular immunity cells, which are regarded to have important role, i.e. the CD4+ and CD8+ count performed directly on liver biopsies available at the Division of Hepatology, Department of Internal Medicine and Anatomical Pathology Laboratory in Cipto Mangunkusumo Hospital.

METHOD

This study was a cross-sectional study with analytical technique to find mean differences between CD4+ counts of T helper lymphocytes and CD8+ cytotoxic T lymphocytes in portal tracts and hepatic lobules as well as the correlation with fibrosis and necroinflammatory grades in untreated hepatitis C patients. The population of study was patients infected with HCV who lived in Jakarta (pre-treatment) and visited Cipto Mangunkusumo Hospital to have antiviral treatment between January 2008 and February 2010. Male or female patients aged between 18 and 70 years with positive HCV serology based on ELISA examination confirmed with polymerase chain reaction (PCR) were included in our

study. The exclusion criteria were history of alcohol consumption in the last 6 months period prior to liver biopsy, HIV infection, previous hepatitis C treatment (using interferon or ribavirin), having another liver disease other than chronic hepatitis C and those who refused to be participated in our study.

The study was conducted at Division of Hepatology, Department of Internal Medicine and Department of Anatomical Pathology, Cipto Mangunkusumo Hospital between March 2010 and July 2010. We obtained data of liver biopsy speciments and laboratory data of patients with chronic hepatitis C who visited the Outpatient Clinic of Hepatology Unit at Cipto Mangunkusumo Hospital between January 2008 and July 2010.

Approximately 33 patients were participated in this study. The collection of baseline data included age, sex and basic history taking and physical examination according to the available forms. All patients had given their informed consent prior to the study. Paraffin blocks of patients' liver biopsy were taken randomly according to the storing number at the Department of Anatomical Pathology. Each block was cut and made into two specimens for immunohistochemistry examination, stained with primer antibody of CD4+ and CD8+ and subsequently examined under light microscope with 400 x magnification. Each count of CD4+ and CD8+ in five portal tracts and five hepatic lobules of each specimen were measured. Afterward, the total counts of both portal tracts and lobules were calculated for determining the mean value. The mean value of total counts in portal tracts and the lobules is intrahepatic CD4+ and CD8+ counts. Since CD4+ and CD8+ cells in liver tissues are predominated by specific cells, we hope that our techniques may represent the overall liver-specific CD4+ and CD8+ counts and minimize the possibility of uncounted CD4+ and CD8+.

Statistical analysis using unpaired T-test for both groups (lobules and portal) for each CD4+ and CD8+ count was used to obtain mean differences of CD4+ and CD8+ counts between the lobules and portal tracts. Moreover, the correlation between portal and lobules CD4+ and CD8+ count and necroinflammatory grading as well as fibrosis staging in keeping with METAVIR was analyzed by using Spearman correlation test. Furthermore, to determine which variable of cellular immunity that may have role in liver damage process, the results of Spearman correlation test with $p < 0.25$ were included in multivariate analysis of multinomial logistic regression test. All analysis considered the value of 5% as significant and used 95% confidence interval. Data was processed using computer program of SPSS 17.

RESULTS

Of 33 subjects included in the study, about 3 (9%) subjects were excluded since the results of their liver biopsies did not meet the minimal number requirement of portal tracts and lobules; therefore, only 30 subjects were analyzed at the end of the study. There were more female subjects, i.e. 17 people; while the age of subjects ranged between 24-69 years with median age of 58 years. Most subjects had genotype 1. Subject characteristics and laboratory results are presented in Table 1.

Table 1. Subject characteristics (n = 30)

Characteristic	n or median (range)
Age (year)	58 (24-69)
Sex	
Male	13
Female	17
Genotype	
1	23
2	2
3	1
4	2
Not available	2
Subtype	
1a	6
1b	9
1a/1b	1
1c	2
2a/2c	2
3k	1
Not available	9
Complete peripheral blood	
Hemoglobin (g/dL)	13.36 (1.51)
Hematocryte (%)	39.60 (5.25)
Leukocyte (x10^3/µL)	5.5 (3.4-11.5)
Thrombocyte (x10^3/µL)	177 (78-403)
AST (U/L)	89 (8-300)
ALT (U/L)	100.5 (9-415)
HCV-RNA (x 10^4 copy/mL)	56.5 (0.07-768)
Degree of fibrosis (METAVIR)	
F2	11
F3	9
F4	10
Degree of necroinflammatory (METAVIR)	
A1	1
A2	12
A3	17

AST: aspartate transaminase; ALT: alanin transaminase; HCV-RNA: hepatitis C virus-ribonucleic acid

The results of mean differences of CD4+ and CD8+ count in portal tracts and lobules along with its significant level are shown in Table 2; however the CD4+ and CD8+ counts, both in portal tracts and lobules, did not correlate with fibrosis or necroinflammatory grades (Table 3).

Table 2. Mean difference of CD4+ and CD8+ count in portal tracts and lobules

	Average (SD)	Δ mean (SE)	95% CI	p*
CD4+ count (cell/LPB amount)		11.1 (3.4)	4.3-17.9	0.002
Portal	31.41 (15.43)			
Lobulus	20.31 (10.42)			
CD8+ count (cell/LPB amount)		25.5 (5.0)	15.4-35.6	< 0.001
Portal	59.25 (23.60)			
Lobulus	33.72 (14.01)			

*unpaired T-test; SD: standard deviation; SE: standard error; LPB: leukocyte-poor blood

Table 3. Correlation between portal tracts and lobules (CD4+ and CD8+ count) for the grades of fibrosis and necroinflammation

	Fibrosis		Necroinflammatory	
	r	p*	r	p*
CD4+				
Portal	0.02	0.91	0.26	0.17
Lobulus	-0.12	0.53	0.16	0.41
CD8+				
Portal	-0.12	0.53	-0.05	0.80
Lobulus	-0.35	0.06	0.15	0.42

*Chi-square test

Multivariate analysis was not performed since there was only one variable with $p < 0.25$ for each variable depended on fibrosis and necroinflamation, i.e. the correlation between the lobule CD8+ count and fibrosis staging ($p = 0.06$) and portal CD4+ count and necroinflammatory grading ($p = 0.17$).

DISCUSSION

The study was a cross-sectional study conducted in 30 patients with chronic hepatitis C and most of them were female subjects, i.e. 17 people aged 24-69 years. Some studies have demonstrated that there is no significant difference on the prevalence of chronic hepatitis C between male and female subjects. This study found that predominant genotype was genotype 1 with most subtype of 1b, which is consistent with the data of genotype distribution in Indonesia.[7] HCV genotype has been frequently used as a significant epidemiologic marker of independent predictors to evaluate response to antiviral treatment. Worldwide studies have been conducted to retrieved data about hepatitis C patients with genotypes 1, 2, 3 and there are even special guidelines for determining the type of antiviral treatment and duration of treatment.[8]

The genotypes have been extensively studied to determine the persistence level of HCV infection. Amoroso et al have been specifically studied the role of HCV genotype on persistence level of HCV infection after initial exposure. They found that the evolution grade of becoming chronic infection after exposure of HCV was 92% when the subjects were exposed to genotype 1b, compared to 33-50% when they were exposed to other genotypes.[9] Such results are consistent with our study that found subtype 1b as the most frequently found subtype. In our study, we found that there were greater number of subjects with more severe necroinflammatory grading since there may be a correlation between genotype 1b and the more severe grade of liver damage; however, it has not been specifically analyzed in our study.

The study demonstrated significant mean differences ($p = 0.002$) between CD4+ and CD8+, either in the portal tracts or lobules (95% CI = 4.3-17.9; 95% CI = 15.4-35.6; $p < 0.001$). Canchis et al conducted a study on liver biopsies of 38 patients with chronic hepatitis C who had also had co-infection with HIV and 41 patients with monoinfection of chronic hepatitis C. They also evaluated CD4+ and CD8+ counts separately.[10] However, the study did not analyze statistically whether there was significant difference between CD4+ and CD8+ counts in the portal and in the lobules. They had lesser number of CD4+ and CD8+ counts than our results, which may be due to separation of proliferative apoptosis T lymphocytes from the T lymphocytes stained with antibody against CD4+.

A study conducted by Vrolijk et al in 17 subjects who had antiviral treatment of IFN-α2b (3 MU 3 x/ week) and ribavirin (1,000–1,200 mg/day) for 26 weeks, evaluated the CD8+ count in portal tracts and lobules before and after treatment. The study found that the mean value of CD8+ counts before treatment in portal tracts and lobules were 47 (16.04%) dan 18 (7.91%) with statistically significant mean difference between portal tracts and lobules ($p < 0.001$), i.e. 28.99 (95% CI = 19.4-38.6).[11] The results of their study are similar to our results.

This study showed that there was greater mean value of CD8+ than CD4+ count and both counts are greater in portal tracts compared to those in the lobules. It suggests that CD8+ count in the liver is overall greater than CD4+ count. Different results have been

found by Fiore et al who demonstrated that there was greater amount of CD4+ cells in portal and periportal area; while the CD8+ cells were predominantly found in lobules infiltrates. However, the study by Fiore et al also supported the hypothesis that the number of intrahepatic CD8+ is overall greater than CD4+.[12] The greater number of CD4+ and CD8+ in portal tracts may be associated with diffuse inflammatory manifestation in portal and expanded portal area, which has been a characteristic histopathologic manifestation in chronic hepatitis C.[13]

In chronic infection, the uninfected surrounding hepatocytes will secrete cytokines such as IFN-γ and will modulate local immune responses, which may cause chronic inflammation. Lymphoid neogenesis, a developmental process of lymphoid follicles which will take place in portal area, is a characteristic manifestation of HCV chronic infection, which also explains the migration of T cells that have not been exposed to antigens from peripheral to the liver. It explains why the majority of CD4+ and CD8+ cells were in portal area. T cells from portal area will also migrate to the lobules and undergo recirculation process in the liver to maintain liver immunity through various mechanisms of apoptosis and proliferation; however, the mechanism explaining recirculation process from parenchymal tissue to local portal area associated with lymphoid tissue and its drainage process has not been defined yet.[13]

Another study as a comparison to this study is the study conducted by Viso et al which included all samples of the mildest to the most severe grades; however, they used the Ishak score for staging and grading system, which is quite different from our study which utilized the METAVIR score.[14]

Statistically, only CD8+ count in the lobules that are likely to have weak negative correlation with liver fibrosis staging ($r = -0.35$; $p = 0.06$); while the CD4+ count in portal area was likely to have weak positive correlation with necroinflammatory grading ($r = 0.26$; $p = 0.17$). Overall, CD4+ and CD8+ were likely to have negative correlation with fibrosis staging and have positive correlation with necroinflammatory grading. Our study results are slightly different from the study conducted by Bonacini et al which demonstrated that CD8+ count had negative correlation with fibrosis score ($r = -0.65$), so did the CD4+ count in portal and periportal area.[15]

Hepatocytes, cholangiocytes and sinusoid endothelial cells, stellate cells and Kuppfer cells express the Fas (CD95/Apo-1), which will be activated when it binds to FasL on the membrane or the soluble FasL. FasL is expressed on T lymphocytes cell and natural killer (NK) cells. The expression of FasL and Fas receptors will increase in patients with chronic liver disease. In chronic hepatitis C, the infected hepatocytes will undergo apoptosis and apoptosis process will be increasing and leading to fibrosis. Simultaneously, the apoptosis process facilitated by Fas will also increase in accordance with the increased inflammatory grades. The expression of Fas will continuously increase on infected hepatocytes which will lead to specific-HCV T cells apoptosis, which also occurs through FasL expression. The theory can explain the overall tendency of negative correlation between CD4+ and CD8+ counts and fibrosis grades associated with the apoptosis process of T lymphocytes (CD4+ and CD8+), which also increased in response to increased FasL expression.[16]

In this study, CD4+ and CD8+ generally had positive correlation with necroinflammatory grading. It indicates that the greater amount of CD4+ and CD8+, the more severe necroinflammatory grade, which is directly associated with necroinflammation in the lobules. Such fact is consistent with the developing theory about the role of CD4+ and CD8+ on liver inflammation. CD4+ of T cells will be stimulated when recognizing viral peptide on class II HLA bound to APC, which subsequently will produce various cytokines i.e. IFN-γ,IL-2 and TNF-α which function as inflammatory mediators, activate CD8+ cells to produce IFN-γ and directly destroy the infected cells. Our study has also been consistent with other study reports that demonstrated direct correlation between CD8+ cells and the marker of hepatocellular damage (histological inflammatory activity and serum level of liver function test).[17]

A study conducted by Roger et al in 28 patients with chronic hepatitis C may explain the positive correlation between CD4+ count in portal area and necroinflammatory grading. They studied the number of CD4+ cells which has undergone apoptosis in the liver correlated to histopathological manifestation of chronic hepatitis C based on METAVIR score. The study indicated negative correlation between the number of CD4+ undergoing apoptosis and METAVIR score. The greater CD4+ count experiencing apoptosis, the lower score of METAVIR is (in regard of the necroinflammatory grade). Efforts of CD4+ undergoing apoptosis, which occurs through Fas signal transmission process is regarded as protective value to reduce liver damage. It may explain the small number

of CD4+ count in portal area which may occur since most of CD4+ experiencing apoptosis and it is also associated with lesser METAVIR score.[18]

Further studies are necessary to find other factors other than CD4+ and CD8+ that may be directly involved with necroinflammation and liver fibrosis. The results of our study showed that there is lesser number of lymphocytes in more severe fibrosis, particularly the CD4+, which can be assumed that it has not been confirmed that T lymphocytes causes fibrosis but rather likewise. However, we have not provided statistic evidences in this study.

The roles of immune system in chronic and acute hepatitis C are different. Several hypothetical mechanisms have tried to explain the cause of incapability of CD4+ and CD8+ to run their optimal function in eradicating the virus. The available IFN-based standard treatment combined with ribavirin is necessary since there are many other factors excluding CD4+ and CD8+ are components of cellular immunity accounts for the pathogenesis of chronic hepatitis C.

CONCLUSION

There are greater CD4+ and CD8+ counts in portal area than hepatic lobules and the study showed more significant number of CD8+ than CD4+ both in portal tracts and hepatic lobules. There was no correlation between CD4+ or CD8+ in portal and the severity of liver damage in chronic hepatitis C. Although CD4+ and CD8+ have important role in pathogenesis of hepatitis C, but CD8+ and CD4+ T cells counts themselves both in portal tracts or hepatic lobules can not be used as predictors for liver damage.

SUGGESTION

Various other factors involved in the pathogenesis of hepatitis C excluding the cellular immunity need further studies to evaluate the possibility of correlation with fibrosis and liver necroinflammatory grades.

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