POLYMORPHISM OF INTERLEUKIN-10 (-819) GENE IS NOT ASSOCIATED WITH LIVER DAMAGE AMONG CHRONIC HEPATITIS B PATIENTS

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ABSTRACT

Introduction: Chronic hepatitis B (CHB) is characterized by chronic liver inflammation and hepatocellular damage caused by hepatitis B virus (HBV), leading to fatal liver diseases such as cirrhosis and hepatocellular carcinoma (HCC). Liver damage causes elevated levels of serum alanine aminotransferase (ALT). Previous studies have shown that IL-10 gene polymorphism (-819 C/T) is linked to several diseases, including inflammatory, autoimmune diseases, and cancer.

Purpose: To investigate the relationship between IL-10 gene polymorphisms (-819 C/T) and liver damages in chronic hepatitis B patients at Arifin Achmad Regional Hospital, Riau Province.

Method: This study was an analytical study with a cross-sectional approach. Liver damages were examined by measuring ALT levels, and IL-10 gene polymorphisms were analyzed using Amplification-Refractory Mutation System Polymerase Chain Reaction (ARMS-PCR).

Results: There were 74 subjects included in this study, aged 45.47±13.42 years old, consisting of 43 males (58.1%) and 31 females (41.9%). Of 74 subjects, 35 CHB patients were presented without complication, 26 CHB patients were complicated with cirrhosis, and 13 CHB patients were complicated with hepatoma. The genotype of the IL-10 (-819) gene polymorphism in this study was found as follows: CT genotype in 34 subjects (45.9%), TT genotype in 30 subjects (40.5%), and CC genotype in 10 subjects (13.5%). There was no significant relationship between IL-10 gene polymorphisms (- 819) and liver damage in chronic hepatitis B patients ($p > 0.05$).

Conclusion: IL-10 gene polymorphism (-819) may not contribute to liver damages among CHB patients.

Keywords: chronic hepatitis B, liver damage, IL-10 gene polymorphisms (- 819)

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INTRODUCTION

Hepatitis B virus (HBV) is a hepatotropic virus that causes inflammation and damage to the liver. Patients with chronic hepatitis B (CHB) with persistent inflammation and liver damage are at increased risk of developing fatal liver diseases such as cirrhosis and hepatocellular carcinoma (HCC). HCC is the most common liver cancer and is one of the second-highest death rates in the world (Hepatitis B, 2017).

Chronic hepatitis B is a global health problem. The World Health Organization (WHO) estimates that approximately 3.5% of the world's population suffers from chronic hepatitis B and causes 887,000 deaths each year (Hepatitis B, 2017). The Asia Pacific region is the most significant contributor to hepatitis B and liver cancer burden worldwide, with 74% of global liver cancer deaths occurring in Asia (World Health Organization, 2019). Previous studies reported that 40-50% of hepatic cirrhosis patients in Indonesia were underlined with chronic hepatitis B infection (World Health Organization, 2019). In addition, the Global Cancer Observatory (GLOBOCAN) stated that in 2018 there were 18,468 new cases of liver cancer and 18,148 deaths due to liver cancer in Indonesia (World Health Organization, 2019).

Based on the 2013 Basic Health Research (RISKESDAS) quoted from Muljono (Muljono, 2017), Indonesia is classified as moderate endemicity for hepatitis B infection with a positive HBsAg prevalence of 7.1 (Muljono, 2017). The prevalence of positive HBsAg in Indonesia varies geographically, ranging from 4-20.3%, where the prevalence of hepatitis B patients outside Java Island is more significant than in Java island (Mulyanto et al., 2010).

Hepatitis B virus is a DNA virus of 42 nm in diameter with a double-layered protein coat belonging to the hepadnaviridae family. The outer sheath contains HBsAg, which can be detected in the circulation, while the inside consists of a nucleocapsid composed of HBcAg (Soemoharjo, 2008). HBV is a non-cytopathic virus, which means that the virus does not directly cause the liver damage in chronic hepatitis B patients but is due to the body's immune response to HBV infected hepatocytes (Soemoharjo, 2008).

The liver inflammation within chronic hepatitis B patients can cause liver damage as indicated by abnormal levels of liver enzymes, namely

aspartate aminotransferase (AST/SGOT) and alanine aminotransferase (ALT/SGPT). Hepatocellular injury releases ALT from the damaged liver cells leading to an increase in serum ALT levels. In acute hepatocellular injury, usually, AST levels will increase faster, even higher than ALT levels, because AST activity is higher in hepatocytes. However, in the next 24 to 48 hours, ALT levels will be higher than AST. Chronic hepatitis B infection is the most common cause of elevated ALT levels (Kim et al., 2008).

Interleukin-10 (IL-10) is an anti-inflammatory cytokine that plays an essential role in regulating inflammatory responses to prevent excessive liver cell damage and maintain organ function. Polymorphisms within the promoter of the IL-10 gene have been widely reported, and although they do not cause changes in protein structure, they are known to affect the expression of IL-10 proteins. These include single nucleotide polymorphism (SNP) at positions -592 (C/A), -819 C/T, and -1082 A/G) (Gao et al., 2016). A previous study showed that the IL-10 gene polymorphism (-819 C/T) was frequently found in chronic hepatitis B patients with liver cirrhosis (Baghi et al., 2015). Another study has demonstrated that IL-10 gene polymorphisms were associated with the risk of periodontitis (Distribution and Periodontitis, 2016), some enterovirus infections (Zhao et al., 2017), and humane immune deficiency (HIV) (Sobti et al., 2010). Given the vital role of the immune response on the pathogenesis of chronic hepatitis B, this study aimed to analyze the association between IL-10 gene polymorphisms (-819 C/T) and liver damage in chronic hepatitis B patients.

METHOD

This study was an analytical study with a cross-sectional approach to seeking the association between IL-10 gene polymorphisms (-819 C/T) and liver damage in chronic hepatitis B patients. Study subjects were recruited from March to October 2019 at the Internal Medicine Outpatient Clinic at the Arifin Achmad Regional Hospital, Riau Province. The inclusion criteria were chronic hepatitis B patients aged 18 years old and agreed to participate in this study. Serum ALT levels were examined at one of the clinical laboratories in Pekanbaru. IL-10 gene polymorphisms (-819 C/T) were characterized at LONTAR Integrated Biomedical Laboratory, Faculty of

Medicine, Riau University, between December 2020 and March 2021.

Genomic DNA was isolated from the blood fraction of EDTA-treated blood that was stored at -80oC until further procedures. DNA isolation was carried out using the Wizard Genomic DNA Purification kit (Promega, Madison, WI, USA). Genomic DNA was stored at -20oC until the next process.

IL-10 gene (-819) polymorphism was carried out using the Amplification Refractory Mutation System - Polymerase Chain Reaction (ARMS-PCR) method (Gao et al., 2016), which uses specific primers to detect polymorphism alleles. The primary sequences were adopted from Perrey et al. (Perrey et al., 1999). PCR conditions consisted of initial denaturation at 95^{o}C for 1 minute, followed by ten cycles of denaturation at 94oC for 15 seconds; annealing at 59^{o}C for 50 seconds, extension at 72oC for 50 seconds, and final extension at 72^{o}C for 7 minutes. PCR products were run on 2% agarose stained with GelâRed (Biotium, USA). Electrophoresis results were visualized using GelDoc (Bio-Rad, USA) (Al-Mohaya et al., 2015).

Univariate analysis was carried out to describe categorical data presented in proportions, while numerical data (age) was presented as mean±SD. The relationship between IL-10 gene polymorphism (-819) and liver damage in chronic hepatitis B patients was analyzed using the Pearson Chi-square test, and p-value <0.05 was deemed significant.

RESULTS AND DISCUSSION

This study analyzed 74 CHB patients at Arifin Achmad Regional Hospital, Riau Province, recruited from March to October 2019. The characteristic of study subjects can be seen in Table 1.

Table 1 Profiles of Chronic Hepatitis B Patients by Age, Sex and Diagnosis

Variable	CHB without complications n = 35	CHB with Cirrhosis n = 26	CHB with Hepatoma n = 13
Age (Mean±SD, year)	38.17±13.17	51.19±9.22	53.69±11.29
Gender			
Male (n /%)	14 / 40.0	20 / 76.9	9 / 69.2
Female (n /%)	21 / 60.0	6 / 23.1	4 / 30.8

Based on Table 1, the mean age of CHB patients without complications in this study was 38.17±13.17 years. This result is in line with Bahgi et al. study conducted on the Iranian population, which found that the average age of chronic hepatitis B patients was 38.5.±13.7 years (Baghi et al., 2015). Different results were reported by Talaat et al. in the Egyptian population, where the mean age of CHB patients was 42.66 ± 12.59 years (Talaat et al., 2014) and Zhang et al. in the Indian population, with a mean age of 44.2 years (Zhang et al., 2014). Differences in the mean age of CHB patients can be due to differences in the study population where the patients may have different disease progression and complications caused by chronic hepatitis B. This age difference can also be due to differences in the mode of hepatitis B transmission between regions. Hepatitis B infection is mainly transmitted from mother to baby during the perinatal period in Indonesia (vertical transmission). Primary HBV infection in neonates has a 95% risk for developing chronic hepatitis B. This may lead to a younger population of chronic hepatitis B patients. The mean age of chronic hepatitis B patients with cirrhosis in this study was 51.19±9.22 years. This result is similar to a study by Arfianti et al. 2011 conducted in Pekanbaru, showing chronic hepatitis B patients aged 52.9 ± 11.3 years (Arfianti et al., 2011). Likewise, the mean age of chronic hepatitis B patients with hepatoma was 53.69±11.29 years. Similar findings were also reported in a study in Pekanbaru with an average age of 48.2 ± 8.7 years (Arfianti et al., 2011).

In this study, CHB patients with cirrhosis and hepatoma were mostly males, with the proportion of 76.9% and 70%, respectively. On the other hand, CHB patients without complication are predominantly females (60%), slightly higher than males (40%). The difference in the risk of complications of cirrhosis and hepatoma in CHB patients has been reported in various literature. This is in line with previous studies, which stated that in areas with vertical HBV transmission patterns such as Indonesia, males with chronic hepatitis B had a 50% greater risk of developing hepatoma progression than females (Ince et al., 1999). The mechanisms underlying the gender disparity in the risk of hepatoma are still not fully understood. Several studies suggest that testosterone increases the risk of developing hepatoma while progesterone is protective against the

development of hepatoma (EL-Serag et al., 1999).

Table 2 Profiles of Liver Damage in Chronic Hepatitis B patients

ALT levels	n	%
Normal	56	71.8
Elevated	10	12.8
No data	8	10.3

The profiles of liver damage in CHB patients based on ALT levels are presented in Table 2. Most chronic hepatitis B patients demonstrated normal levels of serum ALT (71.8%), only 12.8% of them having elevated ALT levels (>2x upper limit of normal). The remaining 10.3% of chronic hepatitis B patients did not have any ALT values in their medical records. The normal levels of serum ALT in this study indicate no liver damage associated with the risk of cirrhosis and hepatoma. Similar results were reported in the study of Trautwein et al. (Trautwein et al., 2015). Among 120 chronic hepatitis B patients in the United Kingdom. This study found no significant relationship between chronic hepatitis B and liver damage (Samant, 2016). However, one study reported that chronic hepatitis B patients presented with ALT levels 20 times higher than normal values (Thoni et al., 2017). This difference in ALT levels among chronic hepatitis B patients could be due to differences in the disease progression and complications in the study population. Chronic hepatitis B patients presenting with acute exacerbations tend to have higher ALT levels than those who do not (Liang, 2009).

IL-10 gene electrophoresis showing genotype of IL-10 polymorphism (-819 C/T) in chronic hepatitis B patients is presented in Figure 1.

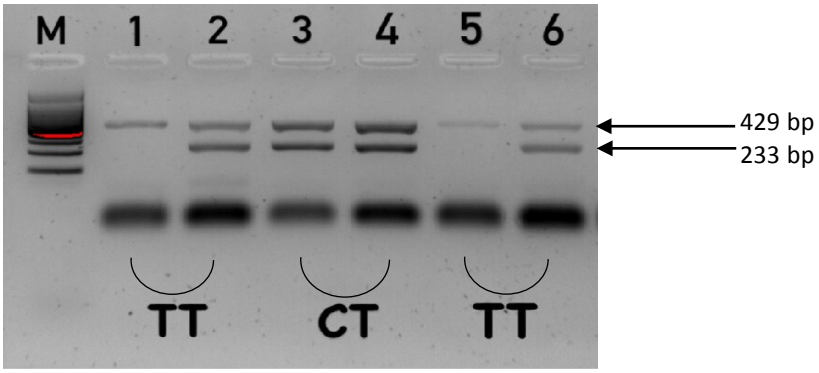

Figure 1. Gel Electrophoresis of IL-10 (819 C / T) gene fragment. M= DNA Marker. Row 1,3,5= C allele. Row 2,4,6= T. 429 bp fragment =Internal Control. 233 bp fragment: IL-10 gene.

Genotype distribution of IL-10 gene polymorphisms (819 C/T) in CHB patients is presented in Table 3. Based on Table 3, 45.9% of CHB patients had CT genotype, 40.5% TT genotype, and 13.5% CC genotype. Comparing the genotype distribution of the IL-10 gene polymorphism (-819 C/T) in this study with previous studies can be seen in Table 4.

Table 3 Genotype Distribution of IL-10 (-819) Gene Polymorphisms in Chronic Hepatitis B Patients

Genotype IL-10 gene polymorphism (-819 C/T)	n	%
CC	10	13.5
CT	34	45.9
TT	30	40.5

Table 4 Comparison of Genotype Distribution of IL-10 Gene Polymorphisms (-819) between Different Population

Research	Population	Genotype		
		CC	CT	TT
Aina *et al*	Indonesia	13.5 %	**45.9%**	40.5 %
Baghi *et al* (Baghi *et al*., 2015)	Iran	11.1 %	**88.9%**	-
Talaat *et al (Talaat et al*., 2014)	Egypt	**60.0%**	34.8%	5.2%
Sofian *et al* (Sofian *et*	Iran	**46.9**	6.2%	**46.9**

al., 2013)		%		%
Srivastava *et al* (Srivastava *et al*., 2014)	India	29.4 %	**47.8%**	22.8 %
Liu *et al* (Liu Q *et al*., 2007)	China	8.7%	**47.7%**	43.6 %

The relationship of IL-10 gene polymorphism (-819) with liver damage based on ALT levels in CHB patients can be seen in Table 5.

Table 5 Relationship between IL-10 (-819) Gene Polymorphisms and Liver Damage in Chronic Hepatitis B Patients.

Genotype of the IL-10 Gene Polymorphism (-819)	ALT levels				*P value*
	Elevated		Normal		
	n	%	n	%	
CC	1	10.0	9	90.0	
CT	5	14.7	29	85.3	0.929
TT	4	13.3	26	86.7	

Based on Table 5, most CHB patients had ALT levels within normal limits regardless of the IL-10 (-819) genotype. The statistical test showed that there was no significant relationship between the genotype distribution of the IL-10 gene polymorphism (-819C/T) with liver damage in chronic hepatitis B patients ($p > 0.05$). Research conducted by Saxena et al. reported that IL-10 (-819) gene polymorphism alleles have a linkage with the IL-10 (-592) gene polymorphism and the haplotype of these two polymorphisms has a significant relationship with the progression or risk of developing hepatoma in chronic hepatitis B patients (Saxena et al., 2014). Another study by Baghi et al. reported that the IL-10 (-819) and (-592) gene haplotypes were associated with the risk of cirrhosis in patients with chronic hepatitis B. Although this study also did not find any association between IL-10 gene polymorphisms (-819) and the degree of liver damage in chronic hepatitis B patients (Baghi et al., 2015),(Talaat et al., 2014). In contrast, a study conducted by Ren et al. found a significant relationship between the IL-10 gene polymorphism (-819) with the degree of liver damage and its progression in the Asian

population (Ren, Zhang, and Hu, 2015).

This study did not find a significant relationship between the IL-10 gene polymorphism (-819) and liver damage, possibly because the sample size was relatively small, leading to limiting study power to find significant differences. Most of the previous studies included > 200 study subjects. In addition, several factors that are known to affect liver damage in chronic hepatitis B sufferers in this study were not controlled, such as genetic variants of HBV, host genetic susceptibility (HLA genes), comorbid diseases, use of other drugs, age or ethnic background.

Acknowledgement

The author would like to thank the management of Arifin Achmad Regional Hospital, which has facilitated the study. This study was funded by a Research Grant provided by the Faculty of Medicine, Universitas Riau (Grant no B/28/UN19.5.1.1.8/PT.01.05/UPPM/2020).

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