Int J Clin Exp Pathol 2015;8(9):11680-11684
www.ijcep.com /ISSN:1936-2625/IJCEP0011439

Original Article

Association between interleukin-10 gene promoter polymorphisms and susceptibility to liver cirrhosis

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Received June 14, 2015; Accepted July 26, 2015; Epub September 1, 2015; Published September 15, 2015

Abstract: We conducted a case-control study to investigate the association between three common SNPs in *IL-10* gene (rs1800896, rs1800871 and rs1800872) and the development of liver cirrhosis in a Chinese population. Between January 2013 and December 2014, a total of 318 patients with liver cirrhosis and 318 health control subjects were enrolled into our study. The *IL-10* rs1800896, rs1800871 and rs1800872 polymorphisms were analyzed using polymerase chain reaction (PCR) coupled with restriction fragment length polymorphism (RFLP). By multivariate logistic regression analysis, we found that individuals with the AA genotype and GA+AA genotype of *IL-10* rs1800896 were more likely to have an increased risk of liver cirrhosis when compared with the GG genotype, and the ORs (95% CI) for the AA genotype and GA+AA genotype were 2.04 (1.20-3.50) and 1.41 (1.02-1.96), respectively. We found that the GA+AA genotype of *IL-10* rs1800896 had higher risk of liver cirrhosis in individuals with chronic hepatitis B when compared with the GG genotype (OR = 1.95, 95% CI = 1.01-3.59). In conclusion, we found that *IL-10* rs1800896 polymorphism was correlated with an increased risk of liver cirrhosis, especially in individuals with chronic hepatitis B.

Keywords: *Interleukin-10*, polymorphism, liver cirrhosis

Introduction

Liver cirrhosis is a severe public health problem worldwide, which is correlated with higher morbidity and mortality worldwide [1-3]. It is reported that about 2 billion population are infected with HBV, and 3/4 of them are in Asia-Pacific region [4]. It is well known that long-term infection with HBV is associated with the development of liver cirrhosis, and other lifestyle factors may influence the susceptibility to liver cirrhosis, such as long-term heavy alcohol drinking [5, 6]. However, not all of the individuals who exposed to long-term infection with HBV and heavy alcohol drinking would suffer from liver cirrhosis, which suggests that molecular and cellular may contribute to the development of liver cirrhosis.

It is reported that cytokines play a fundamental role in the immunopathogenesis of HBV related diseases [7-9]. Previous studies have reported that cytokines gene polymorphisms, such as *Interleukin-1β (IL-1β)*, *IL-28B*, *IL-17* and *IL-35*, are associated with the development of HBV-related liver cirrhosis [10-12]. *Interleukin-10* (*IL-10*) is an immunoregulatory cytokine, which is produced by Th2 cells, regulatory T cells, and monocytes/macrophages. The encoding gene of *IL-10* is located on chromosome 1 (1q31-1q32). *IL-10* is an anti-inflammatory cytokine, which could inhibit the synthesis of cytokines such as *IL-6*, *IL-1β*, *IL-1α* and *TNF-α* in activated macrophage and IFNγ by T cells [13]. Few studies investigated the role of *IL-10* gene polymorphisms in the susceptibility to liver cirrhosis, and the results were inconsistent [14-17]. Therefore, we conducted a case-control study to investigate the association between three common SNPs in *IL-10* gene (rs1800896, rs1800871 and rs1800872) and the development of liver cirrhosis in a Chinese population.

Materials and methods

Study subjects

A case-control study was conducted in our study. Between January 2013 and December 2014, a total of 352 patients with liver cirrhosis

Table 1. Characteristics of included patients with liver cirrhosis and controls

Variables	Patients	%	Controls	%	t or χ^2	P value
Age, years						
< 55	150	47.17	157	49.37		
≥ 55	168	52.83	161	50.63	0.31	0.58
Sex						
Females	100	31.45	100	31.45		
Males	218	68.55	218	68.55	0.00	1.00
Chronic hepatitis B						
No	148	46.54	291	91.51		
Yes	170	53.46	27	8.49	118.71	< 0.001
Chronic hepatitis C						
No	293	92.14	315	99.06		
Yes	25	7.86	3	0.94	18.09	< 0.001
Alcohol status						
No	132	41.51	253	79.56		
Yes	186	58.49	65	20.44	96.36	< 0.001
Child-Pugh score						
A	163	51.26				
B	128	40.25				
C	27	8.49				

were enrolled into our study. Liver cirrhosis was diagnosed either by histopathologically diagnosis, ultrasound, computer tomography (CT) or magnetic resonance imaging (MRI). Patients who had organ transplantation were excluded from this study. Finally, 318 patients were enrolled in our study, and the participation rate was 90.35%.

For the frequency-matched controls on sex and age, 318 health control subjects were randomly selected from individuals who came to our hospital for health check-up. Controls that had a history of liver cirrhosis and other HBV-related diseases were excluded from our study. All patients with liver cirrhosis and control subjects signed written informed consents. The signed written informed consents were obtained from patients with liver cirrhosis and control subjects. Our study was approved by the ethics committee of our hospital.

Genotyping assays

Each subject was asked to provide 5ml peripheral blood samples, and the blood samples were kept in -80°C before DNA extraction. Genomic DNA was selected from 5 mL peripheral blood of each sample, using the TIANamp Blood DNA Kit (Tiangen Biotech, Beijing, China). Therefore, the DNA was stored at -80°C for following genotype analysis. DNA was extracted from peripheral blood samples 100 collected from patients and controls using the TIANamp Blood DNA Kit (Tiangen, Beijing, China), according to the manufacturer's instructions. After extraction, genomic DNA was diluted to a final concentration of 15-20 ng/μl for the genotyping assays. The *IL-10* rs1800896, rs1800871 and rs1800872 polymorphisms were analyzed using polymerase chain reaction (PCR) coupled with restriction fragment length polymorphism (RFLP). Primers for amplification and extension reactions were designed using Sequenom Assay Design 3.1 software (Sequenom, San Diego, CA, USA). The Primers for *IL-10* rs1800896, rs1800871 and rs1800872 were as follows: for *IL-10* rs1800896, 5'-CTACTAAGGCTTCTTTGGGAG-3' (forward) and 5'-ACTACTAAGGCTTCTTTGGGAA-3' (reverse); for rs1800871, 5'-TCATTCTATGTGCTGGAGATGG-3' (forward) and 5'-TGGGGGAAGTGGGTAAGAGT-3' (reverse); for rs1800872, 5'-GGTGAGCACTACCTGACTAGC-3' (forward) and 5'-CCTAGGTCACAGTGACGTGG-3' (reverse). The restriction enzymes for *IL-10* rs1800896, rs1800871 and rs1800872 were *Bse*RI, *Ms*II and *Rsa*I, respectively. The amplification conditions were 95°C for 5 min, then 30 cycles of 94°C for 0.5 min, 60°C for 0.5 min and 72°C for 1 min, at last 72°C for 10 min. Digestion products were separated by electrophoresis on ethidium bromide stained agarose gel and visualized under UV light.

Statistical methods

The statistical difference between cases and controls was analyzed by A Chi-squared test and t test. The Hardy-Weinberg equilibrium (HWE) was tested by Fisher's exact test for each SNP in controls. The odds ratios (ORs) and 95% confidence intervals (CIs) were calculated using logistic regression models adjusted for

Table 2. Association between IL-10 rs1800896, rs1800871 and rs1800872 polymorphisms and risk of liver cirrhosis

SNPs	Patients	%	Controls	%	HWE	OR (95% CI)	*P* value
rs1800896							
GG	125	39.31	152	47.80	0.90	1.0 (Ref.)	-
GA	141	44.34	135	42.45		1.27 (0.90-1.80)	0.16
AA	52	16.35	31	9.75		2.04 (1.20-3.50)	0.005
GA+AA	193	60.69	166	52.20		1.41 (1.02-1.96)	0.03
rs1800871							
TT	128	40.25	139	43.71	0.23	1.0 (Ref.)	-
CT	139	43.71	135	42.45		1.12 (0.79-1.59)	0.52
CC	51	16.04	44	13.84		1.26 (0.77-2.07)	0.34
CT+CC	190	59.75	179	56.29		1.15 (0.83-1.60)	0.38
rs1800872							
AA	110	34.59	122	38.36	0.65	1.0 (Ref.)	-
AC	159	40.25	153	48.11		1.15 (0.81-1.64)	0.41
CC	49	25.16	43	13.52		1.26 (0.76-2.11)	0.34
AC+CC	208	65.41	196	61.64		1.18 (0.84-1.65)	0.32

confounding factors for genotype analysis. Additional regression models were designed where subjects were stratified on drinking status, chronic hepatitis B status and genetic common type and polymorphism carriers of the studied variations. A *P*-value of less than 0.05 was considered to be statistically significant. All statistical analyses were analyzed using the SPSS statistical package software, version 17.0 (SPSS Inc, Chicago, IL, USA).

Results

Population characteristics

The demographic and clinical characteristics of patients with liver cirrhosis and controls were shown in **Table 1**. There were no significant differences between patients and controls in terms of age and sex ($P > 0.05$). By Chi-squared test, we found that patients with liver cirrhosis were more likely to be infected with chronic hepatitis B and chronic hepatitis C, and have a habit of alcohol drinking. Of 318 patients, 163 (51.26%) were at A of Child-Pugh score, 128 (40.25%) were at B and 27 (8.49%) were at C.

The genotype distributions of *IL-10* rs1800896, rs1800871 and rs1800872 were in line with HWE, and the *P* values for HWE were 0.90, 0.23 and 0.65, respectively (**Table 2**). The genotype distribution of *IL-10* rs1800896 showed significant difference between patients with liver cirrhosis and controls ($\chi^2 = 8.08$, *P* value = 0.02). By multivariate logistic regression analysis, we found that individuals with the AA genotype and GA+AA genotype of *IL-10* rs1800896 were more likely to have an increased risk of liver cirrhosis when compared with the GG genotype, and the ORs (95% CI) for the AA genotype and GA+AA genotype were 2.04 (1.20-3.50) and 1.41 (1.02-1.96), respectively. Moreover, there was no significant association between *IL-10* rs1800871 and rs1800872 polymorphisms and development of liver cirrhosis.

We conducted analysis on the association between *IL-10* rs1800896 polymorphism and risk of liver cirrhosis stratified by drinking and chronic hepatitis B status (**Table 3**). We found that the GA+AA genotype of *IL-10* rs1800896 had higher risk of liver cirrhosis in individuals with chronic hepatitis B when compared with the GG genotype (OR = 1.95, 95% CI = 1.01-3.59). However, no significant interaction was found between *IL-10* rs1800896 polymorphism and alcohol drinking in the risk of liver cirrhosis.

Discussion

Genetic susceptibility to cancers has obtained a growing attention to investigate the gene polymorphisms in the development of several diseases. Inflammation and related cytokines play an important role in activating stellate cells and causing liver fibrosis [17], and the inflammatory responses of immune cells and following cytokine expression in the liver could contribute to the development of liver cirrhosis. In our study, we found that *IL-10* rs1800896 polymorphism was correlated with an increased risk of liver cirrhosis, and had interaction with chronic hepatitis B in the pathogenesis of liver cirrhosis.

Table 3. Association between IL-10 rs1800896 polymorphism and risk of liver cirrhosis stratified by drinking and chronic hepatitis B status

Variables	GG		GA+AA		OR (95% CI)	*P* value
	Patients	Controls	Patients	Controls		
Chronic hepatitis B	69	137	79	154	1.02 (0.67-1.55)	0.93
No	56	15	114	12	2.54 (1.03-6.36)	0.02
Yes						
Alcohol drinking						
No	52	118	80	135	1.34 (0.86-2.11)	0.17
Yes	73	34	113	31	1.70 (0.92-3.12)	0.07

Previous studies have reported that *IL-10* gene polymorphisms are associated with the development of hepatocellular carcinoma, and could influence the long-term infection of chronic hepatitis B and C [18-21]. Gao et al. assessed the relationship between the polymorphisms of *IL-10* gene at position rs1800896 and rs1800872 and long-term infection of HBV and/or HCV, and they reported that *IL-10* rs1800896 polymorphism could influence the chronic infection of HBV and HCV replication [18]. Gong et al. conducted a study in a Chinese population, and they indicated that *IL-10*-producing regulatory B cells were associated with the development of impaired anti-HBV immunity and the pathogenesis of chronic hepatitis B [19]. Ren et al. conducted a meta-analysis with 24 studies, and they reported that *IL-10* rs1800872 was associated with an increased susceptibility to HBV infection, but *IL-10* rs1800896 and rs1800871 were not [20]. Saxena et al. reported that *IL-10* rs1800871 and rs1800872 gene polymorphisms were associated with the progression of HBV infection related disease, and they could promote the inactive carrier state to malignancy in an Indian population [21]. In our study, we found that *IL-10* rs1800896 polymorphism had interaction with chronic hepatitis B in the pathogenesis of liver cirrhosis, which is in line with previous studies.

In our study, we found that *IL-10* rs1800896 polymorphism was correlated with an increased risk of liver cirrhosis. Several studies reported their association and the results are inconsistent [14, 16, 17, 20]. Yang et al. conducted a study in a Taiwanese population, and they reported that the *IL-10* promoter haplotype is correlated with alcoholic liver cirrhosis [17]. Jin et al. suggested that *IL-10* rs1800872 gene polymorphism would promote the risk for liver cirrhosis, and this gene has interaction with chronic hepatitis B infection in the development of liver cirrhosis [16]. Liu et al. reported the association between *IL-10* rs1800896, rs1800871 and rs1800872 polymorphisms and risk of liver cirrhosis, and they found that *IL-10* rs1800896 polymorphism slightly enhanced the risk of liver cirrhosis [14]. The discrepancies of the above mentioned results could be explained by differences in ethnicities, study design and disease status as well as sample size.

There were several limitations in our study. First, selection bias may exist in our study due to the hospital-based subjects in our study, but the matched on age and sex could reduce the bias in this study. Second, the sample size of our study is relatively small, which may limit the statistical power to find difference between groups. Therefore, further large-scale studies in different ethnic groups are greatly needed to confirm our results.

In conclusion, we found that *IL-10* rs1800896 polymorphism was correlated with an increased risk of liver cirrhosis; especially in individuals with chronic hepatitis B. Future studies with larger sample size may contribute to elucidate the impact of *IL-10* polymorphisms on the risk of liver cirrhosis.

Disclosure of conflict of interest

None.

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