Associations between P53, Transforming Growth Factor Beta-1, and Interleukin-10 Serum Levels with Advanced Liver Disease and Hepatitis B Virus Infection

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Abstract

Background. Hepatitis B infection can lead to advanced liver disease (ALD) which causes serious health problems. Several factors that are thought to play a role in advanced liver disease are P53, Transforming Growth Factor-β1 (TGF-β1), and Interleukin-10 (IL-10). Therefore, this study aimed to determine the association between P53, TGF-β1, and IL-10 serum levels with ALD.

Methods. We collected 68 sera from patients with HBV infection. P53, TGF-β1, and IL-10 serum levels were measured by ELISA. We also detected SNPs from P53 and TGF-b1 genes to determines their associations.

Results and Conclusions: In this study, we obtained 41.18% CH patients and 58.82% ALD patients. Male patients outnumbered female in all groups. There was a significant relationship between serum P53 and TGF-b1 levels with ALD (p = 0.03 and 0.01, respectively), but not serum IL-10 levels. However, there was no significant relationship between SNP gene P53 and serum P53 levels nor between SNP gene TGF-b1 and serum TGF-b1 levels (p = 0.73 and 0.23, respectively).Both P53 and TGF-b1 serum leveles can act as biomarkers of prognosis and target therapy for ALD patients.

Keywords: *P53,Transforming Growth Factor Beta-1, interleukin-10, advanced liver disease, Hepatitis B Virus infection*

Introduction

Hepatitis B Virus (HBV) infectionisglobalhealth problem, especially in developingcountries[1,2]. Untreated early HBV infection can lead to persistent chronic infection with manifestations range from asymptomatic to cirrhosis, decompensated liver disease, and hepatocellular carcinoma (HCC) in up to 40% of patients[3,4]. Annually, 3% patients with chronic hepatitis B develop liver cirrhosis and 5% develop decompensated liver disease. The main therapy for end-stage liver disease is liver transplantation, however, this treatment modality cannot be accessed by the majority of patients [4]. Liver cirrhosis and HCC are advanced liver disease (ALD) conditions with complex molecular pathogenesis, involving the interactions of various cells and cytokines[5,6]. Several factors that are thought to play a role in advanced liver disease are Transforming Growth Factor-β1 (TGF-β1), P53, and Interleukin-10 (IL-10).

TGF-b1 is a cytokine that can trigger apoptosis and control cell growth, but on the other hand, this cytokine can cause the transition of Hepatic Stellate Cell (HSC) to myofibroblasts which stimulate extracellular matrix synthesis and inhibit its degradation[7,8]. Chronic

inflammatory conditions cause hepatocytes to become resistant to the anti-tumor activity of TGF-β1 and a change in the signaling pathway converts TGF-β1 to be pro-fibrotic and pro-carcinogenic [9,10]. Changes in TGF-β1 levelsplayanimportantrole in the pathogenesis of various diseases, includingliver disease[11]. TGF-β1 isalsosuggested to be a morespecificbiomarkerthan Alpha Feto Protein (AFP) forHCC[12].

P53 also plays an important role in the pathogenes is of ALD, as the gene can be defective and mutated in HBV infection[13,14]. This protein acts as a regulator whichsuppresses the cellcycleandinducedapoptosis, thus preventingcarcinogenesis, including liver cancer. As P53 protein has an important role in theprogressionofALD, its expressioncanbeusedto monitor andidentifyALDatanearlystage.[13,15,16].

Interleukin-10 (IL-10) is a cytokine produced by macrophages and dendriticcellsto inhibit the activation of thesetwocells. Experimental data from animal models and clinical data from patients suggest that IL-10 is involved in the development of liver injury[17]. EventhoughIL-10inhibits the production ofvariousinflammatorycytokines (Th1 cytokines), including IL-1, TNF, and IL-12[18], it suppresses immune system against HBV infection(rolisaxena). IL-10 inhibits T cell activity, either directly or indirectly. The direct effect of Il-10 inhibition on T cells is by inhibiting their proliferation, functional differentiation, and activity, while the indirect inhibition is by reducing the molecular expression of presenting cell antigens, thus interfering with T cell maturation[19].

To date, data regardingtherelationshipbetween TGF-β1, P53, and IL-10 levels and the incidence of CLD in patients with chronic HBV infection, especially in the Indonesian population, are stilllimited. Severalother studies alsolinkedtheSingle NucleotidePolymorphism (SNP)s of TGF-b1 and P53 genes with the irexpression, even though this yield conflicting results[20–23]. Therefore, this study aimedtoanalyzetherelationshipbetween serum TGF-β1, P53, and IL-10 levelsand HBV-relatedadvancedliverdisease in the Indonesian population.

Materials and Methods

Sampling

This was a cross-sectional study that used 68stored blood samples from chronically infected HBVpatients. Samples were taken at Inpatient/Outpatient Clinics of Internal Medicine Department, Dr.SoetomoGeneral Hospital, Surabaya, Indonesia in 2017 and stored in -80°C freezer at Hepatitis laboratory, Institute of Tropical Disease, Surabaya, Indonesia. Inclusion criteriawere: Adult patients with chronic HBV infection (HBsAg was positive for more than 6 months), clinicallystable, and never had Hepatitis B vaccination. Exclusion criteria were: HCV and HIV coinfection, diabetes mellitus/ autoimmune diseases. Patients were categorized into chronic hepatitis without ALD if there were no sign of liver cirrhosis or hepatocellular carcinoma through physical, laboratory, and radiologic examination, while they were categorized into ALD if liver cirrhosis or HCC was found.

This study hadreceived approval from the Ethical Committee of Faculty of Medicine, Universitas Airlangga, Surabaya (Ethical clearance No. 186/EC/ KEPK/FKUA/2020). Informed consent was explained andvoluntarily signed by participants beforehands, in accordance with the declaration of Helsinki.

Measurement of TGF-b1, P53, and Il-10 serum levels.TGF-b1serum level was measured by: Human TGF-b1 ELISA Kit with Catalog No: E-EL-H0110 (Elabscience Biotechnology Inc, USA) according to the manufacturer's instructions. P53 serum level was measured by: Human TP53 (Tumor Protein p53) ELISA Kitwith Catalog No: E-EL-H0910 (Elabscience Biotechnology Inc, USA). IL-10 serum level was measured by: Human TGF-b1 ELISA Kit with Catalog No: E-EL-H0103 (Elabscience Biotechnology Inc, USA). Optical Density for TGF-b1, P53, and IL-10 were measured by Microplate Reader: iMark (Biorad) S/N 12908.

Host DNA Extraction

Host DNA was extracted using QIAamp DNA Extraction kit (Qiagen, Germany) Cat.No.51104 according to the manufacturer's instructions.

PCR-RFLPofTGF-β1 and p53 Genes

PCR was carried out as our previous work [24]. TGF-β1 gene SNP was checked at locus -509, whileP53

gene SNP was checked at codon 72.PCR productsof TGF-β1-509 SNP were incubated using DdeI, while p53 SNPusedBstUI.

TGF-β1 -509 SNP showed 120 bp (T allele), while wild type (C allele) showed 74 and 46 bp fragments. p53 codon 72 SNP showed showed 231 and 165 bp fragments(G allele), while wild type showed 396 bp (C allele).

Statistical Analysis

Associations between TGF-b1, P53, and IL-10 with ALDwere analyzed for each parameter by Mann-Whitney test, while the associations between SNPs of TGF-b1 and P53 with their expression were analyzed by Kruskal Wallis test.A P-value < 0.05 following a two-tailed analysis was considered to be statistically significant. All statistical analyses were performed using SPSS version 23.

Results

Characteristics of the participants

This study involved 68 participants with HBV infection, consisting of 41.18% CH patients and 58.82% ALD patients. Data summary regarding gender, mean ± SD age and age range of theparticipants can be seen in Table 1. The youngest patients with HBV infection in this study were 16 years old and the oldest was 72 years old, with a mean age of 47 years. There were more males (79.41%) than females (20.59%). The age range of male patients in this study was not much different from that of women, with the mean value being almost the same.

Table 1. Characteristics of the participants

Characteristics	CH (n=28)	ALD (n=40)	Total (n=68)	P-value
Gender Female Male	 7 21	 7 33	 14 54	0.55
Age, years	44.36±16.31	49.23±9.52	47.22±12.87	0.16
P53 SNP Genotype CC CG GG Allele C G	 9 15 4 33 23	 8 22 10 38 42	 17 37 14 71 65	0.17 0.28
TGF-b1 SNP Genotype CC CT TT	 8 14 6	 8 18 14	 16 32 20	0.44
Allele C T	 30 26	 34 46	 64 72	0.22
P53 serum level (pg/mL)	114.99±164.73	244.75±374.62	191.32±311.07	0.03*
TGF-b1 serum level (ng/mL)	0.16±0.39	0.29±0.27	0.21±0.35	0.01*
IL-10 serum level (pg/mL)	9.38±9.14	14.34±34	12.3±26.69	0.76

* statistically significant

In both CH and ALD, more patients were male than female, although there was no significant difference in gender distribution in the CH and ALD groups. From statistical analysis, there was no significant difference between gender and age with ALD. For both SNPs, heterozygote genotype were predominant in either CH or ALD. SNP analysis showed that there was no difference in the distribution of genotypes or SNP alleles of TGF-β1 and p53 between CH and ALD. Serum P53, TGF-b1, and IL-10 levels in ALD patients were higher than those of CH patients. There was a significant relationship between serum P53 and TGF-b1 levels with ALD, but not serum IL-10 levels. However, there was no significant relationship between SNP gene P53 and serum P53 levels nor between SNP gene TGF-b1 and serum TGF-b1 levels p = 0.73 and 0.23, respectively).

Discussion

In this study, 68 samples of patients with HBV infection were obtained, consisting of CH and ALD. The number of male patientswere more than women. This is in accordance with several previous studies which stated that there were more people with cirrhosis of the liver with HBV infection in men than in women[25–27]. This is due to the protective role of estrogen for the liver. Estrogen can inhibit stellate cell proliferation and fibrogenesis which plays an important role in the course of cirrhosis. In experimental animal models with liver cirrhosis, administration of estradiol causes a decrease in collagen types I and III and proliferation of stellate cells.[28].Besides being able to reduce viral RNA transcription and HBx expression, estrogen is also able to regulate HBV activity through direct inhibition of the binding of Hepatocye Nuclear Factor (HNF)-4α with HBV EnhI, thus estrogen signals can suppress the production of pro-inflammatory cytokines in the liver[29,30].ALD patients in this study were dominated by the age range 45-50 years. It is said that the mean age of incidence of HCC associated with HBV infection is 50 years, except in high-risk populations which is 40 years [31].

In this study, it was found that the levels of P53, TGF-b1, and IL-10 were higher in men than women. Levels of P53, TGF-b1, and IL-10 were also found to be higher in ALD than in CH. In statistical analysis, there were significant differences in serum P53 and TGF-b1 levels between the ALD and CH ($p < 0.05$). It was said that the expression of P53 increased in malignancy and pre-malignancy, even overexpression of P53 was not uncommon in malignant conditions, including in terms of it's in liver cancer. In liver cancer there may also be a mutation in the P53 gene that increases expression and cannot be distinguished from normal P53 expression[32]. TGF-b1 levels also indicate hepatic function impairment, which is increasing in number in patients with cirrhosis of the liver. In the liver, high levels of TGF-b1 play a role in the occurrence of hepatic fibrogenesis, regulation of cell growth, and tumor development. [33].In a study by Kohla et al (2017), it was also stated that TGF-b1 levels increased with disease severity and progression in hepatocellular carcinoma, and TGF-b1 level could be complementary to alpha fetoprotein (AFP) in the diagnosis of HCC. [34]. In this study, there was no significant relationship between serum IL-10 levels and ALD. This is in line with the meta-analysis conducted by Shakiba et al (2018) which states that there are differences in IL-10 levels between HCC patients and normal people, but there is no difference between HCC and CH patients. In that study, it was also stated that the increase in IL-10 levels in ALD was due more to the cirrhosis process than tumor load [35].

When associated with previous studies, there was no relationship between Single Nucleotide Polymorphism (SNP) TGF-β1 -509T/C and P53 Arg72Pro genes with TGF-β1 and P53 serum levels. In vitro, it is known that there are several external factors that can influence the expression of the five TGF-β isoforms, including TGF-β1. In addition, the TGF-β1 signaling process is quite complex, involving the intracellular protein Smad which acts on various transcription factors in the nucleus. The expression of TGF-β1 is also influenced by a variety of different cytokines and chemical compounds. Other locus polymorphisms such as C466T and T869C can also affect TGF-β1 expression [36].Likewise, P53 levels are not only affected by the Arg72Pro polymorphism, but also SNPs in other P53 gene regions, including non-coding regions that can affect gene splicing [37].

This study has several limitations, the first, it did not compare between ALD and CH with healthy controls.The second, itcould not explain the role of other cytokines and epigenetic pathways in relation to

the pathogenesis of ALD. Further research is needed to understand the mechanism pathways for increasing levels of P53 and TGF-b1 in ALD patients other than through gene expression and polymorphisms.Studies regarding the various cytokines and molecular signaling involved can help understand the pathogenesis and are used as therapeutic pathways in ALD patients with HBV infection.

Conclusion

P53 and TGF-b1 levels were significantly elevated in ALD patients with HBV infection. Both parameters can act as biomarkers of prognosis and target therapy for ALD patients.

Ethical approval: Ethical Committee of Faculty of Medicine, Universitas Airlangga, Surabaya (Ethical clearance No. 186/EC/KEPK/FKUA/2020).

Patient' Informed Consent: Patient's informed consent has been obtained.

Conflict of Interest: Nil

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