FAS and FASL polymorphisms and susceptibility to hepatitis B virus infection in Javanese individuals

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Abstract. Hepatitis B virus infection is still a major global health problem. The polymorphisms in FAS and FASL genes may involve with susceptibility to hepatitis B virus infection for Javanese. To investigate the association of FAS and FASL polymorphisms with the susceptibility to hepatitis B virus infection in Javanese individuals, blood samples with hepatitis B virus infection was subjected for polymerase chain reaction-restriction fragment length polymorphism to genotype the FAS-670 A/G and FASL-124A/G polymorphism status, respectively. The frequencies of G/G genotype and G allele of FAS gene in the hepatitis B virus-infected blood sample were higher than that of the healthy sample (OR 3.5, 95%CI: 1.658 - 7.390, p = 0.001 and OR 2.3, 95%CI: 1.352 - 3.779, p = 0.002, respectively). It is proposed that the hepatitis B virus infection outcome in Japanese individuals might be influenced by FAS-670 A/G polymorphism through alteration in apoptosis of hepatocytes.

1. Introduction
Hepatitis B virus (an Orthohepadnavirus with features like retroviruses) infection stills a major global health threat in the world due to its heterogeneous endemicity, including in Indonesian Javanese people [1-3]. The patients infected with the hepatitis B virus would have a strong risk-factor to develop liver cirrhosis or even hepatocellular carcinoma with high morbidity and mortality rate [4, 5]. At present, there is no ideal hepatitis B virus infection therapy due to the difficulty to clearance the covalently closed circular deoxyribonucleic acid of the virus from the patient’s body [6]. None of the present therapeutic regiments successfully eliminate hepatitis B virus from the infected hepatocytes [7].

The death receptor FAS (apoptosis antigen 1), a member of the tumor necrosis factor receptor superfamily, has a central role in the apoptotic signaling regulation in several cell types including hepatocytes [8]. The FAS is a type I transmembrane protein with extracellular amino-terminal cysteine-rich domains that transmits the apoptotic signal through protein-protein interactions. The FAS receptor induces an apoptotic signal by binding to its ligand, FASL (CD95L) [9]. Apoptosis is important to control the persistent hepatitis B virus infection [10]. However, the hepatitis B virus could interfere the apoptosis signaling to promote hepatitis B virus replication and even hepatocellular carcinoma progression [11]. Polymorphisms in FAS and FASL genes would affect the apoptosis process and have been associated with susceptibility to diseases [8-9]. Since hepatitis B virus pathogenesis may influence by the complex interplay between host genomic polymorphisms status and viral factors [3], the present study intended to investigate the occurrence of FAS and FASL polymorphisms and its role in the susceptibility to hepatitis B virus infection in Javanese individuals.
2. Materials and methods
Blood samples collected previously from the high-risk communities for human bloodborne pathogens [2, 3, 12-14] were screened by serological and molecular assays to find out the human bloodborne pathogens infection status. For the present study, a total of 45 blood samples confirmed positive for hepatitis B virus but negative for other bloodborne pathogens were used. As a control, a randomly 100 blood samples derived from healthy adult negative for all bloodborne pathogens with similar high-risk histories [12] were used.

The molecular detection techniques for $-670A>G$ polymorphism in the FAS gene and $-124A>G$ in the FASL gene polymorphisms status were performed by polymerase chain reaction-restriction fragment length polymorphism assays as described previously (primers and endonuclease restriction enzymes used) with minor modifications [9]. In the present study, the genomic DNA was extracted from whole blood by using the Genomic DNA Mini Kit (Geneaid, New Taipei City, Taiwan). The polymerase chain reactions were performed using the MyTaq HS Red Mix (Bioline, London, UK). Corresponding positive controls and double distilled water as negative control were included for each group. To prevent polymerase chain reaction contamination, the reagent preparation, sample processing, and polymerase chain reaction assays were performed in rooms separate from where the amplified products were analyzed. Aerosol-resistant pipette tips were used throughout the assays. The polymerase chain reaction products were subjected to electrophoresis in 4% agarose gels containing SYBR Green and visualized under ultraviolet illumination using transillumination with a source of ultra-violet light. All samples were tested in duplicate. The genotypes were defined according to generated fragment patterns in the agarose gel electrophoresis analysis of polymerase chain reaction products as described previously [9].

3. Results and Discussion
In the present study, 45 blood samples found positive for hepatitis B virus but negative for other bloodborne pathogens (human immunodeficiency virus, hepatitis C virus, hepatitis D virus, Torque Teno virus, GB virus C, human T-cell lymphotropic viruses, and *Toxoplasma gondii*) by serological and molecular assays were used. The epidemiological data of the hepatitis B virus-infected individuals and the molecular characterization of the hepatitis B virus already partially published elsewhere [2, 3, 12-14]. All 45 blood samples used for the present study were subjected for the molecular detection of FAS$-670A>G$ (rs 1800682) and FASL INV2nt $-124A>G$ (rs 5030772) genes polymorphisms status, respectively. For control, 100 adults’ blood samples negative for all bloodborne pathogens (human immunodeficiency virus, hepatitis B virus, hepatitis C virus, hepatitis D virus, Torque Teno virus, GB virus C, human T-cell lymphotropic viruses, and *Toxoplasma gondii*) with similar high-risk histories for human bloodborne pathogens [12] were randomly selected and then used for the molecular detection for FAS$-670A>G$ (rs 1800682) and FASL INV2nt $-124 A>G$ (rs 5030772) genes polymorphisms status, respectively (Table 1).

The present study examined the occurrence of single nucleotide polymorphism in FAS and FASL genes, and their association with the susceptibility to hepatitis B virus infection. The association of the genotypes for both FAS and FASL polymorphisms may contribute to the prediction of some effector immune response to eliminate the hepatitis B virus [8-9]. In Brazilian and Iranian people, FAS and FASL polymorphisms were not statistically different between hepatitis B virus patients and healthy controls [15, 16]. In the present study, the frequencies of G/G genotype of FAS gene in the hepatitis B virus-infected blood sample was higher than that of the healthy sample (OR 3.5, 95%CI: 1.658 - 7.390, p = 0.001). Moreover, the frequencies of G allele of FAS gene in the hepatitis B virus-infected sample was higher than that of the healthy sample (OR 2.3, 95%CI: 1.352 - 3.779, p = 0.002) (Table 1).

As a non-cytopathic virus (cannot cause injury of the hepatocytes), hepatitis B virus modulates the activation of FAS/FASL through the MLK3-MKK7-JNK3-c-Jun signaling pathway [17, 18]. Moreover, hepatitis B virus protein reported has the ability to reduce the expression of FAS [19]. It is possible that the physical location of a single nucleotide polymorphism of FAS ($-670A/G$) may favor its binding to the site of the signal transducer and activator of transcription, and it would be enough for the up and down-regulation of the expression of the FAS gene. The results presented herein suggest that hepatitis B virus-infected subjects carrying by the FAS $-670GG$ genotype may have a lower affinity to the signal transducer and activator of transcription binding as compared to subjects carrying...
the FAS −670AA genotype. This could lead to a decrease or increase of the apoptotic potential of the FAS receptor among patients carrying FAS −670GG and AA genotypes, respectively [8, 9].

Taken all data together, the G allele (the G/G genotype) of the FAS -670 genes were associated with susceptibility to hepatitis B virus infection in Javanese individuals. However, the present study had several limitations. First, the sample size was limited. Second, we only assessed one polymorphism in the FAS (rs 1800682) and FASL IN V2nt (rs 5030772) gene, respectively; therefore, we cannot rule out that other polymorphisms or haplotypes in this gene might be implicated in the susceptibility to hepatitis B virus infection in Javanese individuals.

Table 1. The frequency of the FAS −670A>G (rs 1800682) and FASL INV2nt −124A>G (rs 5030772) genotypes.

| Alleles and Genotype | Case (%) | Control (%) |
|----------------------|----------|-------------|
| FAS−670A>G (rs 1800682) |          |             |
| A/A                  | 22.2 (10/45) | 34.0(34/100) |
| A/G                  | 26.7 (12/45) | 43.0(43/100) |
| G/G                  | 51.1 (23/45) | 23.0(23/100) |
| A/G + A/A            | 48.9 (22/45) | 77.0(77/100) |
| A/G + G/G            | 77.7 (35/45) | 66.0(66/100) |
| A                     | 35.6 (32/90) | 55.5 (111/200) |
| G                     | 64.4 (38/90) | 44.5 (89/200) |
| FASL INV2nt −124 A>G (rs 5030772) |          |             |
| A/A                  | 80.0 (36/45) | 85.0(85/100) |
| A/G                  | 11.1 (5/45)  | 10.0(10/100) |
| G/G                  | 8.9 (4/45)   | 5.0(5/100)   |
| A/G + A/A            | 91.1 (41/45) | 95.0(95/100) |
| A/G + G/G            | 20.0 (9/45)  | 15.0(15/100) |
| A                     | 85.6 (77/90) | 90.0 (180/200) |
| G                     | 14.4 (13/90) | 10.0(20/200) |

4. Conclusions
The present finding suggests that the G allele (the G/G genotype) of the FAS −670 gene was associated with susceptibility to hepatitis B virus infection in Javanese individuals.

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