Research Article

FAS and FAS-Ligand Promoter Polymorphisms in Hepatitis B Virus Infection

Asadollah Mohammadi,[1] Nader Tajik,[1,*] Alireza Shah-Hosseini,[1] Seyed Moayed Alavian,[2] Zohreh Sharifi,[3] and Lida Jarahi[4]

[1]Immunology Research Center (IRC), Iran University of Medical Sciences, Tehran, IR Iran
[2]Baqiyatallah Research Center for Gastroenterology and Liver Diseases, Baqiyatallah University of Medical Sciences, Tehran, IR Iran
[3]Blood Transfusion Research Center, High Institute for Research and Education in Transfusion Medicine, Tehran, IR Iran
[4]Community Medicine Department, Addiction Research Center, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, IR Iran

Corresponding Author: Nader Tajik, Immunology Research Center (IRC), Iran University of Medical Sciences, Tehran, IR Iran. Tel: +98-9123250344, Fax: +98-2188622652, E-mail: nadertajik@yahoo.com

Abstract

Background: The FAS and FAS-Ligand (FASL) system is an important apoptosis pathway in the liver. The FAS-mediated pathway functions by binding the FASL on the activated cytotoxic T lymphocytes and Natural Killer (NK) cells to the FAS receptor on infected hepatocytes. FAS and FASL polymorphisms, which are related to apoptosis, might influence the outcome of Hepatitis B Virus (HBV) infection.

Objectives: Thus, the present study aimed to determine if FAS and FASL promoter polymorphisms are associated with the clinical outcome of HBV infection.

Patients and Methods: DNA samples were obtained from the infected individuals including chronic carrier (n = 50), chronic hepatitis (n = 50), cirrhosis (n = 25), naturally recovered (n = 26) and compared with those of their matched healthy controls (n = 100). Genotyping for polymorphisms of FAS-670 A/G and -1377 G/A, and FASL -844 C/T was performed using polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) assays.

Results: Multiple analyses for genetic association of FAS and FASL polymorphisms were not statistically different between HBV patients (n = 125) and healthy controls (n = 100). However, genotype and allele frequencies of FASL-844 C/T were significantly different between recovered individuals and patients with cirrhosis (P = 0.02 and P=0.01, respectively). Whereas, FAS-670A/G and -1377G/A polymorphisms were similarly distributed in these two groups (P = 0.8 and P = 0.47, respectively).

Conclusions: The current study results showed that bearing -844T allele in FASL promoter region has a protective effect on cirrhosis and is involved in recovery from infection. In conclusion, it is proposed that HBV infection outcome might be influenced by FASL-844C/T polymorphism through alteration in apoptosis of hepatocytes.

Keywords: FAS, FAS Ligand, Polymorphism, Hepatitis B Virus Infection

1. Background

Hepatitis B Virus (HBV) is a hepatotropic and non-cytopathic DNA virus from Hepadnaviridae family that causes various problems in infected human (1-3). One third of the world's population, namely about two billion people, are infected with HBV and more than 350 million persons are chronically infected and suffer from clinical complications of this disease. It is estimated that HBV infection causes more than 600,000 deaths annually as a result of interactions between this virus and the host's immune system (4, 5).

HBV is not directly cytopathic for infected hepatocytes, but the host immune response to the virus determines if HBV infection is cleared or persists and contributes to liver pathogenesis. HBV infection causes clinical manifestations that vary from spontaneous recovery after an acute hepatitis to asymptomatic carrier or chronic infection, and finally liver cirrhosis (6-8). Cytotoxic T Lymphocytes (CTLs) and Natural Killer (NK) cells are essential components of immune response in the liver (9-11). NK cells contribute to early defense against viral infections, but the control of HBV replication and also elimination of the infected cells from liver tissue of patients depends on the adaptive immune response, especially cell mediated immunity and CTLs effector function (9-13). Cell mediated immune response, in addition to its crucial role to control HBV replication, is also responsible for liver injury and disease pathogenesis (2, 8, 14). It is shown that FAS and FAS Ligand (FASL) expressed on CTLs and NK cells, account for cell-mediated cytotoxicity and are known to be involved in apoptosis of infected hepatocytes. Beside NK cells and CTLs, cancer cells including hepatocytes in the hepatocellular carcinoma also express FASL to escape from immune responses and induce apoptosis in infiltrated lymphocyte to the liver (15-17). During an inflammatory response, liver infiltrating and resident lymphocytes become activated and express FASL; on the other hand, hepatocytes infected

by virus exhibit increase in FAS expression and become susceptible to FASL mediated apoptosis (18, 19).

FAS (CD95 or APO-1) is a type-I membrane protein and its gene consists of nine exons mapped on the chromosome 10q23 (20). FASL (CD95L or CD178) is a type-II membrane protein which its gene is mapped on chromosome 1q23 in humans with four exons (21). There are several Single Nucleotide Polymorphisms (SNPs) in the promoter region of FAS gene including -670 (A/G) (rs1800682) and -1377 (G/A) (rs2234767) that change stimulatory protein-1 (SP-1) and to the signal transducer and activator of transcription-1 (STAT-1) binding sites (22), and in FASL gene in the promoter region at position -844 (C/T) (rs763110) that reduces the interaction of transcription factor CAAT/ enhancer binding protein β (C/EBPβ) with promoter (23). FAS -670 (GG), -1377 (AA) and FASL -844 (TT) genotype diminish promoter activity and decrease FAS and FASL gene expression (22, 23). The -844C allele has twice the basal activity of the -844T allele and results in a significantly higher basal expression of FASL (23). Alteration in the levels of FAS and FASL expression is implicated in the pathogenesis of several liver diseases including viral hepatitis by B and C viruses (24-26), autoimmune hepatitis (18, 27), and alcoholic liver disease (18).

2. Objectives

The current study aimed to investigate if FAS and FASL promoter polymorphisms which alter FAS and FASL gene expression, influence the clearance of HBV infection or have a role in the persistence, pathogenesis and progression of liver disease.

3. Patients and Methods

3.1. Patients and Controls

The study population included 151 unrelated individuals with HBV infection and 100 healthy matched unrelated controls. All HBV cases were randomly selected from Tehran Hepatitis Center, Tehran, Iran, from 2011 to 2013. These patients with HBV infection were further divided into four groups based on the clinical presentations, serological markers, and liver biopsies. These groups were 50 cases with asymptomatic carrier state, 50 patients

with chronic hepatitis B, 25 patients with liver cirrhosis and 26 individuals that naturally recovered from HBV infection. There was no gender and age restriction in selection of patients. The age range of HBV cases and controls was from 20 to 60 years. Patients with HBV infection and a history of autoimmunity and co-infection with other viral infection including HDV, HCV, HIV and patients with alcohol addiction were excluded from the study. The selection criteria for the control subjects were no medical history of hepatitis B, C or D viruses and HIV infection, autoimmunity and alcohol consumption. At recruitment, written informed consent was obtained from each subject and personal data such as gender and age were collected via questionnaire.

3.2. Genotyping and Polymorphism Analysis

About 5 mL of peripheral blood samples were collected from the patients and healthy controls and drawn in Ethylenediaminetetraacetic acid (EDTA) tubes for genotyping. Genomic DNA was then extracted from whole blood samples of all controls and patients, using a salting out method (28). Genomic DNA concentration was determined using BioPhotometer (Eppendorf-Germany). Genotyping for FAS -670 A/G and -1377 G/A, and FASL -844 C/T polymorphisms was performed by Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) assays (29). The PCR primers for amplification of the FAS and FASL promoter variants, specificities, restriction enzymes and digestion patterns are shown in Table 1.

The reactions were amplified by the Mastercycler apparatus (Eppendorf, Germany) under the following conditions: a 25-ML reaction mixture containing approximately 100 Mg of template DNA, 0.5 μM of each primer, 0.2 mM of each deoxy-nucleoside 5' triphosphates, 2.0 mM $MgCl_2$, and 1.0 unit of Taq DNA polymerase (CinnaGen, Iran), in 1 × ammonium sulfate PCR reaction buffer. The thermal cycling was carried out with an initial melting step of two minutes at 94°C; followed by 10 cycles of 10 seconds at 94°C, 60 seconds at 65°C, and followed by 20 cycles of 10 seconds at 94°C and 50 seconds at 61°C and 30 seconds at 72°C, with final extension step of seven minutes at 72°C. The restriction endonucleases ScrFI, BstUI, and BsrDI (New England Biolabs, USA) were used

Table 1. FAS and FASL Promoter Single Nucleotide Polymorphism (SNP) Genotyping

Location	Primer Sequences (5' →3')	Annealing Temperature Tm, °C	PCR Product, bp	Restriction Enzyme	Genotype
FAS-670 A/G		61	193	ScrFI	AA: 193 bp; AG: 193 + 136 + 57 bp; GG: 136 + 57 bp
F	ATAGCTGGGGCTATGCGATT				
R	CATTTGACTGGGCTGTCCAT				
FAS-1377 G/A		61	122	BstUI	GG: 104 + 18 bp; GA: 122 + 104 + 18 bp; AA: 122 bp
F	TGTGTGCACAAGGCTGGCGC				
R	GCATCTGTCACTGCACTTACCACCA				
FASL-844 C/T		61	401	BsrDI	CC: 233 + 168 bp; CT:401 + 233 + 168 bp; TT: 401 bp
F	CAGCTACTCGGAGGCCAAG				
R	GCTCTGAGGGGAGAGACCAT				

to digest the FAS-670 A/G, FAS -1377 G/A, and FASL-844 C/T PCR amplicons respectively. The FAS-670AA product had no restriction site for ScrFI and amplicon remained undigested (193 bp). Inversely, two fragments of 136 and 57-bp were produced by ScrFI digestion on FAS -670GG PCR amplicon. BstUI digestion for FAS -1377 G/A polymorphism generated two fragments of 104 and 18 bp for FAS -1377GG genotype, and a 122 bp undigested fragment for FAS-1377AA. The FASL-844C allele had a BsrDI restriction endonuclease site that resulted in two fragments of 233 and 168 bp, but the T allele lacked this site and therefore only a 401 bp undigested band was generated (Table 1). The digested PCR products were separated on 3% agarose gel containing ethidium bromide and visualized under the UV transilluminator.

3.3. Statistical Analysis

Statistical analysis was performed by SPSS software version 11.5. Genotype and allele frequency differences of FAS and FASL promoter polymorphisms were analyzed between the cases and controls, and the group with HBV infection using Chi-squared test. Logistic regression analysis was used to assess the association of haplotype and combined genotype effects of these polymorphisms between the cases and controls. P values less than 0.05 were regarded statistically significant. The genetic trait association between the groups was measured by odds ratio (OR) and the exact confidence intervals (CI) of 95% were obtained. To assess the consistency of genotype distributions with the Hardy-Weinberg equilibrium, Chi-squared test was used.

4. Results

4.1. Distribution of FAS and FASL Polymorphisms in Patients With HBV Infection and Controls

The frequencies of AA, AG, and GG genotypes for FAS-670 A/G were 32.0%, 52.2%, and 12.8% among the patients with HBV infection (n = 125; carrier, chronic hepatitis, and cirrhosis groups), respectively and 27.0%, 51.0%, and 22.0%, in controls respectively. For FAS-1377G/A polymorphism, frequencies of GG, AG, and AA genotypes were 72.0, 26.4 and 1.6% in patients with HBV respectively and 72.0%, 25.0%, and 3.0% among the controls, respectively. Similarly, for FASL-844C/T polymorphism, frequencies of CC, CT, and TT genotypes were 31.2%, 47.2%, and 21.6% among patients with HBV respectively and 25.0%, 51.0%, and 24.0% in the controls respectively. Distribution of genotype and allele frequencies for FAS and FASL polymorphisms was not statistically different between patients with HBV infection and the controls (Table 2).

Table 2. Genotype and Allele Frequencies of FAS-670, FAS-1377 and FASL-844

Genotypes and Alleles	Controls (n = 100)	Patients With HBV (n = 125)	P-Value	OR	95% CI
FAS-670 A/G					
Genotype					
AA	27 (27.0%)	40 (32.0%)	1.0	1.0	
AG	51 (51.0%)	69 (55.2%)	0.67	0.88	0.49 - 1.58
GG	22 (22.0%)	16 (12.8%)	0.16	0.58	0.27 - 1.24
AG + GG	73 (73.0%)	85 (68.0%)	0.50	0.80	0.41 - 1.53
Allele					
A	105 (52.5%)	149 (59.6%)	1.0	1.0	
G	95 (47.5%)	101 (40.4%)	0.13	0.78	0.54 - 1.11
FAS -1377 G/A					
Genotype					
GG	72 (72.0%)	90 (72.0%)	1.0	1.0	
GA	25 (25.0%)	33 (26.4%)	0.76	1.09	0.61 - 1.95
AA	3 (3.0%)	2 (1.6%)	0.38	0.44	0.07 - 2.72
GA + AA	28 (28.0%)	35 (28.0%)	0.93	1.02	0.58 - 1.79
Allele					
G	169 (84.5%)	213 (85.2%)	1.0	1.0	
A	31 (15.5%)	37 (14.8%)	0.83	1.00	0.70 - 1.43
FASL -844 C/T					
Genotype					
CC	25 (25.0%)	39 (31.2%)	1.0	1.0	
CT	51 (51.0%)	59 (47.2%)	0.85	0.94	0.50 - 1.74
TT	24 (24.0%)	27 (21.6%)	0.97	0.98	0.48 - 2.02
CT + TT	75 (75.0%)	86 (68.8%)	0.88	0.95	0.53 - 1.71
Allele					
C	101 (50.5%)	137 (54.8%)	1.0	1.0	
T	99 (49.5%)	113 (45.2%)	0.36	1.04	0.63 - 1.71

4.2. Distribution of FAS and FASL Polymorphisms in Different Groups With HBV Infection and the Controls

The distribution of genotype and allele frequencies of both FAS and FASL polymorphisms were compared among individuals with HBV infection with different clinical states and the control subjects. The frequencies of AA, AG, and GG genotypes for FAS-670 A/G were 34.0, 52.0 and 14.0% among carrier and chronic hepatitis patients, 30.8%, 42.3% and 26.9% in the recovered group, 24.0%, 68.0% and 8.0% among those with cirrhosis and 27.0%, 51.0% and 22.0% in the controls, respectively. For FAS-1377 G/A polymorphism, the frequencies of GG, AG, and AA genotypes were 70.0%, 28.0% and 2.0% among the carriers, 80.0%, 18.0%, and 2.0%, in patients with chronic hepatitis, 60.0%, 40.0% and 0% among those with cirrhosis, 69.2%, 30.8% and 0% in the recovered individuals, and 72.0%, 25.0% and 3.0% among the controls, respectively. Regarding, FASL-844 C/T polymorphism, the frequencies of CC, CT, and TT genotypes were 20.0%, 56.0%, and 24.0% in carriers, 38.0%, 40.0%, and 22.0% among the patients with chronic hepatitis , 0%, 61.5%, and 38.5% in recovered group, 40.0%, 44.0%, and 16.0% among patients with cirrhosis, and 25.0%, 51.0% and 24.0% in the controls, respectively (Table 3). The distribution of FAS polymorphisms was not statistically different between different groups of individuals with HBV infection and controls (P = 0.51 for -670 A/G, P = 0.65 for -1377 G/A). In contrast, significant dissimilarity in FASL-844 C/T genotypes was observed between the recovered group and patients with cirrhosis (P = 0.02). Moreover, patients with liver cirrhosis had a lower frequency of FASL-844T allele and higher frequency of FASL-844C allele compared to the recovered individuals (P = 0.01, Table 3).

Table 3. Genotype and Allele Frequencies of FAS -670, FAS -1377 and FASL-844 Gene Polymorphisms Among the Controls and the Individuals With HBV Infection and Different Clinical States

Genotypes and Alleles	Controls (n = 100)	Recovered Individuals (n = 26)	Asymptomatic Carriers (n = 50)	Chronic Hepatitis (n = 50)	Liver Cirrhosis (n = 25)	P-Value
FAS -670 A/G						
Genotype						
AA	27 (27.0%)	8 (30.8%)	17 (34.0%)	17 (34.0%)	6 (24.0%)	0.51
AG	51 (51.0%)	11 (42.3%)	26 (52.0%)	26 (52.0%)	17 (68.0%)	
GG	22 (22.0%)	7 (26.9%)	7 (14.0%)	7 (14.0%)	2 (8.0%)	
AG+GG	73 (73.0%)	18 (69.2%)	33 (66.0%)	33 (66.0%)	19 (76.0%)	0.80
Allele						
A	105 (52.5%)	27 (51.9%)	60 (60.0%)	60 (60.0%)	29 (58.0%)	
G	95 (47.5%)	25 (48.1%)	40 (40.0%)	40 (40.0%)	21 (42.0%)	0.61
FAS -1377 G/A						
Genotype						
GG	72 (72.0%)	18 (69.2%)	35 (70.0%)	40 (80.0%)	15 (60.0%)	0.65
GA	25 (25.0%)	8 (30.8%)	14 (28.0%)	9 (18.0%)	10 (40.0%)	
AA	3 (3.0%)	0 (0.0%)	1 (2.0%)	1 (2.0%)	0 (0.0%)	
GA + AA	28 (28.0%)	8 (30.8%)	15 (30.0%)	10 (20.0%)	10 (40.0%)	0.47
Allele						
G	169 (84.5%)	44 (84.6%)	84 (84.0%)	89 (89.0%)	40 (80.0%)	
A	31 (15.5%)	8 (15.4%)	16 (16.0%)	11 (11.0%)	10 (20.0%)	0.67
FASL -844 C/T [a]						
Genotype						
CC	25 (25.0%)	0 (0.0%) [a]	10 (20.0%)	19 (38.0%)	10 (40.0%)	0.02 [a]
CT	51 (51.0%)	16 (61.5%) [a]	28 (56.0%)	20 (40.0%)	11 (44.0%)	
TT	24 (24.0%)	10 (38.5%) [a]	12 (24.0%)	11 (22.0%)	4 (16.0%)	
CT + TT	75 (75.0%)	26 (100.0%) [a]	40 (80.0%)	31 (62.0%)	15 (60.0%)	0.002 [a]
Allele						
C	101 (50.5%)	16 (30.8%) [a]	48 (48.0%)	58 (58.0%)	31 (62.0%)	
T	99 (49.5%)	36 (69.2%) [a]	52 (52.0%)	42 (42.0%)	19 (38.0%)	0.01 [a]

[a]Significant differences.

4.3. Haplotype Frequency Analysis of FAS Gene Polymorphisms in the Patients With HBV Infection and the Controls

For FAS-670A/G and FAS-1377G/A, four possible haplotypes: GA, GG, AA, and AG were derived from known genotypes. According to FAS-670G/FAS-1377A, distribution of haplotypes was not significantly different between the patients with HBV infection and the controls. Haplotype analysis of two SNPs in FAS gene is shown in Table 4.

4.4. Combined Genotype Analysis of FAS Gene Polymorphisms in the Patients With HBV Infection and the Controls

To study the gene-gene interactions, nine combinations of FAS-670 and -1377 genotypes were evaluated among the patients with HBV infection and the controls. Taking the combined genotypes of -670AA/-1377GG as the reference decreased the frequency of -670GG/-1377GG genotypes in the patient group compared to controls (P = 0.03, OR = 0.34; 95% CI = 0.12 - 0.97). The combined genotype frequencies of FAS-1377 and FAS-670 polymorphisms in the patients with HBV infection and the controls are summarized in Table 5.

4.5. Interaction Between FAS and FASL Polymorphisms in the Patients With HBV Infection and the Controls

Although the FASL-844 C/T gene polymorphism and the presence of CC genotype and C allele increased among the patients with cirrhosis, analysis of FAS and FASL genetic interaction, indicated the lack of an additive effect between FAS-670 and -1377 with FASL-844CC genotypes in the groups under study. The interaction between FAS and FASL gene polymorphisms in HBV infected patients and the controls is shown in Table 6.

Table 4. FAS-670 and FAS-1377 Haplotype Analysis in the Patients with Hepatitis B Virus Infection and Controls

FAS-670	FAS-1377	Control (n = 100)	Patients With HBV (n = 125)	P-Value	Odds Ratio	95% CI
G	A	64 (32%)	67 (26.8%)	1.0	1.0	
G	G	31 (15.5%)	33 (13.2%)	0.98	1.00	0.57 - 1.77
A	A	105 (52.5%)	145 (58.0%)	0.29	1.28	0.83 - 1.87
A	G	0 (0.0%)	5 (2.0%)	0.08	-	-

Table 5. Combined Genotype Frequencies of FAS-670 and FAS-1377 Polymorphisms in the Patients With HBV Infection and Controls

Combined Genotype		Controls (n = 100)	Patients With HBV Infection (n = 125)	P-Value	OR	95% CI
FAS-670 A/G	FAS-1377 G/A					
AA	GG	27 (27.0%)	38 (30.4%)	1.0	1.0	
AG	GG	33 (33.0%)	48 (38.4%)	0.95	0.97	0.51 - 1.85
GG	GG	12 (12.0%)	4 (3.2%)	0.03 [a]	0.34 [a]	0.12 - 0.97 [a]
AA	GA	0 (0.0%)	2 (1.6%)	0.55	-	-
AG	GA	18 (18.0%)	20 (16.0%)	0.54	0.78	0.36 - 1.69
GG	GA	7 (7.0%)	11 (8.8%)	0.67	1.25	0.45 - 3.47
AA	AA	-	-	-	-	-
AG	AA	0 (0.0%)	1 (0.8%)	0.65	-	-
GG	AA	3 (3.0%)	1 (0.8%)	0.29	0.19	0.01 - 1.97

[a]Significant differences.

Table 6. Joint Effect of FAS and FASL Gene Polymorphisms Among the Patients with HBV Infection and the Controls

Combined Genotype		Controls (n = 100)	Patients With HBV Infection (n = 125)	P-Value	OR	95% CI
FASL-844	FAS-670					
TT + CT	GG + GA	55 (55.0%)	59 (47.2%)	1.0	1.0	
TT + CT	AA	20 (20.0%)	27 (21.6%)	0.50	1.25	0.65 - 2.39
CC	GG + GA	18 (18.0%)	26 (20.8%)	0.93	1.03	0.51 - 2.06
CC	AA	7 (7.0%)	13 (10.4%)	0.57	1.32	0.49 - 3.54
FASL-844	FAS-1377					
TT + CT	AA + GA	22 (22.0%)	20 (16.0%)	1.0	1.0	
TT + CT	GG	53 (53.0%)	66 (52.8%)	0.51	1.24	0.64 - 2.39
CC	AA + AG	6 (6.0%)	15 (12.0%)	0.22	1.96	0.65 - 5.89
CC	GG	19 (19.0%)	24 (19.2%)	0.98	0.99	0.43 - 2.25

5. Discussion

Elimination and removal of unwanted cells, such as senescent, damaged, genetically mutated and virus infected cells, is important for the maintenance of liver homeostasis. This process naturally occurs through a highly regulated form of cell death called apoptosis. FAS and FASL system is an important pathway of apoptosis in the liver (18, 27). There are several SNPs in these apoptosis related genes that change promoter activity and thus, gene expression (22, 23). Therefore, the present study focused on genetic change of FAS and FASL genes to determine if variations in these genes influence the outcome of HBV infection. Several studies have shown that FAS -670 AA genotype and A allele were associated with liver diseases such as autoimmune hepatitis and development of cirrhosis (30, 31), Liver graft rejection (32) and Hepatitis C (33). Zamani et al. (34) showed that FAS-670 A/G polymorphism was associated with chronic HBV infection, while FASL IVS2nt-124 A/G polymorphism was not; also FAS -1377 G/A and FASL-844 C/T genotypes were likely to play a substantial role in HBV infection outcome.

The current study found that the presence of FAS-670G allele (FAS-670 A/G or G/G) and FAS -1377A allele (FAS-1377 G/A or A/A) variants were not associated with the outcome of HBV infection or progression to liver cirrhosis. This result was in accordance with that of a study by Jung et al. performed on HBV infection (35). In the present study, although the FASL-844 C/T polymorphism was not different between the patients with HBV infection and the controls, it was significantly dissimilar in naturally recovered group and patients with HBV infection and liver cirrhosis. Individuals with cirrhotic HBV had high frequencies of CC genotype (P = 0.02), and C allele (P = 0.01) at FASL -844 SNP locus in comparison with the recovered group. In contrast, bearing FASL-844T allele or CT and TT genotypes was found to be associated with recovery from HBV infection. It is shown that the G/A transition at position-1377 and the A/G transition at position -670 in the promoter region of FAS gene, disrupt SP-1 and STAT-1 protein-binding element and therefore, diminish promoter activity and ultimately reduce FAS gene expression (22). With regard to the FASL gene, -844C/T polymorphism in promoter region is located in a binding motif for transcription factor C/EBPβ. A two fold higher basal expression of FASL is associated with the presence of the FASL-844C allele rather than the FASL -844T allele (23). Although no association was found between FAS polymorphisms and the resolution of HBV infection, it was found that FASL-844T allele, which decreases FASL gene expression, may be associated with HBV infection recovery.

It is widely accepted that CTLs response clears viral infections by destruction of the infected cells through FASL and release of granules containing granzyme B and perforin (6, 7). Although immune responses to viral antigens initiated by T cells are fundamental for viral clearance, it may not be possible for CTLs to kill all the infected cells if they are greatly activated and outnumbered. During HBV infection up to 100% of hepatocytes can be infected (2, 3) and massive cell destruction might be pathologic rather than protective. Therefore, viral clearance may require more efficient CTLs function than direct killing (2, 8, 14). With regard to non-cytopathic action of HBV, the liver disease in this infection is clearly due to immunopathologic activity of CTL response. Several studies suggested that non-cytopathic viral inactivation through cytokines released by virus specific CTLs could have an important role in HBV clearance without killing of the all infected cells. Virus specific CTLs were capable of eliminating viral DNA from cytoplasm of hepatocytes via a mechanism that was at least partly mediated by non-cytopathic antiviral activities of cytokines such as IFN-γ and TNF-α (2, 14).

With regard to the importance of FAS/FASL apoptosis system in the liver, previous studies showed that a progressive increase in FAS/FASL protein expression was associated with the severity of liver disease in chronic hepatitis and cirrhosis (19, 36). In addition, the increases in FAS and FASL mRNA expression in the liver tissue was correlated with the degree of liver cirrhosis (25) and all the stages of viral liver disease (25, 37). The increase in FASL mRNA expression in hepatic tissue causes an enhanced local inflammatory response and contributes to neutrophil recruitment and interleukin-1 beta (IL-1β) release. This process is crucial to chronic liver damage, persistence of viral infection and progression of liver cirrhosis (25, 26). Although apoptosis and cellular immune responses are vital for maintenance of liver homeostasis and clearance or persistence of viral infections, cirrhosis is the outcome of a long time chronic liver inflammation and damage. Therefore, changes in FAS/FASL expression in liver may be harmful and destructive (18, 27).

In conclusion, the patients with liver cirrhosis had higher frequency of FASL-844 CC genotypes and C allele than the other groups. Thus, increased FASL gene expression in chronically damaged liver tissue may make hepatocytes more susceptible to apoptosis and consequently liver destruction. On the other hand, FASL-844T allele was associated with HBV clearance or protection against cirrhosis. Finally it was noted that, further clinical and functional studies are needed to confirm the possibility of association between FASL-844C/T polymorphism and HBV infection outcome.

Acknowledgments

Footnote

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based on clinical and laboratory work-up and contribute to patient selection. Lida Jarahi performed data analysis. Nader Tajik designed the project, super-vised the study, and critically reviewed and revised the manuscript.

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