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Genetic polymorphisms in the *IFNL4*, *MxA*, and *MxB* genes were associated with biochemical index of chronic HBV patients in Yunnan, China

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Hepatitis B virus (HBV) infection lead to Hepatitis B, which was one of the most common causes of hepatocellular carcinoma (HCC). Single Nucleotide Polymorphisms (SNPs) of host immune genes could influence HBV infection, viral clearance, and treatment effect. In order to investigate the role of *IFNL4* and its downstream genes (*MxA* and *MxB*) in Yunnan HBV infected persons, whole blood and biochemical index of 448 HBV patients and controls were collected. Seven SNPs were genotyped to analyze the frequency of genotypes, alleles, and haplotypes between HBV patients and controls. However, no association was identified between SNPs and HBV infection. Then, biochemical indexes levels were studied among HBV patients with different genotypes of seven SNPs. The results showed that liver function indexes level (including ALT, AST, TBIL, DBIL, IBIL, and ALB) could be influenced by genotypes of SNPs in HBV patients. When HBV patients were divided into HBsAg-positive and -negative groups, the association between genotypes of SNP and biochemical indexes still existed. Above all, although the genetic polymorphisms were not associated with HBV infection in Yunnan, it might indirectly influence disease progress by relating with biochemical indexes level of HBV patients in Yunnan.

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Abstract

Hepatitis B virus (HBV) infection lead to Hepatitis B, which was one of the most common causes of hepatocellular carcinoma (HCC). Single Nucleotide Polymorphisms (SNPs) of host immune genes could influence HBV infection, viral clearance, and treatment effect. In order to investigate the role of *IFNL4* and its downstream genes (*MxA* and *MxB*) in Yunnan HBV infected persons, whole blood and biochemical index of 448 HBV patients and controls were collected. Seven SNPs were genotyped to analyze the frequency of genotypes, alleles, and haplotypes between HBV patients and controls. However, no association was identified between SNPs and HBV infection. Then, biochemical indexes levels were studied among HBV patients with different genotypes of seven SNPs. The results showed that liver function indexes level (including ALT, AST, TBIL, DBIL, IBIL, and ALB) could be influenced by genotypes of SNPs in HBV patients. When HBV patients were divided into HBsAg-positive and -negative groups, the association between genotypes of SNP and biochemical indexes still existed. Above all, although the genetic polymorphisms were not associated with HBV infection in Yunnan, it might indirectly influence disease progress by relating with biochemical indexes level of HBV patients in Yunnan.

Keywords: *IFNL4*, *MxA*, *MxB*, SNPs, HBV infection, biochemical index

Introduction

In 60s and 70s of the 20th century, hepatitis B virus (HBV) was identified to be the cause of hepatitis B disease, which was one of the most popular reasons for serious hepatitis diseases (Glebe et al. 2021). In adults, 5% of HBV infected persons developed chronic HBV (CHB) infection, and 20-30% CHB might develop cirrhosis and/or hepatocellular carcinoma (HCC). According to World Health Organization (WHO) reports, 275 million persons (about 3.5% of population) were living with CHB. The epidemic regions of CHB mainly located in Western Pacific Region and African (W.H.O. 2017). Although usage of vaccine greatly protected persons from HBV infection, the number of adult HBV patients was still large.

Interferons (IFNs) were commonly used for HBV therapy in clinic, and interferon pathway played important role in CHB infection through activating expression of interferon-stimulated genes (ISGs) (Mani & Andrisani 2019). However, the treatment effect showed great difference among various HBV patients, and host immunologically genetic factors were necessary for antiviral response (Brouwer et al. 2019). Distinguishing from other members of IFN family, IFNL4 was reported to impair hepatitis C virus (HCV) clearance (Prokunina-Olsson et al. 2013), and its genetic polymorphisms could influence viral clearance of HCV patients treated by pegylated-IFN-α/ribavirin (O'Brien et al. 2015). Similarly, the genetic polymorphisms in the *IFNL4* gene were also considered to associated with HBV viral load and producing protective antibody (Chihab et al. 2021; Grzegorzewska et al. 2020).

Mx genes, including the *MX dynamin like GTPase 1* gene (*Mx1* or *MxA*) and the *MX dynamin like GTPase 2* gene (*Mx2* or *MxB*), belonged to IFN-stimulated gene (ISG). The amino

acid homology between *MxA* and *MxB* reached to about 63%, but the antiviral activity seemed greatly different. Although MxA was well known as a wide-spectrum antiviral factor (Haller et al. 2015), both MxA and MxB could inhibit HBV replication. However, whether genetic polymorphisms of these two genes could influence HBV infection and pathogenesis were not well studied.

In this study, frequency of genetic polymorphisms of the *IFNL4*, *MxA*, and *MxB* gene were analyzed in HBV patients from Yunnan, China. The association between biochemical index and genotypes of polymorphisms were identified.

Material and methods

Individuals and biochemical index

448 HBV infected persons, who were identified as CHB patients without any treatment, and 448 general controls were collected by doctors in The First People's Hospital of Yunnan Province. All patients were infected with Hepatitis B virus (HBV) but without HCV and/or Human Immunodeficiency virus (HIV) infection. All controls were not infected by any virus and without any disease. 3 mL whole blood of each individual was obtained to extract the genomic DNA (gDNA) for single nucleotide polymorphisms (SNP) analysis.

Biochemical information of each person was collected, which included alanine transaminase (ALT), aspartate transaminase (AST), total bilirubin (TBIL), direct bilirubin (DBIL), indirect bilirubin (IBIL), total protein (TP), albumin (ALB), globin (GLOB), blood urea nitrogen (BUN), serum creatinine (CREA), serum uric acid (UA), blood glucose (GLU), white

77 blood cells (WBC), neutrophilic granulocyte (NEUT), lymphocytes (LYM), monocytes
78 (MONO), eosinophil granulocyte (EO), and basophile granulocyte (BASO). Whether hepatitis B
79 surface antigen (HBsAg) was positive or negative was detected in each HBV sample.
80 Written informed consent conforming to the tenets of the Declaration of Helsinki was
81 obtained from each participant prior to the study. This study was approved by the Institutional
82 Review Board of Kunming University of Science and Technology. (Approval No. 2014SK027)
83
84 **SNP genotyping and haplotype construction**
85 Two SNPs (rs11322783 and rs117648444) in the *IFNL4* gene, two SNPs (rs2071430 and
86 rs17000900) in the *MxA* gene, and three SNPs (rs9982944, rs408825, and rs2838029) in the *MxB*
87 gene were genotyped by using SnapShot method. All seven SNPs were tag SNP or functional
88 SNP.
89 Haplotypes were constructed by using seven SNPs of HBV patients and controls in SHEsis
90 software platform (http://analysis.bio-x.cn/myAnalysis.php). The Lowest frequency threshold
91 (LFT) for haplotype analysis was 0.05. Linkage Disequilibrium (LD) was calculated among
92 seven SNPs.
93 **Data analysis**
94 The data of each biochemical index was presented by Mean ± SEM in HBV patients or
95 controls. Biochemical indexes between HBV patients and controls were compared by Student's t
96 test (two-tailed). The frequencies of genotype, allele, and haplotypes were compared between
97 HBV patients and controls by using Chi-square test with Yates' correction. The association

between genotypes and biochemical index was analyzed by using Student's t test (two-tailed). Genotype and allele frequencies of each SNP were compared between HBV patients with HBsAg-positive and -negative. When the *P*-value was less than 0.05, it was considered significant difference.

Results

Basic information

The mean age of HBV patients and controls were 42.12 ± 0.38 and 40.58 ± 0.53 years old, respectively. Although the numbers of male individuals were somewhat more in controls (N= 275, 61.38%) than in HBV patients (N= 245, 54.69%), it showed no significance. The ratio of male: female was 1.2: 1 and 1.6:1 in HBV patients and controls, respectively. Excluding IBIL, BUN, CREA, UA, WBC, MONO, and EO, all other biochemical indexes showed significantly different (Table 1). These results suggested liver function were impaired in HBV patients.

No association between SNPs in three genes and HBV infection was identified

Genotype and allele frequencies of seven SNPs showed no significant difference between HBV patients and controls (Table 2). No individual carried with genotype AA of rs117648444. The D' value of SNPs rs2071430 and rs17000900 in the *MxA* gene and D' value of SNPs rs408825 and rs2838029 in the *MxB* gene was 0.96 and 0.97, respectively. However, the r^2 value showed no Linkage disequilibrium among these SNPs (Fig. 1). This indicated that all SNPs could not tag each other. Totally 31 and 35 haplotypes were constructed in HBV patients and controls, respectively. The frequencies of seven haplotypes were more than 0.05, but no

haplotype showed statistical difference between HBV patients and controls (Table 3).

Biochemical indexes showed significant difference among HBV patients carried various genotypes

Genotypes of 6 SNPs were associated with biochemical indexes of HBV patients. Because genotype ΔG of rs11322783 and genotype AA in rs2838029 existed in only one HBV patient and/or one control person, genotype ΔG was combined with genotype ΔG/T of rs11322783 and genotype AA was combined with genotype AG in rs2838029 for further analysis. Biochemical indexes showed significantly different among three genotypes of each SNP (Fig. 2). In the *IFNL4* gene, LYM (P= 0.007) and MONO (P= 0.010) levels were significantly higher in patients with genotype TT than those with genotype GG and GT of rs11322783. Genotype TT of rs2071430 seemed to decrease ALT and CREA level of patients. The ALT, AST, and CREA level was statistically lower in patients carried genotype AA of rs17000900. The AST, TBIL, DBIL, IBIL, and MONO level was higher in patients with genotype GG of rs2838029 than the patients with other two genotypes. Genotype TT of rs408825 seemed to be the risk factor for DBIL, IBIL, and EO level in Yunnan HBV patients. ALB level showed significantly higher level in patients with genotype AA of rs9982944 than the other patients.

Rs17000900 was associated with HBsAg seroclearance of patients

HBsAg was a marker for HBV cccDNA replication in clinic, so genotype and allele frequencies were compared between HBV patients with HBsAg-positive and HBsAg-negative (Table 4). The results showed that the frequency of genotype AC in rs17000900 was statistically higher in HBsAg-negative patients (69/238, 28.99%) than in HBsAg-positive patients (43/210,

20.48%).

Biochemical indexes also expressed significant difference between HBsAg-positive and HBsAg-negative HBV patients (Fig. 3). The AST and TBIL level were higher in HBsAg-positive patients than in HBsAg-negative patients carried genotype AC of rs17000900. Similarly, the DBIL and IBIL level also showed higher levels in HBsAg-positive patients than in HBsAg-negative patients with genotype CC of rs17000900. However, ALB, WBC, and NEUT level significantly decreased in HBsAg-positive patients with genotype AC of rs17000900.

Discussion

Although the contradictory role of pegylated-interferon (Peg-IFN) was found, it was commonly used to treat HBV infection in clinic for immune modulator because it could boost the immune system and stimulate ISGs expression (Mani & Andrisani 2019). HBV could interfere in IFN signaling pathway via various mechanisms, for example HBV core protein could inhibit expression of the *MxA* gene stimulated by Peg-IFN (Yu et al. 2010). Moreover, the treatment effect showed significant difference among HBV patients, due to HBV genotype, HBV viral load, and host genetic factors. There are many studies to analyze the role of genetic polymorphisms in the *IFN* genes in HBV patients.

SNPs of the *IFNL4* gene was widely studied in different HBV populations. Grzegorzewska *et al.* found that ΔG allele of rs368234815 (merged into rs11322783) was the risk factor for developing anti-HBs, and individuals with genotype ΔG/ΔG showed lower responsiveness to HBV vaccination (Grzegorzewska et al. 2020). SNP rs12979860 located at both the *IFNL3* and *IFNL4* genes, and genotypes of rs12979860 could modulate HBV cccDNA levels (Chihab et al.

161 2021). In our previous study, LYM level was much higher in HBV patients with genotype CC of
162 rs12979860 than patients with genotype AA (Song et al. 2017). These reports suggested genetic
163 polymorphisms in the *IFNL4* gene could influence HBV disease progress by modulate immune
164 reaction. Haplotype constructed by three SNPs (rs12971396-rs8113007-rs7248668: GTA) were
165 more frequent in HCC patients caused by HBV infection than in HBV patients, but no difference
166 was found between HBV patients and controls (Ma et al. 2018). In this study, the similar
167 numbers of haplotypes constructed by seven SNPs in three genes were obtained among two
168 groups. Thus, we supported that genetic polymorphisms of *IFNL4* gene could not influence HBV
169 infection in Yunnan population.

170 *MxA* and *MxB* owned direct anti-virus function. HBV replication was downregulated in
171 *MxA* transgenic mice (Peltekian et al. 2005), and *MxB* decreased HBV RNA level and indirectly
172 impaired HBV cccDNA (Wang et al. 2020). However, the relationship between genetic
173 polymorphisms in the *MxA* and *MxB* gene and HBV infection were rarely reported. SNP of -88
174 nt (G/T) in *MxA* gene promoter expressed higher frequency in HBV sustained patients than in
175 non-responders. Unfortunately, our results did not identify the association between genotypes of
176 SNPs and HBV infection, it might due to different SNPs selection.

177 The *IFNL3* gene played important roles in HBV infection, viral clearance, treatment effect,
178 and response to HBV vaccine (Zhao et al. 2020). Due to the limited investigation, genetic
179 function of the *IFNL4* gene in HBV patients was needed. Although polymorphisms in the *IFNL4*
180 gene was not associated with HBV infection and naturally viral clearance, three-way interaction
181 was identified between IFNL4/ HLA-DQ and HBV infection by using multifactor dimensionality

reduction test (Fan et al. 2016). Two SNPs rs368234815 and rs117648444 in the *IFNL4* gene, which were also analyzed in this study, might be as predictor for IFN treatment response in HBeAg-negative HBV patients (Galmozzi et al. 2018). In Thai HBV patients, SNPs in the *IFNL4* gene could not influence response to PEG-IFN of patients (Limothai et al. 2015). Similarly, no association was found between the *IFNL4, MxA, MxB* genes and Yunnan HBV patients, but the biochemical index level showed significantly different among HBV patients with different genotypes of SNPs in three genes. Thus, we suggested that SNPs in *IFNL4* might not directly influence HBV infection but predict the disease progress or treatment effect. Because there were contradict results among various populations, further studies could be performed.

HBsAg level was considered as recovery of hepatitis activity and a better outcome, and host genetic factors associated with HBsAg loss. SNPs in the *HLA-DPA1* and *HLA-DPB1* genes could influence spontaneous HBsAg seroclearance in male HBV patients (Cheng et al. 2013). In addition, serum HBsAg level could reflected the interferon treatment effect (Su et al. 2014). In this study, genotype AC of rs17000900 was suggested as the protective factor for HBsAg seroclearance in HBV patients. Moreover, biochemical indexes showed significantly different between HBV patients with HBsAg-positive and -negative. These results further indicated that host genetic polymorphisms were associated with HBsAg seroclearance in HBV patients.

Conclusion

In summary, genetic polymorphisms of the *IFNL4*, *MxA*, and *MxB* genes were not association with HBV infection, but could influence the biochemical index levels of HBV patients in Yunnan.

Competing Interests

The authors report no conflict of interest.

Acknowledgement

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288

Figure 1

Figure 1. Linkage Disequilibrium map of seven SNPs in three genes.

A: D’ value; B: r^2 value.

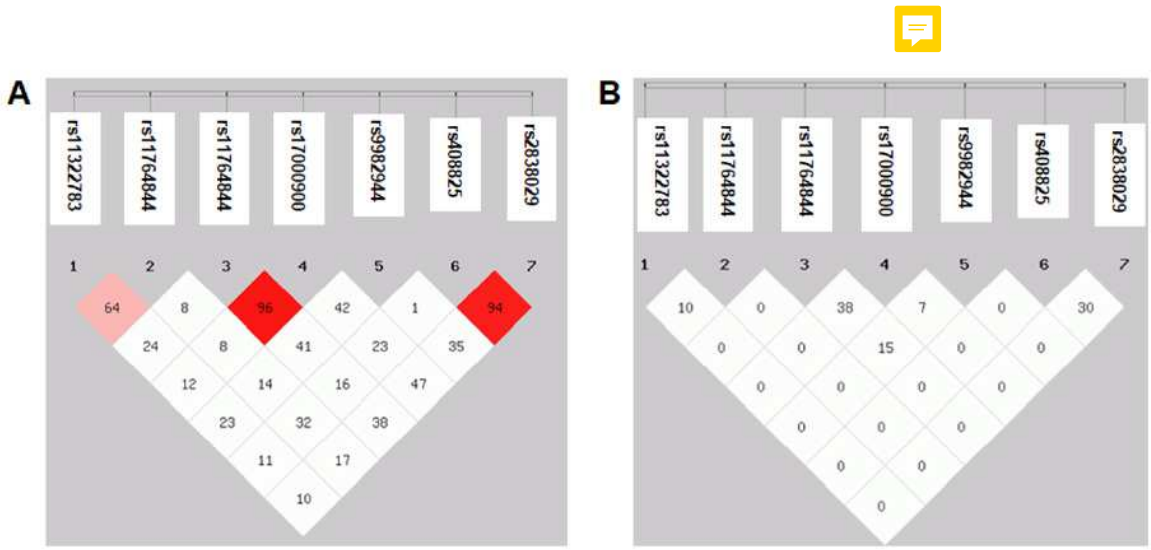

Figure 2

Figure 2. Comparison of biochemical index of HBV patients with different genotypes of each SNP.

Rs11322783 located in the *IFNL4* gene; rs2071430 and rs17000900 was in the region of *MxA* gene; rs9982944, rs408825, and rs2838029 were located in the *MxB* gene.

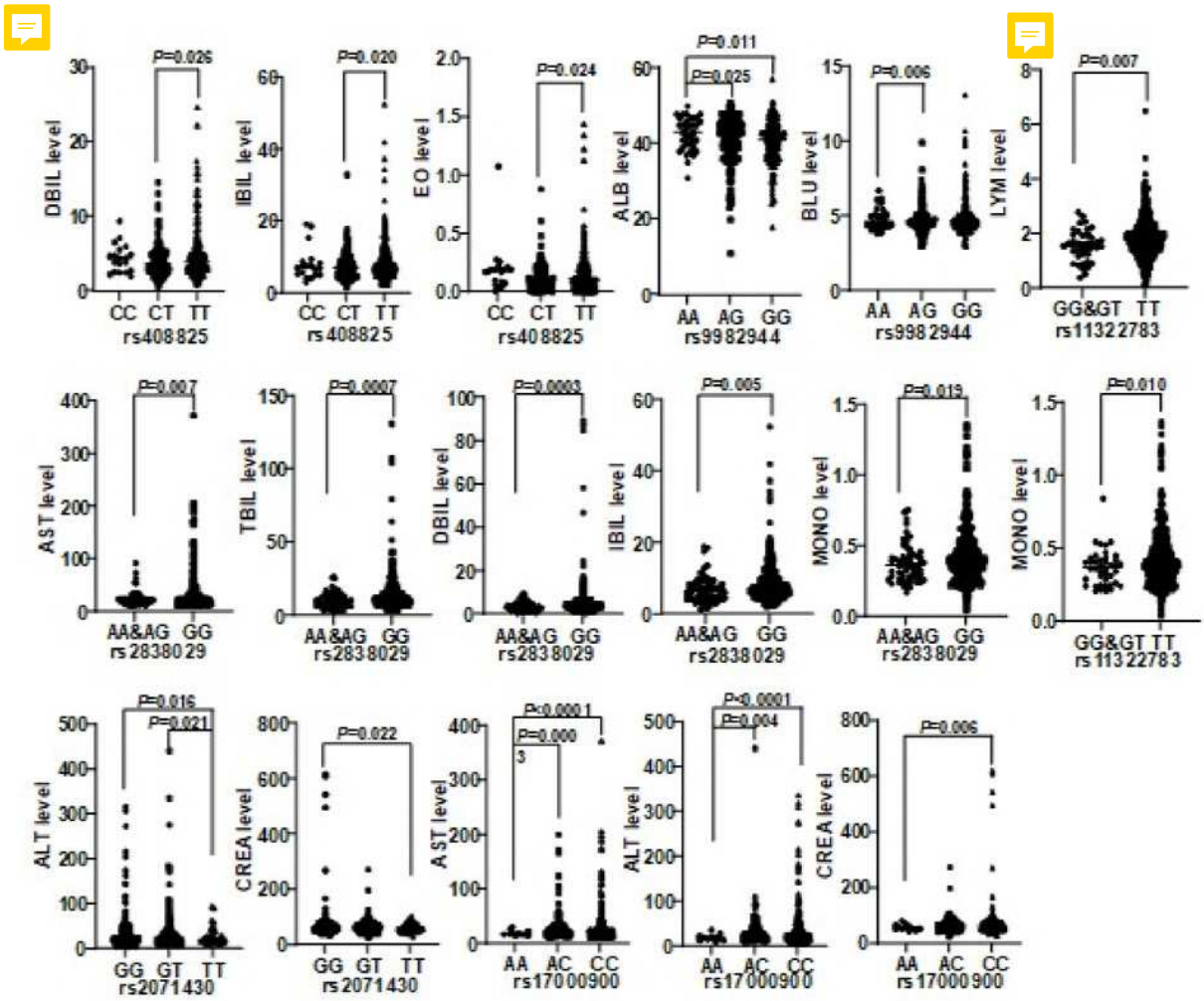

Figure 3

Figure 3. comparison of biochemical indexes between HBsAg-positive and -negative HBV patients with various genotypes of rs17000900.

The AST and TBIL level were significantly higher in patients with HBsAg-positive and genotype AC; The DBIL and IBIL level were significantly higher in patients with HBsAg-positive and genotype CC; The ALB was significantly lower in patients with HBsAg-positive and genotype AC/CC; The WBC and NEUT level were significantly lower in patients with HBsAg-positive and genotype AC.

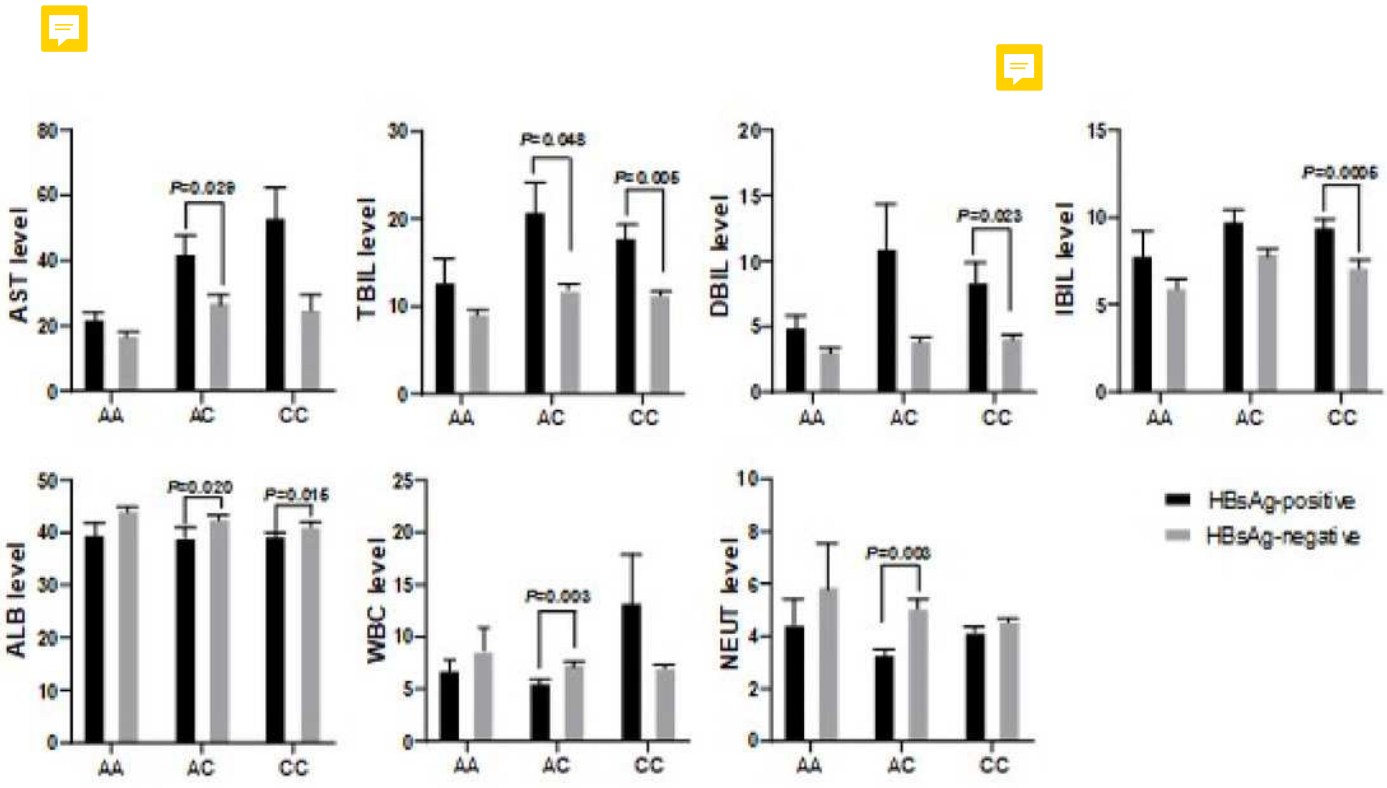

Table 1(on next page)

Table 1. Analysis of biochemical index between HBV infected persons and controls.

Table 1. Analysis of biochemical index between HBV infected persons and controls.

	HBV patients	controls	*P*-value
Gender			
Male (%)	245 (54.69%)	275 (61.38%)	>0.05
Female (%)	203 (45.31%)	173 (38.62%)	
Age	42.12 ± 0.38	40.58 ± 0.53	>0.05
AST (U/L)	37.37 ± 4.07	24.63 ± 0.53	0.002
ALT (U/L)	47.49 ± 7.30	29.00 ± 1.04	0.013
TBIL (μmol/L)	14.78 ± 0.89	11.66 ± 0.28	0.0009
DBIL (μmol/L)	6.40 ± 0.69	3.86 ± 0.09	0.0003
IBIL (μmol/L)	8.39 ± 0.25	7.78 ± 0.19	0.053
TP (g/L)	73.12 ± 0.37	78.57 ± 0.20	< 0.0001
ALB (g/L)	40.78 ± 0.29	47.18 ± 0.13	< 0.0001
GLOB (g/L)	32.25 ± 0.26	31.53 ± 0.18	0.010
GLU (mmol/L)	4.88 ± 0.07	5.34 ± 0.06	< 0.0001
BUN (mmol/L)	4.94 ± 0.13	5.04 ± 0.06	0.491
CREA (μmol/L)	75.86 ± 4.22	71.56 ± 0.79	0.317
UA (μmol/L)	344.6 ± 4.91	352.1 ± 4.59	0.265
WBC (10^9/L)	9.28 ± 2.42	6.70 ± 0.12	0.288
NEUC (10^9/L)	4.41 ± 0.13	3.77 ± 0.06	< 0.0001
LYM (10^9/L)	1.82 ± 0.03	2.25 ± 0.03	< 0.0001
MONO (10^9/L)	0.43 ± 0.01	0.57 ± 0.11	0.199
EO (10^9/L)	0.15 ± 0.01	0.14 ± 0.005	0.361
BASO (10^9/L)	0.03 ± 0.001	0.03 ± 0.001	0.0005

Table 2(on next page)

Table 2. Analysis of genotypes and alleles in the *IFNL4*, *MxA*, and *MxB* genes between HBV infected persons and controls.

Table 2. Analysis of genotypes and alleles in the *IFNL4*, *MxA*, and *MxB* genes between HBV infected persons and controls.

SNP		HBV patients (N= 448)	Controls (N=448)	*P*-value	OR (95% CI)
rs11322783 (*IFNL4*)					
Genotype	ΔG	1	1	0.479	1.000 (0.053-19.04)
	ΔG/T	40	25	0.071	1.659 (1.000-2.812)
	TT	407	422	0.075	0.612 (0.368-1.000)
Allele	ΔG	42	27	0.086	1.583 (0.970-2.566)
	T	854	869		0.632 (0.390-1.031)
rs117648444 (*IFNL4*)					
Genotype	AA	0	0	-	-
	AG	11	7	0.475	1.586 (0.627-4.119)
	GG	437	441	0.475	0.631 (0.243-1.595)
Allele	A	11	7	0.477	1.579 (0.630-4.115)
	G	885	889		0.634 (0.243-1.588)
rs2071430 (*MxA*)					
Genotype	GG	230	217	0.423	1.123 (0.867-1.456)
	GT	168	188	0.195	0.830 (0.632-1.087)
	TT	50	43	0.511	1.183 (0.767-1.801)
Allele	G	628	622	0.797	1.032 (0.844-1.262)
	T	268	274		0.969 (0.792-1.185)
rs17000900 (*MxA*)					
Genotype	AA	12	13	0.999	0.921 (0.414-1.988)
	AC	112	114	0.939	0.977 (0.726-1.314)
	CC	324	321	0.882	1.034 (0.775-1.380)
Allele	A	136	140	0.844	0.966 (0.746-1.250)
	C	760	756		1.035 (0.800-1.340)
rs9982944 (*MxB*)					
Genotype	AA	37	40	0.812	0.918 (0.576-1.452)
	AG	204	222	0.255	0.851 (0.656-1.103)
	GG	207	186	0.178	1.210 (0.930-1.576)
Allele	A	278	302	0.246	0.885 (0.725-1.079)
	G	618	594		1.130 (0.926-1.380)
rs408825 (*MxB*)					
Genotype	CC	19	18	0.999	1.058 (0.560-2.024)
	CT	126	131	0.768	0.947 (0.712-1.260)
	TT	303	299	0.831	1.041 (0.784-1.370)
Allele	C	164	167	0.903	0.978 (0.771-1.240)
	T	732	729		1.022 (0.806-1.297)

rs2838029 (*MxB*)					
Genotype	AA	1	5	0.219	0.198 (0.017-1.434)
	AG	60	58	0.921	1.040 (0.702-1.544)
	GG	387	385	0.923	1.038 (0.712-1.516)
Allele	A	62	68	0.649	0.905 (0.635-1.284)
	G	834	828		1.105 (0.779-1.576)

Table 3(on next page)

Table 3. haplotype analysis constructed by seven SNPs between HBV infected persons and controls.

Table 3. haplotype analysis constructed by seven SNPs between HBV infected persons and controls.

haplotype	HBV patients	controls	*P*-value	OR (95% CI)
TGGCATG	85	97	0.386	0.872 (0.639-1.189)
TGGCGCA	42	54	0.238	0.778 (0.512-1.182)
TGGCGTG	405	378	0.094	1.189 (0.971-1.456)
TGTAATG	68	66	0.822	1.042 (0.730-1.486)
TGTAGTG	34	51	0.065	0.660 (0.423-1.030)
TGTCATG	63	72	0.456	0.874 (0.612-1.246)
TGTCGTG	49	38	0.184	1.344 (0.868-2.081)
others	150	140	-	-

Table 4(on next page)

Table 4. Genotype and allele frequency in patients with HBsAg-positive and -negative.

Table 4. Genotype and allele frequency in patients with HBsAg-positive and -negative.

SNP		HBsAg-positive HBV patients (N= 210)	HBsAg-negative HBV patients (N= 238)	*P*-value	OR (95% CI)
rs11322783					
Genotype	ΔG	0	1	0.999	0.000 (0.000-10.20)
	ΔG/T	16	24	0.455	0.735 (0.369-1.431)
	TT	194	213	0.372	1.423 (0.741-2.819)
Allele	ΔG	16	26	0.313	0.686 (0.359-1.260)
	T	404	450		1.459 (0.793-2.789)
rs117648444					
Genotype	AA	0	0	-	-
	AG	6	5	0.833	1.371 (0.400-3.986)
	GG	204	233	0.833	0.730 (0.251-2.503)
Allele	A	6	5	0.835	1.365 (0.404-3.931)
	G	414	471		0.733 (0.254-2.478
rs2071430					
Genotype	GG	110	120	0.749	1.082 (0.742-1.579)
	GT	75	93	0.525	0.866 (0.588-1.269)
	TT	25	25	0.749	1.151 (0.632-2.098)
Allele	G	295	333	0.985	1.013 (0.760-1.355)
	T	125	143		0.987 (0.738-1.316)
rs17000900					
Genotype	AA	6	6	0.942	1.137 (0.354-3.648)
	AC	43	69	0.049	0.631 (0.407-0.976)
	CC	161	163	0.068	1.512 (0.984-2.318)
Allele	A	55	81	0.124	0.735 (0.507-1.063)
	C	365	395		1.361 (0.941-1.971)
rs9982944					
Genotype	AA	18	19	0.957	1.081 (0.549-2.100)
	AG	94	110	0.831	0.943 (0.644-1.378)
	GG	98	109	0.929	1.036 (0.709-1.513)
Allele	A	130	148	0.978	0.994 (0.748-1.317)
	G	290	328		1.007 (0.759-1.338)
rs408825					
Genotype	CC	9	10	0.849	1.021 (0.422-2.585)
	CT	54	72	0.337	0.798 (0.529-1.217)
	TT	147	156	0.366	1.226 (0.831-1.826)
Allele	C	72	92	0.449	0.864 (0.615-1.219)

	T	348	384		1.158 (0.820-1.625)
rs2838029					
Genotype	AA	0	1	0.950	0.000 (0.000-10.02)
	AG	25	35	0.466	0.784 (0.457-1.348)
	GG	185	202	0.393	1.319 (0.772-2.253)
Allele	A	25	37	0.347	0.751 (0.438-1.270)
	G	395	439		1.332 (0.788-2.285)