

Factors associated with disease progression and viral replication in patients with chronic hepatitis B virus infection

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Abstract. Hepatitis B virus (HBV) infection remains a severe clinical concern in China. Of note, the progression of HBV infection varies between different populations. To identify the factors that influence the disease progression and prognosis, a total of 478 chronic HBV-infected patients were enrolled, and liver function parameters, HBV DNA levels and hepatic fibrosis indices were analyzed. First, the results demonstrated a significant difference in hepatitis B e antigen (HBeAg) expression between male and female patients ($\chi^2=4.061$, $P=0.044$). Furthermore, when comparing either HBeAg-negative or -positive male and female patients, males exhibited a greater variation in HBV DNA levels. Although significant differences between male and female patients in certain abnormal ratios of liver function parameters were identified, a trend in the differences was observed in the HBeAg-negative and -positive groups. When considering age, the results of the present study confirmed that HBV DNA levels decreased with advanced age, and the values of the majority of biomarkers exhibited an evident decreasing trend

with increasing age. In addition, it was demonstrated that all HBeAg seropositive patients had higher levels of hepatic fibrosis indexes and higher abnormal ratios of hepatic fibrosis values in their serum when compared with those of HBeAg seronegative patients, particularly with regard to serum IV collagen. The present results revealed that HBV DNA replication was closely associated with liver function; however, it was notable that in HBeAg-negative patients, the association between HBV DNA levels and liver function was particularly significant among subjects aged <61. Furthermore, this result was not observed in HBeAg-positive patients. In conclusion, the present study indicated the importance of host factors (including sex and age) and viral factors (including HBeAg expression pattern and HBV DNA levels) in the progression of chronic HBV infection, and its influence regarding prognosis and treatment. The present results provide a foundation for clinical management strategies for chronic HBV infection, particularly in individual schemes.

Introduction

Hepatitis B virus (HBV) infection remains a severe clinical issue worldwide, particularly in China (1), despite the number of prophylactic vaccines and effective anti-viral medicines that are available to treat HBV. The social and economic burden remains serious, but no concerted efforts have been made by communities to increase awareness and improve access to care (2-4). In China, the rates of HBV infection vary greatly across different regions and populations (5), and the incurred liver damage also varies in each individual due to physical differences.

HBV leads to a variety of clinical symptoms, ranging from the affected individual being an asymptomatic carrier to hepatocellular carcinoma (HCC). Progression of HBV infection is as a consequence of combined factors, including the host immune response, as well as age, sex, viral genotypes and environmental factors (6-8). According to a statistical survey, male and female patients present with different responses to HBV infection and males have a greater risk of developing HCC than females (9). This may be due to the opposite effects of the sex hormones androgen and estrogen (8). In addition, age appears to serve an important role in predicting significant fibrosis progression in patients and in chronic hepatitis B

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Abbreviations: HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; TP, total protein; ALB, albumin; GLB, globulin; A/G, albumin/globulin; TBIL, total bilirubin; DBIL, direct bilirubin; IBIL, indirect bilirubin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, γ -glutamyl transpeptidase; ALP, alkaline phosphatase; TBA, total biliary acid; HA, hyaluronic acid; LN, laminin; PCIIINP, procollagen III N-terminal peptide; IV-C, serum IV collagen

Key words: hepatitis B, hepatitis B e antigen expression, gender, age, precision medicine

treatment (10,11). It was therefore hypothesized that the host's characteristics may be critical regarding the progression of HBV infection, which should be taken into consideration by physicians when selecting the correct treatment and prevention strategy.

The progression of HBV infection may be detected by histological observation of the accumulation of fibrosis and physiologically by liver function injury (12). Certain studies have demonstrated that liver function markers, including albumin (ALB), bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and AST/ALT exhibited a marked variation among different patients infected with HBV (13). In addition, HBV DNA serves a central role in maintaining persistent infection (14). A number of previous studies have revealed that the HBV DNA levels are associated with the extent of liver damage and liver fibrosis severity (15,16). It may be used as an independent factor to predict the response to anti-viral treatment (17-19). In addition, HBV DNA is a virological marker that reflects HBV replication levels.

Hepatic fibrosis is the pathological change caused by chronic liver damage. It is a necessary developmental stage from chronic liver disease to liver cirrhosis (20). The early stage of hepatic fibrosis is reversible, and therefore the prevention and control of early liver fibrosis are of great significance. Although numerous anti-viral drugs have been introduced to control HBV replication, the subsequent emergence of HBV drug resistance has made it harder to control HBV infection and prevent liver fibrosis (21). The exploration of biomarkers of early liver fibrosis remains a feasible strategy to identify and possibly prevent disease progression in chronic HBV infection in the future.

Hepatitis B e antigen (HBeAg) is a critical indicator to determine the severity of the disease and it is associated with the activity of viral replication and infectivity (22). In addition, HBeAg analysis is useful for predicting seroconversion and disease progression (23). However, how HBeAg expression influences HBV DNA levels, liver function and hepatic fibrosis remains elusive.

The present study investigated the potential role of sex and age in HBV infection depending on different HBeAg statuses, providing a further reference for clinical treatment strategies in different patients. A total of 478 patients were enrolled and were then divided into 2 groups according to their HBeAg serum levels. The serum biomarkers of liver function, HBV DNA levels and hepatic fibrosis indices were then analyzed and compared between different subgroups. In addition, the roles of the HBeAg expression status in HBV infection progression were explored. The correlation between HBeAg expression status and either HBV DNA levels, liver function biomarkers or fibrosis index values were also determined. The results demonstrated that sex, age and HBeAg expression status markedly influenced the progression of the HBV infection as well as the viral load represented by HBV DNA.

Materials and methods

Study population. A total of 478 patients admitted to Gansu Provincial Hospital (Gansu, China) between March 2015 and July 2016 were enrolled in the present study. Participants were permanent residents of Gansu province, recruited from rural

and urban areas. The diagnostic criteria used were based on the Guidelines for the Prevention and Treatment of Chronic Hepatitis B from 2015, which was issued by Chinese Society of Hepatology of Chinese Medical Association and the Society of Infectious Diseases from the Chinese Medical Association (24). The present study was approved by the Ethics Review Committee of Gansu Provincial Hospital (Lanzhou, China) and all patients provided their written informed and consent.

All of the patients exhibited impaired liver function or extremely high viral load and had been serum hepatitis B surface antigen (HBsAg)-positive for >6 months without hepatitis C virus or human immunodeficiency virus coinfection. Patients exhibited no evidence of alcohol abuse at the time of diagnosis and initiation of the study. The baseline values of each patient was collected and analyzed. There were no differences between age/sex either for the duration of treatment or that of infection. However, there may be some errors in data due to patients incorrectly recalling the time of infection. Blood samples were obtained from all of the patients following treatment, and the majority of the enrolled patients had received entecavir (ETV) and/or tenofovir (TDF) therapy, the first-line drugs according to the abovementioned guidelines. The number of patients treated with different drugs to ETV and TDF was relatively small. Demographic characteristics, including gender and age, were recorded during interviews.

Virological and serological marker tests. Quantitation of HBV DNA and 13 liver function biomarkers, as well as determination of the HBsAg and HBeAg status, were performed in all of the patients. The detection of HBV DNA was performed by using real-time fluorescent-quantitative (q)PCR on a Roche Light Cycler 480II (Roche Diagnostics). The HBV DNA PCR-Fluorescence Quantitative Diagnostic kit (Shenzhen Qiagen Bio-Engineering, Shenzhen, China) was used for HBV DNA extraction. The reaction volume was 50 μ l. The thermocycling conditions were as follows: 37°C for 5 min, then 95°C for 5 min, followed by 45 cycles of 95°C for 15 sec and 60°C for 40 sec, and then a final extension at 72°C for 5 min.

Liver function biomarkers, including total protein (TP), ALB, globulin (GLB), total bilirubin (TBIL), direct bilirubin (DBIL), indirect bilirubin (IBIL), ALT, AST, γ -glutamyl transpeptidase (GGT), alkaline phosphatase (ALP) and total biliary acid (TBA) were measured using an Olympus AU5400 auto biochemical analyzer (Olympus Corp.) using the manufacturer's reagents. The ALB vs. GLB ratio (A/G) and AST/ALT were calculated according to the values of ALB, GLB, ALT and AST. The hepatic fibrosis indexes were quantified using chemiluminescence (AutoLumo A2000; AutoBio Diagnostics Co., Ltd.). The experimental methods and reference range of each parameter are summarized in Table I. HBsAg and HBeAg levels were detected by I2000 immunoassay (Abbott Pharmaceutical Co., Ltd.) in accordance with the manufacturer's protocols. All serological markers were tested by the Clinical Laboratory Center of Gansu Provincial Hospital (Lanzhou, China).

Statistical analysis. SPSS statistical software (version 19.0; IBM Corp.) was used for statistical analysis. Descriptive statistics, including the mean, standard deviation, frequency and rate were used. All values are expressed as the mean \pm standard

Table I. Summary of test methods and reference ranges for each parameter.

Parameter	Method	Reference range
TP (g/l)	Biuret method	65.0-85.0
ALB (g/l)	Bromocresol green method	40.0-55.0
TBIL (μ mol/l)	Diazonium salt method	5.1-29.6
DBIL (μ mol/l)	Diazonium salt method	0.0-6.8
ALT (U/l)	Alanine substrate method	9.0-50.0
AST (U/l)	Aspartic acid substrate method	15.0-40.0
GGT (U/l)	GCANA substrate method	10.0-60.0
ALP (U/l)	NPP substrate-AMP buffer method	45.0-125.0
TBA (μ mol/l)	Enzyme colorimetry	0-15.0
HA (ng/ml)	Magnetic particle chemiluminescence	<120.0
LN (ng/ml)	Magnetic particle chemiluminescence	<130.0
PCIIINP (ng/ml)	Magnetic particle chemiluminescence	<15.0
IV-C (ng/ml)	Magnetic particle chemiluminescence	<95.0

TP, total protein; ALB, albumin; TBIL, total bilirubin; DBIL, direct bilirubin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, γ -glutamyl transpeptidase; ALP, alkaline phosphatase; TBA, total biliary acid; HA, hyaluronic acid; LN, laminin; PCIIINP, procollagen III N-terminal peptide; IV-C, serum IV collagen.

deviation. Furthermore, the Chi-square test and independent-samples Student's t-test were used to compare parameters among different groups. Spearman correlation coefficient analysis was employed to explore the correlation between different DNA levels and the patients' liver function. One-way analysis of variance and an independent-samples Student's t-test were used to analyze the differences between two groups following correction via the Bonferroni method in various age groups. The 95% confidence intervals were determined for all results. $P < 0.05$ was considered to indicate a statistically significant difference.

Results

Population characteristics. A total of 478 HBsAg-positive patients were selected for the present study. The baseline demographic data of the cohort were obtained through patient interviews, written records and the hospital's electronic database. Laboratory data were obtained from the database of Gansu Province Hospital (Lanzhou, China). As summarized in Table II, of the 478 patients aged from 4 to 90 years (mean age, 41.01 ± 14.31 years), 254 were male (53.1%) and 224 were female (46.9%). Furthermore, 254 patients (53.1%) were HBeAg-negative and 224 (46.9%) were HBeAg-positive. All patients donated blood samples and had undergone HBV DNA detection. The mean peripheral HBV viral load in \log_{10} was 4.45 ± 1.87 IU/ml. HBV DNA levels were undetectable in 32.6% (156/478) of patients.

The laboratory parameters of all patients and the proportion of patients beyond the normal range were calculated and are presented in Table III. The majority of patients in the present study exhibited reduced liver function and the rates of hepatic fibrosis abnormalities were also lower. Only procollagen III N-terminal peptide (PCIIINP) was observed to be increased in 62.4% of patients infected with HBV.

Table II. Characteristics of the patients with hepatitis B virus (n=478).

Characteristic	Value
Sex	
Male	254 (53.1)
Female	224 (46.9)
Age (years)	41.01 ± 14.31 (4-90)
HBeAg	
Negative	254 (53.1)
Positive	224 (46.9)
HBV-DNA	
Negative	156 (32.6)
Positive	322 (67.4)
(\log_{10} IU/ml)	4.45 ± 1.87 (2.77-8.00)

Values are expressed as either the mean \pm standard deviation (min-max) or n (%). HBeAg, hepatitis B e antigen; HBV, hepatitis B virus.

Clinical measurements based on the expression of HBeAg.

In general, HBeAg is a critical serological marker used to assess the infectivity and prognosis of a patient (23). To investigate whether qualitative HBeAg may serve as a marker to indicate the degree of liver injury in HBV carriers, all of the 478 patients were divided into 2 groups according to their HBeAg status (Table III), and the association between HBeAg and HBV replication levels, as well as parameters of liver function, were analyzed.

First, the population demographics were analyzed based on gender and age. Of note, it was indicated that a larger proportion of male patients were HBeAg-positive when

Table III. Comparison of hepatic fibrosis and liver function parameters between HBeAg-positive and -negative groups.

Parameter	Value	Ratio of patients beyond the normal range (%)	HBeAg-negative (n=254)	HBeAg-positive (n=224)	P-value
Sex ^a					0.044
Male (n=254)	N/A	N/A	124 (49)	130 (51)	
Female (n=224)	N/A	N/A	130 (58)	94 (42)	
Age (years) ^b	N/A	N/A	44.37±14.78	37.21±12.75	<0.001
HBV DNA ^a					<0.001
Negative	N/A	N/A	125 (49.2)	31 (13.8)	
Positive	N/A	N/A	129 (50.8)	193 (86.2)	
(log ₁₀ IU/ml)	N/A	N/A	3.50±1.22	5.53±1.90	<0.001
Liver function parameters ^b					
TP (g/l)	69.07±7.14 (37-86)	22.6	68.26±7.77	70.00±6.24	0.007
ALB (g/l)	42.40±6.54 (4.0-52.8)	29.9	42.46±6.30	42.34±6.82	0.837
GLB (g/l)	26.58±5.01 (15.0-49.5)	8.8	24.33±3.37	28.19±4.89	<0.001
A/G	1.66±0.41 (0.57-3.46)	16.9	1.79±0.36	1.56±0.38	<0.001
TBIL (μmol/l)	25.60±44.41 (4.7-627.2)	17.4	24.52±45.15	26.83±43.62	0.573
DBIL (μmol/l)	8.64±25.54 (0.3-385.2)	23.8	8.25±27.06	9.09±23.74	0.722
IBIL (μmol/l)	16.96±20.69 (2.3-242.0)	13.6	16.27±18.83	17.74±22.63	0.440
ALT (U/l)	83.93±233.27 (7-3404)	34.5	65.73±189.82	104.58±273.35	0.070
AST (U/l)	76.74±225.06 (8-2360)	37.7	55.85±175.61	102.55±272.62	0.081
AST/ALT	1.05±1.01 (0.18-15.1)	NA	1.04±0.68	1.06±1.30	0.801
GGT (U/l)	51.49±73.18 (6.5-718.3)	27.8	41.92±62.20	63.45±83.65	0.012
ALP (U/l)	104.38±65.23 (3-755)	21.4	97.28±43.73	113.21±84.05	0.044
TBA (μmol/l)	15.08±38.30 (0.1-331)	18.8	9.71±21.41	21.74±51.48	0.011
Hepatic fibrosis ^b					
HA (ng/ml)	178.50±171.47 (15-725)	47.1	159.83±143.02	220.32±220.23	0.216
LN (ng/ml)	106.25±69.11 (19-520)	23.5	101.64±51.77	115.64±95.72	0.474
PCIIINP (ng/ml)	58.16±60.04 (5-295)	62.4	57.69±52.44	59.11±74.25	0.919
IV-C (ng/ml)	87.68±67.46 (16-362)	27.1	75.51±52.62	112.46±86.40	0.044

Values are expressed as either the mean ± standard deviation (min-max) or mean ± standard deviation; the limit from min to max was calculated from the enrolled patients' test results. ^aStatistical analysis was performed using Chi-square test, $\chi^2=4.061$ for sex and $\chi^2=67.746$ for HBV DNA; ^bStatistical analysis was performed using independent-samples Student's t-test. TP, total protein; ALB, albumin; GLB, globulin; A/G, albumin/globulin; TBIL, total bilirubin; DBIL, direct bilirubin; IBIL, indirect bilirubin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, γ -glutamyl transpeptidase; ALP, alkaline phosphatase; TBA, total biliary acid; HA, hyaluronic acid; LN, laminin; PCIIINP, procollagen III N-terminal peptide; IV-C, serum IV collagen.

compared with female patients, while more female than male patients were HBeAg-negative ($\chi^2=4.061$, $P=0.044$; Table III). The average age of HBeAg-positive patients was 37.21 ± 12.75 years, which was significantly lower than that in patients with undetectable HBeAg in their peripheral blood ($P<0.05$; Table III). In the HBeAg-positive group, 193 patients (86.2%) were HBV DNA-positive, with log₁₀ values ranging from 2.97 to 8.00 IU/ml (the mean log₁₀ of the viral load was 5.53 ± 1.90 IU/ml). The cutoff for HBV DNA replication was defined as 500 IU/ml in all patients. Only 50.8% of patients had viral replication in the HBeAg-negative group, and the viral load (mean viral load, 3.50 ± 1.22 log₁₀IU/ml) was significantly lower than that in the HBeAg-positive group ($P<0.05$).

Regardless of whether any statistically significant differences were observed, all of the patients with seropositive

HBeAg had increased liver functional proteins and higher levels of hepatic fibrosis indexes in their serum than those in the seronegative HBeAg group, except for ALB and A/G. In particular, the levels of TP, GLB, GGT, ALP and TBA were significantly higher in the HBeAg-positive group compared with those in the HBeAg-negative group ($P<0.05$). In addition, the ratios of patients with abnormal liver function were calculated in the two groups (data not shown), and the proportion of patients with hepatic fibrosis and liver function values above the normal range was higher in the HBeAg-positive group than that in the HBeAg-negative group, following blood tests for ALT, AST, GGT and TBA. However, the abnormality rates of TP, ALB and GLB were much higher in the HBeAg-negative patients compared with those in HBeAg-positive individuals.

Table IV. Differences in liver function markers between males and females in the HBeAg-positive and -negative groups.

Parameter	HBeAg-negative (n=254)			HBeAg-positive (n=224)		
	Males (n=124)	Females (n=130)	P-value	Males (n=130)	Females (n=94)	P-value
Age (years)	44.03±14.55	44.69±15.03	0.723	37.90±11.59	36.24±14.20	0.353
HBV DNA (log ₁₀ IU/ml)	3.68±1.36	3.32±1.04	0.019	5.21±1.88	5.97±1.85	0.003
Liver function parameters						
TP (g/l)	68.03±7.92	68.46±7.65	0.663	69.96±5.69	70.05±6.93	0.918
ALB (g/l)	43.33±6.37	41.64±6.14	0.033	43.15±6.55	41.23±7.05	0.038
GLB (g/l)	24.70±4.65	26.83±4.45	<0.001	26.81±5.41	28.38±4.85	0.027
A/G	1.81±0.42	1.59±0.34	<0.001	1.69±0.44	1.51±0.34	0.001
TBIL (μmol/l)	23.67±21.74	25.32±59.54	0.773	25.82±32.63	28.18±55.27	0.692
DBIL (μmol/l)	7.18±11.52	9.27±36.15	0.540	8.75±17.54	9.54±30.27	0.809
IBIL (μmol/l)	16.49±11.27	16.05±23.95	0.851	17.06±15.63	18.64±29.64	0.609
ALT (U/l)	73.44±131.59	58.42±232.17	0.530	120.90±326.13	82.18±176.34	0.297
AST (U/l)	58.18±187.88	53.84±165.32	0.872	117.13±319.67	85.23±204.36	0.492
AST/ALT	0.86±0.45	1.19±0.80	0.001	0.85±0.44	1.32±1.84	0.031
GGT (U/l)	47.96±40.02	36.58±76.45	0.228	85.28±102.63	37.51±40.46	<0.001
ALP (U/l)	97.04±34.88	97.51±50.58	0.944	126.05±103.63	98.16±49.33	0.051
TBA (μmol/l)	10.81±25.26	8.75±17.48	0.534	24.70±57.58	18.26±43.44	0.467
Hepatic fibrosis						
HA (ng/ml)	171.77±132.36	178.71±158.17	0.888	317.36±340.44	238.56±236.65	0.592
LN (ng/ml)	94.83±50.48	106.24±54.00	0.420	145.18±137.02	96.53±52.32	0.194
PCIIINP (ng/ml)	56.69±43.78	59.16±64.18	0.863	65.36±83.64	61.06±69.89	0.727
IV-C (ng/ml)	71.98±37.00	80.72±70.32	0.588	136.27±84.50	97.06±86.56	0.248

Values are expressed as the mean ± standard deviation. Statistical analysis was performed using independent Student's t-test in the HBeAg-negative and -positive groups. TP, total protein; ALB, albumin; GLB, globulin; A/G, albumin/globulin; TBIL, total bilirubin; DBIL, direct bilirubin; IBIL, indirect bilirubin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, γ-glutamyl transpeptidase; ALP, alkaline phosphatase; TBA, total biliary acid; HA, hyaluronic acid; LN, laminin; PCIIINP, procollagen III N-terminal peptide; IV-C, serum IV collagen; HBeAg, hepatitis B e antigen.

Different responses to chronic HBV infection in female and male patients. The sex disparity of HBV-associated liver diseases has been noted for a number of years (19). This may be due to the different effects of sex hormones (25). In order to evaluate the contribution of sex to the progression of chronic HBV infection, the patients were divided into two groups by gender in the HBeAg seronegative and seropositive groups (Table IV). There was no age difference between males and females in the seropositive and seronegative HBeAg groups (P=0.723 in the HBeAg-negative group and P=0.353 in the HBeAg-positive group). The male patients exhibited increased ALB and A/G levels when compared with females, regardless of their HBeAg status. By contrast, GLB and AST/ALT values were higher in females than in males (GLB, P<0.001 in the HBeAg-negative group and P=0.027 in the HBeAg-positive group; AST/ALT, P=0.001 in the HBeAg-negative group and P=0.031 in the HBeAg-positive group).

Among the HBeAg-negative patients, males had higher HBV DNA copies than females (t=-2.368, P=0.019) and a greater number of male patients had increased abnormal ratios of liver function biomarkers (TP, GLB, A/G, ALT, GGT, TBA, TBIL, DBIL and IBIL) than females (data not shown). By contrast, female patients with seropositive HBeAg had higher HBV DNA

copies than males (t=3.017, P=0.003), and a greater number of male patients had abnormal levels of GLB, A/G, ALT, AST, GGT, ALP and TBA than female patients, even if the differences were not statistically significant (data not shown). Regarding hepatic fibrosis, female patients also had higher levels of the majority of fibrosis indicators than male patients in the HBeAg-negative group. In addition, among the HBeAg-positive patients, males exhibited more severe liver damage than females, as indicated by the levels of hyaluronic acid (HA), laminin (LN), PCIIINP and serum IV collagen (IV-C; Table III).

Age is closely associated with the patients' response to chronic HBV infection. Age has been reported to be an important factor in the progression of chronic HBV infection. The natural course of HBV infection is complex, and is highly influenced by the age at infection (26). Therefore, the present study investigated the HBV clinical virological characteristics and liver function parameters of 478 patients with chronic HBV infection from Gansu province in different age groups (≤30, 31-60 and ≥61 years of age). The majority of patients were aged 31 to 60 years. Within the HBeAg-negative and -positive groups, the difference in levels of certain biomarkers, including HBV DNA, TP, ALB and A/G were significant in

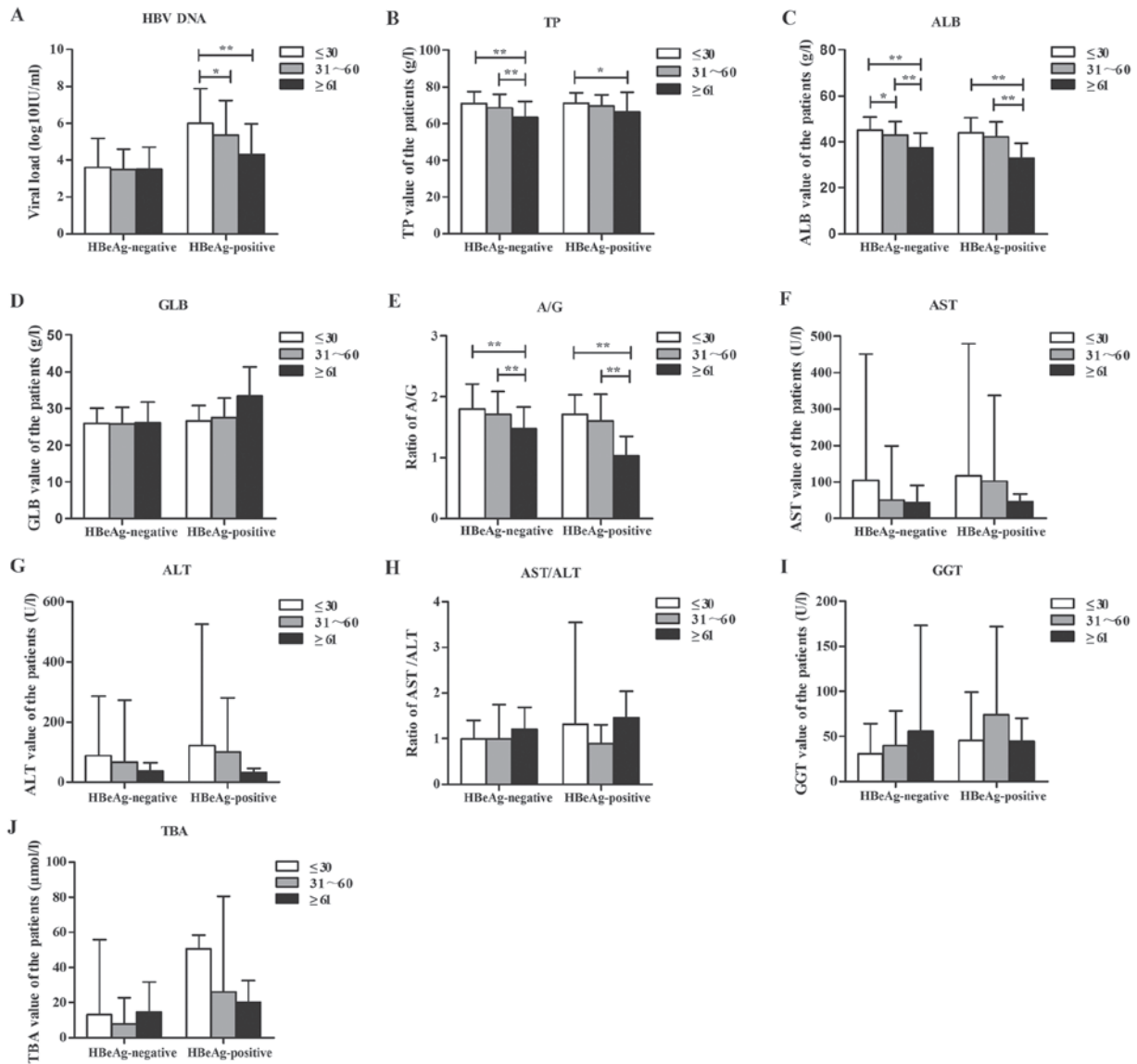


Figure 1. Patients in different age groups exhibit different reactions to HBV infection. A total of 478 patients were divided into 2 groups based on their HBeAg status (negative or positive), which were further subdivided into 3 groups according to their age (≤ 30 , 31~60 or ≥ 61 years). The HBeAg-negative group contained 43, 175 and 36 patients, while the HBeAg-positive group contained 75, 139 and 10 patients in the respective abovementioned age groups. The HBV clinical virological characteristics and liver function indexes were compared among the different age groups of HBeAg-positive and -negative subjects. (A) Viral load of HBV in the different groups. (B-J) Liver function biomarkers, including (B) TP, (C) ALB, (D) GLB, (E) the ratio of A/G, (F) AST, (G) ALT, (H) the ratio of AST/ALT, (I) GGT and (J) TBA. Statistical analysis between different groups was performed using one-way analysis of variance, and an independent Student's t-test was used to analyze the differences between two groups following correction via the Bonferroni method. * $P<0.05$ and ** $P<0.01$. HBV, hepatitis B virus; HBeAg, hepatitis B e antigen; TP, total protein; ALB, albumin; GLB, globulin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, γ -glutamyl transpeptidase; TBA, total biliary acid.

patients <30 years old or between 31-60 years, when compared with those ≥ 61 years. The values of the majority of biomarkers exhibited an evident decreasing trend with increasing age, although the differences were not statistically significant in certain cases, except for GLB (Fig. 1).

DNA replication levels are associated with HBeAg expression. To further identify the association of DNA replication levels with HBeAg expression, the patients were divided into 3 groups ($\leq 5.00 \times 10^2$, $5.01 \times 10^2 - 10^4$ and $10^5 - 1.0 \times 10^8$ IU/ml), according to HBV DNA levels. Low levels of viral load ($5.01 \times 10^2 - 10^4$ IU/ml) were predominantly observed in HBeAg-negative patients who had less viral replication, while patients with seropositive HBeAg had a higher

number of viral copies ($10^5 - 10^8$ IU/ml; $\chi^2=117.302$, $P<0.001$; data not shown). Furthermore, to investigate how different levels of DNA replication affected the patients' liver function, the present study explored the liver function indexes that were closely associated with HBV DNA levels using Spearman correlation coefficient analysis (Table V). The results revealed that, in all of the patients, HBV DNA levels were positively associated with GLB, ALT, AST, GGT and TBA, and negatively associated with A/G and AST/ALT. The patients were then divided into different groups according to sex and age based on the different HBeAg status. The ALT and AST values increased with rising HBV DNA levels in all male and female patients with negative HBeAg status and decreased in female patients with HBeAg positivity. Of

Table V. Correlation of HBV DNA and liver function parameters in all patients.

Liver function parameter	r	P-value
TP	0.078 ^a	0.090
ALB	-0.080 ^b	0.083
GLB	0.161 ^a	<0.001
A/G	-0.130 ^b	0.005
TBIL	0.029 ^a	0.534
DBIL	0.025 ^a	0.589
IBIL	0.036 ^a	0.438
ALT	0.305 ^a	<0.001
AST	0.307 ^a	<0.001
AST/ALT	-0.115 ^b	0.043
GGT	0.166 ^a	0.003
ALP	0.035 ^a	0.537
TBA	0.147 ^a	0.010

Significance was determined using Spearman correlation coefficient analysis (n=478). r is the correlation coefficient of HBV DNA and liver function parameters. ^aPositive correlation between HBV DNA and the respective liver function parameter; ^bNegative correlation between HBV DNA and the respective liver function parameter. TP, total protein; ALB, albumin; GLB, globulin; A/G, albumin/globulin; TBIL, total bilirubin; DBIL, direct bilirubin; IBIL, indirect bilirubin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, γ -glutamyl transpeptidase; ALP, alkaline phosphatase; TBA, total biliary acid.

note, in the HBeAg-negative group, numerous parameters (e.g. TBA), exhibited a positive correlation with HBV DNA levels in female patients, whereas in HBeAg-positive female patients, TBA had a negative association with HBV DNA levels, as presented in Table VI. Finally, the same analysis was also performed for different age groups in the HBeAg seronegative and -positive groups. The results revealed no marked correlation between liver function indexes and HBV DNA when the patients were aged ≥ 61 years (Tables VII and VIII). Furthermore, in HBeAg-negative patients, the liver function parameters were affected the most by the HBV DNA replication levels in the age groups of ≤ 30 and 31-60 years, while this result was not observed in the HBeAg-positive patients.

Discussion

HBV infection has been a great public health concern for a number of years. It may develop into cirrhosis and HCC if it is not treated in a timely and effective manner. The natural course of HBV infection is complex and is highly influenced by the host and virological factors. In the present study, it was observed that HBV progression and liver damage were not only associated with HBeAg expression patterns and HBV activity, but also host characteristics, including gender and age at diagnosis.

HBeAg is a soluble protein in the core particles of HBV, which is processed from the precore protein (27). A number

of studies have reported that HBeAg serves a critical role in chronic HBV infection treatment, prognosis and disease progression (28,29). Patients with seropositive HBeAg have an increased risk to develop cirrhosis and HCC. Therefore, HBeAg has been regarded as an important indicator of disease severity; it also contributes to disease diagnosis and the formulation of treatment strategies. However, the results of the present and other studies have demonstrated that serum HBeAg patterns are not always positively associated with HBV DNA levels (23). To be more precise, several factors, including patients' demographic characteristics, should be considered in the treatment, prognostication and assessment of disease progression. In the present study, gender and age were critical in the diagnosis, selection of therapeutic options and determination of liver injury. Of note, the present results revealed that more male patients expressed HBeAg, while less female patients had detectable HBeAg in the serum. However, females with seropositive HBeAg had higher HBV DNA copies than male patients. It is possible that an unhealthy lifestyle, including smoking (30), alcohol consumption and a nocturnal daily rhythm, which have been noted to be more common in men than in women, may have a negative effect resulting in active hepatitis and the promotion of liver disease progression. Females may present with an increased activity of HBV infection as a result of the different effects of estrogen and androgen on the host's immune response (25,31,32). In addition, males and females exhibited a difference in HBV DNA levels, indicators of hepatic fibrosis and abnormal ratios of liver function parameters in the HBeAg-negative and -positive groups. In the HBeAg-positive group, the differences between male and female patients were much greater regarding HBV DNA levels and liver function parameters. When considering age, the present study confirmed that HBV DNA levels decreased with advanced age, but there was no evident correlation between liver function indexes and HBV DNA in patients aged ≥ 61 years. Certain previous studies have indicated that liver regeneration and cellular maintenance may be impaired with advanced age (33,34). Furthermore, in another study, a senior population exhibited impaired endocytosis of the liver sinusoidal endothelial cells and increased leukocyte adhesion, which may reduce hepatic perfusion (35). All of these factors probably contribute to the changes in liver function in the older population. HBV replication may be restrained at the same time. Therefore, the HBeAg serum level should not be considered as a sole, independent indicator for disease diagnosis and treatment. It is necessary to determine more effective treatment strategies by considering a variety of factors in patients, e.g., gender and age, in addition to DNA replication levels and liver function parameters.

Hepatic fibrosis indexes, including HA, LN, PCIIINP and IV-C, are considered to have a better correlation with liver fibrosis severity when compared with other available tests (35). In the present study, all HBeAg-positive patients had higher levels of hepatic fibrosis indexes and higher abnormal ratios of hepatic fibrosis values in their serum when compared with HBeAg-negative patients, particularly with regard to IV-C. However, gender had no marked effect on liver fibrosis. Thus, HBeAg may be considered as an independent and effective factor for the prognosis and early diagnosis of liver fibrosis.

Table VI. Correlation of HBV DNA and liver function parameters in different groups.

Parameter	HBeAg-negative				HBeAg-positive			
	Males (n=124)		Females (n=130)		Males (n=130)		Females (n=94)	
	r	P-value	r	P-value	r	P-value	r	P-value
TP	-0.120 ^a	0.187	0.169 ^b	0.056	0.118 ^b	0.187	0.026 ^b	0.803
ALB	-0.203 ^a	0.024	0.050 ^b	0.577	-0.136 ^a	0.127	0.067 ^b	0.521
GLB	0.050 ^b	0.583	0.210 ^b	0.017	0.153 ^b	0.085	-0.165 ^a	0.111
A/G	-0.128 ^a	0.157	-0.094 ^a	0.292	-0.159 ^a	0.074	0.172 ^b	0.098
TBIL	-0.153 ^a	0.092	0.180 ^b	0.041	-0.119 ^a	0.184	-0.106 ^a	0.308
DBIL	0.166 ^b	0.066	0.161 ^b	0.069	-0.127 ^a	0.156	-0.228 ^a	0.027
IBIL	0.134 ^b	0.141	0.188 ^b	0.033	-0.088 ^a	0.325	-0.023 ^a	0.826
ALT	0.341 ^b	<0.001	0.300 ^b	0.001	0.319 ^b	<0.001	-0.016 ^a	0.877
AST	0.395 ^b	<0.001	0.317 ^b	0.002	0.337 ^b	0.003	-0.168 ^a	0.186
AST/ALT	0.029 ^b	0.797	-0.149 ^a	0.160	-0.245 ^a	0.033	-0.174 ^a	0.168
GGT	0.116 ^b	0.298	0.192 ^b	0.065	0.281 ^b	0.014	-0.309 ^a	0.013
ALP	0.140 ^b	0.210	0.192 ^b	0.069	-0.007 ^a	0.952	-0.403 ^a	0.001
TBA	0.238 ^b	0.035	0.305 ^b	0.003	-0.071 ^a	0.546	-0.296 ^a	0.018

Significance was determined using Spearman correlation coefficient analysis in each group. r is the correlation coefficient of HBV DNA and liver function parameters. ^aNegative correlation between HBV DNA and the respective liver function parameter; ^bpositive correlation between HBV DNA and the respective liver function parameter. TP, total protein; ALB, albumin; GLB, globulin; A/G, albumin/globulin; TBIL, total bilirubin; DBIL, direct bilirubin; IBIL, indirect bilirubin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, γ -glutamyl transpeptidase; ALP, alkaline phosphatase; TBA, total biliary acid; HBeAg, hepatitis B e antigen.

Table VII. Correlation of HBV DNA and liver function parameters in hepatitis B e antigen-negative patients in different age groups.

Parameters	≤ 30 years (n=43)		31-60 years (n=175)		≥ 61 years (n=36)	
	r	P-value	r	P-value	r	P-value
TP	-0.098 ^a	0.537	0.058 ^b	0.446	0.014 ^b	0.938
ALB	-0.044 ^a	0.056	-0.059 ^a	0.435	-0.212 ^a	0.220
GLB	-0.056 ^a	0.726	0.119 ^b	0.116	0.204 ^b	0.240
A/G	0.091 ^b	0.566	-0.082 ^a	0.283	-0.335 ^a	0.049
TBIL	0.280 ^b	0.260	0.159 ^b	0.035	0.107 ^b	0.540
DBIL	0.280 ^b	0.072	0.173 ^b	0.022	0.003 ^b	0.988
IBIL	0.260 ^b	0.096	0.154 ^b	0.042	0.154 ^b	0.377
ALT	0.385 ^b	0.012	0.318 ^b	<0.001	0.277 ^b	0.102
AST	0.638 ^b	0.001	0.317 ^b	0.001	0.238 ^b	0.169
AST/ALT	0.112 ^b	0.619	-0.113 ^a	0.232	-0.204 ^a	0.239
GGT	0.213 ^b	0.329	0.188 ^b	0.042	0.233 ^b	0.179
ALP	0.476 ^b	0.022	0.187 ^b	0.045	0.008 ^b	0.962
TBA	0.523 ^b	0.010	0.317 ^b	0.001	0.043 ^b	0.808

Significance was determined using Spearman correlation coefficient analysis. r is the correlation coefficient of HBV DNA and liver function parameters. ^aNegative correlation between HBV DNA and the respective liver function parameter; ^bpositive correlation between HBV DNA and the respective liver function parameter. TP, total protein; ALB, albumin; GLB, globulin; A/G, albumin/globulin; TBIL, total bilirubin; DBIL, direct bilirubin; IBIL, indirect bilirubin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, γ -glutamyl transpeptidase; ALP, alkaline phosphatase; TBA, total biliary acid.

HBeAg expression is closely associated with the activity of the virus and may be further influence liver disease progression.

For the majority of the HBeAg seropositive patients of the present study, the liver function values exhibited an increasing

Table VIII. Correlation of HBV DNA and liver function parameters in hepatitis B e antigen-positive patients of different ages.

Parameter	<30 years (n=75)		31-60 years (n=139)		≥61 years (n=10)	
	r	P-value	r	P-value	r	P-value
TP	0.068 ^a	0.569	-0.035 ^b	0.683	-0.153 ^b	0.672
ALB	-0.160 ^b	0.176	-0.199 ^b	0.019	-0.362 ^b	0.304
GLB	0.161 ^a	0.174	0.104 ^a	0.224	-0.399 ^b	0.254
A/G	-0.142 ^b	0.230	-0.166 ^b	0.051	-0.055 ^b	0.880
TBIL	0.062 ^a	0.600	-0.134 ^b	0.116	-0.271 ^b	0.449
DBIL	0.020 ^a	0.866	-0.199 ^b	0.019	-0.431 ^b	0.214
IBIL	0.079 ^a	0.505	-0.085 ^b	0.322	-0.215 ^b	0.551
ALT	0.245 ^a	0.034	0.110 ^a	0.198	0.252 ^a	0.483
AST	-0.017 ^b	0.913	0.207 ^a	0.055	0.239 ^a	0.506
AST/ALT	-0.268 ^b	0.082	-0.004 ^b	0.973	-0.018 ^b	0.960
GGT	-0.214 ^b	0.169	0.070 ^a	0.520	0.632 ^a	0.050
ALP	-0.400 ^b	0.008	-0.166 ^b	0.126	-0.362 ^b	0.304
TBA	0.041 ^a	0.795	-0.121 ^b	0.268	-0.067 ^b	0.853

Significance was determined using Spearman correlation coefficient analysis. r is the correlation coefficient of HBV DNA and liver function parameters. ^aPositive correlation between HBV DNA and the respective liver function parameter; ^bnegative correlation between HBV DNA and the respective liver function parameter. TP, total protein; ALB, albumin; GLB, globulin; A/G, albumin/globulin; TBIL, total bilirubin; DBIL, direct bilirubin; IBIL, indirect bilirubin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, γ -glutamyl transpeptidase; ALP, alkaline phosphatase; TBA, total biliary acid.

trend, regardless of the viral replication levels. However, ~87% of HBeAg-negative patients had low-level viral replication, while the abnormal ratios of TP, ALB and GLB were higher than those in HBeAg-positive patients. This may be due to different host immune responses being activated by a varied viral load (36), and low-level viral replication not activating effective immune responses, and thereby, the virus may not be cleared in time, resulting in more severe injury of liver cells when compared with that in patients with higher replication levels of HBV DNA. In addition, the patients with lower HBV DNA levels had a higher abnormal ratio regarding PCIIINP compared with the patients with higher HBV DNA levels, which was closely associated with the activity of inflammation and the formation of liver fibrosis; the possible reasons may be associated with insufficient immune responses and the lack of effective virus elimination. A larger cohort study is underway to verify this hypothesis.

As a complicated and chronic disease, hepatitis B treatment efficacy is also affected by many additional factors, including the type of drug treatment, duration of drug treatment and the time of hepatitis B infection (37-40). In the present study, perhaps due to the limitation of patient cohort size, no differences between age, sex, duration of treatment or the time of infection were observed between patients. According to the Guidelines for the Prevention and Treatment of Chronic Hepatitis B from 2015 (24), the inpatients were treated with ETV and/or TDF, and only a small number were treated with other drugs, including LAM, FTC, LDT and ADV. Besides the different responses to viral infection, patients may respond differently to the various drugs administered, which may also affect disease progression. Although the current authors detected the gene mutations that cause resistance/sensitization

to different drug treatments within a previous study with more detail, the association between drug treatment and liver function requires further investigation.

In conclusion, the present study explored the importance of host factors, including sex and age, and viral factors, including HBeAg expression pattern and HBV DNA levels, in chronic HBV infection and progression. All of the results provide a foundation for clinical management strategies for HBV infection, promote the future development of precise medicine and be conducive to the selection of individual treatment schemes.

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Availability of data and materials

The datasets used during the present study are available from the corresponding author on reasonable request.

Authors' contributions

JJ performed most of the experiments. YL and CW performed the statistical analysis. RG, HX and YJ contributed to the study design and drafting of the manuscript. YW, YL, ZW, XQ and

ZL performed the qPCR analyses. XG critically revised the manuscript for important intellectual content.

Ethics approval and consent to participate

The present study was approved by the Ethics Review Committee of Gansu Provincial Hospital (Lanzhou, China) and written informed consent was obtained from all patients.

Patient consent for publication

All patients informed and signature for all conditions in informed consent.

Competing interests

The authors declare that they have no competing interests.

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