Virus Research 325 (2023) 199036

Contents lists available at ScienceDirect

Virus Research

journal homepage: www.elsevier.com/locate/virusres

Association of HLADQ-B1 polymorphisms in three generations of chronic hepatitis B patients

Malihe Naderi [a,b], Seyed Masoud Hosseini [a,1,*], Naser Behnampour [c], Iraj Shahramian [d], Abdolvahab Moradi [b,1,**]

[a] Department of Microbiology and Microbial Biotechnology, Faculty of Life Sciences and Biotechnology, Shahid Beheshti University, Tehran, Iran

[b] Department of Microbiology, Faculty of Medicine, Golestan University of Medical Sciences, Gorgan, Iran

[c] Biostatistics and Epidemiology Department, Faculty of Health, Health Management and Social Development Research Center, Golestan University of Medical Sciences, Golestan, Iran

[d] Department of Pediatrics, Faculty of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran

ARTICLE INFO

Keywords:
HLA-DQB1
HBV
Polymorphisms, anti-HCV, anti-HDV

ABSTRACT

The presence of polymorphisms in the human leukocyte antigen (HLA)-DQB1 gene, along with its expression, has been demonstrated to be correlated with spontaneous clearance and susceptibility to HBV infection. The present study aimed to evaluate the possible role of genetic polymorphisms in HLA-DQB1 in three generations of patients with chronic hepatitis B (CHB). Based on the inclusion criteria, 90 CHB patients, 18 individuals recovered from HBV infection, and 40 healthy subjects were chosen. The DNA contents of the whole blood samples were extracted in order to perform HLA-DQB1 typing by the PCR technique. Besides whole blood samples, sera were applied to measure liver function tests (LFTs), as well as the titers of anti-HDV and anti-HCV. Also, in all CHB patients were measured liver stiffness (LSM) by Fibro Scan. The results of HLA-DQB1 polymorphisms (rs2856718 and rs7453920) demonstrated that the majority of polymorphisms in CHB patients were HLA-DQB1*03, HLA-DQB1*05, HLA-DQB1*04:01 and HLA-DQB1*03:01 that associated with HBV persistence and chronicity. Among the patients who showed these polymorphisms, the mean±SD, LSM was 4±1.57 KPa and most of them, F grade was reported as F2, which was a sign of disease progression towards chronicity. HLA polymorphisms imputation revealed that HLA-DQB1*06:04 (3.4%, *P-Value*= 0.2) was detected only in healthy subjects as protective polymorphism, while the allele HLA-DQB1*03:03 was reported in both healthy subjects (*P-Value*= 0.06) and recovered patients (*P-Value*= 0.1) as suppressor of CHB formation. The allele HLA-DQB1*05:02 was found in both healthy subjects (3.4%) and CHB patients (4.5%) which was associated with risk to liver cirrhosis (*P-Value*= 0, OR: 0.002 0.95CI: 0.000-0.15). HLA polymorphism analysis indicated that 17.39% of patients who were seropositive for anti-HCV carried the HLA-DQB1*03:01. HBV resistance or infection risk could be assessed by DBQ1 typing. The existence of polymorphisms in HLA gene could influence the clearance (HLA-DQB1*03:03) or susceptibility and persistence of infection (HLA-DQB1*03, HLA-DQB1*05, HLA-DQB1*04:01 and HLA-DQB1*03:01). These results have the potential to improve personalized therapy and prognosis for HBV infection.

1. Introduction

Infection with the hepatitis B virus (HBV) remains the main cause of liver-related morbidity and death worldwide and a key contributor to hepatocellular carcinoma, liver cirrhosis, and chronic hepatitis (Yengo et al., 2020; Wang et al., 2021; Lavanchy, 2004). As a result, there are large individual differences in the severity of chronic HBV infection, which points to a complicated biological process for which the cellular processes and genetic factors involved in pathogenesis are yet unclear. These data aid in developing personalized treatment, diagnosis, or prognosis, reducing health inequities among patients (Huang et al., 2016; Xu et al., 2018).

* Corresponding author. Seyed Masoud Hosseini: Department of Microbiology and Microbial Biotechnology, Faculty of Life Sciences and Biotechnology, Shahid Beheshti University, Tehran, Iran.

** Corresponding author: Department of Microbiology, Golestan University of Medical Sciences, Gorgan, Iran.

E-mail addresses: ma_Hosseini@sbu.ac.ir (S.M. Hosseini), abmoradi@gmail.com (A. Moradi).

[1] These authors contributed equally to this work and shared the corresponding authorship.

https://doi.org/10.1016/j.virusres.2022.199036
Received 7 December 2022; Received in revised form 28 December 2022; Accepted 29 December 2022
Available online 30 December 2022
0168-1702/© 2023 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

The pathogenic mechanisms used by these viruses to achieve lifetime persistence and avoid immune surveillance in humans remain elusive (Mbarek et al., 2011). From HBV clearance to chronic infection, viral (Kim et al., 2012), host and environmental factors (Thursz, 2011) are taken into account in the clinical course of HBV infection (Jiang et al., 2014; Ochi et al., 2016). Both the susceptibility to HBV and the progress of infection rely on a complicated interplay between environmental, pathogenic, and host genetic factors (Segal & Hill, 2003; Frodsham, 2005). It is believed that a number of host factors, including co-infection with other hepatitis viruses, gender, and age at the time of infection, are capable of influencing viral persistence (McCormack & Boffetta, 2011; Matsuura et al., 2016). Furthermore, genetic components and host immunological elements are thought to be effectively involved in the persistence of chronic HBV infection and susceptibility to HBV (Yan et al., 2012).

Several studies have reported that different variations in the human leukocyte antigen (HLA) class I and class II genes are an essential part of the immune response and an important genetic control gene for immune characteristics involved in the persistence or clearance of HBV (Yan et al., 2012). This is why the outcome of HBV infection is highly dependent on the host's immune response (Godkin et al., 2005; Guo et al., 2011). HLA interacts with molecules and other immunoregulatory cells in a variety of ways to influence the outcome of HBV infection. These interactions may also be population-specific (Yengo et al., 2020; Al-Qahtani et al., 2017). Although genetic variations at multiple HLA loci have been linked to CHB, none of these links have been confirmed satisfactorily (Matsuura et al., 2016). Also, the mechanism of susceptibility to chronic persistent HBV infection is not well clarified (Godkin et al., 2005).

Several polymorphisms in HLA loci, particularly HLA-II (rs2856718 and rs7453920), are linked with spontaneous clearance or susceptibility to HBV infection (Thomas et al., 2012; Wong et al., 2013). It is now known that multiple polymorphisms in the HLA-DQB1 gene are linked with the development of human immunodeficiency virus (HIV) infection, spontaneous clearance of HBV, susceptibility to HCV infection, and other diseases (Li et al., 2017; Waldron et al., 2016; Xiong et al., 2017). Such polymorphisms are encoded by three loci: HLA-DQ, -DP, and –DR (Blackwell et al., 2009). Some alleles of HLA class II have been attributed to HBV infection persistence, such as HLA-DQB1*03:01 (susceptible to chronic HBV infection), HLA-DQA1*05:01, HLA-DQA1*03:02, HLA-DPA1*02:02, and HLA–DPB1*05:01 or to the HBV resistance to therapies, such as HLA-DQB1*06:04 and HLA-DQB1*05:01 (Singh et al., 2007; Lu et al., 2006; Jiang et al., 2003; Kamatani et al., 2009). The current study aimed to look into distinct HLA-DQB1 alleles and their associations with HBV susceptibility and spontaneous clearance in the third and second generations of patients, intra-family members, recovered patients, and healthy subjects.

2. Materials and methods

2.1. Sample collection

Among 4250 patients with CHB who were resident in the North-East region of Iran, based on pre-defined inclusion criteria (Table 1), this cross-sectional study recruited 90 patients with CHB, 18 individuals who recovered from HBV, and 40 healthy subjects between September 2019 and April 2020. The entire experimental procedure of the current research, as well as its purpose, was explained to participants, and then informed consent was obtained from all individuals.

Participants' baseline data, including age, gender, and marital status, were gathered via a standardized questionnaire. Two sets of 5 ml blood specimens were obtained from each participant. One of the two collected samples was utilized to separate sera to detect HBV-related markers using commercial ELISA kits, as well as indices of liver functionality. Another sample was applied to extract the DNA contents of the whole blood specimens to analyze HLA-DQB1 genotypes. Besides, the history of hepatitis B vaccination and the use of antiviral drugs were documented for all subjects.

2.2. Liver Stiffness Measurement (LSM) of FibroScan

In the end of the study, using the FibroScan 502 machine (*EchoSense, Paris, France, and 5 MHz*) and by transient elastography was measured Liver stiffness. The M probe and XL probe were used for subjects with thoracic perimeter less than 110 cm and 110 cm and above, respectively. The probes were placed on the patient's skin, overlying the right lobe of the liver, through the intercostal spaces. For each patient sample, 10 repetitions were performed and their median value was recorded. Valid values are interquartile ranges (IQR) less than 30% of the mean reading.

2.3. HLA genotyping

By means of a DNA extraction kit, 200 µl of the whole blood from each individual was drawn into EDTA-coated tubes. Following the measurement of DNA purity at A260/280 of 1.6–2.0, the DNA concentration was 30 ng/µL. using the PCR, the Olerup SSP® typing kit was employed to identify HLA-DQB1 alleles. PCR was carried out at a volume of 10 µl per well according to the manufacturer's instructions, with 15 wells constructed for each sample (*Applied Biosystems, CA and the USA*). The fragments were then separated using gel electrophoresis using 2% agarose on the amplified DNA products. Each sample's PCR-SSP results were assessed using Score software (*ver.5.00.80.02 T*).

2.4. Statistical analysis

The chi-squared test was used to determine the genotype distribution of the HLA gene in patients with CHB, recovered patients, and healthy

Table 1
Inclusion criteria for participating in the study.

Groups	Number			HBV Serum-Markers				
				HBsAg	HBs-Ab	HBc-Ab	HBe-Ag	HBe-Ab
Patients groups	*Group 1*	Three generations	Grandmother N=10	Positive	Negative	Positive	Positive/ Negative	Positive/ Negative
			Mother N=10	Positive	Negative	Positive	Positive/ Negative	Positive/ Negative
			Offspring N=10	Positive	Negative	Positive	Positive/ Negative	Positive/ Negative
	Group 2	Two generations	Mother N=20	Positive	Negative	Positive	Positive/ Negative	Positive/ Negative
			Child N=20	Positive	Negative	Positive	Positive/ Negative	Positive/Negative
	Group 3	Interfamilial member	N=20	Positive	Negative	Positive	Positive/ Negative	Positive/ Negative
Controls groups	*Group 4*	Recovered patients	N=18	Negative	Positive	Positive	Negative	Positive/ Negative
	Group 5	Healthy control	N= 30	Negative	Negative	Negative	Negative	Negative

individuals. The Hardy–Weinberg equilibrium (HWE) was applied to analyze the correlation between the disease status and the presence of HLA polymorphisms. The p-value of less than 0.05 was statistically considered significant. The statistical analysis was performed with the adjusted odds ratio (OR) at a 95% confidence interval (CI).

3. Results

3.1. Laboratory and demographic parameters of participants

The average age of the participants in this study was 33.35±18.32 years, and 68.8% of them were female. Age was compared among CHB patients, and it was shown that there was a statistically significant difference between age and hepatitis B infection (*P-Value=0.02*) but not between gender and marital status (Table 2). The analysis of liver-specific enzymes indicated that the mean ALP levels were above the standard range (360-80 U/I) in three generations (first group), two generations (second group), and family members, whereas the mean ALT/AST levels were within the normal range (>40 U/I). The examination of the serological markers of CHB patients revealed that HBeAg was reported to be negative in 88.9% of those who were HBsAg positive. In all groups, the mean level of HBc-Ab (>1.1) in mothers and their children was 9.9±3.37 and 10.49±3.30, respectively. As shown in Table 2, grandmothers (13±4.6) and children (10.37±2.33) had higher average titers of HBcAb than in other cases. Besides, the mean titer of HBcAb in mothers and their offspring were determined, and the results revealed that this marker was greater in offspring (10.22±2.73) than in mothers (9.38±3.2). Additionally, the mean HBc-Ab titer differed from the control group in a statistically significant way (*P-Value=0.001*). The control group had the largest percentage of vaccinated individuals (56.7%), followed by the recovered patients (38.9%) and the two-generation CHB group (42.5%), according to an analysis of the history of vaccination among the various groups. According to the testing, none of the five groups' individuals had anti-HIV antibodies. Anti-HCV and anti-HDV serological positivity rates in all subjects were 8% and 11.6%, respectively. As depicted in Table 2, neither anti-HCV nor anti-HDV positive cases were found among the individuals in the control group or those who had recovered. Among grandmothers, mothers, and children in the first group, there were no cases of anti-HCV positivity; however, in the second group, 20% of moms and children, and in the third group, 25% of intra-familial members, there were anti-HCV positive cases. Likewise, there was no statistically significant distinction between hepatitis B and anti-HCV infection (*P-Value*: 0.12). There was a statistically significant discrepancy between anti-HDV and HBV infection, with 6.7% of the first group, 25% of the second group, and 20% of the third group testing positive for hepatitis D antibodies. Table 2 displays the demographic and laboratory data of the patient groups, recovered subjects, and control participants.

3.2. Liver stiffness and its association with HBV infection

Liver stiffness measurement results were available in 48 of the 90 (53.4%) patients with LSM values between 1.2 and 7.7 KPa, within a delay of less than 6 months. Eighteen (37.5%) of the 48 patients had absent/mild fibrosis (METAVIR F0–F1) which, LSM values ranged from 1.2 to 3.2 KPa and, 30 (62.5%) had significant fibrosis (F2) which, LSM ranged from 3.2 to 7.7 KPa in them. There was no report of F3 and F4 stage. Comparing different groups showed that the highest mean LSM was observed in the first group (4.3±1.7 KPa) (Chart 1).

Also, no statistically significant correlation was observed between LSM and different polymorphisms in the three generations *(P-value = 0.4)*, two generations *(P-value = 0.3)*, and intrafamilial members *(P-value = 0.4)*. Among the patients who showed HLA-DQB1*03, HLA-DQB1*05, HLA-DQB1*04:01 and HLA-DQB1*03:01 polymorphisms, the mean±SD, LSM was 4±1.57 KPa and in the most of them; F grade was reported as F2, which was a sign of disease progression towards chronicity. The allele HLA-DQB1*05:02 was found in CHB patients (4.5%), which was associated with progression disease to liver cirrhosis (P-Value= 0, OR: 0.002 0.95CI: 0.000-0.15), and mean Liver stiffness in them was 4.9 KPa (F2 grade). Based on the results, no statistically significant correlation was observed between Liver stiffness and Body mass index (BMI), (*P-value* = 0.4), gender (*P-value*= 0.1) and age (*P-value*= 0.1).

3.3. HLA distribution and its association with HBV infection

15 alleles from the HLA-DQB1 area were examined for each person by PCR-SSP, as seen in Fig. 1. By using a 2% agarose gel electrophoresis picture, HLA-DQB1 alleles were analyzed.

The two-generation group showed the highest prevalence of the HLA-DQB1 allele, particularly in mothers (51.72%). The HLA-DQB1 variants with the highest frequency were HLA-DQB1*05 (72.2%), HLA-DQB1*04:01 (23.4%), HLA-DQB1*03 (43.7%), and HLA-DQB1*06 (30%) among CHB patients. HLA-DQB1*04:01 was different from the control group in a statistically significant way (*P-Value* = 0.03; 0.95CI: 1.87-46.3). As displayed in Table 3, in comparison to recovered patients, the prevalence of HLA-DQB1*05 alleles in CHB patients was statistically significant (*P-Value = 0.05*, 0.95CI: 1-19.8). The Hardy Weinberg equilibrium was used to match the distributions of the HLA-DQB1 alleles in the HBV and healthy control groups. The greatest frequencies identified in the CHB group were HLA-DQB1*05 and HLA-DQB1*06, which were statistically significant in the recovered group (*P-Value* =0.005 and

Table 2
The demographic and laboratory parameters between patient's groups and control.

Variable	Three generations (N=30)	Two generations (N=40)	Interfamilial members (N=20)	Recovered patients (N=18)	Healthy control (N=40)	*P-Value*
Age (years, mean ± SD)	45.23±22.7	35.83±17.6	35.50±15.2	24.83±25.4	29.33±17.1	0.02
Gender (%)	16.7 (male)83.3 (female)	30 (male)70 (female)	40 (male) 60 (female)	33.3 (male) 66.7 (female)	43.3 (male) 56.7 (female)	0.12
Marital status (%)	63.4 (married) 36.6 (single)	65 (married) 35 (single)	40 (married) 60 (single)	22.2 (married) 77.8 (single)	57.5 (married) 42.5 (single)	.007
LSM (KPa)	22.44 ± 6.3	21.85 ± 5.6	18.19 ± 4.4	-	-	0.3
BMI (kg/m^2)	4.26 ± 1.6	3.76 ± 1.43	3.83 ± 0.85	23.56±3.5	26.7±4.5	0.06
Vaccination (%)	30 (n=9)	42.5 (n=17)	30 (n=6)	38.9 (n=7)	56.7 (n=17)	-
ALT (mean ± SD)	24.6±14.3	20.35±16.5	25.80± 10.1	11.39±4.9	24.57±2.4	0.7
AST (mean ± SD)	25.47±13.6	23.52±9.8	23.8±8.9	22.3±7.3	27±16.2	0.1
ALP (mean ± SD)	419.57±25.97	435.80±38.9	550.67±30	316.25±26.73	301.83±26.3	.008
Anti-HCV (%)	0	20	15	0	0	0.12
Anti-HDV (%)	6.7	25	20	0	0	.001

SD – standard deviation; LSM- liver stiffness measurement; BMI- Body Mass Index; ALT – alanine aminotransferase, AST – aspartate aminotransferase; ALP- Alkaline phosphatase.

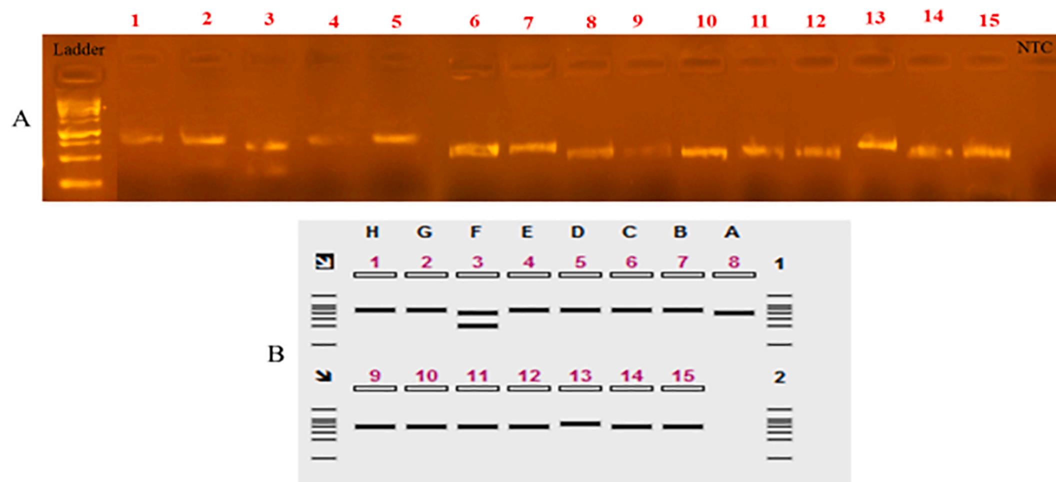

Fig. 1. Image of the PCR-SSP products of the HLA-DQB1 alleles on a 2% agarose gel electrophoresis analyzed by the Score software

Table 3
. The distribution of HLA-DQB1 alleles in HBV- infection patients, recovered individuals, and healthy control groups.

HLA-DQB1	Description	HBV group vs. Healthy controlHBV group Healthy control N=90			N=30 %		*P-value*	0.95 CI	CHB group vs. Recovered patients N=18 %		*P-value*	0.95 CI
		Three generations group N (%)	Two generations group N (%)	Interfamilial members N (%)								
DQB1*05	Was associated with HBV persistence and development of chronic active hepatitis.	23 (76.7)	28 (70)	14 (70)	22	73.4	0.7	0.8-1.2	17	94.5	0.05	1-19.8
DQB1*06	Associated with markedly increased antibody response to hepatitis B vaccine.	8 (26.7)	12 (30)	6 (33.4)	13	43.4	0.1	0.82-3.62	13	72.3	0.001	2-17.4
DQB1*03	Was associated with HBV persistence of HBV and development of chronic active hepatitis.	9 (30)	16 (40)	11 (61.2)	10	33.4	0.7	0.31-1.7	12	66.7	0.06	1.03-8.7
DQB1*02	Correlated with no responsiveness to the vaccine and were related to persistent HBV infections.	5 (16.7)	4 (10)	5 (25)	2	6.7	0.3	0.09-1.95	-	-	0.1	-
DQB1*05:01	Resistance to treatment.	4 (13.4)	3 (7.5)	1 (5)	2	6.7	1	0.16-3.37	1	5.6	1	0.07-5.3
DQB1*04:01	Risk factor for HBV infection susceptibility.	3 (10)	10 (25)	7 (35)	1	3.4	0.03	1.87-46.3	8	44.5	0.09	1.08-7
DQB1*03:01	Related to persistent HBV infections.	2 (6.7)	4 (10)	5 (25)	2	6.7	0.78	0.16-3.91	1	5.6	0.42	0.05-3.49
DQB1*06:04	Protected against HBV infection and Spontaneous HBV clearance.	-	-	-	1	3.4	0.2	-	-	-	-	-
DQB1*06:03	Protected against HBV infection.	-	-	-	2	6.7	0.06	-	-	-	-	-
DQB1*03:03	Correlated with viral clearance and suppression of CHB formation.	-	-	-	2	6.7	0.06	-	1	5.6	0.1	-
DQB1*05:02	Was associated with risk to liver cirrhosis.	1 (3.4)	2 (5)	1 (5)	1	3.4	0	0.000-0.15	-	-	1	-
DQB1*05/ DQB1*05:01	Development of chronic active hepatitis /Resistance to treatment.	4 (13.4)	2 (5)	1 (5)	2	6.7	1	0.17-4.55	1	5.6	1	0.08-6.23
DQB1*05/ DQB1*03	Development of chronic active hepatitis.	3 (10)	9 (22.5)	1 (5)	4	13.4	1	0.29-3.22	1	5.6	0.46	0.04-2.95

0.0001, respectively) when compared to the recovered and control patients. The Hardy Weinberg equilibrium was used to match the distributions of HLA-DQB1 alleles in the HBV and recovered patient groups. The carriers of HLA-DQB1*04:01 in CHB patients were 6.3 times more likely than healthy persons (0.95: 1.87-46.3) and one time more likely than recovered patients (0.95 CI: 1.08-7) as susceptibility factors to HBV infection. Furthermore, the probability of acquiring chronic active HBV and its persistence is 1.2 times greater in CHB patients with HLA-DQB1*05 than in recovered cases (0.95 CI: 1- 19.8), as depicted in Table 3. HLA polymorphisms, on the other hand, revealed that HLA-DQB1*06:04 (3.4%) was identified in just one individual and HLA-DQB1*06:03 (6.7%) in two individuals in the control group. In

addition, compared to the CHB group, the prevalence of HLA-DQB1*06 was highest in control (43.4%) and recovered (72.3%) groups. Between the recovered and CHB groups, there was a statistically significant difference in the frequency of this polymorphism (*P-Value* = *0.001*, 0.95CI: 17.4-2). HLA-DQB1*03:03 was not found in CHB patients and was only found in 5.6% of recovered individuals (N=1) and 6.7% of healthy individuals (N=2), according to the examination of HLA polymorphisms. The control group had the greatest frequency of HLA-DQB1*06, as indicated in Table 3, and there was no statistically significant difference found. Contrarily, this polymorphism occurs often in recovered individuals, which is statistically distinct from CHB patients (*P-Value* = *0.001*). The frequency of the HLA-DQB1*02 allele was higher in CHB patients (17.2%) in comparison to the control (6.7%) and recovered groups; however, such a difference was not statistically significant.

The HLA-DQB1*05:02 was found in 4.5% of CHB patients (one individual in intra-familial members, two mothers in the two-generation group, and one child in the three-generation group) and 3.4% of controls, according to the frequencies presented in Table 3 (*P-Value* = *0.00*, 0.95CI=0.000-0.15). This polymorphism was not detected in those who had recovered.

As displayed in Fig. 2, HLA-DQB1*02, HLA-DQB1*03, HLA-DQB1*04:01, HLA-DQB1*05, and HLA-DQB1*06 (50%), HLA-DQB1*03, HLA-DQB1*04:01, HLA-DQB1*05 and HLA-DQB1*06 (11.2%) were concurrently more common than other investigated groups, according to the analysis of different HLA polymorphisms in recovered patients. The findings of the research revealed that the concurrence of HLA polymorphisms in the five groups tested differed. The Hardy Weinberg equilibrium was used to fit the HLA-DQB1 allele distributions in the HBV and healthy control groups. The prevalence of HLA-DQB1*05 alone (13.4%), HLA-DQB1*05 and HLA-DQB1*05:01 concurrently (13.4%), and HLA-DQB1*05 and HLA-DQB1*06 simultaneously (20%) was greatest in the three-generation group compared to other groups. In addition, HLA-DQB1*05 and HLA-DQB1*03 (22.5%) concurrently had the greatest frequency compared to other groups, according to the analysis of the various polymorphisms, which revealed that HLA-DQB1*05, HLA-DQB1*04:01, and HLA-DQB1*03 (5%) were simultaneously seen exclusively in the two-generation group. Fig. 2 shows that among the five groups examined, HLA-DQB1*05 and HLA-DQB1*03:01 (13.4%) in the healthy control group and HLA-DQB1*05 and HLA-DQB1*02 (10%) in the intra-familial group had the greatest frequency. According to the findings, four families in the two-generation group and six families in the three-generation group had HLA-DQB1 polymorphisms.

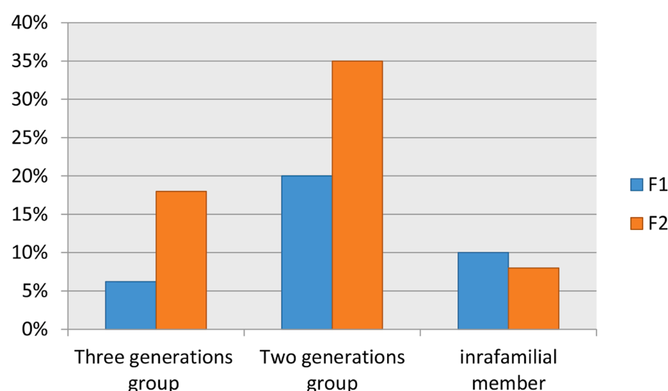

Chart 1. Liver stiffness Results in CHB patients.

Based on the presented results, six families in the first group (three generations) and 4 families in two generations (second group) they had relatively similar polymorphisms in terms of HLA-DQB1.

Analysis of HLA polymorphisms in patients who were positive for anti-HCV revealed that 17.39% of patients had HLA-DQB1*03:01, with no statistically significant difference between patients with and without this polymorphism. Other polymorphisms discovered had no significant impact on disease prognosis or therapy.

4. Discussion

The main agents that control the continuing development of viral diseases include host immunogenetic, viral, and environmental factors (Chisari, 2000). The findings of this study demonstrated that the polymorphisms that are most prevalent in the studied groups—HLA-DQB1*05, HLA-DQB1*03, and HLA-DQB1*03:01—as well as those that are most prevalent in the CHB patient group—are those that increase susceptibility to HBV infection and contribute to the spread of the infection and the emergence of chronic active hepatitis. Different HLA-DQB1 polymorphisms were analyzed in all groups of patients, namely three-generation, two-generation, and intra-familial groups in our study, and HLA-DQB1*05, HLA-DQB1*03, HLA-DQB1*04:01, and HLA-DQB103:01, which are connected to infection susceptibility in three groups of patients, had the highest frequency compared to the other two groups. *Yengo* and colleagues discovered that the likelihood of

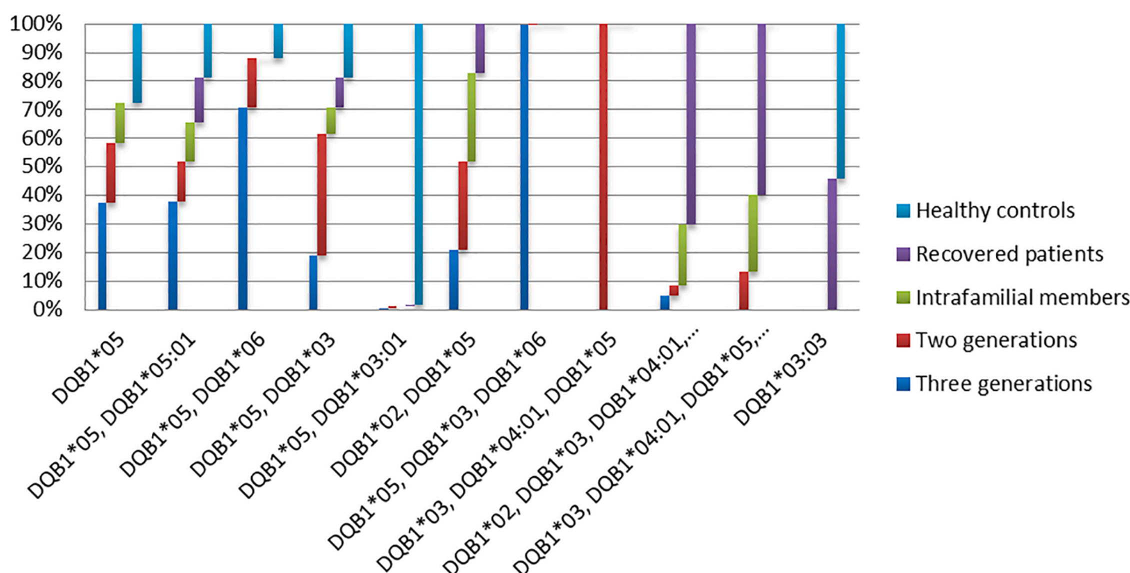

Fig. 2. Concurrent frequency distribution of HLA-DQB1 polymorphisms in various populations.

being an HLA-A*30:01 carrier was six times greater in hepatitis patients than in uninfected controls. Similarly, HLA-C*17:01 carriers were found to be overexpressed in the HBV-infected group compared to the uninfected control group, indicating that this allele may have a role in HBV infection susceptibility (Yengo et al., 2020).

In the study by Ou *et al.*, they discovered that HLA-DQB1*06:03 protected against HBV illness and that the HBV group had greater DQB1 mRNA expression than the healthy control group with HLA-DQB1*05:03 and HLA-DQB1*06:02 (Ou et al., 2018). HLA-DQB1*02:01, HLA-DQB1*03:01, and HLA-DQB1*05:02 polymorphisms were shown to be strongly related to an elevated risk of CHB, according to *Huang et al.* However, polymorphisms at the HLA-DQB1 loci*03:03 and HLA-DQB1 loci *06:04 were linked to a lower incidence of CHB (Huang et al., 2018). The current study's assessment of HLA polymorphisms revealed that among the variants that contribute to protection against HBV infection and spontaneous clearance, HLA-DQB1*06:04 and HLA-DQB1*06:03 were only found in the control group. HLA-DQB1*03:03, which inhibits CHB production, was found in recovered cases as well as controls. The HLA-DQB1*03:01 allele, which is related to chronic HBV infection and persistent HBV infections, was more common in three-generation, two-generation, and intra-familial groups than in recovered individuals and healthy controls; however, such a discrepancy was not statistically significant. These findings provide credence to the idea that HLA-DQB1 allele variation may affect an individual's susceptibility or resistance to CHB infection. *Matei* and colleagues found a strong relationship between HLA-DRB1*03 and HLA-DQB1*05 alleles in infected people, whereas HLA-DRB1*01 was shown to protect against HBV infection (Matei et al., 2018). The current study found that HLA-DQB1*05 and HLA-DQB1*03 were linked to the development of chronic active hepatitis, HLA-DQB1*05:01 led to treatment resistance, and HLA-DQB1*04:01 was a risk factor for HBV infection susceptibility. These polymorphism that mentioned above were more prevalent among patients than among those who have recovered. These findings suggest that alterations in HLA genes might influence patient susceptibility and clearance of HBV infection. Furthermore, frequency analysis in patient groups revealed that HLA-DQB1*05 is the most prevalent variant across three-generation, two-generation, and intra-familial groups, resulting in infection persistence and chronicity. The distribution frequency of HLA-DQB1*02:01 was found to be greater in the recovered group compared to the patient group and higher in the CHB group compared to the chronic HBV carrier group in the research by *Zhang et al.* In comparison to the chronic HBV carrier group, the CHB group had a greater distribution frequency of HLA-DQB1*0301. Other groups' allele frequencies did not differ statistically significantly from one another (Zhang et al., 2015). According to *Akcam et al.*, HLA-DQB1*05 and HLA-DQB1*02 are associated with patients who have chronic HBV infection (Akcam et al., 2002). According to the findings of several HLA polymorphisms, none of the tested groups had HLA-DQB1*02:01. Additionally, a larger number of patients in the patient group carried the HLA-DQB1*02 gene, which is frequently linked to chronic infection and vaccination failure.

Doganay et al. demonstrated that individuals with active illness had higher concentrations of HLA-DQB1*03:01 and HLA-DQB1*05:01 than those with inactive disease (Doganay et al., 2014). According to our study, CHB patients had the greatest prevalence of HLA-DQB1*05:01, which promotes treatment resistance. Additionally, they demonstrated that HLA-DQB1*03:01 has the highest frequency in the control group, which has an impact on hepatitis B infection susceptibility. A growing amount of research demonstrates that particular HLA polymorphism variations are linked to lower or greater antibody responses to hepatitis B vaccinations in different people (Lee et al., 2018). Several investigations on the influence of HLA on the immunological response to hepatitis B vaccinations in healthy people found that HLA-DQB1*06 and HLA-DQB1*06:02 were related with a considerable increase in the antibody response to the hepatitis B vaccine, but HLA-DQB1*02 had the reverse effect (Mert et al., 2014). The outcomes of the aforementioned study were comparable to our findings, and in control and recovered people, vaccination frequency was greater than in patients, which was linked to a considerable rise in antibody response to the hepatitis B vaccine. Additionally, HLA-DQB1*02 was more common in the patient group than it was in the other study populations. HLA-DQB1*02 was associated with persistent HBV infections and non-responsiveness to the vaccination. Therefore, various HLA types should be taken into account when assessing the response to HBV immunization. This study showed a substantial correlation between HBV susceptibility in the CHB group and genetic variants in the HLA-DQ gene. Furthermore, a key result of the current investigation is the connection between HBV viral clearance and SNPs that belong to HLA-DQ variations.

In the study conducted by *Ramezani et al.* on 94 patients with hepatitis B, it was shown that DRB1*13 alleles were significantly related to the outcome of HBV infection. As a result, host HLA polymorphism is an important factor for determining the outcome of HBV infection. HLA-A*33:03 and HLA-DRB1*13:01 are dominant subtypes of HLA-A*33 and HLA-DRB1*13 alleles was found in Iranian patients infected with HBV (Ramezani A., et al. 2009). The results of this study showed that the highest frequency percentage of polymorphisms HLA-DQB1*05, HLA-DQB1*06 and HLA-DQB1*03, which are related to the persistence of infection, had the highest frequency percentage among patients compared to the other two groups.

The study conducted on 50 patients infected with hepatitis B in the northeast of Iran by Baniaghil and his colleagues showed that the frequency of alleles HLA-DQB1*03:01, HLA-DQA1*05:01 and HLA-DQB1*06:04 in the patient group was higher than the control group, while the frequency of HLA-DRB1*13:01, HLA-DRB1*15:01 and HLA-DQB1*04:01 and HLA-DQA1*04:01, HLA-DQA1*01:02 were lower in patients than in the control group. Also, the results of the study reported that in the Iranian Turkmen population, HLA-DRB1*03:01, HLA-DQA1*05:01 and HLA-DQB1*06:04 play an important role in susceptibility to chronic hepatitis B infection, and HLA-DRB1*13:01, HLA-DRB1*15:01, HLA-DQB1*04:01 are associated with protection against chronic hepatitis B infection (Baniaghil S., et al. 2007). The present study, which was conducted in the northeastern region of Iran on 90 patients, 18 people recovered patients and forty healthy people as a control group, and unlike Baniaghil's study, it was found that HLA-DQB1*06:04, which leads to protection against hepatitis B, there was with frequency 4.3% in the control group and HLA-DQB1*04:01 with the highest frequency in the patient groups is considered as a risk factor for hepatitis B.

5. Conclusion

This study for the first time in the three generations group (Grandmother/mother and child) in the Iran population. With the limitation in the study, such as the limited sample size and lacked data on liver stiffness at Grandmothers, which caused limitation in assessing the possible association between the baseline and follow up markers. Finding clinical and genetic indicators to assist in identifying those at increased risk of developing CHB and poorer outcomes, such as hepatocellular carcinoma, is crucial, given the history of the complicated nature of HBV infection. The results of this study demonstrate that HLA gene variants are linked to HBV clearance spontaneously, disease progression, antiviral medication effectiveness, and responsiveness to HBV vaccinations, in addition to vulnerability or resistance to HBV infection. Likewise, various groups may experience varied clinical outcomes from persistent HBV infections depending on certain HLADQB1 variants. The identification of the connection of distinct HLA allele types with disease progression or viral clearance in chronic HBV infections in diverse ethnic communities will require larger-scale examination in controlled clinical studies.

CRediT authorship contribution statement

A.M, SMH, I.SH: Conception and design of the experiments, A.M, NB, MN: data analysis, A.M, SMH, MN: drafting the manuscript, MN: performance of the experiments, A.M, SMH, MN; Reading and confirming the final version of the manuscript, A.M, MN: Revision of the manuscript.

Funding statement

No specific grant was awarded to this research by any funding organization in the public, private, or nonprofit sectors.

Ethical Approval

The study was approved by the Ethics Committee of Golestan University of Medical Sciences (Ethical code: IR. 105. GOUMS.REC.1399).

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The data that has been used is confidential.

Acknowledgments

This study was part of a research project dedicated to achieving the Ph.D. dissertation of Malihe Naderi, who is affiliated with the Golestan University of Medical Sciences and Shahid Beheshti University. The authors thank all individuals who participated in this study.

References

Akcam, Z., Sunbul, M., Durupinar, B., Eroglu, C., Esen, S., Leblebicioglu, H., 2002. Tissue types as prognostic risk factor in hepatitis B virus infection. Indian J Gastroententerol 21 (4), 139–141. PMID: 12385540.

Al-Qahtani, A.A., Al-Anazi, M.R., Nazir, N., Ghai, R., Abdo, A.A., Sanai, F.M., et al., 2017. Hepatitis B virus (HBV) X gene mutations and their association with liver disease progression in HBV-infected patients. Oncotarget 8 (62), 105115–105125. https://doi.org/10.18632/oncotarget.22428.

Baniaghil, S., Sarafnejad, A., Amirzargar, A., Khosravi, F., Ansaripour, B., Moradi, B., et al., 2007. HLA-DRB, DQA and DQB allele frequencies in Iranian patients with chronic hepatitis B by PCR-SSP. Tehran University Medical Journal 64 (11), 11–17 issn: 1683-1764, eissn: 1735-7322.

Blackwell, J.M., Jamieson, S.E., Burgner, D., 2009. HLA and infectious diseases. Clin Microbiol Rev 22 (2), 370–385. https://doi.org/10.1128/CMR.00048-08.

Chisari, F.V., 2000. Viruses, immunity, and cancer: Lessons from hepatitis B. Am J Pathol 156 (4), 1117–1132. https://doi.org/10.1016/s0002-9440(10)64980-2.

Doganay, L., Fejzullahu, A., Katrinli, S., Yilmaz Enc, F., Ozturk, O., Colak, Y., et al., 2014. Association of human leukocyte antigen DQB1 and DRB1 alleles with chronic hepatitis B. World J Gastroenterol 20 (25), 8179–8186. https://doi.org/10.3748/wjg.v20.i25.8179.

Frodsham, A.J., 2005. Host genetics and the outcome of hepatitis B viral infection. Transpl Immunol 14 (3-4), 183–186. https://doi.org/10.1016/j.trim.2005.03.006.

Godkin, A., Davenport, M., Hill, A.V., 2005. Molecular analysis of HLA class II associations with hepatitis B virus clearance and vaccine non-responsiveness. Hepatology 41 (6), 1383–1390. https://doi.org/10.1002/hep.20716.

Guo, X., Zhang, Y., Li, J., Ma, J., Wei, Z., Tan, W., O'Brien, S.J., 2011. Strong influence of human leukocyte antigen (HLA)-DP gene variants on development of persistent chronic hepatitis B virus carriers in the Han Chinese population. Hepatology 53, 422–428. https://doi.org/10.1002/hep.24048.

Huang, J., Xiong, L., Wang, J., Liu, Y., Zhu, Q., Lei, J., Zhou, Z., 2016. Association between the HLA-DQB1 polymorphisms and the susceptibility of chronic hepatitis B: a comprehensive meta-analysis. Biomed Rep 4 (5), 557–566. https://doi.org/10.3892/br.2016.632.

Jiang, Y.G., Wang, Y.M., Liu, T.H., Liu, J., 2003. Association between HLA class II gene and susceptibility or resistance to chronic hepatitis B. World J Gastroenterol 9 (10), 2221–2225. https://doi.org/10.3748/wjg.v9.i10.2221.

Jiang, X., Ma, Y., Cui, W., Li, M.D., 2014. Association of variants in HLA-DP on chromosome 6 with chronic hepatitis B virus infection and related phenotypes. Amino Acids 46 (8), 1819–1826. https://doi.org/10.1007/s00726-014-1767-2.

Kamatani, Y., Wattanapokayakit, S., Ochi, H., Kawaguchi, T., Takahashi, A., et al., 2009. A genome-wide association study identifies variants in the HLA-DP locus associated with chronic hepatitis B in Asians. Nat Genet 41 (5), 591–595. https://doi.org/10.1038/ng.348.

Kim, D.W., Lee, S.A., Hwang, E.S., Kook, Y.H., Kim, B.J., 2012. naturally occurring precore/core region mutations of hepatitis B virus genotype C related to hepatocellular carcinoma. PloS one 7, e47372. https://doi.org/10.1371/journal.pone.0047372.

Lavanchy, D., 2004. Hepatitis B virus epidemiology, disease burden, treatment, and current and emerging prevention and control measures. J Viral Hepat 11 (2), 97–107. https://doi.org/10.1046/j.1365-2893.2003.00487.x.

Li, W., Li, C., Xia, W., Li, X., 2017. HLA-DQB1*06 and breadth of Nef core region-specific T-cell response are associated with slow disease progression in antiretroviral therapy-naive Chinese HIV-1 subtype B patients. Hum Vaccin Immunother 13 (10), 2341–2347. https://doi.org/10.1080/21645515.2017.1340138.

Lu, L.P., Li, X.W., Liu, Y., Sun, G.C., Chen, Z.H., et al., 2006. Association of haplotype formed on HLA-DRB1 and HLA-DQA1 alleles with outcomes of hepatitis B virus infection. Zhonghua Yi Xue Yi Chuan Xue Za Zhi 23, 427–430. PMID: 16883532.

Horea Vladi Matei, Mihaela Laura Vica & Costel Vasile Siserman., 2018. Association between HLA class II alleles and hepatitis B virus infection in Transylvania, Romania. *Immunological Investigations*. 92. 10.1080/088 20139.2018.1489832.

Matsuura, K., Isogawa, M., Tanaka, Y., 2016. Host genetic variants influencing the clinical course of hepatitis B virus infection. J Med Virol 88 (3), 371–379. https://doi.org/10.1002/jmv.24350.

Mbarek, H., Ochi, H., Urabe, Y., Kumar, V., Kubo, M., et al., 2011. A genome-wide association study of chronic hepatitis B identified novel risk locus in a Japanese population. Hum Mol Genet 20 (19), 3884–3892. https://doi.org/10.1093/hmg/ddr301.

McCormack, V.A., Boffetta, P., 2011. Today's lifestyles, tomorrow's cancers: trends in lifestyle risk factors for cancer in low-and middle-income countries. Ann. Oncol. 22 (11), 2349–2357. https://doi.org/10.1093/annonc/mdq763.

Mert, G., Sengul, A., Gul, H.C., Karakas, A., Eyigun, C.P., 2014. The role of human leukocyte antigen tissue groups in hepatitis B virus vaccination in Turkey. Journal of Microbiology, Immunology and Infection 47 (1), 9–14. https://doi.org/10.1016/j.jmii.2013.01.004.

Ochi, Y., Hashimoto, S., Kawabe, N., Murao, M., Nakano, T., 2016. HLA-DQ gene polymorphisms are associated with hepatocellular carcinoma and hepatitis B surface antigen in chronic hepatitis B virus infection. Hepatol Res. https://doi.org/10.1111/hepr.12812.

Ou, G., Xu, H., Yu, H., Liu, X., Yang, L., Ji, X., et al., 2018. The roles of HLA-DQB1 gene polymorphisms in hepatitis B virus infection. J Transl Med 16 (1), 362. https://doi.org/10.1186/s12967-018-1716-z.

Ramezani, A., Eslamifar, A., Aghakhani, A., Kalantar, E., Banifazl, M., Velayati, A., 2009. Association between Hepatitis B Virus Infection Outcome and HLA-A and DRB1 Genotyping in North Part of Iran. Iranian Journal of Pathology 4 (2), 71–74.

Segal, S., Hill, A.V., 2003. Genetic susceptibility to infectious disease. Trends Microbiol 11 (9), 445–448. https://doi.org/10.1016/s0966-842x (03)00207-5.

Singh, R., Kaul, R., Kaul, A., Khan, K., 2007. A comparative review of HLA associations with hepatitis B and C viral infections across global populations. World J Gastroenterol 13 (12), 1770–1787. https://doi.org/10.3748/wjg.v13.i12.1770.

Thomas, R., Thio, C.L., Apps, R., Qi, Y., Gao, X., Marti, D., Stein, J.L., Soderberg, K.A., Moody, M.A., Goedert, J.J., et al., 2012. A novel variant marking HLA-DP expression levels predicts recovery from hepatitis B virus infection. J Virol 86 (12), 6979–6985. https://doi.org/10.1128/JVI.00406-12.

Waldron, P.R., Belitskaya-Levy, I., Chary, A., Won, J., Winters, M., Monto, A., Ryan, J., Lazzeroni, L.C., Holodniy, M., 2016. Genetic variation in the IL-6 and HLA-DQB1genes is associated with spontaneous clearance of hepatitis C virus infection. J Immunol Res, 6530436. https://doi.org/10.1155/2016/6530436.

Wang, W.-C., Lin, Y.-S., Chang, Y.-F., Yeh, C.-C., Su, C.-T., Wu, J.-S., Su, F.-H., 2021. Association of HLA-DPA1, HLA-DPB1, and HLA-DQB1 Alleles with the Long-Term and Booster Immune Responses of Young Adults Vaccinated Against the Hepatitis B Virus as Neonates. Front. Immunol. 12, 710414 https://doi.org/10.3389/fimmu.2021.710414.

Wong, D.K., Watanabe, T., Tanaka, Y., Seto, W.K., Lee, C.K., Fung, J., Lin, C.K., Huang, F. Y., Lai, C.L., Yuen, M.F., 2013. Role of HLA-DP polymorphisms on chronicity and disease activity of hepatitis B infection in Southern Chinese. PLoS ONE 8 (6), e66920. https://doi.org/10.1371/journal.pone.0066920.

Xiong, Y., Wang, L., Mo, P., Huang, G., Li, A., Chai, R., Lin, X., Zhong, Y., Liu, B., Ou, W., et al., 2017. Association between HLA-DQB1 alleles and susceptibility to coronary artery disease in Southern Han Chinese. Hum Immunol 78 (9), 540–546. https://doi.org/10.1016/j.humimm.2017.06.003.

Xu, T., Zhu, A., Sun, M., Lv, J., Qian, Z., Wang, X., Wang, T., Wang, H., 2018. Quantitative assessment of HLA-DQ gene polymorphisms with the development of hepatitis B virus infection, clearance, liver cirrhosis, and hepatocellular carcinoma. Oncotarget 9 (1), 96–109. https://doi.org/10.18632/oncotarget.22941.

Yan, Z.H., Fan, Y., Wang, X.H., Mao, Q., Deng, G.H., Wang, Y.M., 2012. Relationship between HLA-DR gene polymorphisms and outcomes of hepatitis B viral infections: A

meta-analysis. World J Gastroenterol 18 (24), 3119–3128. https://doi.org/10.3748/wjg.v18.i24.3119.

Yengo, C.K., Torimiro, J., Kowo, M., Lebon, P.A., Tiedeu, B.A., Luma, H., et al., 2020. Variation of HLA class I (- A and -C) genes in individuals infected with hepatitis B or hepatitis C virus in Cameroon. Heliyon 6 (10). https://doi.org/10.1016/j.heliyon.2020.

Zhang, Y., Zhao, F., Lan, L., Qin, Z., Jun, L., 2015. Correlation of HLA-DQB1 gene polymorphism of Xinjiang Uygur with outcome of HBV infection. Int J Clin Exp Med 8 (4), 6067–6072. PMID: 26131205.