Communication

Chronic Hepatitis B Virus Infection and HLA Variations in a Greek Population

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Chronic Hepatitis B Virus Infection and HLA Variations in a Greek Population

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Abstract: Chronic hepatitis B is linked with considerable liver-related morbidity and mortality globally. Human leukocyte antigens (HLAs) polymorphisms affect the susceptibility and outcome of many immune-mediated diseases and infections. Our aim was to study HLA alleles' impact on HVB-infected individuals in a Greek population. 107 patients with chronic HBV infection (cHBV group) and 101 with spontaneous clearance (SC-group) of hepatitis B surface antigen (HBsAg) were genotyped for HLA-A, HLA-B, HLA-C, HLA-DRB1, and HLA-DQB1 loci by single-specific primer polymerase chain reaction (PCR-SSP). The HLA alleles' frequencies were compared between the two patients' groups and healthy individuals from the North Greece Bone Marrow Donor Registry (14506 samples). We found a significantly increased frequency of HLA-C*01 and HLA-DRB1*16 alleles in cHBV group versus SC-group. HLA-A*01, HLA-B*08, HLA-C*01, HLA-C*08, HLA-DRB1*03, and HLA-DQB1*05 alleles frequencies were significantly higher in cHBV patients versus healthy individuals, while allele HLA-B*38 frequency was significantly lower. Our study showed an association of specific HLA alleles with either susceptibility or protection against chronic HBV infection.

Keywords: HLA class I alleles; HLA class II alleles; chronic HBV infection; HBV clearance; HBV virus

1. Introduction

Chronic hepatitis B (CHB) is a serious public health problem, affecting 296 million people worldwide [1]. Although hepatitis B virus usually causes an acute and self-limiting disease, 5% of infected adults and up to 95% of individuals infected in early childhood will acquire chronic HBV infection. HBV persistence can lead to liver cirrhosis and even hepatocellular carcinoma (HCC) [2].

Chronic hepatitis B prevalence varies significantly in different geographic regions, with central/east Asia, sub-Saharan Africa, and the Pacific region reporting a prevalence of about 5-8%, in contrast to the United States, where the respective number is close to 0.3% [3]. In Greece, CHB prevalence in the general population is 1,88%, but there are cluster populations that present higher rates[4,5]. Host genetic, viral, socioeconomic, and environmental factors contribute to these regional differences in HBV infection incidence and outcomes[6].

The Major Histocompatibility Complex (MHC) encodes the human leukocyte antigens (HLAs) vital in the immune system's response to different pathogens. HLA class I molecules present endogenous peptides to CD8+ T cells that cytotoxically attack infected cells. In contrast, HLA class II molecules present exogenous antigens leading to the activation of T helper lymphocytes[7]. HLA genes polymorphism at the genome, allele, or haplotype level has been intensively investigated in correlation with various diseases in different populations. According to the literature, several HLA

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polymorphisms have been associated with either susceptibility or protection against HBV virus persistence. Research in this field would provide more insights into disease etiology, risk assessment, and prognosis. The aim of this study was to assess the prevalence of HLA alleles in patients with chronic HBV infection and compare these findings to those of healthy individuals to determine the association between HLA polymorphism and chronic HBV risk[8–10].

2. Materials and Methods

We enrolled patients ≥ 18 years of age diagnosed with HBV infection at the outpatient clinic of Hippokration General Hospital of Thessaloniki. This study was approved by the Institutional Review Board of Hippokration General Hospital (decision No. 29075/20-6-2024). All patients provided written informed consent following the Helsinki Declaration and were subsequently enrolled in the study.

The study population was divided into two groups: a) The **spontaneous clearance (SC) group** included 101 HBV-infected individuals who spontaneously cleared the hepatitis B surface antigen (HBsAg) and acquired natural immunity to the virus. Laboratory workup in the SC group was consistent with negative HBsAg testing, positive testing for the anti-hepatitis B surface antibody (anti-HBs) and anti-hepatitis B core antibody (anti-HBc), and normal liver function biochemical tests values; b) **The chronic HBV (cHBV) group** included 107 individuals with chronic HBV infection. Laboratory workup in the cHBV group consistent with positive HBsAg for at least 6 months before study entry, positive testing for anti-HBc and undetectable anti-hepatitis B core immunoglobulin M (anti-HBc IgM) antibody. Finally, we used **as a control group** adults with a healthy medical record from the Northern Greece Bone Marrow Donor Registry (**14506 samples)**.

Exclusion criteria for this study were infection with other hepatotropic viruses, diagnosis of alcoholic hepatitis, steatohepatitis, autoimmune hepatitis, toxic hepatitis, primary biliary cirrhosis, and non-HBV-related HCC.

We collected peripheral blood samples for DNA extraction using the iPrep™ PureLink™ gDNA Blood kit (Invitrogen, USA). HLA-A, -B, -C, -DRB1, and -DQB1 alleles of study participants were genotyped in low-resolution analyses by single-specific primer polymerase chain reaction (SSP-PCR). Statistical analysis was performed by using R programming language, version 4.2.2.[11] The detected HLA alleles frequencies were compared among the groups of our study with the 2-sided Fisher's exact test. The Haldane-Anscombe correction was used when needed [20]. Results with p-value < 0.05 were considered statistically significant. Odds ratios (OR) with 95% confidence intervals (CI) were calculated to further strengthen the results.

3. Results

Patient demographic characteristics are *shown in* Table 1. In Table 2, we present the HLA analysis using Fisher's exact test assessing the prevalence of HLA alleles between the SC and cHBV group. Results revealed that the prevalence of alleles **HLA-C*01** (8.82% vs 0.91%, P = 0.005 < 0.05, OR = 0.1, 95% CI[0.0-0.62]) and **HLA-DRB1*16** (13.73% vs 7.14%, P = 0.03< 0.05, OR = 0.48, 95% CI[0.23-0.99]) was significantly higher in the cHBV group compared to SC group. No other significant differences regarding the prevalence of HLA alleles were observed between the two groups.

Table 1. Baseline characteristics of patients' groups included in the study.

Variables	cHBV group (n = 107)	SC group (n = 101)
Male, number (%)	86 (80.4)	68 (67.3)
Female, number (%)	21 (19.62)	33 (32.7)
Age (years), mean ± SD	60.6 ± 5.1	60.9 ± 12.0

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Table 2. HLA analysis between patients in the cHBV and SC group.

Allele	Patients in the cHBV group (2N = 214)	Patients in the cHBV group, %	Patients in the SC group (2N = 202)	Patients in the SCgroup, %	P value	OR	95%CI
			HLA – A				
A*01	33	16.18	22	11.11	0.15	0.65	[0.35 - 1.2]
A*02	45	22.06	56	28.28	0.17	1.39	[0.86 - 2.25]
A*03	22	10.78	13	6.57	0.16	0.58	[0.26 - 1.25]
A*11	10	4.9	17	8.59	0.16	1.82	[0.76 - 4.57]
A*23	7	3.43	4	2.02	0.54	0.58	[0.12 - 2.33]
A*24	30	14.71	31	15.66	0.89	1.08	[0.6 - 1.93]
A*25	1	0.49	2	1.01	0.62	2.07	[0.11 - 122.71]
A*26	12	5.88	9	4.55	0.66	0.76	[0.28 - 2.02]
A*29	6	2.94	3	1.52	0.5	0.51	[0.08 - 2.42]
A*30	2	0.98	3	1.52	0.68	1.55	[0.18 - 18.76]
A*31	3	1.47	6	3.03	0.33	2.09	[0.44 - 13.1]
A*32	13	6.37	13	6.57	1	1.03	[0.43 - 2.49]
A*33	5	2.45	3	1.52	0.72	0.61	[0.09 - 3.2]
A*66	1	0.49	3	1.52	0.37	3.12	[0.25 - 164.61]
A*68	11	5.39	11	5.56	1	1.03	[0.4 - 2.7]
			HLA – B				
B*07	6	2.94	4	2.02	0.75	0.68	[0.14 - 2.94]
B*08	17	8.33	7	3.54	0.06	0.41	[0.14 - 1.06]
B*13	4	1.96	4	2.02	1	1.04	[0.19 - 5.65]
B*14	10	4.9	7	3.54	0.62	0.72	[0.23 - 2.13]
B*15	8	3.92	4	2.02	0.38	0.51	[0.11 - 1.94]
B*18	17	8.33	24	12.12	0.25	1.52	[0.76 - 3.13]
B*27	6	2.94	5	2.53	1	0.86	[0.2 - 3.44]
B*35	37	18.14	31	15.66	0.59	0.84	[0.48 - 1.47]
B*37	5	2.45	2	1.01	0.45	0.41	[0.04 - 2.53]
B*38	1	0.49	5	2.53	0.12	5.27	[0.58 - 250.94]
B*39	5	2.45	5	2.53	1	1.04	[0.23 - 4.58]
B*40	9	4.41	14	7.07	0.29	1.66	[0.65 - 4.45]
B*41	4	1.96	4	2.02	1	1.04	[0.19 - 5.65]
B*44	14	6.86	15	7.58	0.85	1.12	[0.49 - 2.58]
B*49	3	1.47	3	1.52	1	1.04	[0.14 - 7.83]
B*50	2	0.98	5	2.53	0.28	2.62	[0.42 - 27.87]
B*51	28	13.73	30	15.15	0.67	1.13	[0.62 - 2.05]
B*52	7	3.43	10	5.05	0.46	1.5	[0.5 - 4.76]
B*55	5	2.45	4	2.02	1	0.83	[0.16 - 3.9]
B*56	2	0.98	1	0.51	1	0.52	[0.01 - 9.99]
B*57	6	2.94	4	2.02	0.75	0.68	[0.14 - 2.94]
B*58	3	1.47	1	0.51	0.62	0.34	[0.01 - 4.31]
B*73	1	0.49	1	0.51	1	1.04	[0.01 - 81.65]
			HLA – C				
C*01	**18**	**8.82**	**1**	**0.91**	**0.005**	**0.1**	**[0 - 0.62]**
C*02	14	6.86	6	5.45	0.81	0.78	[0.24 - 2.25]
C*03	14	6.86	8	7.27	1	1.06	[0.37 - 2.83]
C*04	36	17.65	17	15.45	0.75	0.85	[0.42 - 1.66]
C*05	7	3.43	4	3.64	1	1.06	[0.22 - 4.29]
C*06	16	7.84	9	8.18	1	1.05	[0.39 - 2.62]
C*07	37	18.14	18	16.36	0.76	0.88	[0.45 - 1.7]
C*08	12	5.88	5	4.55	0.8	0.76	[0.2 - 2.4]
C*12	25	12.25	17	15.45	0.49	1.31	[0.63 - 2.67]
C*14	2	0.98	2	1.82	0.61	1.87	[0.13 - 26.08]
C*15	12	5.88	14	12.73	0.05	2.33	[0.96 - 5.74]
C*16	6	2.94	6	5.45	0.36	1.9	[0.49 - 7.3]
C*17	4	1.96	2	1.82	1	0.93	[0.08 - 6.58]
			HLA – DRB1				
DRB1*01	21	10.29	23	11.73	0.75	1.16	[0.59 - 2.29]
DRB1*03	21	10.29	17	8.67	0.61	0.83	[0.4 - 1.71]
DRB1*04	12	5.88	19	9.69	0.19	1.72	[0.77 - 3.99]
DRB1*07	13	6.37	9	4.59	0.51	0.71	[0.26 - 1.84]
DRB1*10	2	0.98	4	2.04	0.44	2.1	[0.3 - 23.47]

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DRB1*11	61	29.9	55	28.06	0.74	0.91	[0.58 - 1.44]
DRB1*12	2	0.98	7	3.57	0.1	3.73	[0.7 - 37.27]
DRB1*13	15	7.35	22	11.22	0.23	1.59	[0.76 - 3.41]
DRB1*14	14	6.86	12	6.12	0.84	0.89	[0.36 - 2.12]
DRB1*15	13	6.37	13	6.63	1	1.04	[0.43 - 2.52]
DRB1*16	**28**	**13.73**	**14**	**7.14**	**0.03**	**0.48**	**[0.23 - 0.99]**
			HLA – DQB1				
DQB1*02	31	15.35	7	11.67	0.54	0.73	[0.26 - 1.82]
DQB1*03	82	40.59	33	55	0.05	1.78	[0.96 - 3.34]
DQB1*05	62	30.69	16	26.67	0.63	0.82	[0.4 - 1.62]
DQB1*06	25	12.38	4	6.67	0.25	0.51	[0.12 - 1.56]

Note**:** statistically significant associations are presented in bold. Abbreviations: cHBV: chronic hepatitis B virus; CI: confidence interval; HLA: human leucocyte antigen; SC: spontaneous clearance

In Table 3, we present the HLA analysis using Fisher's exact test assessing the prevalence of HLA alleles between the cHBV and control group. Results revealed that the prevalence of alleles **HLA-A*01** (16.18% vs 9.7%, P = 5.21E-10 < 0.05, OR = 4.33, 95% CI[2.77-6.64]), **HLA-B*08** (8.33% vs 4.3%, P = 0.009 < 0.05, OR = 2.02, 95% CI[1.15-3.34]), HLA-C*01(8.82% vs 4.2%, P = 0.01 < 0.05, OR = 2.18, 95% CI[1.15-4.0]), **HLA C*08** (5.88% vs 2.2%, P = 0.009 < 0.05, OR = 2.8, 95% CI[1.22-6.12]), **HLA-DRB1*03** (10.29% vs 5.8%, P = 0.01 < 0.05, OR = 1.86, 95% CI[1.12-2.95]), and **HLA-DQB1*05** (30.69% vs 22%, P = 0.04 < 0.05, OR = 1.57, 95% CI[1.01-2.46]) was significantly higher in the cHBV group compared to healthy individuals group. On the contrary, allele **HLA-B*38** (3.4% vs 0.5%, P = 0.017 < 0.05, OR = 0.14, 95% CI[0.0-0.79]), was found in a significantly lower frequency in chronically infected patients versus healthy-individuals group. No other significant differences regarding the prevalence of HLA alleles were found between the two groups.

Table 3. HLA analysis between cHBV and healthy adult group.

Allele	Patients in the cHBV group (2N = 214)	Patients in the cHBV group, %	Healthy adults (2N = 29012)	Healthy adults, %	P value	OR	95%CI
			HLA – A				
A*01	**33**	**16.18**	**2814**	**9.7**	**5.21E-10**	**4.33**	**[2.77 - 6.64]**
A*02	45	22.06	7921	27.3	0.1	0.75	[0.53 - 1.06]
A*03	22	10.78	2698	9.3	0.47	1.18	[0.72 - 1.84]
A*11	10	4.9	2002	6.9	0.33	0.7	[0.33 - 1.31]
A*23	7	3.43	928	3.2	0.84	1.08	[0.43 - 2.27]
A*24	30	14.71	4497	15.5	0.85	0.94	[0.61 - 1.39]
A*26	12	5.88	1625	5.6	0.88	1.05	[0.53 - 1.89]
A*32	13	6.37	1770	6.1	0.88	1.05	[0.55 - 1.84]
A*68	11	5.39	1161	4	0.28	1.37	[0.67 - 2.51]
			HLA – B				
B*07	6	2.94	1230	4.2	0.48	0.69	[0.25 - 1.54]
B*08	**17**	**8.33**	**1259**	**4.3**	**0.009**	**2.02**	**[1.15 - 3.34]**
B*18	17	8.33	3396	11.6	0.19	0.69	[0.39 - 1.14]
B*35	37	18.14	5446	18.6	0.93	0.97	[0.66 - 1.39]
B*38	**1**	**0.49**	**995**	**3.4**	**0.017**	**0.14**	**[0 - 0.79]**
B*40	9	4.41	966	3.3	0.33	1.35	[0.61 - 2.63]
B*44	14	6.86	2196	7.5	0.89	0.91	[0.49 - 1.57]
B*51	28	13.73	4157	14.2	0.92	0.96	[0.62 - 1.44]
			HLA – C				
C*01	**18**	**8.82**	**38**	**4.2**	**0.01**	**2.18**	**[1.15 - 4.0]**

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C*02	14	6.86	58	6.3	0.75	1.09	[0.55 - 2.03]
C*03	14	6.86	46	5.0	0.3	1.39	[0.69 - 2.64]
C*04	36	17.65	156	17.0	0.83	1.04	[0.68 - 1.57]
C*05	7	3.43	20	2.2	0.31	1.59	[0.56 - 3.98]
C*06	16	7.84	88	9.6	0.5	0.8	[0.43 - 1.41]
C*07	37	18.14	164	17.9	0.92	1.02	[0.67 - 1.52]
C*08	**12**	**5.88**	**20**	**2.2**	**0.009**	**2.8**	**[1.22 - 6.12]**
C*12	25	12.25	164	17.9	0.06	0.64	[0.39 - 1.02]
C*14	2	0.98	34	3.7	0.05	0.26	[0.03 - 1.02]
C*15	12	5.88	74	8.1	0.38	0.71	[0.34 - 1.35]
C*16	6	2.94	0	0.0	1	60	[3.37 - 1069.83]
C*17	4	1.96	10	1.1	0.3	1.81	[0.41 - 6.36]
C*18	1	0.49	0	0	1	13.5	[0.55 - 332.86]
			HLA - DRB1				
DRB1*01	21	10.29	1283	6.7	0.05	1.6	[0.96 - 2.53]
DRB1*03	**21**	**10.29**	**1110**	**5.8**	**0.01**	**1.86**	**[1.12 - 2.95]**
DRB1*04	12	5.88	1838	9.6	0.07	0.59	[0.3 - 1.05]
DRB1*07	13	6.37	1513	7.9	0.51	0.79	[0.41 - 1.39]
DRB1*11	61	29.9	5169	27	0.34	1.15	[0.84 - 1.57]
DRB1*13	15	7.35	1895	9.9	0.29	0.72	[0.4 - 1.23]
DRB1*14	14	6.86	1149	6	0.55	1.15	[0.62 - 1.99]
DRB1*15	13	6.37	1455	7.6	0.6	0.83	[0.43 - 1.45]
DRB1*16	28	13.73	2259	11.8	0.38	1.19	[0.77 - 1.78]
			HLA – DQB1				
DQB1*02	31	15.35	28	11.2	0.26	1.41	[0.79 - 2.54]
DQB1*03	82	40.59	0	0	1	0	[0 - Inf]
DQB1*05	**62**	**30.69**	**54**	**22**	**0.04**	**1.57**	**[1.01 - 2.46]**
DQB1*06	25	12.38	30	12.2	1	1.02	[0.55 - 1.86]
DQB1*07	0	0	71	28.8	1	0	[0 - Inf]
DQB1*08	0	0	9	3.8	1	0	[0 - Inf]

Note**:** statistically significant associations are presented in bold. Abbreviations: cHBV: chronic hepatitis B virus; CI: confidence interval; HLA: human leucocyte antigen;.

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4. Discussion

In this study, we compared the prevalence of HLA alleles between patients with chronic HBV infection and natural immunity to HBV, and we further compared HLA alleles distribution between healthy adult individuals and patients with chronic HBV.

4.1. HLA Class I Alleles Associations

Regarding HLA class I associations our results showed that the prevalence of the HLA-C*01 allele was significantly higher in patients with cHBV compared with patients with natural immunity to HBV. Also, compared to healthy adult individuals, patients with cHBV had a significantly higher prevalence of HLA-A*01, HLA-B*08, HLA-C*01, HLA C*08, and a significantly lower prevalence of HLA-B*38.

Other researchers have, also, demonstrated HLA class I alleles association with susceptibility or protection of chronic hepatitis B. Thio *et al.* found that HLA-A*03:01 allele frequency was higher in Caucasian individuals with natural immunity while HLA-B*08 and HLA-B*44 allele frequencies were lower versus patients with persistent HBV infection[12]. Ramezani *et al.* in their research correlated HLA-A*33 with chronic HBV infection[13]. A meta-analysis that included 1652 healthy individuals and 659 chronic HBV patients from different geographic regions showed a protective role of HLA-B*07 and HLA-B*58 against HBV persistence[14]. In previous research of our team, HLA-A*01 and B*57 alleles were significantly associated with chronic HBV complications such as cirrhosis and hepatocellular carcinoma (HBV-HCC) while the HLA-C*15 allele was linked with protection against HBV persistence complications[15].

4.2. HLA Class II Alleles Associations

Regarding HLA class II associations our results showed that the prevalence of HLA-DRB1*16 allele was significantly higher in patients with cHBV compared with patients with natural immunity to HBV. Additionally, HLA-DRB1*03 and HLA-DQB1*05 alleles were found in a significantly higher frequency in cHBV patients versus healthy adults' group.

Höhler et al. study on Caucasian patients demonstrated an association of HLA-DRB1*13:01-02 with the protection of chronic HBV infection [16]. Han et al. HLA-genotyped 769 Han Chinese individuals and showed a higher frequency of HLA-DRB1*09 in chronically hepatitis B infected patients vs healthy subjects, while HLA-DRB1*04 and *13 were found in lower frequency [17]. Jin et al. showed an association of HLA-DRB1*140101 allele with an increased risk of complicated CHB [18]. HLA DRB1*04:03 and HLA-DRB1*15:01 have been linked with antibodies to hepatitis B surface antigen (anti-HBsAg) status[19].

Other studies have shown an association of polymorphisms in the HLA-DQB1*05 locus such as HLA-DQB1*05:02 [20] and HLA-DQB1*05:03 [21] with the risk of chronic HBV infection. Further polymorphisms in other HLA-DQB1 regions, including HLA-DQB1*02, HL-DQB1*03, and HLA-DQB1*06, are also associated with the persistence of HBV virus [22]. Huang et al. meta-analysis including 815 chronically HBV-infected patients and 731 healthy individuals associated HLA-DQB1*02:01, *03:01, and *05:02 alleles with CHB risk[23]. Furthermore, we have found in previous research that the HLA-DQB1*05:01 allele in significantly higher frequency in chronically HBV-infected patients with complications [15].

On the other hand, Tălăngescu et al. [24] found polymorphisms within the HLA-DQB1*01, HLA-DQB1*06, HLA-DQB1*13, and HLA-DQB1*15 regions with a protective role against chronic HBV infection. Also, Naderi et al. [25] revealed a protective role of HLA-DQB1*06:04 polymorphism against viral persistence. In Huang J. et al. meta-analysis HLA-DQB1*0303 and *0604 alleles were also associated with protection against hepatitis B virus persistence [23] Furthermore, HLA-DQB1 alleles have also been associated with response to HBV vaccines [26].

All the above findings show that further low and high-resolution analysis is critical to accurately define the genetic regions that positively or negatively influence viral HBV infection's clinical outcome.

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Our study's limitations included the small sample size and the fact that high-resolution HLA analysis could not be carried out due to limited funding or a shortage of stored DNA samples for further analysis.

5. Conclusions

In conclusion, we showed an association of several HLA class I and II alleles with either higher or lower risk of HBV infection persistence. According to the literature, conflicting data between research in different origin populations are often. The identification of factors that could lead to HBV virus persistence is of major importance since they can play a prognostic role or even contribute to early diagnosis and targeted treatment of patients at risk for chronic HBV infection. Validation of such host genetic polymorphisms associations with HBV infection chronicity should be carried out on further large-scale, and multi-center studies.

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Abbreviations

The following abbreviations are used in this manuscript:

cHBV	Chronic Hepatitis B
HLAs	Human leukocyte antigens
PCR-SSP	Single-specific Primer Polymerase Chain Reaction
MHC	Major Histocompatibility Complex
SC	Spontaneous Clearance
HBsAg	hepatitis B surface antigen
anti-HBs	anti-Hepatitis B surface antibody
anti-HBc	anti-Hepatitis B core antibody
OR	Odds Ratio
HBV-HCC	Hepatitis B virus Hepatocellular Carcinoma

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