ORIGINAL ARTICLE

HLA Gene Polymorphism in Patients with Chronic HBV Infection. Fundeni Clinical Institute Experience

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Abstract. Introduction: *Hepatitis B virus (HBV) infection is a serious health problem for the public health systems in many countries worldwide. According to the European Society for the Liver Study, more than 350 million people are diagnosed with hepatitis B virus infection.* ***Chronic*** *viral HBV infection could be caused by the inability of both the cellular and humoral immune systems to eliminate HBV. HLA genes control cellular and humoral immune responses and present the viral antigens to CD8+ (cytotoxic T cells) and CD4+ T (T helper cells).* ***Aim:*** *To look at the HLA allele polymorphisms in chronic hepatitis B-infected patients to search for significant HLA allele associations.* ***Methods:*** *We have included 240 patients with HBV infection from the Gastroenterology and Hepatology ward, at Fundeni Clinical Institute. As a control group, 300 unrelated healthy people with no hepatitis B infection were also included. We have genotyped the HLA class I and class II genes for both patients and the control group with Next Generation Sequencing Illumina (Immucor, Mia Fora NGS Flex, Norcross, GA, USA).* ***Results:*** *Our preliminary data showed that HLA-DQA1*01:02:02 and HLADRB5*02:02:01 alleles are associated with the risk of HBV infection persistence.* ***Conclusions:*** *Our study showed that a specific HLA genotype profile is associated with chronic HBV infection in our Romanian patients.*

Keywords*: HBV, HLA, NGS Illumina.*

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Abbreviations:
HBV: hepatitis B virus
HLA: human leukocyte antigen
NGS: Next Generation Sequencing

Introduction

Chronic hepatitis B infection represents a global health concern. It affects millions of people annually, leading to significant morbidity and mortality. 14,428 cases of hepatitis B virus (HBV) infection were reported by member states of the European Union in 2020. Excluding the five countries that reported only acute cases, the total number of cases (14,137) corresponds to a rate of 4.2 cases per 100,000 inhabitants [1]. Recent reports indicate that chronic HBV infection is prevalent in approximately 4.4% of adults in Romania, with an estimated number of 650,000 infected individuals. Among those who test positive for HBsAg, it has been found that 23.1% also have anti-HDV IgG antibodies [2].

The course of HBV infection is complex, and the host immune response is critical to eliminating the virus. While most adults recover fully from acute hepatitis B, infants and children are more likely to develop a long-lasting chronic infection. Unfortunately, there is no cure for chronic hepatitis B, but preventive measures, including vaccination, can significantly reduce the risk of transmission and complications. The public health aspects of chronic hepatitis B that are mainly on focus are the potential development of liver cirrhosis and hepatocellular carcinoma [3].

HBV spreads through contact with infected blood, semen, or other body fluids. Common modes of transmission include sexual contact, sharing needles, accidental needle sticks (common among healthcare workers), and mother-to-child transmission during childbirth [4].

Vaccination against hepatitis B virus infection is crucial for prevention. While most adults recover, chronic infections persist in some patients. Early diagnosis and effective treatment strategies are essential for the outcome of the disease [5].

Actual research explores correlations between human leukocyte antigens (HLAs) and HVB infection susceptibility, immune response, and disease severity. These genes play a central role in complex immune system interactions [6]. HLA Class I proteins are essential in immune defense against intracellular pathogens. T cells expressing CD8 molecules interact with class I HLA proteins. These lymphocytes often have a cytotoxic effect, enabling them to recognize and eliminate infected cells. All nucleated cells have class I HLA molecules on their surface; any infected cell could function as an antigen-presenting cell for CD8 T cells [7].

The HLA Class II proteins are mainly located on specific cells that can present antigens, including B cells, dendritic cells, macrophages, Langerhans cells, activated T cells, and thymic epithelium. CD4 T cells are connected to class II HLA molecules and serve as activators for CD8 and B cells [8].

Our main objective was to identify specific HLA alleles associated with chronic HBV infection in our patients.

2. Materials and Methods

Patients

Our work was conducted under the Declaration of Helsinki (2013 version) and was approved by the Commission of Bioethics at the Fundeni Clinical Institute, Romania (No. 28640/25.05.2022). All participants signed an informed consent statement for collecting biological samples and all medical procedures performed during the study. At Fundeni Clinical Institute, we have chosen 247 individuals diagnosed with chronic HBV infection. Positive HBsAg and HBV DNA have characterize this condition for over six months. The patients were admitted to Gastroenterology and Hepatology Clinical Ward. Of the total of 247 patients with chronic hepatitis B, 113 were women aged 23 to 81 and 134 men aged 23 to 83. Patients were included in this study if their age was over 18 years. The diagnosis of chronic HBV infection was based on well-established virological and biochemical parameters. Thus, each patient was routinely analysed for biochemical markers such as alanine aminotransferase (ALT), gamma-glutamyl transpeptidase (GGT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total bilirubin (TB), and direct

bilirubin (DB) using well-established enzymatic methods.

The study employed chemiluminescence to measure virological markers such as HBs antigen (HBsAg), anti-HBs antibodies, HBe antigen (HBeAg), anti-HBe antibodies, anti-HBc antibodies (including total anti-HBc antibodies and anti-HBc IgM), and α-fetoprotein (AFP). The eligibility criteria for the study excluded individuals under 18 years of age and those with co-infections of hepatitis D virus (HDV), hepatitis C virus (HCV), human immunodeficiency virus (HIV), hepatitis A virus (HAV), autoimmune hepatitis (AIH), and other liver diseases to ensure the accuracy of the findings.

Controls

The group designated as the control group for the study was made up of 304 Caucasian individuals who voluntarily donated bone marrow. The group consisted of 118 women between the ages of 19 to 48 and 186 men between the ages of 18 to 50.

Sample Collection and DNA Extraction

The following is a summary of a DNA extraction and HLA genotyping procedure. Blood samples were collected from participants, and DNA was extracted using the QIAmp DNA Blood Mini® kit (QIAGEN, Hilden, Germany) based on a silica membrane method. The extracted DNA was purified, and its concentration and purity were assessed using an IMPLEN nanophotometer.

The HLA genotyping was done using next-generation sequencing reagents provided by Immucor (Mia Fora NGS Flex, Norcross, GA, USA) for 11 loci. After long-range PCR amplification of HLA genes, the PCR products were enzymatically cleaved into fragments and attached to unique barcode sequences. These barcode sequences facilitated the attachment of the fragments to beads and identified samples and loci during the final sequence analysis. The raw data generated by the Illumina MiniSeq Sequencer (Illumina, San Diego, CA, USA) was analyzed using the MIA FORA FLEX version 5.2 alignment software, which generated one- or two-phased consensus sequences.

Statistical Analysis

Microsoft Office Version 365 Excel application was used to calculate the number of HLA alleles in both patient and control groups. The laboratory's internal database was the source of demographic data. To determine the association between HLA alleles and genotypes, we used IBM's SPSS v.20. Our analysis included both confidence intervals and measures of statistical significance. To prevent false associations between HLA allele genes and disease associations, we applied the Bonferroni correction method for p-values.

We reported odds ratios to indicate the magnitude and direction of the association, along with confidence intervals for statistical significance. Each allele has a specific decrease in HBV risk, and all featured alleles indicate reduced risk. The "Estimate (95% CI)" column captures effect size and accuracy, with odds ratios less than one, signifying a protective effect against HBV.

3. Results

In our work, from 247 cases of hepatitis B analyzed, 140 were males and 107 were females. This represented a male-to-female ratio of 1.3:1. The male-to-female ratio was chronic cases (1.3:1). Almost half of all cases were among 25–44-year-olds. The age distributions among reported cases of chronic infections are represented in Figure 1. 7% of chronic cases were among people under 25 years of age.

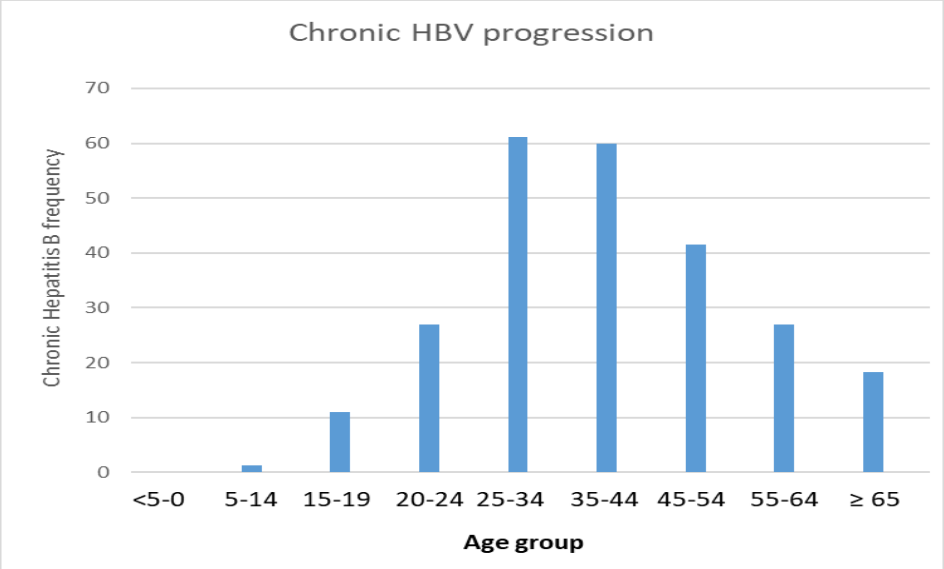

Figure 1. Age distributions among reported cases of chronic infections.

All the patients had anti-HBe antibodies and normal AFP serum values. None of them had anti-HDV antibodies. We have investigated the HLA gene polymorphism by NGS in our chronic hepatitis B patients in comparison with the control samples and we have found certain HLA alleles strongly associated with chronic HBV infection. Thus, our resolution showed that the HLA-DQA1*01:02:02 allele was significantly associated with the disease progression (p = 0.002) with an odds ratio of 2.42 (Figure 2).

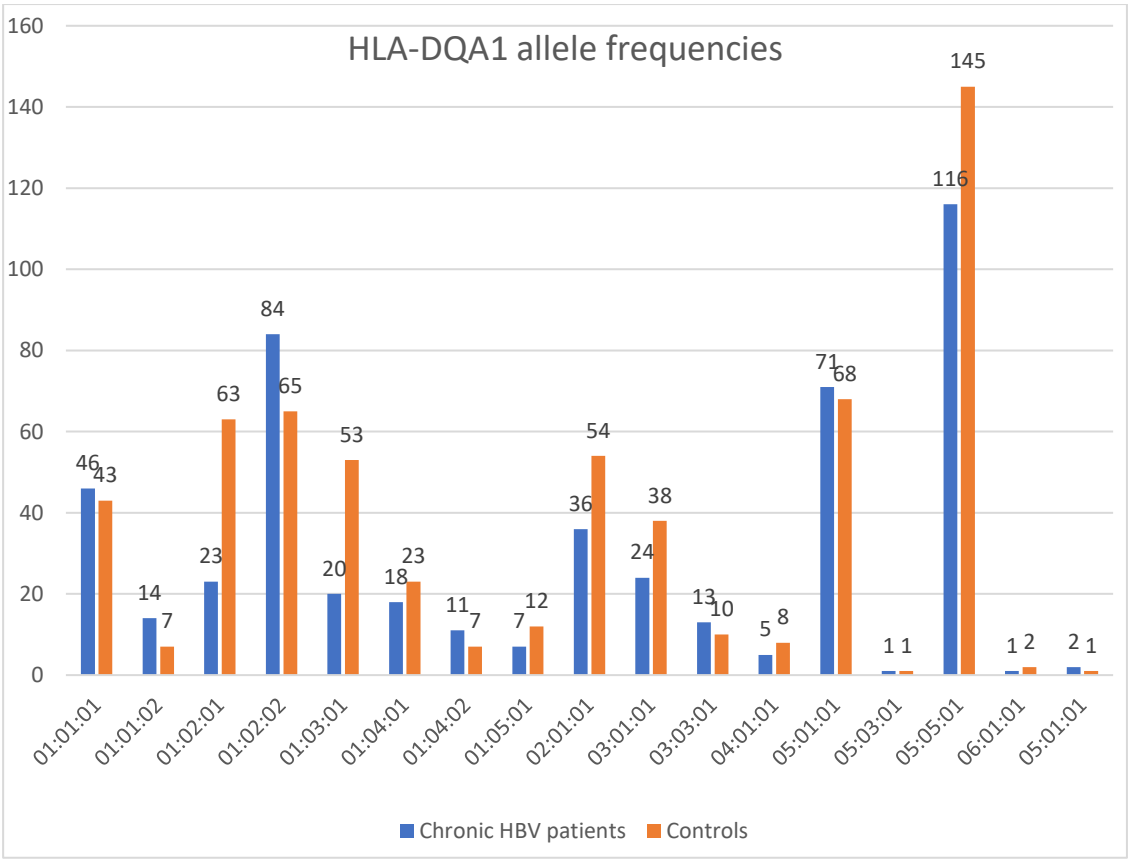

Figure 2. Distribution of HLA DQA1in patients and controls.

HLA DRB5*02:02:01 was also strongly associated with the progression of HBV infection (p-value=0.002) (Figure 3). Both HLA genes maintained their significant association after applying the Bonferroni correction.

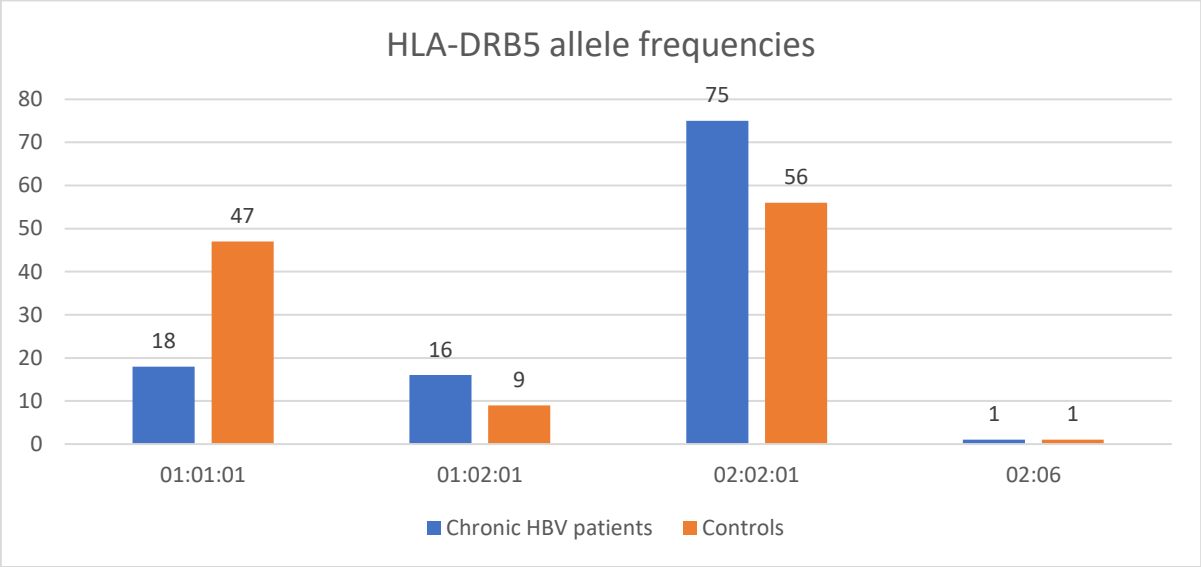

Figure 3. Distribution of HLA DRB5 in patients and controls.

4. Discussion

We conducted a study on Romanian patients with chronic hepatitis B to understand the role of distinct polymorphic variations in the human leukocyte antigen (HLA) class I and II gene clusters in the immunological response. The study used next-generation sequencing (NGS) and successfully achieved three-field HLA for all 11 loci of the HLA genes.

Previous research has shown that certain genetic variations in the HLA loci, especially HLA-II, are connected to the natural clearance of viral infections or immunity against HBV infection. Recent genome-wide association studies (GWAS) have emphasized the impact of genetic variations in HLA genes on HBV infection and disease progression. These associations vary globally across different populations, indicating the complexity of HLA genotypes and their influence on hepatitis outcomes. For instance, specific HLA alleles have distinct effects among Caucasian patients. While HLA-B*08 and HLA-B*44 alleles were associated with increased viral persistence, HLA-B*58, DRB1*13:01, and DRB1*13:02 genotypes provide protective effects against chronic HBV infection [9,10].

In a previous study, our research group found that the most common HLA alleles in the Romanian population were HLA-A*01, HLA-B*18, and HLA-DRB1*01, using a low-resolution typing method [11]. Furthermore, specific HLA haplotypes have been found to correlate with certain pathologies, like celiac disease or end-stage kidney diseases, in the Romanian population. In our Romanian populations, for instance, studies have shown that HLA-DQA1*01:02:02 and HLADRB5*02:02:01 are linked to the progression of hepatitis B. These findings were obtained using NGS technologies, which is a high-resolution method [12,13].

It is possible to explain the different results obtained in this study, when compared to other studies, by taking into account the immunogenetics of different ethnic populations and the sample sizes of patients and controls that were enrolled. Additionally, the use of different genotype technologies that vary in terms of low or high resolution could also result in different outcomes. A study conducted in Transylvania found that certain alleles,

HLA-DRB1*03 and HLA-DQB1*05, were linked to an increased risk of HBV infection. The study used a low-resolution typing methodology [14]. The research methodology employed in this study was low-resolution typing. In Iran, the most common allele found in patients with chronic hepatitis B is HLA-DQB1*04:01 [15].

A study by Baniaghil and colleagues with 50 hepatitis B infected patients, observed distinct allele frequencies. The patients group had higher frequencies of alleles HLA-DQB1*03:01, HLA-DQA1*05:01, and HLA-DQB1*06:04, while the patient group exhibited lower frequencies of alleles HLA-DRB1*13:01, HLA-DRB1*15:01, HLA-DQB1*04:01, HLA-DQA1*04:01, and HLA-DQA1*01:02 [16].

Another study by Yengo and colleagues found that individuals with hepatitis had a sixfold higher likelihood of carrying the HLA-A*30:01 allele compared to uninfected controls. Furthermore, HLA-C*17:01 carriers were overrepresented in the HBV-infected group compared to the uninfected control group, indicating a potential role of this allele in HBV infection susceptibility [17].

5. Conclusions

In conclusion, our work identified using high-resolution methodology, NGS Illumina, two HLA strongly associated alleles with a significant risk effect for chronic HBV infection in Romanian patients. These included HLA-DQA1*01:02:02 and HLADRB5*02:02:01. The importance of these findings is related to the immune response profile against the HBV virus in each patient. Such insights into the immunogenetics of host viral impacts are vital for developing more effective, region-specific strategies for HBV prevention and treatment.

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Compliance with Ethics Requirements: *"The authors declare no conflict of interest regarding this article".*

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