Research Article

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Relationship between cerebrovascular diseases and human leukocyte antigen subgroups

Serebrovasküler hastalıklar ile insan lökosit antijen alt grupları arasındaki ilişki

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Abstract

Purpose: Ischemic stroke is classified as large artery atherosclerosis, small vessel occlusion (lacunar infarcts), ischemic stroke due to other identified causes, and cryptogenic. The human leukocyte antigen (HLA) complex is a group of genes on chromosome six in humans responsible for encoding cell-surface proteins that regulate the immune system. In this study, HLA's roles in the pathophysiology of ischemic stroke, especially the lacunar subgroup, are investigated, and their potential mechanisms are presented.

Materials and methods: This study consisted of 49 patients with ischemic stroke and 50 healthy participants. HLA-A, HLA-B, HLA-C, HLA-DQB, and HLA-DRB subgroup genomes were assessed.

Results: A statistically significant difference in the presence of *HLA-A, HLA-B, HLA-C, HLA-DQB,* and *HLA-DRB* subgroups was found between the control and patient groups. The presence of HLA-A*02, HLA-A*30, *HLA-B*08, HLA-B*15,* and *HLA-DQB*06* genomes was higher in the patient group than in the control group ($p \leq 0.05$). Nevertheless, *HLA-DQB*03* and *HLA-DRB*11* genomes were found more in the control group than the patient group ($p \leq 0.05$)

Conclusion: The results of this study pioneered in scrutinizing HLA alleles in small vascular disease (SVD). *HLA-A*01, HLA-A*30, HLA-B*08, HLA-B*15, HLA-DQB*06, HLA-DQB*03* and *HLA-DRB*11* are associated with HLA alleles of stroke patients with small vessel occlusion. We attempted to provide objective evidence for whether HLA genomes could act as a discriminative factor between SVD patients and control groups, which might hold considerable promise for future therapies.

Key words: Stroke, major histocompatibility complex, genome, small vessel disease, ischemia.

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Öz

Amaç: İskemik inme, büyük arter aterosklerozu, küçük damar tıkanıklığı (laküner enfarktlar), diğer tanımlanmış nedenlere bağlı iskemik inme ve kriptojenik olarak sınıflandırılır. İnsan lökosit antijeni (HLA) kompleksi insanlarda bağışıklık sistemini düzenleyen hücre yüzeyi proteinlerini kodlamaktan sorumlu, altıncı kromozom üzerinde yer alan bir grup gendir. Bu araştırmada, HLA'nın iskemik inme patofizyolojisindeki özellikle laküner alt gruptaki rollerinin araştırılması ve olası mekanizmaların aydınlatması amaçlanmıştır.

Gereç ve yöntem: Bu çalışma iskemik inmeli 49 hasta ve 50 sağlıklı katılımcıdan oluşmaktadır. *HLA-A, HLA-B, HLA-C, HLA-DQB* ve *HLA-DRB* alt grup genomları değerlendirilmiştir.

Bulgular: Kontrol ve hasta gruplarında *HLA-A, HLA-B, HLA-C, HLA-DQB* ve *HLA-DRB* alt gruplarının varlığı arasında istatistiksel olarak anlamlı fark bulunmuştur. *HLA-A*02, HLA-A*30, HLA-B*08, HLA-B*15 ve HLA-DQB*06* genomları hasta grubunda daha fazla bulunmuştur ve istatistiksel olarak anlamlı fark vardır ($p \leq 0.05$). Bununla birlikte *HLA-DQB*03* ve *HLA-DRB*11* genomları kontrol grubunda daha fazla bulunmuştur ($p \leq 0.05$).

Sonuç: Bu araştırma, küçük damar hastalığında (KDH) HLA alellerini incelemede öncüdür. *HLA-A*01, HLA-A*30, HLA-B*08, HLA-B*15, HLA-DQB*06, HLA-DQB*03, HLA-DRB*11,* inme hastalarının HLA alelleri ile ilişkilidir. Küçük damar tıkanıklığı HLA genomlarının, KDH hastaları ve kontrol grupları arasında, gelecekteki tedaviler için umut vaat edebilecek, ayırt edici bir faktör olarak hareket edip edemeyeceğine dair nesnel kanıtlar sağlamaya çalıştık.

Anahtar kelimeler: Strok, major doku uygunluk kompleksi, genom, küçük damar hastalığı, iskemi.

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Introduction

Stroke is a significant health problem and the second most common worldwide mortality cause. It accounts for more than 50% of neurological diseases requiring hospital treatment. According to the 1993 TOAST (Trial of Org 10172 in Acute Stroke Treatment Study), ischemic stroke causes are classified as large artery atherosclerosis (LAA), SVD (lacunar strokes), ischemic stroke due to other determined causes, and cryptogenic ones. The most prevalent subtype is ischemic stroke, and the second is hemorrhagic [1]. A hospital-based, multicenter study investigating the general characteristics and risk factors of stroke patients in Türkiye found that ischemic stroke was 72% and hemorrhagic stroke was 28% [2].

Our study found a statistically significant difference between the control and patient groups in the presence of *HLA-A, HLA-B, HLA-C, HLA-DQB,* and *HLA-DRB* subgroups. *HLA-A*02, HLA-A*30, HLA-B*08, HLA-B*15*, and *HLA-DQB*06* genomes were found more in the patient group, and there was statistically significant difference. ($p \leq 0.05$) Nevertheless, *HLA-DQB*03* and *HLA-DRB*11* genomes were found more in the control group ($p \leq 0.05$). As examined in our study, the relationship between SVD and *HLA* alleles has never been investigated. Therefore, this research is a pioneer in its field.

Although there are some studies about stroke and its relationship with various parameters in the literature, a few studies have evaluated the relationship between ischemic stroke and *HLA* subgroups. To the best of our knowledge, no studies have determined the association between SVD and *HLA* subgroups.

Our study aims to determine any potential evidence for whether *HLA* alleles might act as a discriminative factor between SVD patients and healthy participants, which might pave the way to future therapeutic alternatives.

Materials and methods

This study has a randomized, controlled, cross-sectional clinical research design.

The Pamukkale University's Non-Interventional Clinical Research Ethics Committee approved this study's ethical suitability. All authors signed that they had complied with the Declaration of Helsinki.

Patients were evaluated in the Department of Neurology outpatient clinic at the Pamukkale University Hospital. Forty-nine patients aged 18-75 diagnosed with SVD according to the TOAST classification were selected. Patients had small vascular lesions detected on computerized cranial tomography (CT) or magnetic resonance imaging (MRI), large artery stenosis of less than 50% found by vascular imaging (carotid-vertebral doppler ultrasonography, MRI angiography, or CT angiography). On echocardiography, there was no thrombus, and 24-hour-holter electrocardiograph scans showed no paroxysmal atrial fibrillations. Fifty control group members were healthy people who did not have a stroke or any vascular disease risk factors. Demographic data were recorded from all participants. Risk factors and cranial imaging findings were also noted in the patient group.

Informed consent was obtained from all the participants. After obtaining informed consent, blood samples (5 mL) were drawn from the median cubital vein and transferred into purple-capped hemogram tubes containing ethylenediaminetetraacetic acid (EDTA). Deoxyribonucleic acid (DNA) isolation was performed with EZ 1 kit (Qiagen® Corp., California, USA) from peripheral whole blood samples taken into EDTA tubes. Sequence-specific oligonucleotide method was used. Extracted DNA was typed *HLA-A, -B, -C, DRB*, and *DQB* alleles using Mia For a Flex software with next-generation sequencing (NGS) reagents provided by Immucor® (Mia Fora NGS *HLA* Typing Kit, NJ, USA).

Statistical analysis

Data were analyzed using Statistical Package for the Social Sciences (SPSS)® version 25 (IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0 Armonk, NY, USA). Continuous variables were stated as mean ± standard deviation (SD) and median (minimum and maximum values), and categorical variables were expressed as numbers and percentages. The suitability of data for normal distribution was examined with the Shapiro-Wilk and Kolmogorov-Smirnov tests. Differences between categorical variables were

analyzed with Pearson's Precise chi-square test. In all analyses, $p<0.05$ was considered statistically significant.

Results

The patient group consisted of 49 people. In patients with SVD, 21 (42.9%) women and 28 (57.1%) men are present. There was no statistically significant difference between the patient and control groups regarding mean age and gender distribution. The patient group's mean age was 57.67±11.14, and the median was 60. In both groups, there were 35 participants with a history of cerebrovascular disease (CVD), 30 people with hypertension (HT), 25 people with hyperlipidemia (HPL), and 38 participants with a sedentary lifestyle.

The *HLA-A*02* genome was found in 22 different alleles in the patient group. In comparison, there were 11 different alleles in the control group, and the difference was statistically significant ($p=0.024$). The *HLA-A*30* genome was found in seven different alleles in the patient group and one in the control group, and the difference was statistically significant ($p=0.030$). The *HLA-B*08* genome was in nine different alleles in the patient group, while there were two in the control group, and a statistically significant difference was found ($p=0.027$).

The *HLA-B*15* genome was found in 10 different alleles in the patient group and two in the control group, with a statistically significant difference ($p=0.015$). A statistically insignificant difference was found in the *HLA-C* genome between the two groups.

The *HLA-DQB*03* genome was found in 30 different alleles in the patient group while in 49 different alleles in the control group, and the difference was statistically significant ($p=0.006$).

The *HLA-DQB*06* genome was found in 21 different alleles in the patient group and 11 in the control group. The difference was statistically significant ($p=0.035$). There were 13 different alleles of the *HLA-DRB*11* genomes in the patient group, while there were 26 different alleles in the healthy control group. The difference was statistically significant ($p=0.019$).

While the *HLA-B*08* genome was found in nine different alleles in the patient group, there were two in the control group, and the difference was statistically significant ($p=0.027$). In the patient group, there were ten different alleles of the *HLA-B*15* genome. In the control group, there were two different alleles. The difference was statistically significant ($p=0.015$). The *HLA-DRB*11* genomes were found in 13 different alleles in the patient group, while it was in 26 different alleles in the control group. There was a statistically significant difference ($p=0.019$).

The *HLA* genomes, which had a statistically significant difference between the groups, are demonstrated in Table 1.

Table 1. Comparison of *HLA genome* in patient and control groups

HLA Genome [+]	**Available (%) / Non-available (%)**		**All Groups**	***p*-Value** [++]
	Patient Group	**Control Group**		
HLA-A*02	22 (23.5)/75 (76.5)	11 (13.0)/87 (87.0)	33	0.024
HLA-A*30	7 (7.1)/91 (92.9)	1 (1.0)/99 (99.0)	8	0.030
HLA-B*08	9 (9.2)/89 (90.8)	2 (2.0)/98 (98.0)	11	0.027
HLA-B*15	10 (10.2)/88 (89.8)	2 (2.0)/98 (98.0)	12	0.015
HLA-DQB*03	30 (30.6)/68 (69.4)	49 (49.0)/51 (51.0)	79	0.006
HLA-DQA*06	21 (21.4)/77 (78.6)	11 (11.0)/89 (89.0)	32	0.035

[+]*HLA: Human leukocyte antigen*; [++]Pearson's chi-square test in which, $p<0.050$ was considered statistically significant

Discussion

Cerebrovascular diseases, or strokes, are neuronal dysfunctions caused by the rupture or obstruction of one of the arteries feeding the contralateral hemisphere of the brain (atherosclerosis, thrombosis, etc.) [3]. Pathological changes in blood vessels to the brain, trauma or some cerebrovascular diseases may cause this neurological picture. Although it can occur at any age, it is rare to see before age 40 [4]. Inflammatory mechanisms are essential for forming atherosclerosis in the pathogenesis of CVD [5]. CVD is one of the most critical health

problems that still cause health and workforce loss worldwide [6]. 80% of cerebrovascular diseases are caused by ischemic and 20% by hemorrhagic causes [7]. According to the 1993 TOAST, ischemic stroke causes are classified as large artery atherosclerosis (LAA), cardiac causes, SVD (lacunar), ischemic stroke due to other determined causes, and cryptogenic ones.

HT is the primary risk factor for stroke and is thought to accelerate the atherosclerotic process and the risk of stroke increases with increasing systolic and diastolic blood pressure values [8]. The most common known risk factors for ischemic stroke include HT, diabetes, and high cholesterol [9-11]. High blood pressure, high cholesterol, carotid stenosis, and atrial fibrillation are conclusively shown in randomized clinical trials to be causally related to ischemic stroke, and their treatment reduces the incidence of stroke [11, 12].

This research evaluated the relationship between *HLA* gene polymorphism and patients with SVD. According to the literature, the age of the population in this study was lower [9]. Since the *HLA* gene assets were examined, we assumed that the genetic presence would not pose a problem for the study when it was considered not age-related. The patient group included 28 (57.1%) men and 21 (42.9%) women, and in terms of gender, the female-male ratio was compatible with the literature [9, 13].

The major histocompatibility complex (MHC) locus, known as the human leukocyte antigen locus, is on the short arm of chromosome 6. Molecules encoded by this region participate in antigen presentation, inflammation regulation, the complement system, and immune responses. Therefore, *MHC* is essential in immune-mediated, autoimmune, and infectious diseases [14]. *MHC* plays a role in some neurological disorders [15-19]. The *MHC* genes are subdivided into five regions from the telomeric to the centromeric end: the extended class I, class I, class III, class II, and the extended class II regions. The class I region consists of the three classic *HLA* gene loci: *HLA-A, HLA-B,* and *HLA-C*; three non-classic *HLA-E, HLA-F*, and *HLA-G* gene loci [14]. The class II region comprises the classic gene loci *HLA-DP, HLA-DQ, HLA-DR*, and the non-classic *HLA-DO* and *HLA-DM* loci. The class III region includes genes with roles in inflammation via complement cascades and cytokine production, like tumour necrosis factor (TNF) [14]. So, *MHC I* and *II* molecules supervise T-cells and are crucial for instructing the cellular adaptive immune responses.

Many studies have investigated the *HLA* class I region in CVD. The majority have reported insignificant results for *HLA-A* alleles. For example, some researchers examining Behcet's disease that might cause SVD have detected no significant *HLA-A* allele [20, 21]. Our study showed no specific allele in the *HLA-A*01, HLA-A*26*, or *HLA-A*33* genomes.

Kang et al. [22] revealed that the *HLA-A* alleles *A*02:07, A*26:01*, and *A*30:04* might be CVD susceptibility alleles *HLA-A*33:03* can be a protective agent in the Korean population. They also found that *A*02:07* was associated with skin lesions and arthritis, *A*26:01* with uveitis, and *A*30:04* with vascular lesions, genital ulcers, and positive paternity testing independent of *HLA-B*51. HLA-A*02:07* and *A*26:01* were confirmed to be CVD susceptibility alleles. However, *HLA-A*33:03* was associated with a reduced risk of CVD. In our research, the *HLA-A*02* allele was related to a higher risk of SVD (p=0.024), similar to the literature.

This study found no statistically significant difference between the healthy group and those with small vessel disease in the presence of *HLA-A*01, HLA-A*26 and HLA-A*33* alleles. Hence, this could be due to the small number of participants or might be explained by the absence of a causal relationship, especially in our population. There was only one patient with *HLA-A*33:03* alleles in both groups. Although there was one patient with *HLA-A*33:03* alleles, there were four more patients with *HLA-A*33:01* alleles.

The *HLA-A*30* allele was higher in the patient group, indicating that this allele might be associated with SVD. Comparing our results with other studies might reveal a striking genetic difference. On the other hand, patients with *HLA-A*02:07* alleles should be examined further in terms of arthritis [22].

The prevalence of *HLA-B*15* in the Moroccan population was similar to that observed in other

people in North Africa or Southern Europe. In these populations, the *B*15* genome was twice as abundant in controls as in controls in the same order as in *B*51*. Approximately 56% of patients were shown to express *B*51* or *B*15*, compared to 27% of healthy controls. It was stated that *HLA-B*15* was expressed more frequently in female patients [1, 20]. Our research demonstrated that the *HLA-B*15* genome was found in 10 different alleles in the patient group, and there were two different alleles in the healthy group (p=0.015). This result was undoubtedly compatible with the literature.

In a study on the association of *HLA-B*08* alleles with cerebrovascular diseases, the results of the Caucasian population were compared, and significance was found in small and local vascular disease with stroke aetiology with the control group [23]. In our research, there was a similar result (p=0.027). In another study in the Iranian population, no significant relationship was found between *HLA-B*08 and HLA-B*15* genomes [24]. In conclusion, *HLA-B*08* and *HLA-B*15* in homozygous *HLA-B* patient carriers as additional alleles detected in our study appeared to represent an increased risk for SVD.

In this research, the presence of *HLA-DQB*03, HLA-DQB*04*, and *DQB*06* in control and patient groups was found statistically significant (p<0.05). Interestingly, the *DRB* and *DQB* alleles reveal a stronger association between their observed profile in ischemic stroke and disease expression than the disease itself or individual clinical symptoms. *HLA-DQB*04:02* alleles did not show a statistically significant difference in the relationship between patients with SVD and the control group. However, the frequency of the *DQB* allele was more intense in the patient group. In the *HLA-DQB*06* areas, the patient group was in the majority, with 21 patients, which was statistically significant (p=0.035). The significant difference in these *HLA-DQB*06* alleles in our patient group was associated with SVD.

A significant *HLA-DQB* gene polymorphism, which might be protective, was in *DQB*03* alleles. This gene, found in 49% of the control group, was in around 30.6% of the patient group and could have protective effects based on these results.

A recent high-resolution genotyping study from China revealed higher haplotypes of allele *HLA-DRB*11* and haplotype *HLA-DRB*11:06* and *DQB*03:01* in patients with ischemic stroke [25]. In our research, the *HLA-DRB*11* genomes, even if not of this haplotype, revealed a different result from the previously mentioned study. In this research, *HLA-DRB*11* genomes were found in 13 different alleles in the patient group while in 26 different alleles in the control group (p=0.019), which suggested that *HLA-DRB*11* could have protective effects on SVD.

The significant limitation of this study was that our patient group consisted of only SVD patients, which means that the other types of stroke could not be evaluated. Only *HLA-A, HLA-B, HLA-C, HLA-DQB*, and *HLA-DRB* subgroup genomes were assessed. In the future, all *HLA* subgroup genomes should be addressed in more extensive and clinically different stroke patients.

In conclusion, this research endeavoured to find out whether there was a causal relationship between *HLA-A, HLA-B, HLA-C, HLA-DQB*, and *HLA-DRB* subgroup genomes with small vascular disease. We assumed that *HLA-A*02, HLA-A*30, HLA-B*08, HLA-B*15, HLA-DQB*03, HLA-DQB*06*, and *HLA-DRB*11* were associated with *HLA* alleles of SVD patients. These findings could be promising for developing new therapeutic alternatives. In addition, *HLA* genomes might even be used as a biomarker.

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Authors' contributions to the article

E.T. and U.Ç. constructed the main idea and hypothesis of the study.

E.T., U.Ç., and E.M. developed the theory and arranged the material and method section.

E.T. and U.Ç. evaluated the data in the Results section.

The discussion section of the article was written by E.T. and U.Ç.

E.T., U.Ç., and E.M. reviewed, corrected and approved.

In addition, all authors discussed the entire study and approved the final version.