DOI: 10.4274/MMJ.galenos.2024.89801
Medeni Med J 2024;39:243-253

Original Article

Determination of HLA Tissue Type According to the Etiology of Patients with Chronic Renal Failure

Kronik Böbrek Yetmezliği Hastalarının Etiyolojilerine Göre HLA Doku Tipinin Belirlenmesi

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ABSTRACT

Objective: Chronic kidney disease (CKD) is a prominent public health concern, is defined as functional and structural damage to the kidneys. This study aims to investigate the association between human leukocyte antigen (HLA) alleles individuals with CKD and the different etiological subgroups of diesease.

Methods: Genomic DNA was obtained from peripheral blood samples of 1,079 patients with retrospective CKD and 1,111 healthy control individuals. HLA genotyping was conducted using the Luminex based low-resolution method. Allele frequency distributions were calculated with the help of Arlequin v3.11 population genetics statistics program and SPSS v23.0 program, and p<0.05 values were accepted as significant by chi-square tests.

Results: HLA A*02 (21.83%), B*35 (18.30%), DRB1*11 (21.41%) alleles were observed most frequently in individuals with CKD, respectively. In our study, B*08, B*49, B*50 alleles in the HLA B locus (p=0.002, p=0.012 p=0.009) and DRB1*03, *04 alleles in the HLA DRB1 locus (p<0.001, p<0.001) were found positively associated with CKD. A*02, A*11, A*74 alleles at the HLA A locus (p=0.003, p<0.001, p=0.009) and B*27, B*39, B* alleles at the HLA B locus 40, B*59 (p<0.001, p<0.001, p<0.001, p=0.009), DRB1*07, *08, *09, *13, *16 (p<0.001, p=0.012, p=0.007, p<0.001, p<0.001) alleles were determined as negatively associated with the disease. Among the etiological groups of CKD, cystic kidney disease (36.8%), hypertension (16.8%) and urological anomalies (16.6%) were negatively associated with the HLA-DR*13 allele.

Conclusions: Since CKD shows serious morbidity and mortality, this comprehensive study of HLA subgroups gave an explanatory idea about which alleles associated with the disease in terms of susceptibility and protection.

Keywords: Chronic Renal Insufficiency, DNA Probları, HLA, polymerase chain reaction-sequence-specific oligonucleotide probing

ÖZ

Amaç: Kronik böbrek hastalığı (KBH) belirgin bir halk sağlığı sorunu olup fonksiyonel ve yapısal böbrek hasarı ile tanımlanmaktadır. Bu çalışma, KBH olan bireylerde insan lökosit antijeni (HLA) allel dağılımının, hastalığın farklı etiyolojik alt gruplarıyla olan ilişkisini araştırmayı amaçlamaktadır.

Yöntemler: Retrospektif olarak incelenen, KBH tanılı 1079 hasta ve 1111 kontrol grubundan alınan periferik kan numunelerinden elde edilen DNA'lar, HLA tiplemesi için düşük çözünürlüklü Luminex yöntemi kullanılarak analiz edilmiştir. Allel frekans dağılımları, Arlequin v3.11 popülasyon genetiği istatistik programı ve SPSS v23.0 yazılımı ile hesaplanmış; p<0,05 olan değerler ki-kare testleri ile anlamlı kabul edilmiştir.

Bulgular: KBH'li bireylerde sırasıyla en yaygın HLA A*02 (%21,83), B*35 (%18,30), DRB1*11 (%21,41) alelleri gözlenmiştir. Araştırmamızda, HLA B lokusunda B*08, B*49, B*50 (p=0,002, p=0,012, p=0,009) ve HLA DRB1 lokusunda DRB1*03, DRB1*04 ve DRB1*15(p<0,001,p<0,001,p=0,035) alelleri KBH ile pozitif ilişkili bulunmuşken, HLA A lokusunda A*02, A*11, A*74 (p=0,003, p<0,001, p=0,009) ve HLA B lokusunda B*27, B*39, B*40, B*59 (p<0,001, p<0,001, p<0,001, p=0,009) alelleri ve HLA DRB1 lokusunda DRB1*07, *08, *09, *13, *16 (p<0,001, p=0,012, p=0,007, p<0,001, p<0,001) alelleri ise hastalıkla negatif ilişkili olarak bulunmuştur.

Sonuçlar: KBH'ın ciddi sağlık sorunlarına ve ölüme yol açması nedeniyle, bu çalışmada hastalığa yatkınlık oluşturan ve hastalıktan koruyucu HLA alt grupları belirlenmiştir.

Anahtar kelimeler: Kronik Böbrek yetmezliği, DNA propları, HLA, polimeraz zincir reaksiyonu-diziye özgü oligonükleotid araştırması

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Received: 12 June 2024
Accepted: 16 September 2024
Online First: 04 December 2024

Cite as: Turan BK, Ucar F, Yılmaz T, Kılınc Y, Darbaş Aras S, Kocak H, Sayın Ekinci N, Aydınlı B, Arslan HS. Determination of HLA Tissue Type According to the Etiology of Patients with Chronic Renal Failure. Medeni Med J. 2024;39:243-253

INTRODUCTION

Chronic kidney disease (CKD), which is characterized as a continuous and permanent damage of renal function, diagnosed with glomerular filtration rate (GFR) of 60 mL/min per 1.73m^2 and the constant development of kidney damage-related symptoms such as active urine sediments, proteinuria, structural abnormalities, histological damages or a background of renal transplantation that lasts for more than three months[1]. CKD has historically been a global public health issue and represents a significant healthcare and fiscal strain, as it leads to a decreased GFR, which is commonly associated with a greater change of hospitalization, cognitive problems, cardiovascular events, and total mortality[2].

CKD represents a major public health issue both in Türkiye and worlwide, especially as its frequency is rising in Türkiye, count of individuals with end- stage kidney disease requiring kidney transplantation has grown during the previous two decades. CKD is caused by three major factors: Diabetic nephropathy, chronic glomerulonephritis, and hypertension[3].

Genetic and non-genetic factors are thought to contribute to CKD. Numerous gene mutations that affect the progress of CKD have been found in most genetic investigations, though the consequences of these variants on kidney disease risk are fairly mild[4]. Many gene regions, including APOL1, MYH9, and UMOD, are nowadays implicated for the disease's development[5].

Human leukocyte antigen (HLA) is one of the most significant genetic factors that causes CKD. The major histocompatibility complex (MHC), which is found on chromosome 6p21.3, encodes the HLA, the most polymorphic locus within the human genome. Numerous HLA genes encoded in the MHC are connected to an increased or decreased risk of renal failure[6-9].

In our country, few regional studies have investigated the connection between HLA alleles and CKD. In our study, the objective was to examine the connection between HLA allele distribution and the etiological subtypes of the disease among 1,079 patients diagnosed with CKD and 1,111 healthy control individuals attending the Akdeniz University Hospital Organ Transplantation Center from across Türkiye.

MATERIALS and METHODS

Research Gruop and HLA Genotyping

The study, which was made retrospectively and supported by Akdeniz University Scientific Research Fund (Project:1176/2016) was approved by Akdeniz University Clinical Research Ethical Committee (no:354, date 01.09.2015), was DNA isolated from peripheral blood samples of 1,079 patients diagnosed with CKD who applied to the outpatient clinics of Nephrology and Organ Transplantation Center between 2010-2015. As the control group, healthy bone marrow donors (1,111) of pediatric and adult bone marrow transplant patients without a history of CKD were selected.

All investigations were carried out in HLA Tissue Typing Laboratory of Akdeniz University Hospital, which is constantly going through the external quality control tests from the European Federation of Immunogenetics and the United Kingdom's National External Quality Assessment Service.

EZ1 advanced XL Workstation, employing magnetic bead technology for sample processing, was used to harvest genomic DNA from peripheral venous blood samples (Qiagen, Germany). Luminex technology was utulized for HLA genotyping in each participant using the polymerase chain reaction-SSO method (IMMUCOR-Lifecodes, Georgia).

Statistical Analysis

The allele frequencies (AF) of HLA alleles were calculated in patients with CKD and controls using the following formula: AF (%) = (n/2N) 100, where n defined as the the number of alleles and N indicated as total amount of individuals. By using Arlequin v3.11 population genetics software, the frequencies of HLA class I and II alleles and haplotypes in all participants were performed[10].

SPSS 23.0 software was used to conduct statistical analysis (SPSS Inc, Chicago IL, USA). Cross tabulation with corrected chi-square test or Fisher's exact test was utilized to assess the differences in allele percentages between CKD patients and the control group. The p-value was computed using the expected value in the statistical method. The chi-square test was applied directly when the expected value was grater than 5. The Fisher test was used when one of the predicted values was less than one. Correction chi-square tests were performed in other examples. The degree of the disease association to a specific allele was reflected using an odds ratio (OR) and 95% confidence intervals (CI). Statistical significance was prioritized at a level of $p<0.05$.

RESULTS

Etiology of CKD Patients

As per Turkish Society of Nephrology (TSN) data from 2018, CKD cases in Türkiye were categorized into

9 subgroups based on the underlying etiologies. With the data from our study, we were able to create detailed subgroups of CKD patients[11].

Once patients were separated into 13 groups based on their initial illnesses, CKD arose approximately in 36.8% due to cystic kidney diseases. Following cystic kidney disease, the most frequent main disorders leading to CKD were hypertension (16.8%, n=181), urologic abnormalities (16.6%, n=179) and nephrolithiasis (6.5%, n=71) (Table 1).

HLA A, B, DRB1 AF of 1,079 patients diagnosed with CKD and 1,111 healthy bone marrow donors as the control group is given in Table 2.

When comparing the patient group and the healthy control group, HLA alleles with high allele frequency in the patient group were deemed positively associated with the disease, while HLA alleles with a higher allele frequency in the control group relative to the patient group were considered negatively associated.

In our study, according to the data obtained, HLA A*02 (21.83%), B*35 (18.30%), DRB1*11 (21.41%) alleles were observed most frequently in individuals with CKD,

Table 1. The proportion of core diseases among CKD patients

Etiology of CKD	(N)	(%)
Unknown etiology	70	6.5
Hypertension	181	16.8
Urologic abnormalities	179	16.6
Tubulointerstitial nephritis	21	1.9
Diabetic nephropathy	31	2.9
Diabetic nephropathy and hypertensive nephropathy	18	1.7
Nephrolithiasis	71	6.6
Cyctic kidney disease	397	36.8
Familial mediterranean fever (FMF)	29	2.7
Glomerulonephritis	26	2.4
Amyloidosis	12	1.1
Congenital renal diease	20	1.9
Genetic disorder (Ankylosing spondylitis, Bardet Biedl syndrome, Bartter syndrome, Alport syndrome)	24	2.2
Total	1079	100

N: Patient number, CKD: Chronic kidney disease

Table 2. HLA-A, B, DRB1 allele frequencies in chronic kidney patients and control groups

HLA	CKD (2n=2158)	Control (2n=2222)	Statistical analysis	
	n-AF (%)	n-AF (%)	OR (95 %CI)	p
A* 01	225 (10.43)	207 (9.32)	1.133 (0.929- 1.382)	0.218$_a$
A* 02	471 (21.83)	637 (28.67)	0.695 (0.605- 0.797)	0.003$_a$
A* 03	263 (12.19)	245 (11.03)	1.120 (0.931-1.348)	0.230$_a$
A* 08	-	1 (0.05)	-	-
A* 09	3 (0.14)	-	-	-
A* 10	1 (0.05)	-	-	-
A* 11	1 (0.05)	116 (5.22)	0.008 (0.001-0.060)	<0.001$_a$
A* 13	1 (0.05)	-	-	-
A* 20	1 (0.05)	-	-	-
A* 22	-	1(0.05)	-	-
A* 23	85 (3.94)	70(3.15)	1.261(0.913-1.739)	0.158$_a$
A* 24	362 (16.78)	334(15.03)	1.13 (0.96-1.340)	0.115$_a$
A* 25	15 (0.70)	7(0.32)	2.215 (0.901-5.443)	0.075$_a$
A* 26	126 (5.84)	154 (6.93)	0.833 (0.653-1.062)	0.140$_a$
A* 27	1 (0.05)	-	-	-
A *28	2 (0.09)	1 (0.05)	2.060(0.186-22.738)	0.620$_b$
A* 29	41(1.90)	37 (1.67)	1.144 (0.730-1.791)	0.557$_a$
A* 30	78 (3.61)	75 (3.38)	1.074 (0.777-1.482)	0.667$_a$
A* 31	38 (1.76)	28 (1.26)	1.405 (0.859-2.297)	0.174$_a$
A* 32	98 (4.54)	108 (4.86)	0.931 (0.704-1.232)	0.618$_a$

Table 2. Continued				
HLA	**CKD (2n=2158)**	**Control (2n=2222)**	**Statistical analysis**	
	n-AF (%)	**n-AF (%)**	**OR (95 %CI)**	**p**
A* 33	53 (2.46)	70 (3.15)	0.774 (0.539-1.112)	0.164$_a$
A* 34	3 (0.14)	4 (0.18)	0.772 (0.173-3.453)	1.000$_b$
A* 36	1 (0.05)	-	-	-
A *39	1 (0.05)	3 (0.18)	0.343 (0.036-3.299)	0.625$_b$
A* 66	13 (0.60)	6 (0.27)	2.238 (0.849-5.900)	0.094$_a$
A* 68	91(4.22)	99 (4.46)	0.944 (0.706-1.2639)	0.698$_a$
A* 69	10 (0.46)	4 (0.18)	2.581(0.808-8.243)	0.097$_a$
A *74	3 (0.14)	14 (0.63)	0.220 (0.063-0.756)	0.009$_a$
A *80	-	1 (0.05)	-	-
B* 05	2 (0.09)	-	-	-
B* 06	1 (0.05)	-	-	-
B* 07	88 (4.08)	114 (5.13)	0.786 (0.591-1.045)	0.097$_a$
B* 08	95 (4.40)	60 (2.70)	1.659 (1.194-2.305)	0.002$_a$
B* 11	1 (0.05)	-	-	-
B* 12	2 (0.09)	-	-	-
B* 13	65 (3.01)	84 (3.78)	0.790 (0.569-1.099)	0.161$_a$
B* 14	48 (2.22)	34(1.53)	1.464 (0.940-2.281)	0.090$_a$
B* 15	86 (3.99)	99 (4.46)	0.621 (0.462-0.833)	0.001$_a$
B* 18	138 (6.40)	129 (5.81)	1.108 (0.865-1.420)	0.415$_a$
B* 21	3 (0.14)	4 (0.19)	0.772 (0.173-3.453)	1.000$_b$
B* 22	1 (0.05)	2 (0.09)	0.515 (0.047-5.679)	1.000$_b$
B* 25	-	1 (0.05)	-	-
B* 27	46 (2.13)	92 (4.18)	0.504 (0.352-0.722)	<0.001$_a$
B* 33	1 (0.05)	-	-	-
B* 35	395 (18.30)	374 (16.83)	1.706 (0.606-0.823)	0.200
B* 36	-	1 (0.05)	-	-
B* 37	15 (0.70)	30 (1.35)	0.511 (0.274-.953)	0.032$_a$
B* 38	108 (5.01)	83 (3.74)	1.358 (1.013-1.819)	0.040$_a$
B* 39	19 (0.88)	72 (3.24)	0.265 (0.159-0.441)	<0.001$_a$
B* 40	71 (3.29)	121 (5.45)	0.591 (0.438-0.797)	<0.001$_a$
B* 41	52 (2.41)	42 (1.89)	1.282 (0.850-1.933)	0.236$_a$
B* 42	1 (0.05)	1 (0.05)	1.030 (0.064-16.742)	1.000$_b$
B* 44	154 (7.14)	182 (8.19)	0.861 (0.689-1.077)	0.190$_a$
B* 45	6 (0.28)	1 (0.05)	6,192(0.745-51.478)	0.066$_b$
B* 46	3 (0.14)	-	-	-
B* 47	5 (0.23)	6 (0.27)	0.858 (0.261-2.815)	0.800$_a$
B* 48	13 (0.60)	8 (0.36)	1.677 (0.694-4.055)	0.246$_a$
B* 49	95 (4.40)	66 (2.97)	1.504 (1,093-2,071)	0,012$_a$
B* 50	77 (3.57)	50 (2.25)	1.607 (1.120-2.306)	0.009$_a$
B* 51	313 (14.50)	282 (12.69)	1.167 (0.982-1.388)	0.080$_a$

Table 2. Continued				
HLA	**CKD (2n=2158)**	**Control (2n=2222)**	**Statistical analysis**	
	n-AF (%)	**n-AF (%)**	**OR (95 %CI)**	**p**
B* 52	89 (4.12)	74 (3.33)	1.249 (0.912-1.710)	0.165_a
B* 53	15 (0.70)	20 (0.90)	0.771 (0.394-1.509)	0.446_a
B* 54	4 (0.19)	-	-	-
B* 55	71 (3.29)	60 (2.70)	1.226 (0.865-1.738)	0.252_a
B* 56	5 (0.23)	10 (0.45)	0.514 (0.175-1.505)	0.216_a
B* 57	36 (1.67)	41 (1.85)	0.902 (0.574-1.418)	0.656_a
B* 58	24(1.11)	25(1.13)	0.988 (0.563-1.736)	0.967_a
B* 59	2 (0.09)	12 (0.54)	0.171 (0.038-0. 764)	0.009_a
B* 60	-	4 (0.18)	-	-
DRB1* 01	112 (5.19)	90 (4.05)	1.297 (.976-1.723)	0.072_a
DRB1* 03	228 (10.57)	150 (6.75)	1.632 (1.136-2.024)	$<0.001_a$
DRB1* 04	372 (17.24)	278 (12.51)	1.457(1.231-1.723)	$<0.001_a$
DRB1* 06	1 (0.05)	-	-	-
DRB1* 07	160 (7.41)	237 (10.67)	0.671 (0.544-0.827)	$<0.001_a$
DRB1* 08	39 (1.81)	66 (2.97)	0.601 (0.403-0.897)	0.012_a
DRB1* 09	10 (0.46)	27 (1.22)	0.378 (0.183-.0784)	0.007_a
DRB1* 10	55 (2.55)	64 (2.88)	0.882 (0.612-1.271)	0.500_a
DRB1* 11	462 (21.41)	438 (19.71)	1.110 (0.958-1.285)	0.165_a
DRB1* 12	27 (1.25)	34 (1.53)	0.815 (0.490-1.356)	0.431_a
DRB1* 13	214 (9.92)	350 (15.75)	0.589 (0.491-0.706)	$<0.001_a$
DRB1* 14	153 (7.09)	146 (6.57)	1.085 (0.858.1.372)	0.496_a
DRB1* 15	229 (10.61)	194 (8.73)	1.241 (1.015-1.517)	0.035_a
DRB1* 16	88 (4.08)	147 (6.62)	0.600 (0.458-0.787)	$<0.001_a$
DRB1* 18	3 (0.14)	-	-	-
DRB1* 21	1 (0.05)	-	-	-
DRB1* 23	1 (0.05)	-	-	-
DRB1* 45	1 (0.05)	-	-	-
DRB1* 51	-	1 (0.05)	-	-

*:p<0.05, **:p<0.001. HLA: Human leukocyte antigen, CKD: Chronic kidney disease, AF: Allele frequency, OR: odds ratio, CI: Confidence interval, 2n: each individual was represented by two codominant allelic data, $_a$: Pearson chi-square test, $_b$: Fisher's exact test

respectively. Although the HLA A*02 (p=0.003) allele was statistically significant when compared to the control group, no notable difference was observed for the B*35 (p=0.200) and DRB1*11 (p=0.165) alleles.

Alleles were positively associated with the disease at the HLA B locus B*08, B*49, B*50 [OR; 1.66 (95% CI; 1.19-2.30), p=0.002], [OR; 1.50 (95% CI; 1.09-2.07), p = 0.012], [OR; 1.60 (95% CI; 1.12-2.30), p=0.009] and at the HLA DRB1 locus DRB1*03, * 04, *15 (p =0.035) [OR; 1.63(95% CI; 01.13–2.03, p <0.001], [OR; 1.46 (95% CI; 1.23–1.72), p <0.001], [OR; 1.24 (95% CI; 1.01–1.51), p = 0.035], respectively.

In the HLA A locus, A*02, *11, *74 (p=0.003, p<0.001, p=0.009), and in the HLAB locus B* 27, B* 39, B* 40, B* 59 (p<0.001, p<0.001, p<0.001, p=0.009) alleles and DRB1*07, *08, *09, *13, *16 (p <0.001,) at the HLA DRB1 locus p=0.012, p=0.007, p<0.001, p<0.001) alleles were found to be negatively associated with the disease.

According to the data obtained in our study, the distribution of patients with CKD according to their known etiology is shown in Table 3.

DISCUSSION

It has been reported in publications that CKD is a common disease with high socio-economic cost in our country and in the world, and its incidence is increasing gradually.

Patients with end-stage renal disease (ESRD) necessarily need a kidney transplant to avoid uremia, a life-threatening condition[12]. Nowadays, with the development of molecular techniques, it is becoming increasingly important to investigate the relationships between HLA, which is one of most genetically diverse gene region in the human genome, and diseases[13]. Although there are various publications from previous studies regarding the association between many diseases and HLA in our region and country, there are only a limited number of studies that have investigated the relationship between CKD and HLA[14,15].

HLA polymorphism may be correlated with ESRD due to its association with the causes and progression of renal disease[7]. For example, HLA B*51 was linked to ESRD in Venezuelan and Brazilian subjects[16], whereas A*26 has shown a protective effect against ESRD in Saudi Arabia[17] and Türkiye[14].

Table 3. Allele frequencies of HLA-A*, B*, DRB1* in chronic kidney patients and control group according to etiological subgroups

Etiology of CKD	Gene/or locus	Patient	Control (2n=2222)	Statistical analysis	Etiology of CKD
	HLA	N-AF (%)	N-AF (%)	OR	p-value
Urological anomalies (2n=358)	A* 01	53(14.80)	207 (9.32)	1.69 (1.22-2.341)	0.001_a^*
	A* 02	75 (20.95)	637 (28.67)	0.65 (0.50-0.864)	0.002_a^*
	A* 11	30 (8.38)	116 (5.22)	1.66 (1.09-2.557)	0.016_a^*
	A* 26	12 (3.35)	154 (6.93)	0.46 (0.25-0.847)	0.010_a^*
	B* 08	22 (6.15)	60 (2.70)	2.35 (1.42-3.897)	0.001_a^{**}
	B* 40	7 (1.96)	121 (5.45)	0.34 (0.16-0.748)	0.005_a^*
	B* 44	17 (4.75)	182 (8.19)	0.55 (0.33-0.931)	0.023_a^*
	B* 45	2 (0.56)	1 (0.05)	12.47 (1.12-137.964)	0.008_a^*
	DRB1* 03	40 (11.17)	150 (6.75)	1.73 (1.20-2.511)	0.003_a^*
	DRB1* 13	35 (9.78)	350 (15.75)	0.53 (0.37-0.771)	0.001_a^*
Cystic kidney disease (2n=794)	A* 02	169 (21.29)	637 (28.67)	0.67 (0.55-0.816)	$<0.001_a^{**}$
	A* 11	67 (8.44)	116 (5.22)	1.67 (1.22-2.28)	0.001_a^*
	A* 23	42 (5.29)	70 (3.15)	1.71 (1.16-2.540)	0.006_a^*
	B* 13	17 (2.14)	84 (3.78)	0.55 (0.32-0.944)	0.028_a^*
	B* 27	14 (1.76)	92 (4.14)	0.41 (0.23-0.733)	0.002_a^*
	B* 39	4 (0.50)	72 (3.24)	0.15 (0.05-0.415)	$<0.001_a^{**}$
	B* 40	28 (3.53)	121 (5.45)	0.63 (0.41-0.965)	0.032_a^*
	B* 41	25 (3.15)	42 (1.89)	1.68 (1.02-2.787)	0.039_a^*
	B* 49	50 (6.30)	66 (2.97)	2.19 (1.50-3.200)	$<0.001_a^{**}$
	DRB1* 03	80 (10.08)	150 (6.75)	1.54 (1.16-2.057)	0.002_a^*
	DRB1* 04	140 (17.63)	278 (12.51)	1.49 (1.19-1.898)	$<0.001_a^{**}$
	DRB1* 07	52 (6.55)	237 (10.67)	0.58 (0.43-0.802)	0.001_a^*
	DRB1* 13	78 (9.82)	350 (15.75)	0.58 (0.44-0.756)	$<0.001_a^{**}$
Unknown (2n=140)	A* 02	19 (13.57)	637 (28.67)	0.39 (0.23-0.639)	$<0.001_a^{**}$
	A* 11	18 (12.86)	116 (5.22)	2.67 (1.57-4.476)	$<0.001_a^{**}$
	A* 30	10 (7.14)	75 (3.38)	2.20 (1.11-4.360)	0.020_a^*
	DRB1* 01	5 (3.57)	90 (4.05)	2.42 (1.32-4.456)	0.003_a^*
	DRB1* 04	26 (18.57)	278 (12.51)	1.59 (1.02-2.487)	0.038_a^*

Table 3 (continued)

Etiology of CKD	Gene/or locus	Patient	Control (2n=2222)	Statistical analysis	Etiology of CKD
	HLA	N-AF (%)	N-AF (%)	OR	p-value
Hypertensive Nephropathy (2n=362)	B* 39	2 (0.55)	72 (3.24)	0.16 (0.04-.674)	0.004_a*
	DRB1* 01	25 (6.87)	90 (4.05)	1.57 (1.11-2.778)	0.015_a*
	DRB1* 03	41 (11.26)	150 (6.75)	1.74 (1.22-2.541)	0.002_a*
	DRB1* 13	37 (10.17)	350 (15.75)	0.60 (0.42-0.872)	0.006_a*
	DRB1* 16	12 (3.30)	147 (6.62)	0.48 (0.26-0.881)	0.015_a*
Diabetic nephropathy (2n=62)	A* 29	4 (6.45)	37 (1.67)	4.07 (1.40-11.803)	0.024_b*
	A* 66	2 (3.23)	6 (0.27)	12.31 (2.43-62.254)	0.018_b*
	B* 55	7 (11.29)	60 (2.70)	4.58 (2.00-10.489)	0.002_b*
	DRB1* 03	12 (19.36)	150 (6.75)	3.31 (1.72-6.360)	0.001_b**
	DRB1* 04	17 (27.42)	278 (12.51)	2.64 (1.49-4.680)	0.001_a**
Diabetic nephropathy and Hypertensive Nephropathy (2n=36)	A* 11	6 (16.67)	116 (5.22)	3.63 (1.48-8.897)	0.011_b*
	B* 42	1 (2.78)	1 (0.05)	63.45 (3.89-1035.056)	0.032_b*
	B* 51	9 (25.00)	282 (12.69)	2.29 (1.06-4.926)	0.041_b*
Nephrolithiasis (2n=142)	A* 26	3 (2.06)	154 (6.93)	0.29 (0.91-0.920)	0.025_a*
	B* 18	18 (12.33)	129 (5.81)	2.35 (1.39-3.98)	0.001_a*
	B* 40	2 (1.37)	121 (5.45)	0.24 (0.06-1.014)	0.036_a*
	B* 50	8 (5.48)	50 (2.25)	2.59 (1.20-5.581)	0.021_b*
	B* 52	12 (8.22)	74 (3.33)	2.67 (1.42-5.057)	0.004_a*
	DRB1* 13	12 (8.22)	350 (15.75)	0.49 (0.27-0.902)	0.019_a*
Familial mediterranean fever (2n=58)	B* 48	2 (3.45)	8 (0.36)	9.88 (2.05-47.605)	0.025_b*
	B* 50	4 (6.90)	50 (2.25)	3.21 (1.12- 9.229)	0.046_b*
Amyloidosis (2n=22)	B* 48	2 (8.33)	8(0.36)	25.15 (5.05-125.282)	0.005_b*
	DRB1* 14	5 (20.8)	146 (6.57)	3.74 (1.37-10.165)	0.019_b*
Genetic disorders (2n=48)	B* 52	5 (10.4)	74 (3.33)	3.37 (1.29-8.768)	0.024_b*
	DRB1* 04	12 (25.0)	278 (12.51)	2.33 (1.19-4.534)	0.010_a*
	DRB1* 15	9 (18.8)	194 (8.73)	2.41 (1.15-5.05)	0.034_b*
Congenital kidney diseases (2n=48)	B* 07	6 (12.5)	114 (5.13)	2.64 (1.10-6.343)	0.038_b*
	B* 38	10 (10)	83 (3.74)	2.99 (1.15-7.762)	0.036_b*
Chronic glomerulonephritis (2n=52)	A* 24	19 (36.54)	334 (15.03)	3.25(1.82-5.791)	$<0.001_a$**
Tubulointerstitial nephritis (2n=42)	DRB1* 04	13 (30.95)	278 (12.51)	3.13 (1.61-6.102)	$<0.001_a$*

*:p<0.05, **:p<0.001. HLA: Human leukocyte antigen, CKD: Chronic kidney disease, AF: Allele frequency, OR: odds ratio, CI: Confidence interval, 2n: each individual was represented by two codominant allelic data, $_a$. Pearson chi-square test, $_b$. Fisher's exact test

Our research was carried out to evaluate the etiological distribution and frequency of patients with CKD who applied to the Akdeniz University Hospital Organ Transplantation Center from all over our country, and to investigate the HLA allele distribution of their patients.

Etiological information about CKD in our country is being researched by the TSN. Especially in the last two decades, a relative change in the etiology of CKD has been reported by TSN. While the most important cause of CKD in the past was chronic glomerulonephritis, in a study by Suleymanlar et al.[18] 2009, the leading etiologic cause was diabetes mellitus (35%), followed by hypertension (27%), glomerulonephritis (7%), polycystic kidney disease (7%), pyeolonephritis (3%), amyloidosis (2%) and other causes. The primary disease is unknown in 15% of the patients.

In our study, cystic kidney disease 36.8%, hypertension 16.8%, urological anomalies 16.6% and nephrolithiasis 6.58% were found to be the most common etiological causes of 1079 chronic kidney failure patients who applied to the organ transplantation polyclinics of Akdeniz University Hospital Nephrology Department, respectively.

The literature suggests that the HLA system is linked to the development of various conditions, including autoimmune disorders, inflammatory bowel disease, allergies, and certain kidney diseases such as diabetic nephropathy, immunoglobulin A (IgA) nephropathy, and glomerulonephritis. These connections highlight the involment of HLA in the pathogenesis of these diseases[19]. Detection and examination of HLA polymorphism are tissue typing tests that are critical not just for studies related to ESRD susceptibility but concerning the selection of tissue recipients and donors for tissue transplantation in. In 2014, Cao et al.[12] in his study, HLA A*24, B*55, B*54, B*40, DRB1*04 alleles were associated with ESRD in Asian countries. In another study, DRB1*11 and DRB1*03 alleles were identified as positively associated with CKD in individuals with ESRD, while the HLA DRB1*08 allele was found to be negatively associated the disease [19]. Crispim et al.[20] They reported that the HLA A*78 and DRB1*11 alleles were at a high frequency in patients with ESRD, while the HLA B*14 allele was at a low frequency, but these values were not statistically significant. In Türkiye in 2010, Karahan et al.[14] in his study on patient groups with CKD, the most prevelant HLA alleles were identified as HLA A*02 (43.8%), DRB1*11 (43.8%) and B*35 (32.4%).

In our study, additionally HLA A*02 (21.83%), B*35 (18.30%), DRB1*11 (21.41%) alleles were observed in individuals with CKD, respectively. B*08, B*49, B*50 (p=0.002, p=0.012, p=0.009) in the HLA B locus and DRB1*03, DRB1*04, DRB1*15 (p<0.001, p<0.001, p = 0.035) in the HLA DRB1 locus, respectively While its alleles were positively associated with CKD, A*02, A*11, A*74 (p=0.003, p=0.001, p=0.009) in the HLA A locus and HLA B*27, B*39, B*40, B*59 (p=0.000, p<0.001, p<0.001, p=0.009) alleles and DRB1*07, *08, *09, *13, *16 (The p<0.001, p=0.012, p=0.007, p=0.001, p=0.001) alleles were found to be negatively associated with the disease.

In countries such as China, France, South America, and England, research has indicated that the HLA DR*03 allele is positively associated with the development of membranous and diabetic nephropathy, but has a protective effect in the development of idiopathic IgA nephropathy[21,22].

It has also been stated that HLA DRB1*03 and DRB1*11 are associated with diabetic nephropathy in the Egyptian population[23]. While HLA DRB1*15 and DQB1*05 alleles are positively associated with ESRD due to type 2 diabetes in Mexico, HLA DRB1*04 has been found to be protective in the USA and Mexico[24,25]. In 2009, Karahan et al.[14] showed that HLA B*58 and HLA DRB1*03 alleles were positively associated with diabetic nephropathy and amyloidosis diseases.

In our study, the most common HLA alleles in the CKD group with diabetic nephropathy were found to be HLA A*02 (17.74%), B*35 (17.74%), DRB1*04 (27.42%). As a result of the statistical analysis, HLA A*29, A* 66, B* 55, DRB1* 03, DRB1*04 (p=0.024, p=0.018, p=0.002, p=0.001, p=0.001) respectively, who had alleles were positively associated with the disease. According to the literature, the HLA DRB1*03 (p=0.001) allele was found to be positively associated with diseases, while the negatively associated allele was not found.

HLA-DR4 has been linked to immune complex-mediated glomerulonephritis in studies carried out in populations from China, Italy, the United States, and different countries[26]. In the Han population of China, the HLA DRB1*04 allele was observed with a high frequency in patients with IgA nephropathy[27]. In a study carried out in 2016, nephrotic syndrome and DRB1*07, DQB1*02, alleles were found to be strongly associated, while DRB1*10, DQB1*05, DQB1*06 alleles were found to be protective against the disease[28]. In our study, the most prevelant alleles in the HLA A group in patients in the glomerulonephritis group were found to be HLA A*24 (36.54%), B*35 (17.31%), DRB1*11 (30.77%).

As a result of the statistical analysis, the HLA A*24 (p<0.001) allele was identified as positively associated with the disease. We came to the conclusion that this different situation may be due to polymorphism in populations.

There are not many research in the literature investigating the assocation between hypertension and CKD. In the USA, in individuals with HLA B*35 and DRB1*03 alleles, AF were demostrated to be high in individuals with CKD due to hypertensive nephropathy and were observed to be statistically significant[29]. In our study, the most common alleles in patients in the hypertension group were HLA A*02 (24.73%), B*35 (20.06%), HLA DRB1*11 (19.23%). DRB1*01 (p=0.015) and DRB1*03 (p=0.002) alleles were found to be positively associated with the disease. B*39 (p=0.004), DRB1*13 (p=0.006), DRB1*16 (p=0.015) alleles were found to be negatively associated with the disease. Some of the researches were found to be compatible with the literature and some were not.

In one of the studies in China in which the relationship between PCD and HLA was investigated serologically in a family of 9 people, 4 of whom had a history of PCD, it was found that the HLA-A9-B22-HLA-DR5 haplotype was associated with PCD[13].

In our study, in individuals with cystic kidney disease, HLA A*11 (p=0.001), A*23 (p=0.006), B*41 (p=0.039), B*49 (p<0.001), DRB1*03 (p=0.002), DRB1*04 (p<0.001), alleles were positively associated with the disease, HLA A*02 (p<0.001), B*13 (p=0.028), B*27 (p=0.002), B*39 (p<0.001), B*40 (p=0.032), DRB1*07 (p=0.001), DRB1*13 (p<0.001) allele was found to be identified as having a negative association with the disease.

In a thesis study carried out in KTU Health Sciences Institute Medical Biology Department in 2014, the most common HLA A*02 (33%), HLA B*35 (17.51%) and HLA DRB1*11 (17.51%) alleles have been determined. However, since these alleles are also common in the control group, no relationship was found between CKD and HLA alleles for HLA A*02 (30.54%), HLA B*35 (17.09%), HLA DRB*11 (19.72%). In this study, the HLA A*25, A*69 and B*08 alleles were positively associated with diabetic nephropathy, while the HLA DRB1*03 allele was negatively associated with the disease. The data have been confirmed in the literature, with the HLA B*08 allele positively associated with glomerulonephritis, and the HLA DRB1*04 allele negatively associated. In addition, in patients with CKD due to hypertension from the study, no alleles could be detected, either positively or negatively associated with the disease. In addition, the HLA A*25, A*26 and A*30 alleles were positively associated in PCKD, while the HLA DRB1*11 allele was negatively associated[30].

Other etiological groups of CKD in our study, on the other hand, were found to be positively associated with the HLA B*18 (p=0.001), B*50 (p=0.021), B*52 (p=0.004) alleles in the nephrolithiasis patient groups as a result of statistical analyzes.

HLA A*26 (p=0.025), B*40 (p=0.036), DRB1*13 (p=0.019) allele were found to be negatively associated with the disease. HLA B*48 (p=0.005) and DRB1*14 (p=0.019) alleles were found to be positively associated with the disease as a result of statistical analyzes performed in the patient group with CKD due to amyliodosis. As a result of the statistical analyses performed in the patient group with CKD due to congenital kidney damage, negatively associated allele with the disease was found. HLA B*07 (p=0.038), B*38 (p=0.036) were found to be positively associated. In individuals with CKD due to urological anomaly, according to statistical data, HLA A*01 (p=0.001), A*11 (p=0.016), B*08 (p=0.001), DRB1*03 (p= 0.003) alleles positively associated with disease, HLA A*02 (p=0.002), A*26 (p=0.010), B*40 (p=0.005), B*44 (p=0.023), B*45 (p=0.008), DRB1*13 (p=0.001) alleles were found to be negatively associated with the disease.

According to statistical data, HLA A*24 (p=0.002), B*38 (p=0.021), DRB1*04 (p<0.001) alleles were found to be positively associated with the disease in individuals with CKD due to tubular interstitial nephritis.

In the patient group with CKD due to diabetes and hypertension, according to statistical data, HLA A*11 (p=0.001), A*42 (p=0.032), A*51 (p=0.041), allele positively associated with the disease found. In the group of patients with CKD caused by genetic disorders (Ankylosing Spondylitis, Bardet-Biedl Syndrome, Bartter Syndrome, Alport syndrome) a positive association was found with the HLA B*52 (p=0.024), DR*04 (p=0.010), and DR*15 (p=0.034) alleles.

According to statistical data, HLA B*48 (p=0.025), B*50 (p=0.046) alleles were found to be positively associated with the disease in patient groups with CKD due to Familial Mediterranean Fever. In the patient group with CKD of unknown etiology, according to statistical data, HLA A*11 (p<0.001), A*30 (p=0.020), DRB1*04 (p=0.038), DRB1*15 (p= 0.012), alleles were positively associated with the disease. HLA A*02 (p<0.001), B*40 (p=0.038), DRB1*01 (p=0.003), DRB1*13 (p=0.022) allele were found to be negatively associated with the disease. Since there is no study in the literature investigating the assocation

between these etiologies and CKD, a comparison could not be made.

CONCLUSION

In all CKD patients in our study, B*08, B*49, B*50 in HLA*B locus and DRB1*03, DRB1*04, DRB1*15 alleles in HLA DRB1 locus were positively associated with CKD. HLA A*02, A*11, A*74 at HLA A locus, B*27, B*39, B*40, B*59 alleles at HLA B locus and DRB1*07, DRB1*08, DRB1*09, DRB1*13, DRB1*16 alleles at HLA DRB1 locus were found to be negatively associated with the disease.

When classified according to etiological distributions in the whole patient group, the HLA DR*13 allele was identified as having a negative association with in Hypertensive Nephropathy, Nephrolithiasis, cystic kidney disease, and Urological Anomalies subgroups and it is thought that it may be a protective allele in CKD.

Susceptible alleles can serve as important markers for risk classification. Additionally, in consanguineous kidney transplantation, avoiding these susceptible alleles when selecting optimal donors could significantly improve the extended survival of transplant recipients.

Ethics

Ethics Committee Approval: The study approved by Akdeniz University Clinical Research Ethical Committee (no:354, date 01.09.2015) and was conducted in full compliance with the Declaration of Helsinki.

Informed Consent: This study is retrospective.

Footnotes

Author Contributions

Surgical and Medical Practices: V.T.Y., H.K., B.A., Concept: B.K.T., F.U., V.T.Y., Y.K., H.K., B.A., Design: B.K.T., F.U., Data Collection and/or Processing: B.K.T., V.T.Y., H.K., B.A., Analysis and/or Interpretation: B.K.T., F.U., Y.K., Ş.D.A., N.S.E., H.S.A., Literature Search: B.K.T., Y.K., Ş.D.A., N.S.E., H.S.A., Writing: B.K.T., Y.K., S.D.A., N.S.E., H.S.A.

Conflict of Interest: The authors have no conflict of interest to declare.

Financial Disclosure: Akdeniz University scientific research project. Project no: 1176.

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