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Original scientific paper

HUMAN LEUKOCYTE ANTIGEN POLYMORPHISMS AS SUSCEPTIBILITY RISK FACTORS FOR END STAGE RENAL DISEASE

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Majority of the patients with chronic renal failure develop end-stage renal disease (ESRD). ESRD is a worldwide public health problem. That requires renal transplantation. Currently, many genome-wide association studies have suggested a potential association between human leukocyte antigen (HLA) and ESRD as uncovered relationship. This study is the first report from Serbia to find out the distribution of HLA -A, -B, -DRB1 specificity, two loci disequilibrium linkage between these HLA loci and possible association in renal transplant recipients from the region of Vojvodina, Serbia. From the same region, 230 ESRD patients who were waiting for kidney transplantation and 290 healthy controls were included in this study. Polymerase chain reaction-sequence specific primer (PCR-SSP) method were used to analyze the HLA polymorphisms (including HLA-A, HLA-B and HLA-DRB1 loci) in both ESRD patients and controls. The frequencies of alleles at these loci and two loci disequilibrium linkage coefficients were compared between ESRD patients and controls.The current work suggests that *HLA DRB1*04* allele (odds ratio = 1.6484, 95 % CI = 1.0395–2.6138, P = 0.0325) may represent susceptibility risk factor for the development of ESRD in Serbian individuals. The highest two loci disequilibrium linkage coefficients in ESRD patients were found for B*18~DRB1*11 (Δ= 0.01583) and A*02 ~B*51 (Δ= 0.0145) and in controls for B*08~DRB1*03 (Δ=0.370) and A*01~DRB1*03 (Δ=0.02446), respectively, but without reaching significant levels. The results of our study suggests HLA DRB1*04 as a risk

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marker that might be involved with ESRD development. Further studies should be undertaken to analyze long-term results from kidney transplantation and clarify types and subtypes of alleles involved with ESRD progression.

Keywords: HLA, end stage kidney disease

INTRODUCTION

With a high incidence, end-stage renal disease (ESRD) becomes a worldwide public health problem (CAO *et al*., 2014) Renal transplant has become the standard care for the fatal renal diseases and the success of such transplantations correlates with the degree of HLA compatibility between recipients and donors (AGRAWAL *et al*., 2001). Although no clear risk factors have been defined for these patients it is believed that their demography and proper access to medical care largely contribute to the lack of prevention and poor management of chronic kidney disease (DAI *et al*., 2015). Age, gender, genetics, race, proteinuria, lipids, hypertension and smoking are among the factors associated with ESRD. Histocompatibility testing is of pivotal importance in the selection of kidney recipient candidates and donors for transplantation, particularly HLA-DRB1, HLA-A and HLA-B genes, which code for molecules with a central role in the immune response.

Glycoproteins encoded by genes of the major histocompatibility complex (MHC) – known in humans as HLA (human leukocyte antigens) – specialize in presentation of short peptides to T lymphocytes and play a key role in the body's immune defense. The MHC, also known in humans as the human leukocyte antigen (HLA) region, encompasses ~4 Mb on chromosome 6p21.3 and is the most gene dense region within the human genome containing 252 expressed genes. The region can be subdivided into the extended class I, classical class I, classical class III, classical class II and extended class II regions and contains the largest degree of polymorphism within the genome. It also exhibits the longest linkage disequilibrium (LD) block, extending up to 540kb, which compares with distances of between 1-173kb seen in the rest of the genome. However, it has long been observed that some allelic variants of certain HLA genes appear to betray their assigned duty and, paradoxically, facilitate certain diseases, many of which involve immune-mediated tissue damage. How does the MHC, a critical immune-policing mechanism become a wrongdoer is unclear (CRISPIM *et al*., 2008; IBELS *et al.*, 1994).

Various kidney disorders leading to end-stage renal disease are associated more frequently with certain human leucocyte antigen-HLA alleles. For instance, idiopathic membranous glomerulonephritis is associated with HLA-DR2 in Japanese patients and with HLA-DR3 in Caucasians. Higher frequency of *HLA DRB1*03:01* has been reported in Saudi patients with idiopathic membranous nephropathy. Anti-glomerular basement membrane nephritis in Japanese patients also has a strong association with *DRB1*15:01* (ALMOGREN *et al*., 2012; KITAGAWA *et al*., 2008).

In the present study, HLA -A, -B and -DRB1 polymorphisms and linkage disequilibrium of ESRD patients from region of Vojvodina, Serbia were compared to a healthy control group in an effort to provide a better understanding of the etiology of this disease.

METHODS

Population study

This case-control study included 520 individuals: the patient group comprised 230 adults with ESRD while the control group consisted of 290 healthy adult individuals. All patients

and controls originated from Vojvodina, Serbia. The patient group consisted of 142 males and 88 females (aged from 18 to 61 years) who had attended the Clinic for Nephrology and Clinical Immunology at Clinical Center of Vojvodina in Novi Sad for a kidney transplant. All ESRD patients in the study were on haemodialysis before they underwent kidney transplantation. The group comprised 173 (75.2%) patients with diabetic nephropathy, 33 (14.3%) patients with glomerulonephritis and 24 (10.43%) patients with essential hypertension. The control group consisted of healthy unrelated individuals (136 males and 154 females), aged from 18 to 57 years. Written informed consent was obtained from all participants before collecting blood specimens. The study was approved by the Local Ethics Committees'.

Genotyping

Genomic DNA was extracted by silica-based extraction method, using the GeneJET Genomic DNA Purification Kit (Thermo Fisher Scientific Inc., Vilnius, Lithuania) from 200 µl of buffy-coat obtained from 3 ml of blood collected with EDTA. Polymerase chain reaction-sequence specific primer based typing at HLA-A, -B and -DRB1 loci was performed using a MASTERCYCLER Gradient (Eppendorf, AG, Hamburg,, Germany) PCR system.

Briefly, 102 µl of DNA at a concentration of 25-100 ng/µl with OD_{260}/OD_{280} ratio >1.7 was mixed with 312 µl of Ready PCR buffer, 8.3 µl of Taq Polymerase and 624 µl of distilled water. Ten µl of this mixture was used for each PCR reaction tube with sequence specific primers (OLERUP *et al.*, 1992). According to the manufacturer's instructions, sequence specific primer (SSP) analysis was performed with a mixture of nucleotides, Taq polymerase (AXITAQ-DNA Polymerase, INNO-TRAIN, Kronberg/ Taunus, Germany) by HLA-ABDR kits, INNO-TRAIN DiagnostikGmbH (HLA-REDY GENE, Kronberg/Taunus,Germany). All amplifications were performed in a thermocycler using the following conditions: initial denaturation 96°C for 2 min, followed by 10 cycles of 15 s at 96°C, 60 s at 65°C and 20 cycles of 15 s at 96°C, 50 s at 61°C and 30 s at 72°C.

The PCR products were analyzed in 2% agarose gel stained with ethidium bromide. After amplification, DNA separation by electrophoresis was undertaken at 180 V, for approximately 20 minutes. The amplification was checked on an UV transilluminator and photographed. The exact HLA type was investigated by SCORE TM–evaluation software (Inno-Train Diagnostik GmbH, Kronberg, Germany).

Statistical analysis

Statistical analyses of the association between HLA alleles and ERSD in patient compared to control groups were performed by estimating the odds ratios (OR) and 95 % confidence intervals (95 % CI) using Social Sciences (SPSS; version 23), (SPSS, IBM Corporation, Armonk, New York).Two tailed P values were estimated by Fisher's exact test. A P value less or equal to 0.05 was considered to be significant. Corrected P values (Pc) were also calculated by dividing the P values by the number of antigens represented in the loci (according to the Bonferroni's correction) (PERNEGER, 1998). The linkage disequilibrium coefficient between any two alleles at these loci is measured by Lewontin D (LEWONTIN, 1988).

RESULTS

In 230 ESRD patients, there were 17 HLA-A, 26 HLA-B and 13 HLA-DRB1 alleles to occur. In 290 controls, there were 16 HLA-A, 27 HLA-B and 13 HLA-DRB1 alleles to occur. In

both groups, a total of 57 distinct HLA alleles occurred including 17 HLA-A, 27 HLA-B and 13 HLA-DRB1 alleles (see Table 1). The most frequent genes which occurred at the HLA-A, -B and -DRB1 loci of all subjects were consistent with a previous studies conducted within the Vojvodina population (VOJVODIĆ, *et al.*, 2012; VOJVODIĆ *et al.*, 2013).

Table 1. HLA A, B and DRB1 frequencies in Vojvodina ESRD patients and controls

HLA specificity	**ESRD patients**	**n=230**	**Controls**	**n=290**
	Carrier frequency	Gene frequency	Carrier frequency	Gene Frequency
A*01	22.1	0.128	29.3	0.162
A*02	45.6	0.263	53.7	0.322
A*03	20.0	0.102	21.3	0.110
A*11	15.2	0.078	9.60	0.055
A*23	4.70	0.023	2.40	0.012
A*24	20.8	0.113	18.6	0.096
A*25	4.70	0.023	5.80	0.029
A*26	12.6	0.063	12.0	0.060
A*29	3.90	0.019	2.00	0.010
A*30	6.00	0.030	3.70	0.018
A*31	5.20	0.026	2.00	0.010
A*32	9.10	0.045	9.30	0.048
A*33	5.20	0.026	3.40	0.018
A*34	0.40	0.002	0	0
A*66	1.30	0.006	0.68	0.003
A*68	8.20	0.047	8.20	0.041
B*07	7.80	0.041	10.0	0.050
B*08	12.1	0.067	18.6	0.100
B*13	7.30	0.039	6.50	0.032
B*14	6.00	0.030	4.80	0.025
B*15	10.8	0.054	4.80	0.024
B*18	20.0	0.102	21.3	0.112
B*27	9.10	0.047	10.6	0.056
B*35	20.4	0.110	19.6	0.108
B*37	1.30	0.006	2.70	0.013
B*38	10.4	0.054	12.7	0.063
B*39	6.50	0.032	6.50	0.032
B*40	9.10	0.045	11.0	0.056
B*41	2.60	0.013	4.10	0.020
B*44	15.2	0.084	15.1	0.079
B*45	0.86	0.004	0.68	0.003
B*47	0.43	0.002	0.34	0.001
B*48	0.86	0.004	0.34	0.001
B*49	4.70	0.028	1.70	0.008
B*50	3.00	0.015	3.10	0.015
B*51	26.5	0.143	19.3	0.096

Table 1. HLA A, B and DRB1 frequencies in Vojvodina ESRD patients and controls, continuited

B*52	3.40	0.017	3.70	0.018
B*53	0.43	0.002	0.68	0.003
B*54	0	0	0.34	0.001
B*55	3.40	0.017	4.10	0.020
B*56	2.60	0.013	2.0	0.010
B*57	4.30	0.021	5.10	0.025
B*58	0.43	0.002	1.30	0.006
DRB1*01	20.8	0.115	17.5	0.087
DRB1*03	19.5	0.102	22.4	0.120
DRB1*04	20.8	0.117	13.7	0.070
DRB1*07	17.3	0.089	17.5	0.089
DRB1*08	6.50	0.032	5.10	0.025
DRB1*09	0.86	0.004	1.00	0.005
DRB1*10	2.60	0.013	3.70	0.018
DRB1*11	22.1	0.132	26.8	0.144
DRB1*12	3.40	0.017	3.70	0.018
DRB1*13	19.1	0.102	25.1	0.134
DRB1*14	10.0	0.050	12.0	0.063
DRB1*15	16.9	0.091	14.8	0.075
DRB1*16	23.9	0.128	24.8	0.132

Table 2. Assocations of HLA-A, B and DRB1 genes among ESRD Vojvodina patients and controls

Allele	ODDS ratio	95% Confidence Interval	p value	significance
A*01	0.6872	0.4602 - 1.0260	0.0659	NS
A*02	0.7316	0.5170 - 1.0353	0.0777	NS
A*03	0.9194	0.5992 - 1.4105	0.6985	NS
A*11	1.6795	0.9882 - 2.8554	0.0534	NS
A*23	2.0307	0.7745 - 5.3243	0.1425	NS
A*24	1.1526	0.7466 - 1.7794	0.5219	NS
A*25	0.8066	0.3701 - 1.7578	0.5902	NS
A*26	1.6154	0.9547 - 2.7334	0.0718	NS
A*29	1.9276	0.6760 - 5.4967	0.2116	NS
A*30	1.6439	0.7317 - 3.6934	0.2253	NS
A*31	2.6055	0.9626 - 7.0526	0.0512	NS
A*32	0.9787	0.5379 - 1.7807	1	NS
A*33	1.5413	0.6537 - 3.6339	0.3197	NS
A*66	1.9031	0.3153 - 11.4861	0	NS
A*68	0.9980	0.5324 - 1.8709	1	NS
B*07	0.7642	0.4130 - 1.4140	0.3896	NS
B*08	0.6198	0.3779 - 1.0167	0.0567	NS
B*13	1.1384	0.5776 - 2.2437	0.7082	NS
B*14	1.2778	0.5964 - 2.7375	0.5270	NS
B*15	2.4042	1.2195 - 4.7397	0.0093	NS
B*18	0.9194	0.5992 - 1.4105	0.6985	NS
B*27	0.8395	0.4685 - 1.5041	0.5541	NS
B*35	1.0273	0.6678 - 1.5804	0.8875	NS
B*37	0.4659	0.1222 - 1.7763	0	NS
B*38	0.7966	0.4616 - 1.3748	0.4130	NS
B*39	0.9951	0.4940 - 2.0044	1	NS

Table 2. Assocations of HLA-A, B and DRB1 genes among ESRD Vojvodina patients and controls, conituited

B*40	0.8101	0.4536 - 1.4467	0.4751	NS
B*41	0.6205	0.2293 - 1.6795	0.3427	NS
B*44	1.0035	0.6197 - 1.6251	1	NS
B*45	1.2632	0.1766 - 9.0368	0	NS
B*47	1.2620	0.0785 - 20.2870	0	NS
B*48	2.5351	0.2284 - 28.1345	0	NS
B*49	2.8630	0.9804 - 8.3611	0.0440	NS
B*50	0.9801	0.3594 - 2.6728	1	NS
B*51	1.5422	1.0188 - 2.3346	0.0397	NS
B*52	0.9140	0.3615 - 2.3111	0.8414	NS
B*53	0.6288	0.0567 - 6.9787	0	NS
B*55	0.8348	0.3354 - 2.0778	0.6985	NS
B*56	1.2679	0.4034 - 3.9845	0.6801	NS
B*57	0.8333	0.3672 - 1.8913	0.6629	NS
B*58	0.3122	0.0347 - 2.8129	0	NS
DRB1*01	1.2071	0.7796 - 1.8691	0.3994	NS
DRB1*03	0.8590	0.5599 - 1.3178	0.4839	NS
DRB1*04	**1.6484**	**1.0395 - 2.6138**	**0.0325**	**S***
DRB1*07	0.9866	0.6255 - 1.5561	1	NS
DRB1*08	1.3754	0.6498 - 2.9114	0.4027	NS
DRB1*09	0.8392	0.1390 - 5.0649	0	NS
DRB1*10	0.6794	0.2474 - 1.8656	0.4502	NS
DRB1*11	0.7744	0.5164 - 1.1612	0.2161	NS
DRB1*12	0.9140	0.3615 - 2.3111	0.8414	NS
DRB1*13	0.7032	0.4610 - 1.0726	0.1009	NS
DRB1*14	0.8095	0.4637 - 1.4132	0.4583	NS
DRB1*15	1.1729	0.7310 - 1.8818	0.5071	NS
DRB1*16	0.9516	0.6357 - 1.4245	0.8064	NS

Bonferroni corrected value for A locus is <0.0333, for B locus is <0.00192 and for DRB1 locus is<0.03846

A comparison of the frequency of *HLA-A* alleles in the patients and controls showed higher frequencies of *HLA A*11* and *HLA A*24* (0.078 vs 0.055 and 0.113 vs 0.096), respectively, and *HLA B*15* and *HLA B*51* (0.054 vs 0.024 and .0143 vs 0.096) respectively, in the ESRD patients in comparison to the controls (OR=1.67, 1.15, 2.40 and 1.54, respectively), however, none of them reached the statistical significance, since Bonferroni corrected value for A locus is <0.0333 and for B locus is <0.00192 (Table 2).

On examining the frequency of HLA DRB1 in ESRD patients in comparison to healthy controls, *HLA DRB1*04* was present more frequently in ESRD patients than in controls and the difference between them was statistically significant (OR = 1.6484, 95% CI=1.0395-2.6138, p=0.0325, pc<0.03846). Kidney disorders leading to ESRD in studied group of patients included 173 (75.2%) patients, among which 3 patients or 1.73% with type 1 and 170 patients or 98.26% with type 2 diabetes, 33 (14.3%) patients with glomerulonephritis and 24 (10.43%) patients with essential hypertension which is in accordance with the most common cause of ESRD, diabetic nephropathy, respectively.

Although, the frequencies of *HLA DRB1*01*, *HLA DRB1*08*, and *HLA DRB1*15*, were higher in ESRD Vojvodina patients (OR=1.20, 1.37 and 1.17, respectively), however, none of them reached the statistical significance (p<0.05).

Table 3. Disequilibrium linkage coefficient (Δ) for HLA- A~B, B~DRB1 and A~DRB1 haplotypes (values >0.01) in ESRD Vojvodina patients and controls

ESRD patients			Controls		
HAPLOTYPE	**Δ**	**χ^2**	**HAPLOTYPE**	**Δ**	**χ^2**
A*01~ B*08	0.01423	0.83022	A*01~ B*08	0.0112	0.3456
A*26 ~B*38	0.01070	1.5735	A*26 ~B*38	0.0144	1.9459
A*01~ B*51	0.01287	0.5109	A*02~ B*51	0.01218	0.1971
A*02 ~B*51	0.01450	0.1928	B*08~DRB1*03	0.0370	1.5416
B*07~DRB1*15	0.012569	1.6844	B*13~DRB1*07	0.01085	1.9051
B*13~DRB1*07	0.010629	0.3769	A*01~DRB1*03	0.02446	0.6291
B*18~DRB1*11	0.015836	0.5880	A*02~DRB1*01	0.01251	0.2233
B*38~DRB1*13	0.011792	1.0820			
A*01~DRB1*03	0.011944	0.4574			
A*24~DRB1*11	0.010084	0.3380			

Critical value where degree of freedom=1, $p < 0.05$ is 3.841

A total of 577 HLA- A~B, -B~DRB1 and -A~DRB1 haplotypes were identified in 230 ESRD patients, and 595 haplotypes identified in 290 controls, respectively. Moreover, the frequencies of the most common two loci HLA haplotypes: HLA-A*02~B*51, HLA-A*02~B*18 and HLA-A*01~B*08 were 0.0521, 0.0239 and 0.0228, respectively, HLA-B*08~DRB1*03, B*18~DRB1*11 and B*51~DRB1*01 were 0.0326, 0,0293 and 0.0225, respectively, while for A*02~DRB1*04, A*02~DRB1*03 and A*01~DRB1*03, were 0.0326, 0.0304 and 0.0250, respectively, in ESRD patients. These results did not differ significantly compared to those in the control group.

The disequilibrium linkage coefficient values greater than 0.01 for ESRD patients and controls are presented in Table 3. The analysis of differences between observed and expected HLA haplotype frequencies revealed that Δ values did not reached statistically significant border value of 3.841, $p<0.05$ for degree of freedom=1, for any of HLA- A~B, -B~DRB1 or -A~DRB1 haplotype. This result indicated that there is no strongly associated HLA allele combinations in ESRD Vojvodina patients, respectively.

DISCUSSION

Many studies have been performed worldwide on the HLA complex and disease. The identification of HLA-associated diseases parallels increased understanding of the genetic complexity of the HLA system and its extensive polymorphism. With the passage of time, several hundred diseases have now been reported to occur more frequently in individuals with particular HLA genotypes. These diseases include a broad spectrum of immune-mediated diseases involving all major organ systems, certain malignancies, infectious diseases and more recently, adverse reactions to particular drugs. In the present study an attempt has been made to see whether there is any difference between ESRD patients and controls at individual HLA A, B, DRB1 allele basis.

Note that the polymorphism of HLA-DRB1 is considered as a susceptible genetic marker for several autoimmune conditions and diseases, such as type I diabetes and dilated cardiomyopathy (CAO *et al.,* 2014; ZHANG *et al.,* 2009; JIN *et al.,* 2011). Although there was no

significant difference in the most frequently occurring HLA- A, -B and -DRB1 genes between the ESRD patients and the normal controls, *HLA-DRB1*04* was positively associated with ESRD (OR=.1.6484, 95% Confidence Interval is 1.0395 - 2.6138, p value is 0.0325, p_c<0.03846, respectively). This finding suggest that *HLA-DRB1*04* may be a susceptibly risk factor for the development of ESDR in Vojvodina population. Our results are in accordance with the results of previous study, where *HLA-DRB1*04* was significantly higher than that in the controls, with the frequency of 14.21% in ESRD patients (CAO *et al.*, 2014).

There are studies that investigate the association between HLA specificities and ESRD showing no difference in phenotypic and genotypic frequency of HLA - A, -B, and -DR antigens in donors and recipients (AGRAWAL *et al.*, 2001). In addition, there are numerous studies that point to an association of specific HLA genes with ESRD. One study showed that HLA-B8 was the only HLA of the three studied loci to be significantly higher in ESRD Kuwaiti patients when compared to healthy controls (OR=262, p=0.001, pc=0.038) (MOSAAD *et al.*, 2014). Among the HLA-DR and HLA-DQ specificities assessed among the Saudi patients awaiting transplantation, *HLA-DQB1*03 e.g.* 8(3) was found to be present more frequently in ESRD patients (ALMOGREN *et al.*, 2012).

The study about ESRD Taiwanese individuals suggests that HLA-DR3 (odds ratio = 1.91, 95 % CI = 1.098–3.324, P = 0.024, Pc = 0.312) and HLA-DR11 (odds ratio = 2.06, 95 % CI = 1.133–3.761, P = 0.021, Pc = 0.273) may represent susceptibility risk factors for the development of ESRD (DAI *et al.*, 2015). According to association study investigating the Brazilian patients awaiting kidney transplant, the antigens positively associated with ESRD were: HLA-A78 (RR=30.31 and EF=0.96) and HLA-DR11 (RR=18.87 and EF=0.65) (CRISPIM *et al.*, 2008).

Therefore, the development of ESRD is associated with different HLA in different ethnic population, different races of the same population and sometimes the results may be contradictory. These findings can be explained by the difference in patients and controls number between studies, different techniques used for HLA-typing, different etiologic factors leading to ESRD, different ethnic and genetic background of each population. A further cause may be the unequal relationship between HLA genes and other nearby genes involved in accommodating the immune response. An example of this relates to the statement that polymorphisms in genes encoding certain cytokines, including interleukin (IL)-6, IL-4 and tumor necrosis factor, may be affected in the progression to ESRD (MOSAAD *et al.*, 2014; NASSAR *et al.*, 2015; RANGANATH *et al.*, 2009).

In population genetics, linkage disequilibrium (LD) is the non-random association of alleles at different loci. Loci are said to be in linkage disequilibrium when the frequency of association of their different alleles is higher or lower than what would be expected if the loci were independent and associated randomly. Alleles from different genes are not in random recombination if the genes are located close to each in a chromosome. In fact, this phenomenon, called 'linkage disequilibrium', is used in gene mapping to determine how close two genes are to each other. Insofar as HLA genes are close to each other they are in linkage disequilibrium. Linkage disequilibrium is influenced by many factors, including selection, the rate of recombination, the rate of mutation, genetic drift, the system of mating, population structure, and genetic linkage. As a result, the pattern of linkage disequilibrium in a genome is a powerful signal of the population genetic processes that are structuring it (SLATKIN, 2008).

The pattern of LD across the human genome varies markedly between regions and populations, and is currently a subject of intense interest both as a mapping tool for the identification of disease susceptibility genes, and for what it may reveal about a population's demographic history and the selective forces acting thereon. An understanding of regional LD may be exploited to fine map susceptibility genes within replicated linkage areas (BLOMHOFF *et al.*, 2006).

Our analysis of HLA two loci haplotype frequencies in ESRD patients and controls revealed that there was no significant difference in Δ values for all HLA- A~B, -B~DRB1 and -A~DRB1 haplotype combinations in both of investigated groups. Our results of haplotype frequencies showed no association with ESRD in Vojvodina patients which is not consistent with the results of the study, where the haplotypes positively associated with ESRD were: HLA-A*01~DRB1*13 and HLA-A*30~DRB1*03 (HAMDI *et al.*, 2014). Our study could be the basis for the further investigations and will be of great help in ERSD susceptibility gene localization or identification. Our study support the idea that polymorphisms of HLA Class II may influence the susceptibility to ESRD. HLA polymorphism might be a useful clinical tool for screening patients with high risk of ESRD.

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REFERENCES

AGRAWAL, S., A.K., SINGH, R.K., SHARMA (2001): HLA gene and haplotype frequency in renal transplant recipients and donors of Uttar Pradesh (North India). Indian J. Nephrol., *11*: 88-97.

ALMOGREN, A.A., Z.Z., SHAKOOR, K.D., HAMAM (2012): Human leucocyte antigens: their association with end-stage renal disease in Saudi patients awaiting transplantation. Brit. J. Biomed. Sci., 69:159-163.

BLOMHOFF, A., M., OLSSON, S., JOHANSSON, H.E., AKSELSEN, F., POCIOT, J., NERUP, I., KOCKUM, A., CAMBON-THOMSEN, E., THORSBY, D.E., UNDLIEN, B.A., LIE (2006): Linkage disequilibrium and haplotype blocks in the MHC vary in an HLA haplotype specific manner assessed mainly by DRB1*03 and DRB1*04 haplotypes. Genes Immun., *7*:130–140.

CAO, Q., D., XIE, J., LIU, H., ZOU, Y., ZHANG, H., ZHANGET, Z., ZHANG, H., XUE, J., ZHOU, P., CHEN (2014): HLA Polymorphism and Susceptibility to End-Stage Renal Disease in Cantonese Patients Awaiting Kidney Transplantation. PLoS ONE, *9*(3): e90869.

CRISPIM, J.C., C.T., MENDES-JUNIOR, I.J., WASTOWSKI, G.M., PALOMINO, L.T., SABER, D.M., RASSI, E.A., DONADI (2008): HLA Polymorphisms as Incidence Factor in the Progression to End-Stage Renal Disease in Brazilian Patients Awaiting Kidney Transplant. Transpl. Proc., *40*: 1333–1336.

DAI, C-S., C-C., CHU, S-F., CHEN, C-Y., SUN, M., LIN, C-C., LEE (2015): Association between human leucocyte antigen subtypes and risk of end stage renal disease in Taiwanese: a retrospective study. BMC Nephrology, *16*:177-185.

HAMDI, N.M., F.H., AL-HABABI, A.E., EID (2014): HLA Class I and Class II Associations with ESRD in Saudi Arabian Population. PLoS ONE, *9*(11):e111403.

IBELS, L.S., A.Z., GYORY (1994): IgA nephropathy: analysis of the natural history, important factors in the progression of renal disease, and review of the literature. Medicine, *73*:79-102.

JIN, B., H., NI, Q., GESHANG, Y., LI, W., SHEN, H., SHI (2011): HLA-DR4 antigen and idiopathic dilated cardiomyopathy susceptibility: a meta-analysis involving 11,761 subjects. Tissue Antigens, *77*: 107–111.

KITAGAWA, W., H., IMAI, A., KOMATSUDA, N., MAKI, H., WAKUI, Y., HIKI, S., SUGIYAMA (2008): The HLA-DRB1*1501 allele is prevalent among Japanese patients with antiglomerular basement membrane antibody-mediated disease. Nephrol. Dial Transplant., *23*: 3126–3129.

LEWONTIN, R.C. (1988): On measures of gametic disequilibrium. Genetics, *120*: 849–852.

MOSAAD, Y.M., M., MANSOUR, I., AL-MUZAIRAI, T., AL-OTABI, M., ABDUL-MONEAM, R., AL-ATTIYAH, M., SHAHIN (2014): Association between Human Leukocyte Antigens (HLA-A, -B, and -DR) and end-stage renal disease in Kuwaiti patients awaiting transplantation, Renal Failure, *36*(8): 1317-1321.

NASSAR, M.Y., H.A., AL-SHAMAHY, H.A.A., MASOOD (2015): The Association between Human Leukocyte Antigens and Hypertensive End-Stage Renal Failure among Yemeni Patients. Sultan Qaboos Univ. Med. J., *15*(2): e241–e249.

OLERUP, O., H., ZETTERQUIST (1992): HLA DR typing by PCR amplification with sequence specific primers (PCR SSP) in two hours: An alternative to serological DR typing in clinical practice including donor recipient matching in cadaveric transplantation. Tissue Antigens, *39*:225-235.

PERNEGER, T.V. (1998): What's wrong with Bonferroni adjustments. BMJ, *316*:1236–1238.

RANGANATH, P., G., TRIPATHI, R.K., SHARMA, S.N., SANKHWAR, S., AGRAWAL (2009): Role of non-HLA genetic variants in end-stage renal disease. Tissue Antigens, *74*:147–155.

SLATKIN, M. (2008): Linkage disequilibrium - understanding the evolutionary past and mapping the medical future. Nat. Rev. Genet., *9* (6): 477–485.

VOJVODIĆ, S., D., ADEMOVIĆ-SAZDANIĆ (2012): HLA Class I and class II polymorphism in the population of Vojvodina, Serbia. Coll, Antropol,, *6*(4):1379-1384.

VOJVODIĆ, S., D., ADEMOVIĆ-SAZDANIĆ, I., BUSARČEVIĆ (2013): Association of HLA -A, -B and -DRB1 alleles with hematological diseases in Vojvodina. Genetika, *45*(1):63-74.

ZHANG, X.M., H.Y., WANG, Y.Y., LUO, L.N., JI (2009); HLA-DQ, DR allele polymorphism of type 1 diabetes in the Chinese population: a meta-analysis. Chin. Med. J. (Engl), *122:* 980–986.

HUMANI LEUKOCITNI ANTIGEN POLIMORFIZAM KAO OSETLJIVI FAKTOR RIZIKA KOD ZAVRŠNE FAZE RENALNIH BOLESTI

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Izvod

Većina pacijenata sa hroničnom insuficijencijom bubrega u terminalnom stadijumu bolesti (ESRD) zahteva transplantaciju bubrega. Mnoge studije su pokazale udruženost između sistema humanih leukocitnih antigena (HLA) i ESRD. Cilj ove studije je da se utvrdi povezanost HLA -A, -B, -DRB1 specifičnosti sa ESRD u populacijii Vojvodine, Srbije. U studiji je ispitivano 230 ESRD pacijenata na listi čekanja za transplantaciju bubrega i 290 zdravih kontrolnih ispitanika sa regiona teritorije Vojvodine. Metodom lančane reakcije polimeraze sekvenciono specifičnim prajmerima (PCR-SSP) su analizrane HLA-A, HLA-B i HLA-DRB1 specifičnosti u obe grupe ispitanika. Rezultati istraživanja ukazuju da HLA DRB1*04 (odds ratio = 1,6484, 95% CI = 1.0395-2.6138, P = 0.0325) gen može predstavljati rizični faktor za razvoj ESRD u populaciji Vojvodine. Najviše vrednosti koeficijenta neravnoteže vezivanja alela za dva HLA lokusa u ESRD pacijenata su pronađene za B*18~DRB1*11 (Δ = 0.01583) i A*02~B*51 (Δ = 0.0145) i kontrolnoj grupi za B*08~DRB1*03 (Δ = 0.370) i A*01~DRB1*03 (Δ = 0.02446), ali bez statističke značajnosti. Rezultati našeg istraživanja ukazuju da *HLA DRB1* 04* kao marker rizika može biti povezan sa razvojem ESRD. Potrebno je sprovesti dodatna istraživanja da bi se istražilo koje vrste i podvrste HLA alela su uključene u progresiju ESRD.

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