

HLA Genotyping in Patients with End-Stage Renal Disease Waiting For Cadaveric Renal Transplantation in Federation of Bosnia and Herzegovina

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Abstract

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AIM: The research was conducted by genotyping two Human Leukocyte Antigen (HLA) gene classes. The main objective of this research was to investigate distribution and frequency of the allelic groups, genotypes and haplotypes in the gene loci of HLA class I (HLA-A*, -B*, -C*) and HLA class II (HLA-DRB1*, -DQB1*) in patients included in the program of cadaveric renal transplantation.

MATERIAL AND METHODS: Our study covered 186 blood samples of patients who are registered on the list for cadaveric renal transplantation in Federation of Bosnia and Herzegovina and included 59 control, healthy unrelated individuals. For the HLA typing, we have used three different methods: micro lymphocyte cytotoxicity test (MLCT), Polymerase Chain Reaction (PCR) – Sequence Specific Primers (SSP) and PCR – Sequence-Specific Oligonucleotides (SSO) or Luminex technology. All patients and cadaveric donors were tested using the three methods because the system is polymorphic.

RESULTS: Analysis of the results of genotyping HLA class I gene loci identified dominant HLA-A*02, HLA-B*35, HLA-C*07 allelic groups. Analysis of the HLA class II gene loci genotyping showed that HLA-DRB1*11 and HLA-DQB1*03 loci had the highest incidence in HLA class II.

CONCLUSION: Based on our results and previous research, there were no observed differences between allelic frequencies and genotypes of healthy people and people with ESRD. Differences between allelic groups occurred, but they were not statistically significant, except HLA-C*01 ($p = 0.020$).

Introduction

The main problem with renal transplantation is the lack of suitable dead donors. The improvements in molecular genotyping methods, diagnostics and therapy for maintaining transplants, upgrade the transplantation process, however, the number of dead donors is not increasing, while transplantation lists and the need for dead transplants continues to grow each year [1]. Kidney transplants from live donors produce better results, including faster rehabilitation and better success rate. Another advantage is the

increase in the number of available organs. Higher tissue HLA matching between donors and recipients contributes to the longer survival of transplanted organs [2]. Many transplant centres are limited to living organ donors only. When the donors are divided into groups by degree of HLA compatibility with the organ recipient, a better result in the survival of transplant has been achieved in the cases of higher HLA compatibility. This fact is especially true when the unrelated persons (or distant relatives) are kidney donors [3, 4]. Living organ transplantation should be considered for each organ recipient. MHC antigens, which have proven to be important in kidney

transplantation, are HLA-A, HLA-B and HLA-DR markers. As these antigens were determined by the genes of both parents, at least six antigens of the recipient (2A, 2B and 2DR) must correspond with the antigens of the potential donor [5]. If the kidney has been taken from a family member, parents or siblings, it is necessary that there is a correspondence in three loci, and partial coincidence in less important, but other present genes HLA-C, HLA-DP and HLA-DQ [6]. Value of the haplotype synchronizing (0, 1, 2) was determined clinically: siblings with two identical haplotypes can expect survival of 90% of transplants after one year, parents and siblings with the same haplotype after one year achieved graft survival of 75%, and members of the same family without equal haplotypes achieve survival of 50% of the grafts after one year [7]. The main problem in transplantation is the immune response of T and B lymphocytes of the host [8]. The most preferred method for the prevention of transplant rejection is achieving sufficient antigen matching between donor and recipient, as with identical twins. Good acceptance of the transplanted tissue or organ is already achieved if the donor and recipient are matched in MHC-II class of antigens (in particular HLA-DR) since they directly activate T-helper lymphocytes of the recipient [9, 10]. HLA antigens are the main target of immune response which leads to the rejection of transplanted organs. For the purpose of transplantation, a state of the histocompatibility between donor and recipient exists only when the immune response is absent or controlled to foreign graft survive [11]. The reaction of transplant rejection is an immune response, directed primarily towards the molecules of the main histocompatibility system or MHC antigens and other mismatched graft antigens [12, 13].

The main objective of this research was analysis of distribution and frequency of the allelic groups, genotypes and different haplotypes in the gene loci of HLA class I (HLA-A*, -B, -C*) and HLA class II (HLA-DRB1*, -DQB1*) in patients who have been included in the program of cadaveric renal transplantation in Federation of Bosnia and Herzegovina (FB&H) for period 2007-2012.

Materials and Methods

The sample included 186 patients. All patients were in End Stage Renal Disease (ESRD) who were included in the program of cadaveric kidney transplantation in FB&H, and they are not related by blood kinship relations. Research covered patients from 9 hemodialysis center: Sarajevo (39 male; 16 women), Zenica (29 male; 12 women), Bihać (13 male; 11 women), Tešanj (13 male; 10 women); Travnik (8 male; 5 women), Mostar +Konjic (4 male; 4

women), Živinice + Gračanica (8 male; 1 women), Odžak (6 male; 2 women). Mean age was 49 ± 7 years. Our research on patients was undertaken in compliance with all applicable guidelines, which aim to ensure the proper implementation of the safety of persons participating in the scientific research, including Fundamentals of Good Clinical Practice, Declaration of Helsinki 1975, as revised in 2008, and in the accordance with the approval of the Ethics Committee of the Institute (Approval No. 01-3-3558 23.6.2016). Genotyping included 59 healthy individuals that are not related to the patients; they represented control group.

HLA genotyping

Samples of venous blood were taken into vacutainer tubes with anticoagulant heparin (serological analysis), and samples of venous blood were taken into vacutainer tubes with anticoagulant EDTA (ethylene diamine tetra acetyl acid – molecular analysis). Isolation of DNA was carried out using the kit for DNA, Ready – DNA Spin Kit (Inno-train, Germany). The HLA genotyping was performed by using three different methods: 1) phenotyping of HLA class I (A, B and C) was done by using micro lymphocyte cytotoxicity test (MLCT); 2) genotyping of HLA class I (HLA-A*, -B* and -C*) and HLA class II (HLA-DRB1* and -DQB1*) was performed by low resolution or by using the Polymerase Chain Reaction (PCR) qualitative detection of sequential specific PCR products by agarose gel electrophoresis (method is based on the PCR-SSP or Sequence Specific Primers); 3) genotyping of HLA class I (HLA-A*, -B* and -C*) and HLA class II (HLA-DRB1* and -DQB1*) was also performed by low resolution, using asymmetric PCR with different primers for each sample. After amplification of the PCR products labelled with biotin, they were mixed and bind to complementary probes during hybridization process (method based on PCR-SSO or Sequence-Specific Oligonucleotides). This technology is also called Luminex technology or technology of fluoroanalyser with microspheres.

Statistical analysis

The frequency of genotypes, gene variants was estimated according to estimation-maximization (EM) algorithm which has been implemented in a computer software PowerMarker v3.25 (Bioinformatic program, Raleigh, NC, USA) and OpenEpi v3.01. [14]. Software for calculating risk ratios (OR) using 2x2 contingency tables was also used. To calculate the statistical significance of the differences in the frequencies of gene variants, and genotypes of the control group and the patients, the Fisher accuracy test, with $P > 0.05$ was used.

Results

The frequencies of allelic groups of HLA class I (HLA-A*, -B*, -C*) are shown in Table 1. The highest frequency of the allelic groups of HLA-A* gene locus had HLA-A*02 (0.2930) in patients and control group HLA-A* (0.2797). The lowest frequency allelic groups were HLA-A*30 and A*31, while they weren't recorded in the control group.

Table 1: Frequency of allelic groups HLA-A*, HLA-B* and HLA-C* between control and experimental group

a) HLA-A* allelic groups							
n	Patients	2n	Frequency fa	Control	2n	Frequency fa	p-values
1.	A*01	68	0.1828	A*01	17	0.1441	0.277
2.	A*02	109	0.29301	A*02	33	0.2797	0.717
3.	A*03	39	0.10484	A*03	10	0.0847	0.503
4.	A*11	23	0.06183	A*11	12	0.1017	0.127
5.	A*24	36	0.09677	A*24	13	0.1102	0.654
6.	A*25	13	0.03495	A*25	4	0.0339	0.956
7.	A*26	26	0.06989	A*26	9	0.0763	0.807
8.	A*30	9	0.02419	A*30	/	-	-
9.	A*31	9	0.02419	A*31	/	-	-
10.	A*32	12	0.03226	A*32	4	0.0339	0.929

b) HLA-B* allelic groups							
n	Patients	2n	Frequency fa	Control	2n	Frequency fa	p-values
1.	B*07	21	0.05645	B*07	4	0.0339	0.318
2.	B*08	34	0.0914	B*08	8	0.0678	0.401
3.	B*13	15	0.04032	B*13	2	0.0169	0.218
4.	B*18	27	0.07258	B*18	11	0.0932	0.445
5.	B*27	15	0.04032	B*27	5	0.0424	0.920
6.	B*35	45	0.12097	B*35	17	0.1441	0.477
7.	B*38	17	0.0457	B*38	4	0.0339	0.572
8.	B*44	42	0.1129	B*44	8	0.0678	0.134
9.	B*51	54	0.14516	B*51	15	0.1271	0.591
10.	B*57	17	0.0457	B*57	5	0.0424	0.820

c) HLA-C* allelic groups							
n	Patients	2n	Frequency fa	Control	2n	Frequency fa	p-values
1.	C*01	41	0.11022	C*01	5	0.0424	0.020*
2.	C*02	38	0.10215	C*02	8	0.0678	0.239
3.	C*03	21	0.05645	C*03	8	0.0678	0.638
4.	C*04	66	0.17742	C*04	22	0.1864	0.813
5.	C*05	20	0.05376	C*05	4	0.0339	0.371
6.	C*06	41	0.11022	C*06	12	0.1017	0.781
7.	C*07	120	0.32258	C*07	36	0.3051	0.626
8.	C*08	5	0.01344	C*08	1	0.0085	0.667
9.	C*12	9	0.02419	C*12	15	0.1271	<0.001*
10.	C*14	3	0.00806	C*14	2	0.0169	0.400

*. Statistically different in comparison with control.

Among allelic groups of HLA-B* gene locus the most usual allelic groups were HLA-B*51 (0.1451) in patients group and HLA-B*35 (0.1209) in control group, while allelic groups HLA-B*13 and B*27 in patients group and allelic groups HLA-B*13 in control group had the lowest frequency.

The highest frequency of HLA-C* gene locus had following allelic group HLA-C*04 (0.1774) in patients group and HLA-C*07 (0.3051) at healthy, control group while the lowest frequency value had allelic group C*14 in patients group and C*08 in control group. Significant differences between both groups were recorded for HLA-C*01 and C*12 allelic groups.

Genotyping of HLA class II gene loci determined presence HLA-DRB1*11 allele group with highest frequencies of patients group and HLA-DRB1*13 in control group. Results of analysis of DRB1* gene locus are shown in Table 2.

The highest frequency within the HLA-DQB1* gene locus had allelic group DQB1*03 (0.3333), and HLA-DQB1* 06 is very frequently in both group. It was not noticed an absence of any allelic group in HLA-DQB1* gene locus. Results of analysis of DQB1* allelic groups are shown in Table 2.

Table 2: Frequency of HLA-DRB1* and HLA-DQB1* gene loci between control and experimental group

HLA-DRB1*							
n	Patients	2n	Frequency fa	Control	2n	Frequency fa	p-values
1.	DRB1*01	45	0.12097	DRB1*01	13	0.1102	0.733
2.	DRB1*03(17)	43	0.11559	DRB1*03(17)	16	0.1356	0.531
3.	DRB1*04	47	0.12634	DRB1*04	9	0.0763	0.110
4.	DRB1*07	29	0.07796	DRB1*07	9	0.0763	0.950
5.	DRB1*08	14	0.03763	DRB1*08	5	0.0424	0.812
6.	DRB1*11	57	0.15323	DRB1*11	15	0.1271	0.443
7.	DRB1*13	38	0.10215	DRB1*13	20	0.1695	0.030*
8.	DRB1*14	22	0.05914	DRB1*14	5	0.0424	0.751
9.	DRB1*15	36	0.09677	DRB1*15	13	0.1102	0.654
10.	DRB1*16	29	0.07796	DRB1*16	11	0.0932	0.580

HLA-DQB1*							
n	Patients	2n	Frequency fa	Control	2n	Frequency fa	p-values
1.	DQB1*02	68	0.1828	DQB1*02	22	0.1864	0.919
2.	DQB1*03	124	0.48334	DQB1*03	36	0.3050	0.427
3.	DQB1*04	12	0.03226	DQB1*04	3	0.0254	0.702
4.	DQB1*05	106	0.28495	DQB1*05	28	0.2373	0.200
5.	DQB1*06	62	0.16667	DQB1*06	29	0.2458	0.028*

*. Statistically different in comparison with control.

HLA-A* gene locus analysis revealed 63 different HLA-A* genotypes for 186 observed patients (Table 3.). The most common genotypes were HLA-A*02/A*02 (0.1022), and HLA-A*01/A*02 (0.0753).

Table 3: Frequency of HLA-A* genotypes between control and experimental group

HLA-A* genotypes							
n	Patients	n	Genotype frequency	Control	n	Genotype frequency	p-values
1.	A*01/A*01	7	0.0376	A*01/A*01	/	/	-
2.	A*01/A*02	14	0.0753	A*01/A*02	5	0.0847	0.812
3.	A*01/A*03	11	0.0591	A*01/A*03	/	/	-
4.	A*01/A*24	8	0.043	A*01/A*24	3	0.0508	0.800
5.	A*01/A*26	6	0.0323	A*01/A*26	1	0.0169	0.460
6.	A*02/A*02	19	0.1022	A*02/A*02	4	0.0678	0.424
7.	A*02/A*03	14	0.0753	A*02/A*03	4	0.0678	0.848
8.	A*02/A*11	8	0.043	A*02/A*11	5	0.0847	0.107
9.	A*02/A*24	11	0.0591	A*02/A*24	4	0.0678	0.809
10.	A*02/A*26	7	0.0376	A*02/A*26	3	0.0508	0.654

Within the HLA-B* gene locus, in 186 patients, an analysis revealed 96 different genotypes (Table 4). The most common genotypes were HLA-B*35/B*44 (0.0484) and HLA-B*08/51*B (0.0376). In the control group, the most frequent genotypes in B* locus are HLA-B*08/51 and HLA-B*35/51 (0.0508).

Table 4: Frequency of HLA-B* genotypes between control and experimental group

HLA-B* genotypes							
n	Patients	n	Genotype frequency	Control	n	Genotype frequency	p-values
1.	B*07/B*35	5	0.0269	B*07/B*35	1	0.0169	0.667
2.	B*07/B*44	4	0.0215	B*07/B*44	/	/	-
3.	B*08/B*44	6	0.0323	B*08/B*44	/	/	-
4.	B*08/B*51	7	0.0376	B*08/B*51	3	0.0508	0.654
5.	B*13/B*18	6	0.0323	B*13/B*18	1	0.0169	0.538
6.	B*18/B*51	4	0.0215	B*18/B*51	1	0.0169	0.829
7.	B*35/B*35	5	0.0269	B*35/B*35	1	0.0169	0.667
8.	B*35/B*44	9	0.0484	B*35/B*44	2	0.0339	0.639
9.	B*35/B*51	5	0.0269	B*35/B*51	3	0.0508	0.366
10.	B*38/B*44	4	0.0215	B*38/B*44	/	/	-
11.	B*38/B*51	4	0.0215	B*38/B*51	1	0.0169	0.829
12.	B*39/B*51	4	0.0215	B*39/B*51	1	0.0169	0.829
13.	B*51/B*51	6	0.0323	B*51/B*51	/	/	-

Within the HLA-C* gene locus it was found 37 different genotypes (Table 5), the most common genotypes were HLA-C*07/07*C (0.1774), HLA-C*04/04*C (0.1022) and HLA-C*04/07*C (0.0699) in patients group. In the control group, the most frequent genotype is HLA-C*07/07*C (0.1356).

Table 5: Frequency of HLA-C* genotypes between control and experimental group

HLA-C* genotypes		n	Genotype frequency	Control	n	Genotype frequency	p-values
n	Patients						
1.	C*01/ C*01	10	0.0538	C*01/ C*01	/	/	-
2.	C*02/ C*02	7	0.0376	C*02/ C*02	/	/	-
3.	C*02/ C*07	7	0.0376	C*02/ C*07	1	0.0169	0.436
4.	C*03/ C*07	7	0.0376	C*03/ C*07	1	0.0169	0.436
5.	C*04/ C*04	19	0.1022	C*04/ C*04	3	0.0508	0.230
6.	C*04/ C*07	13	0.0699	C*04/ C*07	5	0.0847	0.703
7.	C*07/ C*07	33	0.1774	C*07/ C*07	8	0.1356	0.453
8.	C*01/ C*06	7	0.0376	C*01/ C*06	/	/	-
9.	C*06/ C*06	7	0.0376	C*06/ C*06	/	/	-
10.	C*06/ C*07	12	0.0645	C*06/ C*07	5	0.0847	0.086

Within the HLA-DRB1* gene locus it was determined the presence of 58 different genotypes, including the most common HLA-DRB1*01/DRB1*11 (0.0430), HLA-DRB1*04/DRB1*11 and HLA-DRB1*13/DRB1*16 genotype (0.0376) in patients group.

Table 6: Frequency of HLA-DRB1* genotypes between control and experimental group

HLA-DRB1* genotypes		n	Genotype frequency	Control	n	Genotype Frequency	p-values
n	Patients						
1.	DRB1*01/ DRB1*11	8	0.043	DRB1*01/ DRB1*11	/	/	-
2.	DRB1*01/ DRB1*04	6	0.0323	DRB1*01/ DRB1*04	1	0.0169	0.538
3.	DRB1*03(17)/ DRB1*11	6	0.0323	DRB1*03(17)/ DRB1*11	1	0.0169	0.538
4.	DRB1*03(17)/ DRB1*16	6	0.0323	DRB1*03(17)/ DRB1*16	2	0.0339	0.950
5.	DRB1*04/DRB1*07	6	0.0323	DRB1*04/DRB1*07	3	0.0508	0.508
6.	DRB1*04/ DRB1*11	7	0.0376	DRB1*04/ DRB1*11	2	0.0339	0.894
7.	DRB1*11/ DRB1*14	6	0.0323	DRB1*11/ DRB1*14	3	0.0508	0.508
8.	DRB1*11/ DRB1*15	6	0.0323	DRB1*11/ DRB1*15	1	0.0169	0.538
9.	DRB1*13/ DRB1*16	7	0.0376	DRB1*13/ DRB1*16	/	/	-

Research results within the HLA-DQB1* gene locus revealed 27 different genotypes. The most usual genotypes were HLA-DQB1*03/DQB1*05 (0.2204) and HLA-DQB1*02/DQB1*03 (0.1667) in patients group. The genotype HLA-DQB1*02/DQB1*03 is the most frequent in the control group.

Table 7: Frequency of HLA-DQB1* genotypes between control and experimental group

HLA-DQB1* genotypes		n	Genotype frequency	Control	n	Genotype frequency	p-values
n	Patients						
1.	DQB1*02/ DQB1*02	8	0.043	DQB1*02/ DQB1*02	/	/	-
2.	DQB1*02/ DQB1*03	31	0.1667	DQB1*02/ DQB1*03	10	0.1694	0.959
3.	DQB1*02/ DQB1*05	21	0.1129	DQB1*02/ DQB1*05	4	0.0678	0.318
4.	DQB1*03/ DQB1*03	21	0.1129	DQB1*03/ DQB1*03	3	0.0508	0.162
5.	DQB1*03/ DQB1*05	41	0.2204	DQB1*03/ DQB1*05	7	0.1186	0.086
6.	DQB1*03/ DQB1*06	22	0.1182	DQB1*03/ DQB1*06	8	0.1356	0.723
7.	DQB1*05/ DQB1*05	13	0.0699	DQB1*05/ DQB1*05	6	0.1017	0.426
8.	DQB1*05/ DQB1*06	14	0.0753	DQB1*05/ DQB1*06	3	0.0508	0.520

Discussion

HLA class I molecules can be found on the surface of all cells that contain the nucleus, while class II of HLA molecules can be constitutively found on the surface of certain types of cells (dendritic cells, macrophages, B-lymphocytes. HLA-DR (not HLA-DQ, -DP, or -DM) is abundantly expressed on the endothelial cells of peritubular and glomerular capillaries [15].

Table 8: Frequency of allelic group with Odds Ratio (OR) and p-values between control and patients group

Allelic groups	2n	Patients		Control		OR	p-values
		Allele frequency	2n	Allele frequency	2n		
HLA-C*01	41	0.11022	5	0.0424	2.799	0.020	
HLA-C*12	9	0.02419	15	0.1271	0.1702	0.0000063	
HLA-DRB1*13	38	0.10215	20	0.1695	0.5575	0.030	
HLA-DQB1*06	62	0.16667	29	0.2458	0.6138	0.0280	

The explanation for the improved survival of kidney allografts in which HLA have good congruence was: lower occurrence of anti-HLA antibodies [16], lower occurrence of alloreactive CD4+ T-cells or absence of direct CD4+ T-cell response to HLA-DR matched graft [17], fewer peptide epitopes stimulate response of T-helper cells of "indirect way" which includes chronic rejection of allograft [18]. HLA matching in HLA-A, HLA-B and HLA-DR loci increases the likelihood of developing a donor antigen-specific regulatory T-cells [19].

Benefits of HLA matching has an impact on different outcomes in terms of number of days spent in hospital, failure of graft function [20, 21], episodes of rejection, the one-year and three-year levels of serum creatinine [22], on prediction of long-term outcome of the disease, on status of patients and on multivariate analysis [23, 24].

Chronic renal failure (CRF) leads in most cases to ESRD, with final result – kidney transplantation process. There is an interest to assess connection of class I and II of HLA antigens with ESRD or CRF renal diseases [25].

In hemodialysis patients that are the part of cadaveric renal transplantation program in FB&H it was observed that the HLA antigens with the greatest frequency were: HLA-A*02 = 0.29301, HLA-B*51 = 0.14516, HLA-C*07 = 0.32258, HLA-DRB1*11 = 0.15323 and HLA-DQB1*03 = 0.48334. The antigens that showed in control group greatest frequency were: HLA-A*02= 0.2797, HLA-B*35= 0.1441, HLA-C* = 0.03051, HLA-DRB1*13 = 0.1695 and HLA-DQB1*03 = 0.3050. In the analysis of allelic groups in each locus, with estimation of the p-value, the allelic group HLA-A* showed no statistically significant difference in the aforementioned allelic groups. Such results were also recorded when comparing the frequencies of allelic groups in HLA-B*. Locus C* showed a statistically significant difference in the frequency of the allelic HLA-C*01 with p = 0.020 and OR = 2.601,

that is considered as an allelic group of high risk, and it showed a difference in the allelic HLA-C*12 with $p = 0.0000679$. Although the OR value is 0.190, this allelic group can't be considered protective. The HLA-DRB1* locus also showed statistical significance in frequencies in the allelic HLA-DRB1*13 ($p = 0.030$) with OR = 0.6027, and it is not at risk of developing ESRD. The allelic group HLA-DQB1*06 showed statistical significance ($p = 0.028$) and OR = 0.6138, and it is not considered as a group of high risk, as shown in Table 3.

Our research covered hemodialysis patients included in program of cadaveric renal transplantation in FB&H. The HLA class I (HLA-A*, -B*, -C*) genotypes with the highest incidence were: A*02/A*02 = 0.1022, B*35/B*44 = 0.0484, C*07/07 = 0.1774. HLA class II genotypes with highest incidence were: DRB1*01/DRB1*11 = 0.043 and DQB1*03/DQB1*05 = 0.2204.

Based on our results and previous research, there were no observed differences between allelic frequencies and genotypes of healthy people and people with ESRD. Differences between allelic groups occurred, but they were not statistically significant, except HLA-C*01, $p = 0.020$.

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