

Histocompatibility Testing for Organ Transplantation Purposes in Albania: A Single Center Experience

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Background: Histocompatibility testing (HT) which includes donor-recipient human leukocyte antigen (HLA) matching, cross-match testing (XMT) and anti-HLA antibody searching are crucial examinations in solid organ transplantation aiming to avoid the hyperacute graft rejection and also to predict the immunological outcome of the graft.

Aims: The aim of this study was to analyse the tissue typing data collected at the Laboratory of Immunology and Histocompatibility of the University Hospital Center of Tirana, Albania, in order to define those actions that should be taken for improvements in the situation of kidney transplantation in Albania.

Study Design: Descriptive study.

Methods: The donor/recipient cross-match testing was performed through a standard complement-dependent cytotoxicity (CDC) assay using separated donor T and B cells that were tested in parallel with the recipient serum sample. All recipient sera were screened for anti-Class I and anti-Class II HLA antibodies using a bead based Luminex anti-HLA antibody screening test. In the case of detected positivity, an allele-specific anti-HLA antibody determination was conducted with the respective Luminex anti-Class I and Class II HLA antibody determination kits.

Results: A total of 174 recipients and 202 donors were typed for the purpose of living donor kidney transplantation at our laboratory between January 2006 and December 2012. The mean age and female

gender proportion of patients were 34.9 years and 34.5%, respectively, and 48.0 years and 65.3% for the donors, respectively. Here, 25.9% of the patients reported a positive complement-dependent cytotoxicity cross-match test and/or a positive anti-HLA antibody testing result. Eighteen patients that were negative for the complement-dependent cytotoxicity cross-match test were positive for anti-HLA antibodies.

Conclusion: The predominant causes of end-stage renal disease (ESRD) in our patient population are chronic pyelonephritis and glomerulonephritis. The female gender is significantly more frequent among donors, which emphasises the need for more gender equity as far as the altruistic willingness for organ donation is concerned. The significant number of patients with Luminex anti-HLA antibody positivity combined with complement-dependent cytotoxicity cross-match negative results underlines the necessity of using additional methods like cell-based flow cytometry or bead-based Luminex anti-HLA antibody assays for the detection of anti-donor-specific antibodies. We also suggest that the number of kidney transplantations in Albania needs to be increased significantly by expanding it with paired exchange living donation and also by implementing an efficient deceased donor kidney transplantation program.

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Key Words: Albanian population, end-stage renal disease, histocompatibility testing, human leukocyte antigens, kidney transplantation

Histocompatibility testing (HT) plays an important role in determining the degree of human leukocyte antigen (HLA) matching between the solid organ recipients and their donors (1, 2). It also includes the donor-recipient cross-match testing (XMT) and anti-HLA antibody searches that are crucial examinations in solid organ transplantation, aiming to avoid hyperacute graft rejection and also to predict the immunological outcome of the graft (3-5).

Histocompatibility testing for solid organ transplantation in Albania began in 2006, when a living donor kidney transplan-

tation program was implemented at a national level. Since all of the patients from this country are referred to a single laboratory specialised in HT testing, the data obtained in this centre represent the real situation for kidney transplantation tissue typing in the entire country.

The aim of this study was to analyse the data collected in this national reference centre in order to determine the actions that should be taken for improvements in the situation of kidney transplantation in Albania.



MATERIAL AND METHODS

In this study we present the data corresponding to a total of 376 individuals (donors and recipients), which have been typed for the purpose of living donor kidney transplantation at the HT reference centre for Albania between January 2006 and December 2012.

The HLA-A, -B and -DRB1 allele genotyping was performed through a generic 2 digit sequence specific priming (SSP) method (Micro SSP™ DNA Typing Trays; One Lambda, Inc., Canoga Park, USA). In cases of ambiguity and for confirmation purposes, a second sequence-specific oligonucleotide (SSO) method was carried out. In this case, an SSO reverse line (INNO-LiPA; Innogenetics, Gent, Belgium) or a Luminex-based method (LIFECODES; Teplnel Lifecodes Corporation, Stamford, USA) was used.

Cross-match testing

The donor/recipient cross-match testing was performed through a standard complement-dependent cytotoxicity (CDC) assay (3). Briefly, donor lymphocytes were isolated and separated into T and B cells (FluoroBeads T and B isolation reagents, One Lambda Inc., Canoga Park, USA). The serum of the recipient was incubated with fresh donor lymphocytes in Terasaki tray wells. A complement source derived from rabbit serum (One Lambda Inc.) was then added, and after proper incubation, a cell staining solution composed of acridine orange, ethidium bromide, and quenching ink (Fluoroquench, One Lambda Inc.) was added into each tray's well. If donor-specific antibodies were present in the patient sera, they would bind to donor cells and the complement cascade would be activated, resulting in the lysis of lymphocytes. The results (as percentage of dead cells) were read using inverted fluorescence microscopy. Positive reactions were reported in the wells where the number of lysed cells is 20% or higher, and negative reactions in the wells with 0-20% cell death. Positive and negative T and B cell reacting control sera were included in each test. The negative control consisted of sera from non-transfused blood donor adult males of the AB group, whereas the positive control was comprised of pooled sera obtained from highly sensitised patients.

Because of the importance of differentiation between IgG and IgM antibodies, we used dithiothreitol (1,4-Dithio-DL-threitol; Sigma-Aldrich, St Louis, USA), a reducing agent, to inactivate IgM but not IgG in the cross-match assay (6). A positive CDC-XMT result followed by a negative result after DTT treatment was considered indicative of the presence of IgM antibodies only, whereas a positive cross-match despite DTT treatment was an indication of IgG antibody positivity.

Anti-HLA antibody screening with Luminex technology

All recipient sera were screened for anti-HLA Class I and Class II antibodies of the IgG isotypes using a Luminex bead-based solid phase immunoassay, according to the manufacturer's instructions (Teplnel Lifecodes Corporation, Stamford, CT, USA). Briefly, an aliquot of the micro-beads coated with purified Class I and/or Class II HLA antigens was allowed to incubate with a small volume of test

serum sample. The sensitised beads were then washed to remove unbound antibody. An anti-human IgG antibody conjugated to phycoerythrin was then added. After further incubation, the test sample was analysed on a Life-Match fluoroanalyser using the software Luminex 100 IS v 2.3 for data acquisition and Quicktype for Lifematch as the analysis software. Negative and positive control beads and a negative control sample were used to quality control the tests. The mean fluorescence intensity (MFI) of the testing beads was compared to the MFI of the negative control beads to determine whether the testing bead was positive or negative for bound anti-HLA antibodies.

Statistical analysis

The Fischer exact test was used for testing the statistical significance of differences between categorical data. The study protocol was approved by the ethics committee of the institution and conformed to the ethical guidelines of the 1975 Helsinki declaration. Written informed consent was obtained from all of the patients and donors participating in this study.

RESULTS

Patients and donors

From the 376 individuals typed for the purpose of living donor kidney transplantation during the 7 year period from 2006 to 2012, 174 (46.3%) were patients and 202 (53.7%) donors. The age of patients ranged from 6 to 64 years (mean: 34.87 years, SD: 13.51 years). Of these, 114 (65.5%) were males and 60 (34.5%) females ($p < 0.001$).

The main aetiological diagnoses of end-stage renal disease (ESRD) of the patients, with the corresponding patient numbers in decreasing order, are shown in Table 1. The age of living donors ranged from 19 to 74 years (mean: 48.05 years, SD: 10.95 years). Of them, 70 (34.7%) were males and 132 (65.3%) females ($p < 0.001$).

There were 161 (79.7%) related donors and 41 (20.3%) unrelated donors ($p < 0.001$). The detailed data concerning the related and unrelated donors are shown in Table 2.

Cross-match and anti-HLA antibody testing results

From all 174 patients tested, 45 (25.9%) had a positive CDC-XMT and/or positive Luminex anti-HLA antibody testing result. Among the 27 donor/recipient positive CDC-XMT patients, 5 (18.5%) were both T and B cell positive and also Luminex anti-HLA Class I and Class II antibody positive. The other 22 patients were only B cell positive. Out of them, 5 patients resulted Luminex anti-HLA Class I and Class II antibody positive, 6 patients were Luminex anti-HLA Class I antibody positive, 2 patients were only anti-HLA Class II antibody positive, and 9 patients showed no positivity with Luminex anti-HLA Class I and Class II antibodies. In these 9 patients, the initial CDC-XMT positivity changed to

TABLE 1. The causative diagnoses of the 174 ESRD Albanian patients studied

ESRD causative diagnoses	Number of patients (%)
Chronic pyelonephritis	88 (50.6%)
Chronic glomerulonephritis	55 (31.6%)
Polycystic kidney disease	12 (6.9%)
Reflux nephropathy	10 (5.7%)
Nephroangiosclerosis	6 (3.4%)
Diabetic nephropathy	2 (1.2%)
Congenital malformation (Lipomyelomeningocele)	1 (0.6%)

ESRD: end-stage renal disease

TABLE 2. Detailed data about the related and unrelated living donors of the 174 ESRD Albanian patients studied

Related donors (n=161)		Unrelated donors (n=41)	
Mothers	56 (34.8%)	Wives	27 (65.9%)
Fathers	36 (22.4%)	Emotional (altruistic) unrelated donors	11 (26.8%)
Sisters	38 (23.6%)	Husbands	3 (7.3%)
Second degree relatives	17 (10.5%)		
Brothers	13 (8.1%)		
Son	1 (0.6%)		

ESRD: end-stage renal disease

TABLE 3. CDC-XMT and Luminex anti-Class I and Class II HLA antibody testing results for the 174 ESRD Albanian patients studied

Luminex anti-HLA antibody results	CDC - XMT negative (n=27)		CDC - XMT positive (n=18)
	T and B cell positive (T+ / B+) (n=5)	B cell positive (T- / B+) (n=22)	T and B cell negative (T- / B-) (n=18)
	Luminex anti-Class I HLA antibody positive	-	6 (3.4%)
Luminex anti-Class II HLA antibody positive	-	2 (1.1%)	1 (0.6%)
Luminex anti-Class I and II HLA antibody positive	5 (2.9%)	5 (2.9%)	4 (2.3%)
Luminex anti-Class I and Class II HLA antibody negative	-	9 (5.2%)	129 (74.1%)

CDC-XMT: complement-dependent cytotoxicity cross-match testing; ESRD: end-stage renal disease

negative after DTT treatment, which suggests that the Luminex anti-HLA antibody negativity could be explained by the presence of only IgM, but not IgG, anti-HLA antibodies in these patients. Only those patients who were CDC-XMT negative underwent kidney transplantation.

Eighteen patients that were positive for anti-HLA antibodies detected by Luminex technology were negative following the CDC-XMT method. From these, 4 were both anti-HLA Class I and II positive, 13 only anti-HLA Class I positive and

one patient was positive for only anti-HLA Class II antibodies. The data concerning CDC-XMT and Luminex anti-HLA antibody results for all patients are shown in Table 3.

At the post-transplantation follow-up of the patients, the six-month and one-year graft survival rates were 96% and 89%, respectively. No correlation was detected between the graft survival and the degree of HLA matching.

DISCUSSION

In this paper, we present our single centre data about histocompatibility testing results in kidney transplantation in Albania. The average rate of patients typed for the purpose of kidney transplantation in our centre is around 25 per year, which reflects the actual living donor availability for ESRD patients in Albania. Taking into account the Albanian population, the rate of kidney transplantations in Albania can be estimated at less than 8 per one million population (pmp). In other European countries, variable but higher rates are reported, ranging from 16.2 kidney transplantations pmp in Greece, 39.4 pmp in Turkey, 49.4 pmp in Austria and 51.4 pmp in The Netherlands (7, 8).

Living donation constitutes 100% of kidney transplantations in Albania. Although the number of kidney transplantations from living donors is increasing continuously worldwide, they constitute approximately 20% of all kidney transplantations in Europe, with a large variation ranging from 13% in Austria, to 51% in The Netherlands and 82% in Turkey (8).

The average age of Albanian ESRD patients undergoing tissue typing in our centre is 35 years old. This is comparable with data for Turkish ESRD patients, where a similar mean age is reported (9), but differs from Western European countries, where the average age of the patients undergoing chronic dialysis is steadily increasing (10). Furthermore, as in Turkish ESRD patients (11), the predominant causative diagnoses in our ESRD patients were chronic pyelonephritis or glomerulonephritis, which is in contrast with Western European or North American countries, where diabetes and hypertension are becoming the leading causes of ESRD (12).

It is interesting to note that while the male gender predominates among the patients, there are significantly more female donors than males. These results are similar to other reports, such as those from Turkish, Italian, Chinese and also North American populations, where comparable female/male ratios are found (11-15). These data emphasise the need for more gender equity as far as the altruistic willingness for organ donation is concerned.

It is known that ABO blood group compatibility is the most important barrier to be overcome in living donor kidney transplantation, while HLA allele matching has only a relative importance (16). In contrast, the donor/recipient XMT as well as the anti-HLA antibody detection among the recipients play very important roles in the prevention of allograft rejection

(5, 17, 18). In this concern, the overall rate of donor/recipient CDC-XMT and/or anti-HLA antibody positivity found in our patients of around 26% is significantly lower than that reported in the USA, where such positivity has been found to be as high as 40% (19). This lower sensitisation rate may reflect the younger age of Albanian ESRD patients.

Through the CDC-XMT assay, we have found a significant number of T cell negative/B cell positive CDC results (59%), even after DTT treatment. The significance of this B cell positivity is to be considered cautiously and other methods for anti-donor-specific antibodies detection must also be performed in these patients (20, 21). Through anti-HLA antibody testing by the Luminex method, we have also detected a significant number of cases which were CDC-XMT negative and Luminex anti-HLA antibody positive. These results highlight the above-mentioned need to use other methods in parallel with CDC-XMT for the detection of anti-donor-specific antibodies. Such methods, like cell-based flow cytometry or bead-based Luminex anti-HLA antibody solid phase assays, have a higher sensitivity than the CDC-XMT assay (22, 23). On the other hand, these methods are also able to detect non-complement fixing antibodies that seem to have a different clinical significance when compared with the complement fixing antibodies which are generally detected using the CDC-XMT method (24).

As far as the six-month and one-year graft survival rates are concerned, in our study, we did not find any significant difference between the patients who were Luminex anti-HLA antibody positive and those who were anti-HLA antibody negative before transplantation. Regardless, further studies, with more patients and with a longer follow-up are needed to investigate any clinical correlations between the presence of non-complement fixing anti-HLA antibodies and the living donor graft outcome in more detail.

It is well known that related living kidney donation alone is unable to meet the need for kidney transplantation. Therefore, it is clear that the number of kidney transplantations in Albania must be increased significantly by expanding this with non-related paired exchange living donation, and also by implementing an efficient deceased donor kidney transplantation program.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of the Faculty of Medicine of Tirana University.

Informed Consent: Written informed consent was obtained from patients who participated in this study.

Peer-review: Externally peer-reviewed.

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