SENSITIZATION STATE OF POTENTIAL LIVE RELATED DONOR RENAL TRANSPLANT PATIENTS

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ABSTRACT

Sensitization state of 119 patients of end-stage renal disease registered for live related donor transplant was assessed by determining the panel reactive antibody (PRA) status and the donor-specific cross-match positivity. Eighty one patients (69.1%) showed no PRA, whereas 27 (22.6%) had PRA less than 10% and the remaining 11 patients (9.2%) displayed PRA more than 10%. The lower level of sensitization was related to the short dialysis period, less number of blood transfusions, fewer female patients, and lesser number of retransplants. Cross-match positive patients were in the higher PRA group (range 32-68%) as compared to 0-24% PRA in cross-match negative group.

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KEY WORDS : Kidney transplantation; Transplantation immunology; Cytotoxicity tests; Histocompatibility testing.

Introduction

Successful transplantation of parenchymal organs like kidney, heart, and liver are being performed with increasing frequency. The state of immunologic sensitization of the recipient greatly influences the success of the transplant [1]. Data from worldwide show that such sensitized patients have significantly lower graft survival than the nonsensitized patients [2,3]. Panel reactive antibodies (PRA) and donor-specific cytotoxic antibodies are used to assess the degree of sensitization. These antibodies may be generated by previous blood transfusions (BT), previous transplants, pregnancies, or infections [4,5]. In the present study, the sensitization state of 119 prospective renal transplant patients registered at All India Institute of Medical Sciences and Army Hospital Delhi Cantt was assessed. The findings were correlated with the number of pretransplant blood transfusions, duration of dialysis, number of renal transplants, and sex of the patients.

Material and Methods

Patients

Serum samples of 119 (males 104, females 15) prospective renal transplant recipients were collected at the time of their HLA typing and matching. The number of pretransplant BT, duration of dialysis, and whether first or retransplant was noted in each case. In females the number of pregnancies were ascertained. All these patients had related donors with atleast 50% HLA match.

Panel reactive antibodies

PRA state of the recipients was measured by using the extended microlymphocytotoxicity test [6] using their most recent serum against nylon-wool-separated T-lymphocytes of a panel of 40 HLA-typed healthy volunteers. A recipient was considered positive for that particular lymphocyte group if strength of the reaction was greater than 10% above the normal background level [7] and the results were expressed as the percentage of the

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panel lymphocytes to which the patients serum was found positive.

Lymphocytotoxic histocompatibility test

Donor-specific histocompatibility test was performed using the patient's most recent serum and donor nylon-wool-separated T-lymphocytes. A positive cross-match test was defined as lysis of more than 10% donor lymphocytes as compared to a negative control [7]. To detect the type of antibodies, viz, IgG or IgM, dithiothreitol (DTT) cross-match was also done [8]. One μ L of patient's serum was treated for 30 minutes with 1 μ L of 2×10^6 /mL of donor T-cells already suspended in 0.01M of DTT solution. One μ L of saturated cystine solution was incubated with the cell suspension for 5 minutes then complement was added and incubation continued for 60 minutes. Trays were read after staining with eosin and fixation with 40% formaldehyde.

Results

Of the 119 patients, 113 were for first transplantation and 6 for re-transplant. Mean age of patients was 32.2 ± 8.6 years (range 14-60 years). The mean dialysis period was 2.56 ± 2.70 months (range 0-10 months). The mean number of units of pretransplant BT per patient was 3.77 ± 4.33 (range 0-25 units). The relationship of PRA with sex, dialysis period, number of BT and status of grafts is shown in Table 1. Eleven patients (9.2%) were in 10-100% PRA positive group, 27 (22.6%) in 1-9% PRA group, and the remaining 81 (69.1%) patients had no PRA reactivity. Of the six patients for second renal transplantation, only one had a PRA positivity of greater than 10%.

Patients with 10-100% PRA positivity had received a higher number of BT than lower PRA group patients but the difference in number of BT fell short of statistical significance. The duration of dialysis did not differ much in the different PRA groups (Table 1). A significantly greater number of females (26.7%) were in 10-100% PRA positivity group when compared to the male patients (6.7%). All these patients had 1-3 pregnan-

cies in the past.

TABLE 1

Correlation of PRA with dialysis period, BT and secondary Tx

No of patients		Dialysis period (months)	Units of blood transfused	II Tx
PRA	Males/ Females	Mean $\pm$ SD ₁	Mean $\pm$ SD ₂	
0%	73/8	2.30 $\pm$ 1.89	3.29 $\pm$ 3.63	3
1-9%	24/3	3.58 $\pm$ 4.58	4.12 $\pm$ 3.83	2
10-100%	7/4	2.82 $\pm$ 2.16	5.81 $\pm$ 8.13	1

1 - $\chi^2 = 9.123$; $P = 0.455$; 2 - $\chi^2 = 3.36$ NS

PRA = Panel reactive antibodies,

BT = Blood transfusion

Tx = Transplant.

The PRA and cross-match relationship is shown in Table 2. The PRA positivity in 114 patients found compatible with their donors (cross-match test negative) ranged from 0-24% with mean of 1.3 ± 3.26 . In cross-match test positive patients (n=5), PRA positivity ranged from 34-65% with a mean of 45.8 ± 12.1 . Two cross-match positive patients with IgM autoantibodies against non-HLA antigens were found to be negative with DTT cross-match technique. Their PRA ranged from 10% to 12% and both these patients were ultimately considered compatible with their donors.

TABLE 2

Distribution of PRA and crossmatch in 119 patients

Patients	No	Cross match - ve	PRA	Cross match + ve	PRA
Males	104	101	Range 0-24%	3	Range 34%-65%
Females	15	13		2	
Total	119	114	Mean 1.3 ± 3.26	5	Mean 45.8 ± 12.1

PRA - Panel reactive antibodies.

Discussion

Various multicentre studies have shown adverse effects of high PRA and tissue

incompatibility on graft survival. Therefore most transplant centres carry out assessment of PRA status in prospective renal transplant patients. In many studies conducted worldwide, the PRA state has been reported to be greater than 50% in 7.5%-57% of patients in the pre-transplant period (9-13). A higher incidence of positivity has been reported in patients receiving more BT, retransplants, and in females. Significantly, patients who had a poorly matched first graft had higher increases in PRA [14]. The high PRA state makes it more difficult to find suitable grafts for these patients.

In our series, the sensitization state of patients awaiting live related donor (LRD) renal transplantation has been found to be much lower than other published studies (9-13), with only 9.24% of patients having positive PRA. This appears to be the cumulative effect of lesser number of pre-transplant BT (mean 3.77 units), and shorter dialysis period (mean 2.56 months). The other factors contributing to the lower sensitization state in our series may be related to the fewer number of females and the small number of retransplant patients. The role of environmental and MHC factors [6] which may also be contributory is being assessed in a larger project.

In a recent study on cardiac transplantation [7], it was seen that 75% of highly sensitized patients (PRA 51-100%) were cross-match positive as compared to 4.5% in the 0-10% PRA group.

In our study, out of 119 patients only 5 were found to be cross-match positive having mean PRA of 45.8 ± 12.1 (range 32-68%) as compared to 1.3 ± 3.26 PRA (range 0-24%) in cross-match negative group (Table 2). This observation may be of pronounced value in future cadaveric transplant programme of our country.

Based on these findings we feel that patients with higher PRA percentages but negative cross-match should be screened with more sensitive techniques like DTT, antihuman globulin test, and flowcytometry to exclude false negative cross-match with the

conventional tests. These patients may also require better HLA matched grafts and more aggressive immunosuppression.

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