CLINICAL APPLICATION OF FLOWCYTOMETRY IN LIVE RELATED DONOR RENAL TRANSPLANTATION

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ABSTRACT

To monitor the activity of the humoral component in graft dysfunction following renal transplantation using live related donors, flowcytometric cross-match procedure was adopted. Antidonor antibodies were detected in the sera of both pre- and post-transplant patients using conventional serological cytotoxicity cross-match and flowcytometric cross-match assays. In the 52 pretransplant samples no significant differences were observed in flowcytometric and cytotoxicity tests except in 2 secondary transplant cases which were negative by cytotoxicity test. However, in post-transplant samples, flowcytometry was found to be a more objective and useful test than cytotoxicity testing in distinguishing 6 out of 7 mild acute-graft-rejection episodes. Both tests were found to be negative in all 5 cases of cyclosporin-A nephrotoxicity, 7 cases of acute tubular necrosis and 10 out of 11 cases of chronic rejection.

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KEY WORDS : Flowcytometry; Cross-match; Graft dysfunction; Kidney transplantation.

Introduction

Replacement of diseased organs by healthy ones has long been one of the objectives in medicine. Data generated from several thousand transplants carried out world-wide has revealed that one of the major factors determining the success of an organ transplant is the degree of HLA match between the donor and recipient [1]. In the well-matched (i.e., 6-antigen matched) transplants, 1-year graft survival rate of 88% and estimated half-life of 17.3 years was observed as compared to 79% graft survival and half-life of 7.8 years in zero antigen matched graft [2]. The success of a transplant is dependent on factors such as the state of immunoreactivity and sensitization of the recipient [3]. The success of a kidney transplant is still limited by rejection episodes. Increased serum creatinine continues to be the gold-standard for the diagnosis of rejection and other causes of renal function impairment like cyclosporin-A nephrotoxicity [4]. In most instances, renal biopsy helps to distinguish between various causes of graft dysfunction. However entities like acute tubular ne-

crosis, cyclosporin-A nephrotoxicity, and infections can interfere with the diagnosis of graft rejection [5].

A cross-match is performed routinely prior to renal transplantation to avoid hyperacute rejection due to high levels of preformed antibodies in the serum of the recipient against HLA antigens of the donor. However, the presence of subthreshold levels of anti-donor antibodies in the recipient also have prognostic value in respect of post-transplant renal function [6].

A number of studies have been carried out worldwide to assess the influence of anti-donor antibodies on the success of a renal transplant [7,8]. Besides the conventional cytotoxicity test (CTXM), the value of flowcytometric cross-match (FCXM) has also been evaluated mostly on cadaver transplantation [9-11]. The results of these studies have been variable and very little work has been carried out in live related donor (LRD) renal transplantation.

The present study was undertaken to evaluate CTXM and FCXM procedures and correlate the

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antibody status of the recipient with his overall post-operative condition.

Material and Methods

Patients

A total of 76 patients from the All India Institute of Medical Sciences and Army Hospital, Delhi Cantt were included in the study. They were in the age group of 15-56 years (mean 28.4 ± 7.2 year) and all received kidneys from live related donors.

The donors were in the age range of 21-62 years and were selected from the family. They were either parents, siblings, or children of the recipient with the minimum criteria of their selection being HLA haploidentity.

HLA typing was performed by the microlymphocytotoxicity technique [12] using monospecific HLA antisera that defined 20 antigens in locus A, 43 in locus B, 6 in locus C, 14 in DR and 4 in DQ.

Detection of antidonor antibodies

Pretransplant sera from 52 prospective (primary (n=43) and secondary (n=9)) LRD renal transplant patients as well as from 54 post-transplant patients on initial triple drug therapy were tested by CTXM and FCXM. The timing of the post-transplant sera samples ranged from 10 days to 1277 days ($3\frac{1}{2}$ years) after the transplant.

Serological cross-match (CTXM)

This was performed by the standard NIH microlymphocytotoxicity technique using the patient's most recent serum and nylon-wool-separated T- and B-lymphocytes from the donor in the presence of rabbit complement. To identify the type of antibody, i.e., whether IgG or IgM, all CTXM positive cases were analyzed by the dithiothreitol (DTT) cross-match test according to the standard method [13]. A positive cross-match was defined as more than 10% killing of the donor lymphocytes in all DTT treated cases as compared to the negative controls.

Flowcytometric cross-match (FCXM)

The flowcytometric cross-match was performed with a FACScan flowcytometer (Beckton, Dickinson) using 1024 channel log scale according to the method described by Ogura et al [11]. A total of

106 (52 pre-transplant and 54 post-transplant) serum samples were tested. Briefly, 20 μ L of patient's serum was incubated with 0.5×10^6 lymphocytes in 30 μ L of phosphate buffer saline (PBS) for 30 minutes at room temperature. The excess serum was washed 3 times with PBS containing 0.1% sodium azide and 2% fetal calf serum. Secondary antibodies i.e., phycoerythrin labelled anti-CD3 monoclonal antibody (Sigma, USA) and goat F(ab)2 antihuman IgG FITC antibody (Jackson, USA) were reacted with washed lymphocytes at 4°C for 30 minutes. The cells were again washed thrice with PBS. The FCXM was considered positive when median channel shifts (the difference of median channel shifts between the test serum and negative control) were more than 50 channels.

Post-transplantation follow-up

Various graft dysfunctions like acute and chronic rejection episodes, acute tubular necrosis (ATN), cyclosporin-A toxicity, and infective episodes were diagnosed clinically assisted by standard bio-chemical or microbiological techniques e.g., serum creatinine, blood urea, serum electrolytes, serum uric acid, serum cyclosporin-A levels and blood and urine culture examination. Biopsy or FNAC was performed wherever applicable. The patient samples were grouped as follows:

- i) Well-functioning grafts (19).
- ii) Acute graft rejection (12).
- iii) Chronic graft rejection (11).
- iv) Cyclosporin-A nephrotoxicity (5).
- v) Acute tubular necrosis (7)

Results

Anti-Donor Antibodies

Out of 52 pretransplant patients studied, 43 were awaiting primary graft and 9 were waiting for a secondary transplantation. The HLA matching criteria of all these patients was a minimum of 50% match grade. The pretransplant serum samples showed no difference in the results of FCXM and CTXM in all the 43 primary transplant patients studied. Amongst those awaiting a second transplant, 2 patients who gave negative results with CTXM were found to be positive with FCXM (Table 1).

TABLE 1

Comparison between CTXM & FCXM in primary and secondary transplant cases

Patients	No	CTXM		FCXM	
		Negative	Positive	Negative	Positive
Primary	43	38	05	38	05
Secondary	09	07	02	05	04
Total	52	45	07	43	09

Out of the 54 post-transplant serum samples, 12 samples were from clinically or biopsy proven acute-rejection-episode cases and 11 from biopsy proven chronic-rejection patients who were on immuno-suppression and yet to be reverted back to haemodialysis. Of the remaining, 7 samples were from patients with ATN and 5 from patients with cyclosporin-A nephrotoxicity. In addition, 19 samples were from well functioning grafts, out of which 2 were taken from patients with sporadic rise of serum creatinine.

FCXM was found to be positive in 6 out of 7 cases with mild acute-rejection episodes in all of whom the CTXM was found to be negative. In the remaining 5 cases with severe acute-rejection episodes, both FCXM and CTXM were found to be positive. Also, in 17 clinically well-functioning grafts, 5 cases of cyclosporin-A nephrotoxicity, 7 cases of ATN, and 10 out of 11 biopsy proven cases of chronic rejection, FCXM and CTXM were found to be negative. However, 2 patients with well-functioning grafts had sporadic increase in their serum creatinine and developed positive FCXM and CTXM. Both these patients settled without any anti-rejection therapy (Table 2).

Discussion

The development of a reliable, noninvasive, and economical diagnostic test for monitoring immunological rejection has been a long time desire in those involved in organ transplantation. In practice, the increase in serum creatinine continues to be the main bedside tool for the diagnosis of graft rejection and a biopsy helps in differentiating rejection from other causes of declining renal function. Recently, techniques have been developed to estimate serum levels of cyclosporin-A and associ-

TABLE 2

Comparison between CTXM and FCXM in different post-transplant conditions

	No	CTXM		FCXM	
		Negative	Positive	Negative	Positive
Well functioning	19	17	2	17	2
Acute graft rejection	12	7	5	1	11
Chronic rejection	11	10	1	10	1
ATN	7	7	0	7	0
Cyclosporin-A nephrotoxicity	5	5	0	5	0

ate the same with resultant nephrotoxicity following transplantation. However, the results have been far from accurate [4].

FCXM is considered to be far more sensitive test than CTXM in detecting donor-specific antibodies [10]. These antibodies may occur at sub-threshold levels or may be of non-complement binding type making their detection difficult by conventional serological cross-match procedures [14]. Previous studies have shown conflicting results on the usefulness of FCXM in cadaver or unrelated renal transplantation situations [3,6,11,15]. However, very little data is available on the usefulness of these assay systems in the context of LRD renal transplantation. In the present study, 7 patients with clinically mild acute-rejection episodes were negative on the CTXM assays while FCXM was found to be positive in 6 of them. It is possible that the antibody titre in such patients was not high enough to be detected by the CTXM procedures. Thus CTXM does not appear to be a sensitive test for the detection of mild rejection episodes. This is evident from the results of 5 severe acute-rejection cases in which cross-match was positive with both CTXM as well as FCXM.

Compared to acute rejection, chronic rejection follows different mechanisms such as repeated immunological endothelial injury and fibrin deposition in glomeruli [16]. It may also be a consequence of rejection episodes some of which can be self-limiting and therefore not always clinically evident [3]. Thus, in our study, no rise in anti-donor antibody was detected in 10 out of 11

biopsy-proven chronic rejection cases who were still on immunosuppressive therapy. The one FCXM and CTXM positive case had rapid onset of chronic rejection within 4 months of transplantation. These results may have clinical relevance particularly in differentiating acute from chronic graft rejection. To the best of our knowledge, no such study has been conducted previously on biopsy proven cases of chronic rejection in LRD renal transplantation.

Interestingly, both CTXM as well as FCXM were negative in all the 5 cases of cyclosporin-A nephrotoxicity and 7 with ATN suggesting that CTXM and FCXM may be a useful test for differentiating acute rejection from other causes of graft dysfunction. The 2 post-transplant cases with sporadic rise in serum creatinine and having CTXM and FCXM positivity were probably due to other causes of sensitization like infections as no anti-rejection therapy was required by them. However, a long-term monitoring of these 2 patients will help in identifying the actual cause.

In conclusion, the present study has provided evidence to support the use of post-transplant flowcytometric cross-match in LRD renal transplant patients. With arrival of flowcytometer apparatus in different immunological centres of the armed forces, no significant cost difference will be found between CTXM and FCXM procedures. These tests may be done in conjunction with renal biopsy and FNAC so as to ultimately arrive at a definitive diagnosis to monitor LRD renal graft dysfunction.

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