Role of Molecular Typing in Live Related Donor Renal Transplantation

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Abstract

In renal transplantation, a good HLA-DR match is associated with higher success rate of graft outcome. It is particularly so in high risk recipients. Serological HLA-DR typing is not always easy due to a number of technical problems. In view of this, a comparison of serological and molecular typing was done in our institutions. A total of 64 live related donor patients of renal transplantation were studied. Serological typing was done by conventional methods. Molecular HLA class II typing was done by polymerase chain reaction (PCR) based sequence specific oligonucleotide probe (SSOP) hybridization technique. An overall discrepancy of 19.5% was observed in the DR typing obtained by serology and PCR-SSOP of all the recipients and donors. 14.5% of cases showed discrepancy in the results of only one DR antigen. Serological typing failure was seen in 10.9% of total cases. In 19.5% cases, only one DR antigen was assigned by PCR-SSOP as compared to two antigens by serological methods. Maximum number of discrepancies were seen in DR 2 antigens. There was no appreciable difference of graft survival shown in the patients typed by both methods. However, higher incidence of acute graft rejection episodes were seen in patients with 1 antigen mismatch as compared to zero mismatch. It is concluded that HLA-DR typing should be carried out by molecular methods as these have been found to be more specific and accurate.

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Key Words :HLA-DR; Molecular typing

Introduction

Successful transplantation of parenchymal organs like kidney, heart and liver are being performed with increasing frequency. What was once an experimental and life threatening emergency procedure, is now technologically advanced form of therapy. Data generated on several thousand transplants carried out world wide has revealed that one of the major factors controlling the success of organ transplant is the HLA match grade between the donor and recipient [1,2]. Transplantation of kidney from a donor who is well matched for his HLA-A, B and DR antigens with the potential recipient gives an appreciably increased graft survival compared to 1, 2 or upto 6 antigens mismatched donor. In HLA well matched six antigen matched transplants, one year graft survival rate of 88% and estimated half life of 17.3 years was observed as compared to 79% graft survival with half life of 7.8 years in zero antigen matched grafts [3]. However, the beneficial effect of HLA matching in renal transplantation on graft survival is obscured by the high rate of error in serological class II typing [4]. Recent studies have demonstrated an average discrepancy rate of 25% between serology and DNA analysis of HLA-DR typing [5]. Due to a number of technical problems, a reliable serological DR typing cannot be always obtained [6]. The ascertainment of donor recipient HLA compatibility normally relies on well de-

fined serological types of the pair [7]. Sometimes during matching, precise HLA typing of the pair can be hampered by the cross reactivity among antigen groups. The varied amount of expressed cell surface antigen and the variant antigen types found, may also be the cause [8]. These problems indicate the need to study donor recipient compatibility with alternate DNA approach. Recently developed technique providing allelic specially amplified DNA's with sequence specific oligonucleotides (SSO), allows the comparison of HLA compatibility at nucleotide level [7]. The aim of the present study was to conduct PCR-SSOP analysis of HLA class II antigens in live related renal transplantation and to assess its usefulness over conventional serological typing.

Material and Methods

HLA Matching

Renal transplant recipient - donor pairs of 64 patients transplanted in three large hospitals were included in this study. Patients with secondary transplantation, ABO incompatibility or graft failure due to non-immunological causes were excluded from the study. All the patients received kidneys from their immediate relations. The age of patients ranged from 14-61 years (mean 29.2±7.4 years). There were 59 male and 6 female recipients. All the transplanted patients received standard triple drug therapy eg Prednisolone, Azathioprine and Cyclosporine A. Antirejection therapy was given to confirmed cases of acute rejection in

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Fig. 1 : DR B1. PCR - SSOP hybridization Dot blot

the form of 1 gm of injection methyl prednisolone for 3-5 days. All the patients had negative (<10%) panel reactive antibodies before the transplantation. Female patients had 1-3 pregnancies in the past and were transplanted only when their PRA status was negative. OKT3 monoclonal antibodies were used in 8 patients who failed to respond to conventional antirejection therapy during acute rejection episode. The number of late rejection episodes in the 1st post transplant year was noted in each case.

HLA Typing

Serological Typing

Peripheral blood lymphocytes were isolated from heparinized whole blood with density gradient centrifugation method using lymphoprep (Nycomed Co, Oslo). Microlymphocytotoxicity technique of Terasaki and McClellan [9] was employed for the determination of HLA class I & II alleles. The basic principle for this assay is to obtain complement mediated lympholysis. A suspension of lymphocytes mixed with a specific antiserum results in the formation of antigen antibody reaction. In the presence of complement, if the antibody used has the potential for complement activation, cells carrying the appropriate antigen are lysed, resulting in increased membrane permeability. On staining with a supravital dye (Eosin), the dead cells get stained. The trays were read using an inverted phase contrast microscope (Nikon, Japan) and the scoring was done by percentage of cell lysis.

Molecular Typing

10 ml of peripheral blood was drawn in 50 ml tubes containing 2% EDTA solution. DNA was extracted from this blood by standard protocol using Red & White cell lysis buffer, Proteinase K (Boehringer Mannheim) solution and Isopropyl alcohol. DNA was purified with 70% ethanol washings and dissolved in TE buffer.

Quantification of DNA was done by diluting 5ul of DNA in 1 ml of TE solution and reading the optical density at a wave length of 260 and 280 OD using a UV spectrophotometer. The quantified DNA was stored at 30°C for

Fig. 2 : Serology versus molecular typing

future use. The quantified DNA was amplified by PCR with generic and group specific primers using 5 P DRBG and 3P DRBG for DRB1 alleles. Group specific amplification was done for HLA-DR2 as it is most frequent HLA type in Indian population [10]. The amplified DNA was dot blotted and fixed to pre-wetted hybond N+membranes. The blots were hybridized in hybridization incubator with different biotinylated SSOP. The detection of biotin labelled probes on the hybridized membranes was done by chemi luminescence method using a commercial kit (Amersham International UK) and filmed on X-ray films. The signals obtained on X-ray films were interpreted accordingly, alongwith positive and negative controls (Fig-1). The dark dots were considered to be positive for a particular used probe and the blanks and weak dots were considered negative. Evaluation of these dots was done for donors and recipients at a later stage while compiling the results.

Results

Molecular versus serological HLA-DR typing

A total of 64 patient pairs were typed for class II alleles by the PCR-SSOP method and their DR antigen results compared with those obtained by conventional serological method. An overall discrepancy rate of 19.5% was observed in the DR typing obtained by serology and PCR-SSOP of all recipients and donors. 14.5% of cases (total 18) showed discrepancy in the results of only one DR antigen. Serological typing failure was seen in 10.9%(14 cases) of the total patients. In these patients and donors, one or even both DR antigens could not be detected by serology but the antigens were assigned by PCR-SSOP. In 25 cases (19.5%), only one DR antigen was assigned by PCR-SSOP, although the serological typing had revealed two DR antigens. Maximum number of differences between PCR-SSOP and serological typing were observed in HLA-DR2 and its splits (25.7% of all differences). (Fig 2)

PCR-SSOP vs graft survival

All the 64 transplanted patients received kidneys from their immediate relations and were matched by serological

methods. Seven of these patients were zero antigen mismatched with their donors while the remaining 57 were one DR antigen mismatched by serology. Results of the PCR-SSOP typing revealed 15 patients to be zero antigen mismatched, 44 one antigen mismatched and 5 patients showed two DR mismatches. Graft survival data in these patients is given in Table 1. There was no appreciable difference in the group with two, one or zero DR antigen mismatch group.

Out of the 64, patients 31.25% developed one or more episodes of acute rejection in the first post transplant year. Few earlier cases where presumptive antirejection therapy was given to the patients but rejection was not confirmed clinically or by biopsy, were excluded from this part of study. These patients were later found to be suffering from other causes of graft dysfunction.

TABLE 1
Comparison of serology vs PCR-SSOP-DRBI typing

	Graft survival at one year	
	Serology	PCR-SSOP
Two antigen mismatch	-	5/5 (100%)
One antigen mismatch	57/54 (94.7%)	44/42 (95.15%)
Zero antigen mismatch	7/7 (100%)	15/14 (93.33%)
p value	2.54	0.72

TABLE 2
Incidence of acute rejection : Serology vs PCR-SSOP matching

	(20 patients) At 1 year post-transplant period		
	2 Ag mismatch	1 Ag mismatch	Zero mismatch
Serology	-	57/18 (31.57%)	7/2 (28.5%)
PCR-SSOP	5/1 (20%)	44/15 (34.09%)	15/4 (26.66%)
p value	-	0.95	0.67

Out of 64 patients studied by molecular typing, 20 developed graft rejection episodes in the first post transplant year. Out of these 20 patients, 17 (29.3%) were males and 3 (50%) females.

When PCR-SSOP-BI typing was compared with their donors, it was found that 1 patient showed 2 antigen mismatch, 13 patients had one antigen mismatch and 3 had zero antigen mismatch in the male group. In the 3 females developing acute rejection episodes, two patients had one antigen mismatch, whereas only one had zero antigen mismatch (Table - 3).

Discussion

In the present study, we observed a mean discrepancy rate of 19.5% between serological and DNA typing of DR antigens. The maximum discrepancy was seen for the assignment of DR2 specificity and its splits which was more than 25%. This was closely followed by DR8 antigen. Our study further confirms the earlier reports indicating the superiority of DNA

TABLE 3

Acute rejection episodes vs sex and PCR-SSOP DRBI

	(Total 64 patients)				
	Total	AR episodes	2 Ag mismatch	1 Ag mismatch	Zero Ag mismatch
Male	58	17 (29.3%)	1	13	3
Female	6	3 (50%)	-	2	1
Total	64	20 (31.2%)	1	15	4

based typing of HLA-DR alleles over serology [11]. The observed discrepancy between serological and molecular typing can be attributed to a number of technical problems like extensive HLA allelic polymorphism, lack of reliable monospecific alloantisera, problems with cell separation in hemodialysed patients, abnormal class II antigen expression or cross reactivity between HLA-DR alleles [6]. Low rate of discrepancy in our study may be due to procurement and use of good monospecific alloantisera and good temperature and quality control of the methodology adopted. Higher discrepancies between serology and molecular typing have recently been reported in cadaver donor transplantation situation [12,13]. This has been attributed to a particular source of lymphocytes i.e. from the spleen which often yields low viability. This is in contrast to the LRD situation where the venous blood is used for lymphocyte isolation [13].

Higher and improved graft survival has been observed in donor recipient pairs who have been matched by molecular techniques [7] in cadaver transplantation. Nojima et al [14] have demonstrated 92% graft survival in DRBI compatible group of patients at 3 years following transplantation as compared to 94% in the DRBI incompatible group. This has been attributed to the less number of patients in each of the two groups since the success rate was found to be reversed to 92% and 81% at 5 years. There are centre-to-centre variables which may alter the graft outcome and influence the impact of HLA matching [15]. This includes patient population selected for transplantation, the type of immunosuppressive therapy given, adequate follow up of patients in post transplant period and sensitivity of cross match test conducted during the pretransplant period. In our study, higher graft survival at one year was seen in zero antigen mismatch group as compared to 1 antigen mismatch group both by serological as well as molecular typing methods. However, highest graft survival was observed in 2 antigen mismatch group typed by PCR-SSOP. This trend has also been observed by others [7] and may be due to variable size of different groups.

In our study, higher incidence of acute rejection episodes at one year was observed in patients with 1 antigen mismatch as compared to the zero antigen mismatched group. However, there was negligible difference in acute rejection episodes when serological and molecular typing methods were compared for each group. Five patients showing two mismatches by the PCR-SSOP technology can be due to either serological typing error or the donors being not immediate relations. Similarly, the observed higher incidence of acute rejection episodes seen in female patients in our study must be due to the cumulative effect of sensitization and discrepant HLA matching. The difference for graft survival for one and zero antigen mismatch as well as acute rejection between PCR-SSOP and serology was not found to be of statistical significance. This may be due to less number of patients studied.

Matching for HLA loci is the most important crucial factor in the selection of donors in transplantation. The techniques utilised to assign HLA type have evolved significantly since the application of PCR based methodologies. A significant challenge for DNA typing methodologies is to be able to maintain high resolution typing with ever increasing number of defined HLA alleles. Over 800 HLA class I and class II alleles have been identified by sequencing analysis [16]. Apart from serologic methods for HLA typing, PCR based methods like sequence specific primers (SSP) or sequence specific oligonucleotide probes (SSOP) are frequently being used [16]. Direct sequencing for HLA typing involves amplification of target HLA sequence and direct sequencing of PCR product. Several approaches have been used for direct sequencing, like both mRNA and genomic DNA have been used as starting material. The advantages of DNA based methods over serology include the ease with which typing reagents (primers, probes) can be synthesized and the interpretation of data directly relating to nucleotide sequence. In addition, the storage of material is simplified for DNA typing as live cells are not required [16].

To conclude, DNA typing in renal transplantation is a good and reliable method for donor recipient matching. Serological typing has got some inherent technical problems necessitating DNA typing. Thus, it is suggested that all HLA laboratories should be equipped with molecular techniques so as to start DNA-HLA typing in near future. The methodology adopted can

be further used for diagnosing infectious and autoimmune disorders.

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