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Pre and Post Transplant Immunological Evaluation for a Long Successful Graft Survival

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ABSTRACT Allograft rejection continues to be a major problem and is the leading cause of graft loss in renal transplant recipients. Histocompatibility testing plays an important role in selection of donors for transplantation. A correct assignment of HLA antigens is considered important given that inadequate HLA matching of patient-donor pairs is associated with rejection in kidney transplantation. The present study was to assess a long and successful graft survival in end stage renal failure patients. 50 live related renal transplant patient-donor pairs were selected at random (n=100). Serological HLA A, B and DR typing results were compared to typing results obtained using sequence-specific primers in the polymerase chain reaction. In spite of using a very large panel of antisera in the serological method, there were 6 blank or undefined antigens (2 in the A locus, 1 in the B locus and 3 in the DR locus). The PCR-SSP low resolution method allowed identification of these blanks. Results reveal that screening test should be carried by serology. Ambiguous or blank antigens by serology should be confirmed by DNA typing. The best graft survival was obtained in patients transplanted with kidneys from HLA identical siblings, while kidneys from haplo-identical donors gave lower graft survival. The HLA matching was apparent in both, 3 months graft survival as well as in long-term, which is >1 year in this study. There was no difference in graft survival when various family members are used as donors like father, mother and sibling. There was no upper recipient age limit for transplantation, although older recipients fared better than younger recipients.

INTRODUCTION

Since the advent of organ transplantation, the transplant centers are aiming at better quality of life with longer graft survival for renal allograft recipients. Host immune response plays the key role in acceptance of the allograft. Detailed immunological investigation can improve the long-term success of transplantation in individual cases of end stage renal failure. Although the success rate of clinical transplantation has improved in the past two decades, allograft rejection continues to be a major problem and is the leading cause of graft loss in renal transplant recipients. A number of factors, including the use of newer immunosuppressive drugs, pre-transplant blood transfusions, better matching of donor-recipient pairs, organized follow-up care and optimal management of the various post-transplant complications have contributed to the recent improvement in clinical results.

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It has been recognized that the specificity of antigens involved in graft rejection is under genetic control. The HLA system plays a significant role in determining the acceptance or rejection of a tissue transplant. HLA typing by serology is the most commonly used method in routine clinical settings. (Dausset 1958; Amos 1966). Serology is a quick and convenient method, but it is hindered in many cases by serological cross reactivity, non-availability of antisera, and decrease in expression of HLA antigens, particularly in immunosuppressed patients. The present study extends the low-resolution system into a serologically equivalent system. The principle of PCR-SSP is that each individual allele (making up a serological specificity) is amplified by a primer pair exactly matched to that region. (Olerup and Zetterquist 1991).The importance of HLA typing was further documented by several reports that pre-existing leukocyte antibodies could induce hyper acute rejection (William 1968). This resulted in the introduction of cross matching before transplantation.

MATERIAL AND METHODS

This study comprised of 50 renal failure patients, who underwent live related renal transplantation and their perspective donors. 15 – 20 ml of defibrinated or EDTA blood of recipient and donor was collected. Lymphocytes were

separated using ficoll-hypaque density gradient (Histopaque 1077, SIGMA, Missouri, U.S.A.). HLA -A, -B and -DR typing **(**Ray 1979) was performed by the standard NIH (National Institute of Health, U.S.A.) microlymphocytotoxicity assay. HLA antiserum were commercially purchased from Biotest Diagnostics, Germany, BAG, Germany, Pelfreez Clinical systems, U.S.A. and GTI, U.S.A. and some rare specificity sera were gifted from Internationally reputed tissue-typing centers.

High molecular weight DNA was isolated from proteinase-K treated peripheral blood leukocytes using Qiagen GmbH kits. Polymerase chain reaction (PCR) (Bodmer 2001) is a novel technique involving primer-directed enzymatic *in vitro* amplification of specific nucleic acid stretches. Conventional PCR-SSP typing kits are available which allows amplification of an extremely small stretch of HLA allele sequence inserted in the genomic DNA by synthesizing over a million copies within few hours, significantly facilitating subsequent analysis of HLA polymorphism. Conventionally extracted DNA's was amplified in cycles in a thermal cycler. Each cycle consisted of denaturation, annealing and extension. Absence or presence of PCR products were visualized by agarose gel electrophoresis and interpreted by Gel documentation.

The DNA solution was added to PCR reaction mixture containing primers designed to give amplification of specific alleles. Lymphocyte cross match test (Bradley 1985) was performed prior to transplantation using recipient's serum and donor's lymphocytes. Patient's serum was also tested against autologous lymphocytes to detect auto-antibodies (Ettiner 1987). Patients with less than 10% lymphocytotoxic kill were considered cross match negative and received kidney graft (Ting 1978).

Immune suppressive therapy for transplanted patients consisted of triple-drug regimen with cyclosporine, prednisolone and azathioprine. OKT3 was given occasionally to those patients who were showing symptoms of acute rejection. The actuarial method of Barnes (1965) as described by Festenstein and Demant (1978) was followed for calculation of graft survival. The level of significance was reported in terms of probability (p) value.

RESULTS

Serological HLA -A, -B and -DR typing results were compared to typing results obtained using sequence-specific primers in the polymerase chain reaction (PCR-SSP) in 100 individuals (50 recipient-donor pairs). In spite of using a very large panel of antisera in the serological method, there were 6 blank or undefined antigens (2 in the A locus, 1 in the B locus and 3 in the DR locus). The PCR-SSP low resolution method allowed identification of these blanks. (Table1)

Table 1: Differences between the results of serological and PCR-SSP typing

Serology	*PCR*
A3, —	A* 03, A* 24
A2, —	A*02, A*68
B7, —	B*07, B*18
DR2, ? DR4/DR7	DRB1*15, DRB1*07
DR2, —	DRB1*01, DRB1*02
DR3, —	DRB1*03, DRB1*13

The resolution of HLA-A and HLA-DR PCR-SSP method was largely unaffected by cross-reactivity and were able to obtain correct and exact results in these loci. In the B locus, there were more problems with cross-reactions, extra reactions and the presence of mixed primers The results in this study demonstrate that HLA antigens are the major histocompatibility or transplantation antigens and that long term graft survival >1 year is appreciably better in recipients with >50% HLA matched kidneys than those with 50% HLA matched or haploidentical grafts.

Four patients received kidney grafts from identical HLA antigens matched donors. The percentage graft survival is 100% at 3 months, 6 months, 9 months, 12 months and >12 months. 41 patients who received grafts from one haplotype HLA matched donors had percentage graft survival of 100% at 3 months, 97.5% at 6 months, 95.1% at 9 months, 12 months and >12 months, whereas 5 patients who received grafts from 3 antigens mismatched had percentage graft survival of 80 % at 3 months, 6 months, 9 months and 60% at 12 months and >12 months. There was no statistically significant difference between HLA matching at all durations as well as in between the durations ($p>0.05$) (Table 2). Out of the 50 patients transplanted, 20 patients received grafts from mothers. The graft survival was 100% at 3 months and 6 months and 95% at 9 months, 12 months and >12 months. Nine patients received allograft from fathers. whose graft survival was 100% at 3 and 6 months and 89% at 9 months, 12 months

Table 2: Effect of HLA matching on kidney graft-survival

Group	*No.*	*% graft survival (months)*				
		3	*6*	*9*	*12*	*> 12*
Identical	4	100	100	100	100	100
Haploidentical	41	100	97.5 (1)	95.1 (2)	95.1 (2)	95.1 (2)
3 antigens mismatch	5	80 (1)	80 (1)	80 (1)	60 (2)	60 (2)

Number in parenthesis represent graft failure

and >12 months. The survival rates of the grafts received from 11 siblings was 100% at 3 months, 91% up to 9 months and 82% at 12 months and >12 months. However, there was no statistically significant difference between the donors at 3, 6 and 9 months ($p>0.05$) but highly significant difference between the parents and sibling donors at 12 months and >12 months ($p<0.01$) (Table 3).

Table 3: Effect of donor-patient's relation on kidney graft-survival

Group	*No.*	*% graft survival (months)*				
		3	*6*	*9*	*12*	*>12*
Mother	20	100	100	95(1)	95(1)	95(1)
Father	9	100	100	89(1)	89(1)	89(1)
Sister	4	100	91(1)	91(1)	82(2)	82(2)
Brother	7	-	-	-	-	-
Wife	6	-	-	-	-	-
Cousin	2	-	-	-	-	-
Nephew	1	-	-	-	-	-
Aunt	1	-	-	-	-	-

Number in parenthesis represent graft failure

Effect of recipient age on graft survival was analysed separately. 29 patients who were >30 years of age gave better graft survival as 100%, 96.6%, 93.2%, 93.2% and 93.2% at 3, 6, 9, 12 and >12 months respectively as compared to 21 patients with age < 30 years with graft survival of 95.3%, 95.3%, 90.5%, 85.8% and 81% at 3, 6, 9, 12 months and >1 year. There was statistically non-significant difference between recipient age and between durations of recipient age ($p>0.05$) (Table 4). Patients transplanted with donors >25 years of age gave better graft survival. The graft survival for the group of patients (N = 3) who were transplanted with kidneys from donors with >25 years of age was 97.7%, 97.7%, 95.4%, 93.1% and 90.7% at 3,6,9,12 months and >1 year as compared to 85.8%, 85.8%, 85.8%, 71.5% and 71.5% in the group (N=7) with donors <25 years. There was no statistically significant difference between age group of donors at all durations ($p>0.05$) and between durations ($p=0.05$) (Table 5).

Male patients (N=32) had better graft survival as 96.9%, 93.8%, 90.7%, 90.7% and 90.7% at 3, 6, 9, 12 months and >1 year respectively compared to female recipients (N=18) with graft survival 94.5%, 88.9% at 3 and 6 months and 83.4% at 9, 12 months and >1 year, but statistically non-significant difference between recipient sex at all durations ($p>0.05$) (Table 6). Male donors (N=19) gave better graft survival as 94.8%, 94.8%, 89.5%, 89.5% and 89.5% at 3, 6, 9, 12 and >12 months respectively whereas female donors (N=31) gave graft

Table 4: Effect of recipient's age on kidney graft survival

Group	*No*	*% graft survival (months)*				
		3	*6*	*9*	*12*	*>12*
>30 years	29	100	96.6 (1)	93.2 (2)	93.2 (2)	93.2 (2)
<30 years	21	95.3 (1)	95.3 (1)	90.5 (2)	85.8 (3)	81 (4)

Number in parenthesis represent graft failure

Table 5: Effect of donor's age on kidney graft survival

Group	*No*	*% graft survival (months)*				
		3	*6*	*9*	*12*	*>12*
<25 years	7	85.8 (1)	85.8(1)	85.8(1)	71.5(2)	71.5(2)
>25 years	43	97.7(1)	97.7(1)	95.4(2)	93.1(3)	90.7(4)

Number in parenthesis represent graft failure

Table 6: Effect of recipient's sex on kidney graft survival

Group	*No*	*% graft survival (months)*				
		3	*6*	*9*	*12*	*>12*
Male	32	96.9(1)	93.8(2)	90.7(3)	90.7(3)	90.7(3)
Female	18	94.5(1)	88.9(2)	83.4(3)	83.4(3)	83.4(3)

Number in parenthesis represent graft failure

Table 7: Effect of donor's sex on kidney graft survival

Group	*No*	*% graft survival (months)*				
		3	*6*	*9*	*12*	*>12*
Male	19	94.8(1)	94.8(1)	89.5(2)	89.5(2)	89.5(2)
Female	31	93.6(2)	90.4(3)	90.4(3)	87.1(4)	87.1(4)

Number in parenthesis represent graft failure

survival of 93.6% at 3 months, 90.4 % at 6 months and 9 months and 87.1 at 12 and >12 months. There was no statistically significant difference between the donors' sex at all durations ($p>0.05$) (Table 7). Lymphocytotoxic cross matching of recipient's serum with donor's lymphocytes was performed. All 50 recipients with negative crossmatch received kidney graft.

DISCUSSION

The present study includes all renal transplants with live related donors. The results in this study demonstrate that HLA antigens are the major histocompatibility or transplantation antigens and that long-term graft survival above 1 year as in this study is better in recipients with HLA identically matched kidneys than those with 50% HLA matched or haplo-identical grafts. Although the data in this study is not statistically significant, the trend observed is, however, similar to that reported by Persij (1982) and Terasaki (1990) in the live related donor transplant group. The results of HLA -A, -B and -DR typing using serology were compared to the results of typing with PCR-SSP of all recipient-donor pairs. In different situations, when the expression of HLA antigens on the cell surface is down-regulated, it is impossible to type by serological methods and it is advisable to use molecular typing such as PCR-SSP. In addition, the PCR-SSP technique does not require the viable cells necessary for serological typing and also allows determination of the subtypes of HLA antigens very clearly.

There was no statistically significant difference between the donors at 3, 6 and 9 months ($p>0.05$) but highly significant difference between the donors as parents and siblings at 12 months and >12 months ($p<0.01$) when donor-patient's relation was studied. There was no evidence to suggest that maternal donor grafts had better outcome than graft from paternal or sibling donors. The eight patients transplanted with kidneys from 50% phenotypically matched or 3 antigen mismatched cousin, wife or nephew donors had a very poor graft survival with two of the grafts failing within the first year. Although the numbers are small for statistical analysis, this might suggest that genotypically matched grafts from within family donors have better survival rates than those from 50% or more phenotypically matched donors. Our data did not show a statistically significant effect of recipient's age or sex on graft survival individually; an additive influence of these factors when combined with factors like HLA matching cannot be ruled out. Our results show that older patients generally give better graft survival than those that are younger in age. It has been suggested by Yuge and Cecka (1991) that younger recipients are immunologically higher responders than older patients.

CONCLUSION

In the present study, male patients had superior graft survival than female recipients although values did not reach a significant level. This difference may be due to repeated pregnancies, which can cause presensitization of female patients leading to lower graft survival. Our study shows that recipients receiving grafts

from male donors had higher graft survival rates than recipients receiving kidneys from female donors for more than one year after transplant.

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