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CLINICAL STUDY

Outcome of renal transplantation from deceased donors: experience from developing country

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Abstract

Background: In India, there are a large number of end-stage renal disease (ESRD) patients waiting for renal transplantation (RT). Organ retrieval from brain dead deceased donor (DD) is getting increased attention as the waiting list for organ recipients far exceeds the organ donor pool. In our country, despite a large population, the number of brain dead donors undergoing organ donation is very less. DDRT is the possible solution to bridge the disparity between organ supply and demand. In India, the potential for DDRT is huge due to the high number of fatal road traffic accidents and this pool is yet to be tapped. *Patients and methods*: We report DDRT outcome in 294 patients (age: 36.5 ± 14.1 years; male:female, 200:94) between 2005 and 2012. All patients received single-dose rabbit-anti-thymocyte globulin for induction and steroids, calcineurin inhibitor, and mycophenolate mofetil/azathioprine for maintenance immunosuppression. *Results*: Our retrospective study in 294 DDRT shows a fairly successful outcome. Over a mean follow-up of 3.93 years, patient and graft survival rates were 81.7% and 92.6%, respectively, with a median serum creatinine of 1.5 mg/dL. 20.7% had biopsy-proven acute rejection. *Conclusion*: Given the widespread organ shortage, DDRT has a potential to expand the donor pool and shorten the waiting list for RT, encouraging the use of this approach even in low-income countries. Aggressive donor management, increasing public awareness about the concept of organ donation, good communication between clinician and the family members, and a well-trained team of transplant coordinators can help in improving the number of organ donations.

Keywords

Deceased donor, developing country, end-stage renal disease, outcome, renal transplantation

History

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Introduction

Poor economics, paucity of renal replacement therapy (RRT), and renal transplantation (RT) facilities in the government sector and high costs in private sector render majority of end-stage renal disease (ESRD) patients disfranchised from RRT and RT in developing country. In India, approximately 175,000 patients are added each year to the pool of ESRD; however, only 10% of these receive RRT and 2.4% patients receive RT.[1] A limited number of live donor (LRD) availabilities are one of the major reasons for this huge demand and supply gap. A deceased donor (DD) RT program is the possible solution to the widening demand supply gap for kidney donors.[1]

The rate of RT performed yearly in India translates to 3.25 per million populations (PMP); the deceased–donation rate is 0.08 PMP per year. This discrepancy between the number of waiting patients and RT performed can be reduced by developing a DDRT program.[2]

The total number of road accidents in India is approximately 90,000 per annum. Nearly 40–50% of fatal road accidents occur in the world, the cause of death is head injury leaving potential organ donors from road traffic accidents alone. Other causes of brain death such as sub-arachnoids hemorrhage, and brain tumors would potentially add more numbers. Even if 5–10% of all these deceased patients became the organ donors, it would mean that there would be no requirement for a living person to donate an organ. Promoting the DDRT program would not only help RT but also liver, heart, pancreas, and lung transplants to thrive in the country.[3]

DD are seldom used because of an absent or ineffective organ procurement network, lack of facilities for taking care of potential donors, lack of an organized DDRT program,

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social issues, poor public education, lack of the awareness of organ donation, involvement of non-governmental organization, and culturally based perspectives influencing organ donation. Affordability for antibody induction and prophylaxis for cytomegalovirus (CMV) infection is another major barrier. The cost of treating steroid-resistant rejections is prohibitive.

Patients are non-adherent with immunosuppressive drugs which lead to high rates of graft loss. The lack of timely issuing of brain death certification, lack of awareness, and high cost of treatment pose the biggest challenges to be managed by these patients. Limited access to tertiary level health care, lack of facilities for chronic dialysis, scarcity of trained personnel, inadequate availability of health insurance, vascular access expertise, parental support, and transplant counselors are the other major obstacles for DDRT in our country.[4–12] In India, very few centers have a viable DDRT program. We present our experience of DDRT over the last 7 years. We also addressed limited organ donation concerns in the developing world.

Materials and methods

This was a retrospective study of 294 DDRT carried out in our institute from 2005 to 2012. This study was performed with the approval of our local hospital research ethics board. All transplants were performed in accordance with the Istanbul convention. A written and informed consent was obtained from all patients. Outcome measures included death censored graft survival, patient survival, and rejection rate.

Organ allocation and recipient selection

We have a transparent method for organ allocation as per waiting time. Human leukocyte antigen (HLA) typing is performed by the institute, but HLA criteria are not used for organ allocation. HLA matching for class I and class II antigens in order to avoid mismatch is performed after RT because of time constraints. Our transplant coordinator is available all the time for communication regarding deceased organ donation as in other developed countries. A team of nephrologists, urologists, anesthetics, pathologists and transplant co-coordinators, and nursing staff were involved in pre-transplant evaluation. Transplant patients were selected on the basis of their cardiac fitness, an estimated life expectancy of at least 5 years based on the clinical evaluation and laboratory reports, the absence of a major contra-indication to immunosuppressive therapy, and an estimated low perioperative risk. Cardiac catheterization was routinely used among all diabetic ESRD patients. Those accepted for RT were encouraged to find LRD. In the absence of suitable LRD, they were enrolled in DDRT waiting list. There is no annual fee except an entry fee of Rs. 10,0000 (USD 1650), which is collected by the hospital as a deposit for use in emergency on day of RT for medical care. Our hospital creates a waiting list of patients awaiting transplantation for each organ and this is frequently updated every 2–3 months, and the telephone numbers and contact details of the potential recipient are kept in the hospital database. Patients were reviewed every 2–4 months while on waiting list for DDRT.

Preparation for DDRT

Organs were recovered from many cities in the state but used only in our transplant center. Organ recovering team consisted of urologist, anesthetic, nephrologist, transplant co-coordinator, nursing staff, and paramedical worker. The team travel by road in the vehicles equipped with all instruments required for organ recovering. Hospitals in our state informed our transplant co-coordinator regarding the potential brain death patient after willingness and consent of relatives for organ donation. We have three mobile ambulances for organ recovering/transport donated by government of Gujarat to avoid delay in arranging ambulances. We have our local members like relatives of transplant patients, social workers, and doctors who have passed out from our Institute and practicing at peripheral centers who support us during organ transport and sending blood samples for lymphocyte cross matching and counseling of potential donors and carrying out brain death certification. Brain-death declaration – brain death is defined by the following criteria: the aim is to establish that the patient has absent brainstem reflexes and is apneic. Two certifications were required 6 h apart from doctors nominated by the appropriate authority with one of the two being an expert in the field of neurology (like neurologists or neuro-surgeons). Ethically these doctors must not have interest in or benefit in any way from transplantation of DD organs.

Donor selection

The required medical management of donors was done so that optimal perfusion of organs could be maintained till our team reached for harvesting. Both kidneys were procured from all donors and preserved in histidine–tryptophan–ketoglutarate solution. Kidneys allocated to dual or single RT according to pre-transplant biopsy from older donors and expanded criterion donors (ECD). ECD refer to older kidney donors ($\geq$60 years) or donors who are aged 50–59 years and have two of the following three features: hypertension, terminal serum creatinine $>$1.5 mg/dL, or death from cerebrovascular accident. No age limit was set as an exclusion criterion. In case of older donors, we use old-for-old allocation systems. Deceased donation was done with altruistic motives and in a charitable manner. The deceased donation was governed by complete transparency to ensure that the sentiments of the donor's relatives are adequately respected. The deceased-donor family was kept informed of the organ utilization procedure and they were assisted with all formalities including police liaison in the case of road traffic accidents and other medico-legal cases. A postmortem examination can be performed if necessary after the organ retrieval as per the new law passed by the government. The postmortem can be performed in the premises of our hospital or the organ retrieval hospital and hence save substantial time and worry for the donor family. In selected scenario, the cost incurred for donor maintenance after declaration of brain death was reimbursed, in the case of private hospitals, by our hospital. However, we did not provide any economic compensation to organ donors.

Immunosuppressive regimen

It constituted induction with methylprednisolone, 500 mg for 3 days + rabbit-antithymocyte globulin (1.5 mg/kg single dose). Maintenance therapy constituted prednisolone (20 mg/day, tapered to 5–10 mg/day at 1–3 months post-transplant and continued thereafter), calcineurin inhibitor (CNI) [tacrolimus (TaC), 0.08 mg/kg/day or cyclosporine (CsA) 5 mg/kg/day] and mycophenolate mofetil (MMF) 1.5–2 g/day or azathioprine (AZA) 1–2 mg/kg/day. The doses of AZA and MMF were adjusted according to complete blood counts. The doses of CNI were adjusted based on the serum trough levels (C0), measured by fluorescence polarization immunoassay (FPIA) technology during the first 2–3 months; subsequently adjustments were made only in case of graft dysfunction. This decision was due to financial constraints. Tacrolimus dosing was adjusted to achieve target C0 concentrations of 5–10 ng/mL for the first 3 months post-transplantation and 4–7 ng/mL thereafter. Cyclosporine dosing was adjusted to achieve target C0 concentration of 200–300 ng/mL during the first 2–3 months post-transplantation, 100–250 ng/mL 3–6 months post-transplantation, and ~100 ng/mL thereafter. Immunosuppressive drugs are given free of cost in our state government-run hospitals to poor patients, children below 18 years. All patients received prophylaxis against CMV infection (ganciclovir 1 g thrice a day × 3 months), fungal infections (fluconazole 100 mg once a day × 6 months), and *Pneumocystis carinii* pneumonia (trimethoprim/sulfamethoxazole 160/800 mg once a day × 9 months). Graft biopsy was performed in cases of acute graft dysfunction, diagnosed as per the modified Banff classification, and treated according to standard guidelines.

Statistical analysis

All statistical analysis was performed using SPSS statistical software version 12 (SPSS Inc., Chicago, IL). Continuous variables are summarized as mean and standard deviations (mean ± SD). Percentages are used to summarize categorical variables. Continuous variables were compared using analysis of variance (ANOVA) for more than two groups. Chi-square test and Fisher exact test were used to assess the effect of change in differences in categorical variables. Survivals were examined using Kaplan–Meier curves and log-rank tests were used to describe and compare the patient and death-censored graft survival rates. The Cox regression model and multivariate analysis were applied for comparing the effect of several dependent variables. $p < 0.05$ was taken to indicate statistical significance.

Results

Recipient characteristics

Out of 294 DDRT performed, 68% ($n = 200$) were males and 32% ($n = 94$) were females, with a mean age of 36.5 ± 14.14 (range 7–76) years. The original diseases leading to ESRD were chronic glomerulonephritis ($n = 117$), hypertension ($n = 43$), diabetic nephropathy ($n = 38$), autosomal dominant polycystic kidney disease ($n = 17$), obstructive nephropathy ($n = 15$), chronic interstitial nephritis ($n = 10$), lupus nephritis ($n = 7$), single unit kidney ($n = 6$), focal segmental glomerulosclerosis ($n = 5$), crescentic glomerulonephritis ($n = 5$), membranoproliferative glomerulonephritis ($n = 5$), chronic pyelonephritis ($n = 3$), IgA nephropathy ($n = 3$), Alport syndrome ($n = 3$), and others ($n = 17$). Totally 9.5% ($n = 28$) patients received dual kidneys. About 124 patients received kidneys recovered from different districts of our state and transported to our hospital by a mobile ambulance. The median HLA match was 0. The average dialysis duration pre-transplantation was 2 years. Regarding geographic location, 19.38% ($n = 57$) patients belonged to states other than Gujarat. These were Rajasthan (8.8%, $n = 26$), Maharashtra (3.7%, $n = 11$), Madhya Pradesh (1.36%, $n = 4$), Uttar Pradesh (1.36%, $n = 4$), and other states of India. Immunosuppression regimen consisted of CsA ($n = 88$), tacrolimus ($n = 168$), sirolimus ($n = 38$), MMF (75%, $n = 227$), and azathioprine ($n = 44$). A total of 29.9% ($n = 88$) patients developed delayed graft function (DGF). About 5.1 % ($n = 15$) patients developed new onset diabetes after transplant.

Donor characteristics

Mean donor age was 45.93 ± 17.41 (range 5–89) years; 61.5% ($n = 181$) were males and 38.5% ($n = 113$) were females. The age of 28 donors was ≥ 70 years. The age of 39 donors was 60–69 years. There was 8.84% ($n = 26$) donation after cardiac death (DCD) out of 31.2% ECD ($n = 92$). The commonest cause of brain death was road traffic/cerebrovascular accident.

Post-transplant outcome data

Over a mean follow-up of 3.93 ± 2.13 years, patient and graft survival rates were 79.2% ($n = 233$) and 90% ($n = 165$), respectively, with a median serum creatinine (SCr) of 1.5 mg/dL. Using the Kaplan–Meier analysis, 1- and 5-year patient survival was 81.7% and 77.5% and the graft survival was 92.6% and 88.3%, respectively. About 20.7% ($n = 61$) patients died due to infection ($n = 38$) [bacterial ($n = 26$), fungal ($n = 6$), and CMV disease ($n = 6$)], acute myocardial infarction ($n = 6$), and cerebrovascular stroke ($n = 7$). About 20.7% ($n = 61$) had biopsy-proven acute rejection (BPAR). Antibody mediated acute B cell rejections (AMR) were noted in 6.4% ($n = 19$), acute cellular T cell rejections (ACR) in 7.8% ($n = 23$), combined AMR and ACR in 6.5% ($n = 19$), chronic T-cell mediated rejection in 1.7% ($n = 5$), chronic B-cell mediated rejection in 2.38% ($n = 7$), and combined chronic T-cell and B cell rejection in 2.38% ($n = 7$). Acute tubular necrosis (ATN) ($n = 72$), CNI toxicity ($n = 31$), unremarkable graft morphology ($n = 5$), acute pyelonephritis ($n = 4$), BK virus nephropathy ($n = 2$), recurrence of membranoproliferative glomerulonephritis with crescentic glomerulonephritis ($n = 1$), CsA-induced hemolytic uremic syndrome ($n = 1$), acute CMV allograft nephropathy ($n = 1$), de novo focal segmental glomerulosclerosis ($n = 1$), ischemic necrosis of the renal allograft ($n = 1$) were other biopsy findings. About 10 (3.4%) patients had primary non-function of graft leading to graft nephrectomy. Table 1 showed demographic features and clinical events among brain dead standard criteria donation (BD-SCD) versus brain dead expanded criteria donation (BD-ECD) versus DCD donors. Multivariate analysis of donor and recipient age, gender, acute

Table 1. Demographic features and clinical outcomes in subgroups.

	BD-SCD ($n=176$)	BD-ECD ($n=92$)	DCD ($n=26$)	p Value
Age (recipient)	34.9 ± 14.1	38.7 ± 13.56	39.9 ± 15.4	0.053
Gender (recipient)	119:57	63:29	18:8	0.980
Age (donor)	35.34 ± 12.1	62.7 ± 6.8	62.7 ± 15.1	<0.0001
Gender (donor)	112:64	54:38	15:11	0.669
Mean follow up (years)	4.88 ± 2.12	4 ± 2.11	5.64 ± 1.68	<0.0001
Acute rejection (%)	21 ($n=37$)	19.5 ($n=18$)	23 ($n=6$)	0.918
ATN	26.1 ($n=46$)	18.5 ($n=17$)	34.6 ($n=9$)	0.174
Patient loss (%)	17.6 ($n=31$)	19.5 ($n=18$)	46.1 ($n=12$)	
Graft loss (%)	9 ($n=16$)	10.8 ($n=10$)	11.5 ($n=3$)	
Patient survival (%)				0.002
1 year	84.7	83.7	57.7	
5 years	81.9	80.2	51.3	
8 years	81.9	80.2	51.3	
Graft survival (%)				0.744
1 year	94.5	89.5	91.3	
5 years	90.2	88.3	83.7	
8 years	88.1	88.3	83.7	

Table 2. Multivariate analysis.

		95% confidence interval		
	p Value	Hazard ratio (HR)	Lower	Upper
Patient survival				
Age (donor)	0.4360	0.9875	0.9568	1.0191
Age (recipient)	0.3848	1.0081	0.9900	1.0266
Gender (donor)	0.9042	0.9679	0.5703	1.6427
Gender (recipient)	0.5299	0.8348	0.4766	1.4622
Acute rejection	0.8396	1.1532	0.2919	4.5558
ATN	0.4358	0.5773	0.1460	2.2824
Graft survival				
Age (donor)	0.6749	1.0106	0.9623	1.0614
Age (recipient)	0.1526	0.9794	0.9521	1.0076
Gender (donor)	0.4495	1.3407	0.6295	2.8555
Gender (recipient)	0.3409	0.6584	0.2798	1.5492
Acute rejection	0.6200	1.6021	0.2511	10.2217
ATN	0.9767	1.0295	0.1479	7.1636

rejection, and ATN in each group showed no impact on patient and graft survivals (Table 2). Patient and death censored graft survival rates are shown in Kaplan–Meier curves (Figures 1 and 2A and B).

Discussion

DDRT is still low in India despite the need and tremendous potential. Our DDRT program can be considered fairly successful in the Indian context. In our study, over a mean follow-up of 3.93 years, patient and graft survival rates were 81.7% and 92.6%, respectively. We had high 1-year post-transplantation mortality with most of these deaths caused by sepsis with functioning graft. It is possible that triple drug immunosuppressive regimen with r-ATG induction, unhygienic living conditions, delayed presentation and diagnosis, tropical climate, limited availability and expense of diagnostic tools, and financial constraints for treatment in majority of patients may have contributed to the high infection rate.

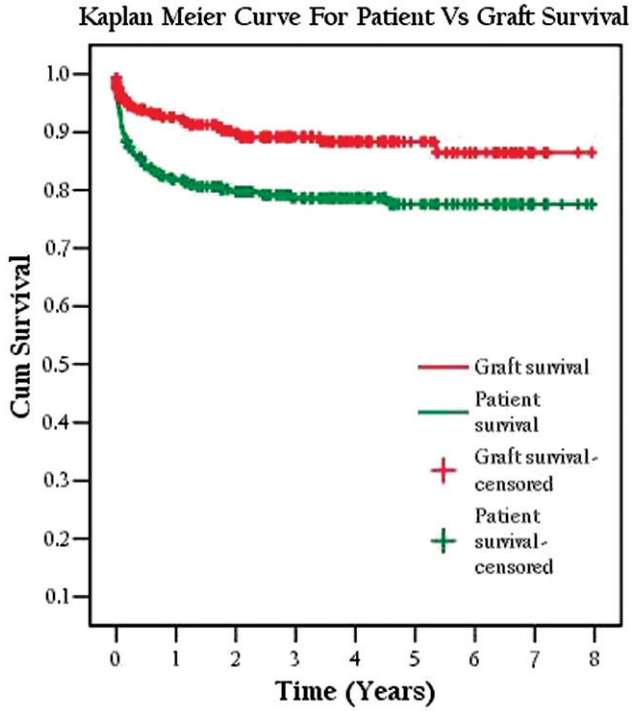

Figure 1. Kaplan–Meier curve for patient and graft survival.

We attribute our success to active steps taken by our institute. Our hospital had created a waiting list of patients awaiting transplantation for each organ, which gets frequently updated, and the telephone numbers and contact addresses of prospective recipients are kept in the hospital record. We have a counseling service for individuals involved in organ transplant and a transplant coordinator is appointed to coordinate all aspects of transplantation on behalf of the hospital. The transplant coordinator is available at all times for purposes of organ-sharing communication. The transplant coordinator also played the role of grief counselor. Because of transportation problems, recipients who are in top 10 number of the waiting list are advised to be available in the vicinity or a short distance from the transplant center. We do the transparent allocation of deceased organs according to the transplant waiting list and age matching. Immunosuppressive drugs are given free of cost to poor patients and all children ≤18 years under Government run School Health Program. As a cost-effective measure, CYP3A4 inhibitor (diltiazem) was used to increase the blood levels of CNI and hence reduce the cost of immunosuppression. On world kidney day, we organized recognition and felicitation of DD families.

We also have growing availability of less-expensive generic immunosuppressive agents, adequate governmental financial resources, and improved clinical training opportunities, governmental and professional guidelines legislating prohibition of commercialization and defining professional standards of ethical practice. We are rarely using the IL2 receptor antagonist due to economic constrains and we are using low-dose rabbit thymoglobulin (1.5 mg/kg). We have initiated satellite dialysis centers in the outskirts of the state, where patients could be dialyzed and eligible, willing patients are referred to us for RT. Majority of transplant centers do not have facilities for basic cross-matching techniques.

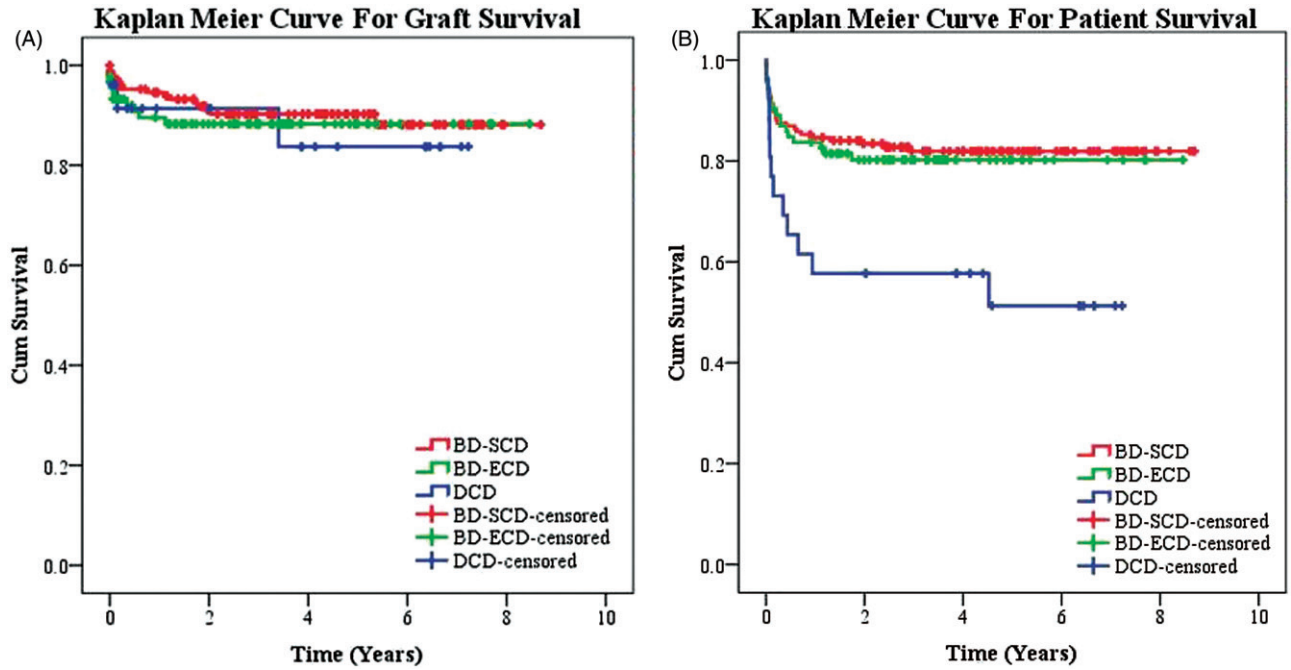

Figure 2 (A) Kaplan–Meier curve for graft survival in subgroups and (B) Kaplan–Meier curve for patient survival in subgroups.

We have our own lab for tissue typing, lymphocytes cross and flow-cross match, and advanced facility for immunological surveillance of recipients such as Luminexx single antigen assay for donor-specific antibodies, panel reactive antibodies, and HLA typing. Because the cost of azathioprine was significantly lower than the cost of MMF with similar long-term outcomes, azathioprine should be preferred over MMF for maintenance of immunosuppression therapy.

Reimbursement for healthcare is available only to a minority. Only the wealthy can afford treatment in private hospitals. The poor typically seek treatment in public sector hospitals where the government subsidizes treatment. A large proportion of ESRD patients in India either do not start or discontinues RRT due to financial reasons. RT is associated with catastrophic out-of-pocket expenditure and pushes a majority of the patients who come for treatment to public hospitals into severe financial crisis. Systematic efforts are required to address these issues. Awareness and changes in attitudes of the public as well as physicians are needed to improve organ donation.

Many transplant centers are reluctant to use kidneys from DCD due to relatively higher incidence of primary non-function (PNF). Issues like uncertainty regarding diagnosis of death on the basis of cessation of cardiac activity (cardiac death), logistics of family consent involved in the procurement of organs, and prolonged warm ischemia all contribute to its slow development. It is important to educate the public, hospitals, and physicians about the possibilities of organ donation from DCD. Public trust is most important in the success of any transplant program.[12]

We report RT outcome between DCD $\geq$70 years (Group 1; $n = 14$; mean age, 75.7 ± 5.81) and DCD $<$ 70 years (Group 2; $n = 19$; mean age, 51.7 ± 10.1) between 1999 and 2012. Patient survival ($p = 0.27$), graft survival ($p = 0.20$), DGF ($p = 0.51$), and BPAR ($p = 0.74$) were similar in two groups. Acceptable outcomes of DCD RT have a potential to expand the donor pool and shorten the waiting list for RT.[13] We describe our institutional experience with outcomes from transplanting deceased-donor kidneys from older donors ($\geq$70 years). DDRT from older donors achieves acceptable graft function with patient/graft survival, provided that organs are allocated to dual or single grafting according to pre-transplant biopsy.[14] We have also shown that because of the organ shortage, DDRT using ECD transplants for younger recipients and brain-dead DD who died from neurotoxic snakebite is a feasible option with acceptable outcomes.[15,16]

Infections remain a major challenge in developing countries due to poor social economic and environmental conditions. In one study, RT outcome did not differ between groups who received different doses (1.5, 3, and 6.0 mg/kg) of thymoglobulin.[17] Hence, we used 1.5 mg/kg (single low dose) thymoglobulin in our study with an acceptable outcome. The favorable outcome with this regimen is very encouraging and cost effective also. The 1-year allograft and patient survivals of 100 DDRT from four major centers in Chennai were 82% and 86%, respectively, with their 2-year allograft and patient survivals of 74% and 80%, respectively.[18] In a study by Mani, 1-year and 4-year graft survivals of 88 DDRT in Chennai were 72% and 63%, respectively, and patient survival was hardly different from graft survival.[19] Five-year patient and graft survivals of 68 DDRT in Chennai were 61.7% and 58.8%, respectively, with biopsy-proven acute rejection in 26.4%, DGF in 50%, and CIT of 5.6 ± 3.2 h.[20] Centers report remarkable differences in the quality of kidney they harvest which may contribute to differences in long-term results. ICU care and skill of the donor maintenance and recovering team may be a contributing factor.

There have been pockets of success with the deceased donation program and organ sharing among various hospitals.

The Multi Organ Harvesting Aid Network (MOHAN) Foundation has improved the deceased organ donation in India.[3,4,8,9,17] The MOHAN Foundation (Non-Governmental Organization based in Tamil Nadu and Andhra Pradesh) has facilitated 400 of the 1300 deceased organ transplants performed in the country over the last 14 years. This private–public partnership promoting DD transplantation has effectively eliminated commercialization in transplantation in the state of Tamil Nadu with a population of 72 million which is a model for other regions of South Asia and developing countries. About 236 kidney and 110 livers were retrieved in the Tamil Nadu program during October 2008 and 2010. A previous study by this group from a single-center experience showed a patient survival of 79.58%, 76.7%, and 74.8% and death censored graft survival was 92.4%, 87.9%, and 87.9% at 1, 2, and 3 years, respectively.[17] Currently, the state of Tamil Nadu has DD rate of 1.2 PMP per year. Although this is low compared with international standards, it is nearly 10 times that of the rate for India as a whole. There is a lack of awareness of organ donation in other states in India. The various positive steps taken by our transplant center and the Tamil Nadu model for successful DD program can be implemented by other new and emerging transplant center.

Health-care providers and patients increasingly turn to the Internet-websites as well as social media platforms-for health-related information and support because of their ease of access and widespread use. FaceBoook, Twitter, online social networks and social media have all been proposed as innovative tools for increasing rates and education about DD. They allow for wide dissemination of information and discussion and could lessen anxiety associated DD. The shortcoming of this analysis included is its retrospective single-center evaluation and variable immunosuppressive regimens.

As per Indian Transplant Registry (data from 48 hospitals), a total of 776 DDRT from 1971 to 2013 are reported in India. About 238 were female patients and 538 were male patients. Our study adds significant information to understand short- and long-term outcomes in the complicated field of transplants. Our model has shown that it is a sustainable option and can be duplicated and developed in other low income countries where DDRT is still in its infantile stage.

Conclusion

Our study demonstrates that DDRT achieves acceptable patient and graft survival, encouraging the use of this approach. Measures like increased public awareness, the recognition and felicitation of DD families, counseling about organ donation and early brain death identification and certification, 24 h services of efficient, committed and trained transplant co-coordinators, transplant team and immunology lab, adequate hospitals infrastructure, and inclusion of expanded criteria donors and marginal donors all helped in the success of our DDRT program.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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