The Frequency and Risk Factors of Delayed Graft Function in living Donor Kidney Transplantation and Its Clinical Impact on Graft and Patient Survival in Part of Middle East

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Purpose: Delayed graft function (DGF) is a form of acute renal failure which results in increased post-transplantation allograft immunogenicity and risk of rejection episodes in addition to decreased long-term survival. Its incidence and risk factors have been extensively studied, especially after deceased donation. However until now, only few data has been published on DGF in living donor kidney transplant recipients. The present study was performed to investigate the frequency and risk factors of DGF among living- kidney transplant recipients.

Material and Methods: In this retrospective study, 500 living kidney transplant recipients recruited and data collected from hospital registries in three countries (Iran, Kingdom of Saudi Arabia (KSA) , and Kuwait ).

Results: Incidence of DGF revealed to be 95% %2.3 CI: 3.6-%0.9). DGF group showed significant older age for the recipients and in “without DGF” group, there were more females, and lower weight for the recipients. It was found that patients with DGF had longer pre transplant dialysis duration, cold ischemic and anastomosis time during surgery.

Conclusion: DGF after living-donor kidney transplantation is a multifactorial complication which donor, recipient, and technical factors would lead toward. Consideration and optimization of these risk factors may drive through better long-term patient and graft outcomes in living kidney transplant recipients.

Keywords: living kidney transplantation; delayed graft function; allograft rejection; slow graft function

INTRODUCTION

There are many different descriptions for delayed graft function in literature as defined by: dialysis requirements within one week after transplantation, urine output less than 1200 ml during first day after transplantation, serum creatinine decrease less than 10% during 48 hours after transplantation, creatinine greater than 2.5 mg/DL at ten days after transplantation, creatinine clearance less than 10 ml/min/1.73m² during 24 hours after transplantation or creatinine did not decrease less than preoperative value1-4. What we have used as the definition of DGF is requirement for dialysis within the first week after surgery. Regarding diversity in definition, the incidence of DGF varies widely among different studies. According to published literature, the incidence of DGF in living donor kidney recipients varies from 1.6% up to 18.3% (3-7) depending on the different studies. Whatever the definition is, the occurrence of delayed graft function has been shown to be a strong risk factor for reduced renal allograft survival8-10. Factors related to the donor and the recipient can contribute to this condition8-10. Although many studies published on predictive factors of DGF after cadaveric kidney transplantation, related factors in living donor kidney recipients are still unclear. As DGF has significant impacts on living donor kidney transplantation outcome, identification of DGF risk factors is critical to improve prognosis in this population. In the present study we aimed to evaluate incidence and related risk factors of DGF after living donor kidney transplant recipients in three different countries of middle east.

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MATERIALS AND METHODS

Study population
No formal sample size calculation was performed, the sample size has not been determined in terms of statistical power, but rather in terms of precision (95% Confidence Interval) associated to event rate estimation.

According to the number of kidney transplantation in each participating site, it was estimated that 500 living donor kidney transplantsations are done over 8-10 months. Accordingly and in order to have 3 years post transplant follow up information, all living donor kidney transplantsations through Oct 2009 - Oct 2011 who met the inclusion criteria were enrolled.

500 patients, from 3 centers in 3 countries were recruited. Distribution of subjects by country was as following: Iran (n = 300), Kuwait (n = 100) and KSA (n = 100) (Figure 1). As it was a retrospective registry with no access or possibility to recall patients, obtaining informed consent from patients was waived up to the local regulatory processes. Approvals were obtained from each site to access patients’ records and collect their data.

Patient Selection and Evaluation
Inclusion criteria was: Living donor kidney recipients with complete history prior transplantation and 3 years post transplantation data, including those who died during the observation period.

Exclusion Criteria was: kidney recipients younger than 18 years, living donor kidney transplantation recipients experiencing DGF among living donor kidney transplantation. DGF defined as need of dialysis within 3 days after transplantation in living donor kidney transplantation. DGF defined as need of dialysis within the first days after transplantation in living donor kidney transplantation recipients. Secondary endpoints were: demonstrating demographic data of donors and recipients, primary causes of end-stage renal disease, pre-transplant time on dialysis, ischemic time, immunosuppressive regimens (induction, if any used), adverse events, acute and chronic biopsy proven graft rejections, graft survival and patient survival at 1, 2 and 3 years follow-up. As a potential important contributor in DGF is timing of introduction of calcineurin inhibitors (CNI), we tried to choose centers with similar immunosuppression protocols post transplantation. Based on this concept CNI was initiated according to same protocol in involved centers when the serum creatinine decreases to ≤ 4 mg/dL or to ≤ 50% of pre-operation value whichever is lower, or within 72 hours after surgery. CNI dose should be maximized within 24 h of the last dose of ATG. Cyclosporine (Neoral®) initiated at 6 mg/kg orally divided to two times per day; or Tacrolimus (Prograf®) 0.15 mg/kg orally divided to two times per day.

Primary endpoint was to assess percentage of recipients experiencing DGF among living donor kidney transplantation. DGF defined as need of dialysis within the first days after transplantation in living donor kidney transplantation recipients.

Secondary endpoints were: demonstrating demographic data of donors and recipients, primary causes of end-stage renal disease, pre-transplant time on dialysis, ischemic time, immunological status, PRA (panel reactive antibody) if it was done, status of HLA matching if available, type of immunosuppressive regimens (induction, if used), adverse events, acute and chronic biopsy proven graft rejections, graft survival and patient survival at 1, 2 and 3 years follow-up. As a potential important contributor in DGF is timing of introduction of calcineurin inhibitors (CNI), we tried to choose centers with similar immunosuppression protocols post transplantation. Based on this concept CNI was initiated according to same protocol in involved centers when the serum creatinine decreases to ≤ 4 mg/dL or to ≤ 50% of pre-operation value whichever is lower, or within 72 hours after surgery. CNI dose should be maximized within 24 h of the last dose of ATG. Cyclosporine (Neoral®) initiated at 6 mg/kg orally divided to two times per day; or Tacrolimus (Prograf®) 0.15 mg/kg orally divided to two times per day.

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and 3 years post transplantation depending on follow-up time and slow graft function (SGF) incidence defined as creatinine reduction ratio (CRR) between time 0 of transplantation and day 7 post-transplantation of <70%. Data was summarized using frequency and percentages for categorical parameters with its 2-sided 95% confidence interval (CI) and mean, median, standard deviation, range and 95% CI for continuous parameters. All statistical tests performed using two-tailed tests at a 5% level of significance. For comparison between patients who experienced DGF and those without DGF regarding all secondary endpoints the appropriate statistical tests for comparison were used according to type of compared parameters (e.g. Chi square tests for categorical data and student t-test for continuous parameters). Using binary logistic regression analysis, the potential risk factors for occurrence of DGF in living donor transplantations were tested. For the 11 patients with DGF, the data was missing for the remaining three patients whose data were available, eight patients regarding to cold ischemia time but for the 11 patients with DGF, the data was missing for complete classification regarding the type of living donation. Among donor population overall, the data was unknown. Among donor population overall, the mean ± SD age of all 480 donors was 29.41 ±5.6 years. Regarding the 11 patients who have developed DGF, only three (27.27%) patients received a graft from genetically related donors. Considering all secondary endpoints the appropriate statistical tests performed using two-tailed tests at a 5% level of significance. When a greater event rate was seen early in the trial rather than toward the end of the trial, the generalized Wilcoxon rank-sum test seemed to be more appropriate test.

RESULTS
The total enrolled patients included in the study sites consisted of 480 patients. Detailed study population, reasons for exclusion and countries distribution are shown in (Figure 1). Overall, DGF found in 11 recipients (2.3%; 95% CI: 0.9%-3.6%) (Table 1). Slow graft function (SGF) defined as creatinine reduction ratio (CRR) between time 0 of transplantation and day 7 post-transplantation of <70% was also calculated and data analysis showed that the overall incidence of SGF in this study was 10.6%. The mean ± SD age of all 480 eligible patients was 42.9 ±13.7 years. The youngest patient was 18 while the oldest patient was 83 years old. The mean ± SD age of the 11 patients with DGF was 44.45 ±12.72 years. Out of the 480 eligible patients; 314 (65.4%) were men and 166 (34.6%) were women. Out of the 11 patients with DGF, eight were men and three (27.3%) were women. The two most common causes of ESRD were hypertension (36.4%) and diabetes mellitus (16.3%). Among diabetic cases, 45.5% had experienced DGF with significant statistical difference (p = 0.021) (Table 2). Among the 11 patients with DGF, five (45.45%) patients had other comorbidities. Hypertension (36.4%) was the main comorbidity found in patients with DGF and without DGF (32.8%). The mean ± SD duration for dialysis before transplantation for the 11 patients who had developed DGF was 26.36 ± 26 months, while for the 434 patients who had not developed DGF it was 15.39 ± 20.60 months. Taking into consideration that a patient could have more than one pre-transplant immunizing event type, it was found that 99 (20.63%) patients had history of blood or blood product transfusion, 17 (3.54%) were pregnant and only four (0.83%) had high panel reactive antibody (PRA). 399 (83.3%) patients have done PRA level test mostly at transplantation time. Out of the 11 patients who have experienced DGF, 9 (81.82%) patients have done PRA test. Taking into account that patient could have more than one PRA method, collected data showed that PRA in DGF patients was determined mainly by cytotoxicity (66.67%), followed by solid phase assays that were performed in the laboratory. Patient survival at 1, 2 and 3 years post transplantation depending on follow-up time was evaluated using Kaplan-Meier survival method or Wilcoxon test at 5% level of significance. When a greater event rate was seen early in the trial rather than toward the end of the trial, the generalized Wilcoxon rank-sum test seemed to be more appropriate test.

Table 3. Type of Living Transplant

<table>
<thead>
<tr>
<th>Type of Living Transplant</th>
<th>With DGF Count</th>
<th>%</th>
<th>Without DGF Count</th>
<th>%</th>
<th>Overall Count</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genetically Related</td>
<td>3</td>
<td>27.2%</td>
<td>107</td>
<td>22.8%</td>
<td>110</td>
<td>22.9%</td>
</tr>
<tr>
<td>Unrelated</td>
<td>8</td>
<td>72.7%</td>
<td>362</td>
<td>77.2%</td>
<td>370</td>
<td>77.08%</td>
</tr>
<tr>
<td>Total</td>
<td>11</td>
<td>100%</td>
<td>469</td>
<td>100%</td>
<td>480</td>
<td>100%</td>
</tr>
</tbody>
</table>

Table 4. Patient Survival at 1,2 and 3 Years Post-Transplant Follow-up

<table>
<thead>
<tr>
<th>Patient Death with Functioning Graft</th>
<th>With DGF (N=11) Count</th>
<th>%</th>
<th>Without DGF (N=469) Count</th>
<th>%</th>
<th>P Value</th>
<th>Overall (N=480) Count</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>0</td>
<td>0%</td>
<td>12</td>
<td>2.6%</td>
<td>1*</td>
<td>12</td>
<td>2.5%</td>
</tr>
<tr>
<td>No</td>
<td>11</td>
<td>100%</td>
<td>457</td>
<td>97.4%</td>
<td>468</td>
<td>97.5%</td>
<td></td>
</tr>
</tbody>
</table>

*Fisher’s Exact Test to Compare the Percentage Between the Study Groups (with DGF and without DGF)
± SD cold ischemia time for harvested kidney preservation was significantly longer \((p = 0.013)\) in patients with DGF than in patients without DGF \((25 \pm 7.9\) min\). Cold ischemia time \((CIT)\) was defined as the duration between the beginning of cold storage and reperfusion of the graft. Regarding warm ischemia time in patients with DGF, the data was missing for six cases but in remaining five patients whose data were available there was insignificant difference \((p = 0.138)\) in the warm time preservation between patients with DGF \((21.8 \pm 22\) min\) and patients without DGF \((18.8 \pm 12.1\) min\). The vascular anastomosis duration was significantly longer \((p = 0.013)\) among patients with DGF \((49 \pm 18\) min\) than in patients without DGF \((31.5 \pm 6.9\) min\). Out of the eligible population \((n = 480)\), the number of patients who have not received induction therapies was 274 \((57.08\%)\) patients. Out of the 11 patients who have developed DGF, data showed that seven \((63.64\%)\) patients have received induction therapies. The most common type of induction therapy administered was Basiliximab followed by Rabbit Anti-Human Thy-mocyte Immunoglobulin. During the first week of post-transplantation phase, 18.18% patients with DGF have experienced surgical complications from which 9.1% fulfilled sepsis criteria and 9.1% experienced other surgical complications. Among patients without DGF 2.13% have experienced surgical complications meanwhile hemorrhage was the most common one \((1.5\%)\) followed by sepsis. Hence, it is shown that the percentage of early surgical complications was significantly lower \((p = 0.028)\) among patients who had not developed DGF \((2.13\%)\) than patient who had developed DGF \((18.18\%)\). Post-transplant early medical complications in recipients were found in 19.1% out of the total eligible population \((n = 480)\) while the percentage of early complications was again significantly lower within the first week post transplantation \((p = 0.002)\) among patients who had not developed DGF \((18.1\%)\) than patient who had developed DGF \((63.6\%)\). Safety results revealed that the percentage of patients who experienced post transplant complications was significantly lower \((p < 0.001)\) in patients without DGF than patients with DGF. The most frequently reported among these adverse events from the total enrolled population was renal tubular necrosis as it had occurred in 10.2% of patients and transplant rejection in 9.4% of patients, followed by thrombocytopenia in 6%, urinary tract infection in 2.6%, cytomegalovirus positive test in 1.8% and graft loss in 1.4% of patients. As it was a retrospective study missing data about the rate and pathologic details of rejections limit concluding significant result however biopsy proven graft rejection had been reported in 60 \((12.5\%)\) of patients from the total eligible population. The rejection was considered early (occu
with less than 6 months) in 44 (75%) cases while it defines as late (after 6 months post transplantation) in 24.1%. Overall, there was no statistical significant difference between both groups (with or without DGF) regarding the graft rejection frequency proven by biopsy (p = 0.636). There was statistically significant higher rate (p < 0.001) of graft loss among patients with DGF (27.3%) than patients without DGF (9.9%) throughout 3 years of follow-up. The 3 years graft survival rate among patients with DGF was 72.7% while among patients without DGF was 99.1%. Thus, the graft survival was significantly shorter (p < 0.001) among patients with DGF (2.26 years) than patients without DGF (4.02 years). (Figure 2) During three years follow-up it was found that, 12(2.5%) patients had died. There was statistically insignificant difference (p = 1) regarding patient survival among patients with DGF (100%) and without DGF (97.4%) throughout these 3 years follow-up post transplantation. (Table 4)

**DISCUSSION**

Incidence of DGF varies a lot according to previous literature with different mentioned risk factors including female donor (6), low donor weight (5), high recipient / donor weight ratio (5), donor age (18), warm ischemia time (8-H), HLA mismatch (5), and non-related donor (6). In one published study (6) conducted between 1994 and 2010 in Iran, DGF complicated 67/385 transplant recipients (17.4%). DGF is not a minor event. Indeed, patients experiencing DGF are more at risk of rejection which can be considered as the main drawback of kidney transplantation and long-term graft survival is significantly impacted in patients experiencing both DGF and rejection (6). In a study published by Kwon (5) evaluating effect of DGF on graft survival in living donor kidney transplantation, the rate of acute rejection in patients experiencing DGF was 70.6%, the 3-year graft survival rate was significantly lower in patients with acute rejection episode complicated by DGF than in patients who experienced acute rejection without DGF (61% vs. 74% respectively, p < 0.002). Moreover, in a retrospective cohort study reported by Narayan (5-6) in 645 patients with first living kidney transplantation over 12 years the cumulative probability of biopsy-proven acute rejection (BPAR) was higher in compensated patients. The 1-, 3-, and 5-year probabilities of BPAR were 16.0% (95% confidence interval (CI): 11.8, 21.3), 21.8% (95% CI: 16.8, 27.9), and 22.6% (95% CI: 17.5, 28.9) in the DGF group and 10.1% (95% CI: 7.6, 13.5), 12.4% (95% CI: 9.5, 16.1), and 15.7% (95% CI: 12.2, 20.1) in the non-DGF group, respectively (P = 0.01). In our study as the primary endpoint 2.3% experienced DGF (n = 11) which can be logical due to short ischemic time in living-donated recipients accompanied by younger and healthier donors. Comparing this demographic data of both recipients and donors this can be shown that, in the cases that had experienced DGF, recipients were significantly older (44.5 ± 12.7 years). As request for kidney transplants outweighs the reserve pool, some transplantation centers have consumed ‘extended criteria kidneys’ particularly those from older donors (6) which are associated with inferior recipient outcomes including reduced short-term and long-term glomerular filtration rate and reduced overall graft survival (10-11). According to a previous experience in Iran conducted on 3716 transplanted cases, donor age was the only statistically significant predictor of graft survival. (12) On the other hand, older recipients are also at an increased risk of death with functioning graft independent of donor age (12-13). The total effect of age matching seems to be little, but as an individualized approach there appears to be benefits. This study confirmed that recipient’s diabetes appeared as one of the important risk factors for poor initial graft function. We believe that this is more than a simple incidental finding and an underlying relationship may exist as diabetes potentiates ischemia/reperfusion injury thus increases the need for early post transplant dialysis. While expressing the role of long-term diabetes remains challenging, controlling hyperglycemia at the time of transplant is entirely achievable. Evaluating the effect of tight versus poor glucose control in both donors and recipients to determine the effect of glucose control at the time of organ procurement and at the time of reperfusion of the transplanted kidney would be the next step to clarify the impact of diabetes on delayed graft function after living kidney transplantation. The mean duration of dialysis pre-transplantation for the patients with DGF was clinically but not statistically longer in DGF group (p = 0.055). These data ascertained that waiting time on dialysis before kidney transplantation is a risk factor for graft dysfunction independent of donor factors (6). Because pre-transplantation dialysis duration is increasing as a result of the widening gap between the increase in the demand for organs and the increase in organ donations, importance of preemptive transplantation should be taken into account more than the last decade. Prolonged vascular anastomosis duration is another risk factor for DGF. According to the United Network for Organ Sharing Registry (UNOS) data, the effect of cold ischemic time continues for years, beyond an average level of about 20 h. (15) According to this study longer cold ischemic and vascular anastomosis time can prone the recipients to develop DGF. More than half of our eligible population did not received induction therapy (57.1%). The mechanism of Thymoglobulin as an induction therapy in reducing ischemia-reperfusion injury (IRI) known to be mainly the result of direct blocking the cell-to-cell interactions and decreasing the degree of leukocyte rolling and adhering along capillary endothelium. (16) Furthermore, Thymo reduces the number of peripheral lymphocytes from the circulating pool by inducing T-cell depletion through complement-related lysis or activation associated apoptosis (17). Lopez et al. showed that the therapeutic effect of Thymo is not only due to T-cell depletion, but also due to generation of regulatory T-cell (18). Hence using Thymoglobulin as pre-transplantation induction would minimize the ischemia reperfusion injury in the grafted organ and subsequently preventing DGF. This study evaluated the very important topic of delayed graft function among recipients of living donor kidney transplants. However, the small sample size limits meaningful subgroup analysis. Meanwhile this retrospective study was mainly based on collecting data from existing local hospital registries facing with incomplete medical records and missing data. Pathological details about the type of rejections were missing data as well which disable us to pulling out any significant results regarding the differences between rejection in DGF and nonDGF groups.
CONCLUSIONS
A number of recipient and donor variables have been identified as DGF risk factors for patients with deceased donor grafts, still, predictive factors associated with DGF following living donor kidney transplantation, remain unknown. The aim of this study was to explore these predictors. It was a, retrospective study on DGF incidence and risk factors among living donor kidney transplant recipients in three countries of Middle East including; Iran, KSA and Kuwait. Incidence of DGF revealed to be 2.3% (95% CI: 0.9%-3.6%) of the 480 eligible enrolled cases. The comparisons between recipients and donors among DGF group showed significant older age for the recipients than donors. In the “without DGF” group, there were significant, more females, and lower weight for the recipients. It was found that patients with DGF had longer pre transplant dialysis duration, cold ischemic and anastomosis time during surgery. DGF after living-donor kidney transplantation is a multifactorial complication which donor, recipient, and technical factors would lead toward. Consideration and optimization of these risk factors may lead to better long-term patient and graft outcomes in living kidney transplant recipients.

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CONFLICT OF INTEREST
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