Steroid and Azathioprine Versus Steroid, Cyclosporine, and Azathioprine Therapies in Primary Haplo-Identical Living Donor Kidney Transplantation

Twenty-Year Experience

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Introduction. Achievements in short-term graft survival since the introduction of cyclosporine has not been matched by improvement in long-term graft function, and chronic allograft nephropathy remains the second commonest cause of graft attrition over time. We aimed to evaluate the long-term results of conventional immunosuppression by steroid and azathioprine in comparison with cyclosporine-based triple therapy in living donor kidney transplants.

Materials and Methods. We evaluated the long-term follow-up data of 369 living related kidney transplant recipients that were on prednisolone-azathioprine immunosuppressive therapy (group 1) or triple therapy by prednisolone, cyclosporine, and azathioprine (group 2). All recipients were followed-up for more than 10 years (mean, 240 ± 12 months). Comparative analyses included patient and graft survival rates, condition at last follow-up, graft rejection, and graft function.

Results. There were 130 patients in group 1 and 239 in group 2. The overall frequency of acute rejection episodes was not significantly different between the two groups. However, the proportion of patients with chronic allograft nephropathy was significantly higher in group 2 (21% versus 35%, $P = .001$). Graft survival rates were 85.3% versus 92.4% at 1 year, 69.9% versus 71.9% at 5 years, and 52.5% versus 50.8% at 10 years in groups 1 and 2, respectively ($P = .03$). The two groups were comparable regarding posttransplant malignancies, diabetes mellitus, serious bacterial infections, and hepatic diseases. However, hypertensive patients were significantly more frequent in group 2.

Conclusions. Chronic allograft nephropathy was significantly higher in patients receiving cyclosporine, possibly due to the risk of drug-induced nephrotoxicity, glomerular disease recurrence, and hypertension. Nowadays, it is possible to achieve excellent calcineurin inhibitors-free regimen using newer maintenance immunosuppressive agents.

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INTRODUCTION

The achievements in short-term kidney allograft survival since the introduction of cyclosporine A has not been matched by improvement in long-term graft function. Chronic allograft nephropathy (CAN) remains the second most common cause of graft

attrition over time after patient mortality.[1] On the other hand, cyclosporine has some serious adverse reactions including nephrotoxicity, hypertension, symptomatic hyperuricemia, hirsutism, and gum hyperplasia.[2] In order to alleviate these adverse reactions, many trials have been adopted to optimize Cyclosporine utilization.[3] Co-administration of calcium channel blockers or ketoconazole was fashioned in order to decrease the dose and achieve acceptable therapeutic window.[3,4] Overall, reduction and possible withdrawal of calcineurin inhibitors may be necessary to slow progressive rate of loss of renal function.[5]

Grimbert and colleagues[6] demonstrated a 12-year graft survival of 56% with steroid and azathioprine versus 59% with calcineurin inhibitor-based triple therapy and showed that a graft half-life of 15 years could be achieved without the primary use of a calcineurin inhibitor in low-risk patients receiving antilymphocyte globulin induction. Interestingly, patients treated with cyclosporine had poorer graft function at 12 years.[6] Moreover, Opelz and Döhler confirmed that maintenance immunosuppression with azathioprine and steroids resulted in good long-term kidney graft survival, provided azathioprine would be administered at a daily dose higher than 1.5 mg/kg.[7] Meanwhile, the available studies among living related kidney transplants are lacking regarding evaluation of long-term efficacy and safety of primary cyclosporine-based immunosuppressive regimens. We designed a prospective study to evaluate the long-term results of conventional (steroid and azathioprine) versus triple (steroid, cyclosporine, and azathioprine) protocols of immunosuppression in living-donor kidney transplantation.

MATERIALS AND METHODS

Study Population

This study was carried out on kidney transplant recipients in Urology and Nephrology Center of Mansoura University, Egypt, from 1983 till 1988. A total of 369 patients were assigned into 2 groups in a ratio of 1 to 2 in order to receive either the primary immunosuppressive protocol as steroid plus azathioprine (130 patients; group 1) or steroid, cyclosporine, and azathioprine triple therapy (239 patients; group 2). Only adult recipients of their first kidney allograft with the age ranged between 18 and 60 years and a maximum of 1 haplotype human leukocyte antigens (HLA) mismatch were included. All of the patients enrolled in this study received their grafts from living related donors and the two groups were matched regarding previous blood transfusions. We excluded patients with HLA mismatches and children (younger than 18 years) from the study.

Methods

All recipients were closely and regularly followed-up for more than 20 years (mean follow-up period, 240 ± 12 months). The follow-up visits were frequent at early posttransplant period and gradually their intervals increased till reaching once every 6 months. On each visit, graft was assessed by serum creatinine, blood urea, creatinine clearance, and urinalysis, in addition to complete blood picture, serum levels of drugs, plasma cholesterol level, and plasma glucose level in diabetics. Conventional and Doppler ultrasonographies of the abdomen were performed if there was clinical suspicion of acute rejection, acute tubular necrosis, or renal artery thrombosis. Kidney allograft biopsy was performed in patients with clinical suspicion of rejection (unexplained rise of serum creatinine level to more than 25% of the baseline level).

In both study groups, prednisolone was started on 1 day prior to transplantation with a dose of 8.5 mg/kg and reduced gradually till the smallest maintenance dose of 0.15 mg/kg by the end of the 9th posttransplant month. Azathioprine was given with a dose of 3 mg/kg/d for the patients of group 1, and a dose of 1.5 mg/kg/d for those in group 2. Cyclosporine was introduced with a dose of 8.5 mg/kg/d for group 2, and it was adjusted to keep the serum trough level between 150 ng/mL and 200 ng/mL during the first 2 months and between 100 ng/mL and 150 ng/mL, thereafter. Antibody-induction therapy was given—according to our policy—to the high-risk patients. Cyclosporine trough level was measured at first using radioimmune assay kits (Sandoz, Basel, Switzerland), and then using monoclonal-specific antibody (Abbott Laboratories, Chicago, Illinois, USA). Before 1997, we were defining acute rejection into 3 grades: mild, moderate, and severe according to the degree of cell infiltration in the kidney tissue. Sine 1997, we have followed the Banff classification.[8] Moreover, we reviewed biopsies of these patients and found that most of

the cases with biopsy-proven chronic allograft nephropathy were matched with mild to moderate degrees according to the Banff 1997.[8]

All acute rejection episodes were biopsy proven and treated by 500 mg of methylprednisolone for 5 days. Steroid-resistant rejection was treated by antithymocyte globulin or OKT3. Plasmapheresis was added to the treatment plan as an adjuvant therapy in cases of accelerated or vascular rejections.

Clinical data of all kidney transplant patients were reviewed. Demographic data included recipient's age and sex; donor's age and sex; causes of end-stage renal disease; HLA-A, HLA-B, and HLA-DR mismatching; and medical complications such as hypertension, diabetes mellitus, infections, malignancies, and hepatic dysfunction. Comparative analyses included patients and graft survival rates, condition at the latest follow-up, rejection (acute and chronic), and graft function (serum creatinine and creatinine clearance).

Statistical Analyses

Statistical analyses were carried out using the SPSS software (Statistical Package for the Social Sciences, version 11.5, SPSS Inc, Chicago, Ill, USA). All values were expressed as means ± standard deviation for continuous parametric data and frequencies for categorical data. The *t* test and the chi-square test were used for comparisons between the two study groups. The Kaplan-Meier actuarial curves were constructed for patient and graft survivals, and the log-rank test was used for survival comparisons. Values of *P* less than .05 were considered significant.

RESULTS

Table 1 illustrates the donors and recipients' characteristics. The majority of recipients were men in their second decade of life, while more than half of the donors were women in their third decade of life. Also, the two groups were homogenous in terms of donor's age and sex, recipient's age and sex, prior blood transfusion, and pretransplant hypertension. In addition, no preformed antibodies against donor antigens were detected in the pretransplant cross-match of any of the study patients. The techniques employed for re-establishment of urinary continuity were also essentially similar.

Rejection Episodes

We found no significant difference between the two groups regarding rejection-free patients or those who experienced acute rejection episodes (Table 2); cases of repeated rejections were more common in the patients of group 2 than group 1, but it did not rank to significance. However, the proportion of patients with CAN was significantly higher in group 2 (n = 28 [21%] versus n = 85 [35%], *P* = .001).

Table 1. Characteristics of Kidney Allograft Donors and Recipients in Patients With Different Immunosuppression Regimens*

Parameter	Group 1	Group 2	*P*
Number of patients	130	239	…
Mean age of donors, y	33.3 ± 10.1	34.0 ± 9.2	.33
Donor sex			
Male	61 (46.9)	115 (48.1)	
Female	69 (53.1)	124 (51.9)	.91
Mean age of recipients, y	29.8 ± 7.9	30.7 ± 10.1	.32
Recipient sex			
Male	95 (73.1)	176 (73.6)	
Female	35 (26.9)	63 (26.4)	.99
Primary kidney disease			
Glomerulonephritis	80 (61.6)	182 (76.2)	.001
Chronic pyelonephritis	3 (2.3)	8 (3.3)	.81
Nephrosclerosis	3 (2.3)	9 (3.4)	.65
ESRD	44 (33.8)	40 (16.7)	.001
Pretransplant hypertension	69 (53.1)	147 (61.5)	.14
Pretransplant blood transfusion	88 (67.7)	166 (69.5)	.81

*Values in parentheses are percents. Patients in group 1 received prednisolone and azathioprine, and those in group 2 had cyclosporine added to their immunosuppressive regimen. ESRD indicates end-stage renal disease and ellipsis, not applicable.

Table 2. Rejection Episodes in Patients With Different Immunosuppression Regimens*

Rejection Episodes	Group 1	Group 2	*P*
0	51 (39.2)	92 (38.4)	.92
1	54 (41.5)	78 (32.6)	.11
≥ 2	25 (19.2)	69 (28.9)	.05

*Values in parentheses are percents. Patients in group 1 received prednisolone and azathioprine, and those in group 2 had cyclosporine added to their immunosuppressive regimen.

Outcomes

There was no statistically significant difference in the survivors with functioning grafts of both groups (P = .14; Table 3). Living patients with graft failure were significantly more in group 2 and in group 1, while mortality cases (with or without functioning grafts) were significantly more in group 1 (P = .001; Table 3). Graft survival rates were 85.3% versus 92.4% at 1 year, 69.9% versus 71.9% at 5 years, and 52.5% versus 50.8% at 10 years in groups 1 and 2, respectively (P = .03; Figure 1). The corresponding patient survival rates were 89.2% versus 95.7% at 1 year, 75.7% versus 85% at 5 years, and 60.9% versus 72.8% at 10 years, respectively (P = .01; Figure 2).

Table 3. Outcome of Patients With Different Immunosuppression Regimens*

Outcome	Group 1	Group 2	*P*
Condition			
Live + functioning graft	51 (39.2)	114 (47.7)	.14
Live + on dialysis	16 (12.3)	64 (26.8)	.001
Died + functioning graft	39 (30.0)	41 (17.2)	.001
Died + failed graft	24 (18.5)	20 (8.4)	.001
Serum Creatinine, mg/dL			
At one year			
< 1.5	111 (85.4)	164 (68.7)	.001
1.5 to 3	16 (12.3)	72 (30.1)	.001
> 3	3 (2.3)	3 (1.3)	.71
Last follow-up			
< 1.5	95 (73.7)	121 (50.6)	.001
1.5 to 3	32 (24.5)	104 (43.7)	.001
> 3	3 (2.8)	14 (5.7)	.12

*Values in parentheses are percents. Patients in group 1 received prednisolone and azathioprine, and those in group 2 had cyclosporine added to their immunosuppressive regimen.

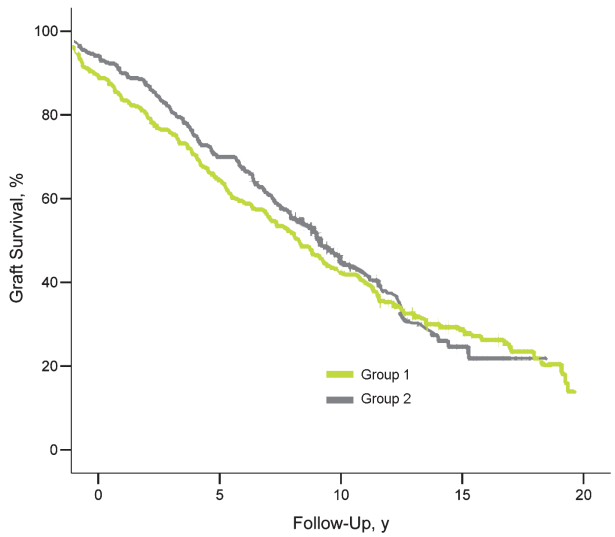

Figure 1. Kaplan-Meier curves for graft survival in the two groups of kidney recipients. Patients in group 1 received prednisolone and azathioprine, and those in group 2 had cyclosporine added to their immunosuppressive regimen.

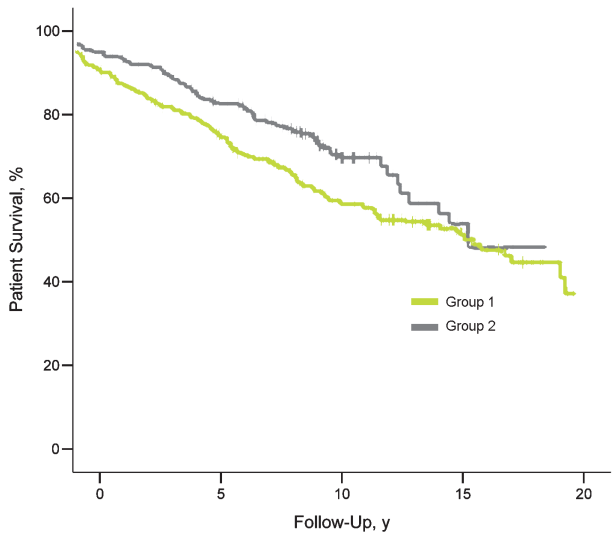

Figure 2. Kaplan-Meier curves for patient survival in the two groups of the study. Patients in group 1 received prednisolone and azathioprine, and those in group 2 had cyclosporine added to their immunosuppressive regimen.

The percentage of patients with grade 1 graft function (serum creatinine level lower than 1.5 mg/dL) was significantly higher in group 1 both at 1 year and at the last follow-up (P = .001), while the percentage of patients with grade 2 graft function (serum creatinine level between 1.5 mg/dL and 3 mg/dL) was significantly higher in group 2 both at 1 year and at the last follow-up (P = .001; Table 3). However, no difference could be detected regarding patients with grade 3 graft function (serum creatinine level higher than 3 mg/dL).

Complications

The two groups were comparable regarding posttransplant malignancies, diabetes mellitus, serious bacterial infections, and hepatic problems. However, hypertensive patients were significantly more frequent in group 2 than in group 1 (P = .001; Table 4).

DISCUSSION

Several strategies have been adopted to improve allograft survival. The introduction of cyclosporine, for instance, has been reported to improve graft survival and decrease the incidence and/or severity of rejection episodes.[9] However, studies among living related kidney transplants are lacking for confirming the long-term effect of cyclosporine. Our study aimed to evaluate the long-term results of triple therapy with steroid, cyclosporine, and azathioprine versus steroid-azathioprine primary protocols after living donor kidney transplantation. We found no significant differences between the two groups regarding rejection-free patients or acute rejection episodes. In the same direction, graft survivals were comparable between the two groups at 5 years (69.9% versus 71.9%) and at 10 years (52.5% versus 50.8%). This is matched with that reported by Bakker and colleagues[10] who found—at 15 years—that graft survival rate tend to be lower in the patients of cyclosporine group (64% versus 76.5%) with higher relative risk of CAN in the cyclosporine group, a finding attributing a role of cyclosporine nephrotoxicity in development of CAN. The conclusion can be replacement of azathioprine by mycophenolate mofetil due to its higher protective effect in prevention of worsening of chronic interstitial fibrosis, either directly or through its immunologic pathway.[9] Moreover, it has been shown that patients treated with cyclosporine have poorer graft function at 12 years.[6] However, experimental work failed to prove significant differences between azathioprine and cyclosporine in development of CAN. It is difficult to be certain as to what extent this model reflect the human disease.[11] Despite the similarity of our two groups regarding acute rejection episodes, the percentage of cases with chronic allograft nephropathy was significantly higher in patients receiving cyclosporine (P = .001) and this is matched with our primary report about the same groups.[12] This result raised the nonimmunological role of cyclosporine or the possible recurrent glomerular lesions.

In our study, patient survival rates were relatively lower in patients with steroid-azathioprine protocol (Table 3), possibly due to higher frequency of bacterial infections, although it did not rank to significance (Table 4). Serum creatinine levels at 1 year and the end of follow-up period tended to be lower in patients who did not receive cyclosporine (Table 3). Again the nephrotoxic effect of cyclosporine might explain this result. Also, we found no significant difference between the two groups regarding posttransplant malignancies, diabetes mellitus, hepatic problems, or serious bacterial infections. Meanwhile, the percentage of hypertensive cases in the patients of cyclosporine group was significantly higher than that among azathioprine group, and this was matched with that reported by Thiel and colleagues who reported

Table 4. Serious Complications in Patients With Different Immunosuppression Regimens*

Complication	Group 1	Group 2	*P*
Malignancies	9 (7.6)	12 (5.3)	.60
Hepatic impairment	12 (9.2)	23 (9.6)	.94
Posttransplant hypertension	67 (56.3)	196 (62.6)	.001
Posttransplant diabetes mellitus	19 (14.6)	34 (14.2)	.92
Bacterial infections	11 (8.5)	9 (3.8)	.09

*Values in parentheses are percents. Patients in group 1 received prednisolone and azathioprine, and those in group 2 had cyclosporine added to their immunosuppressive regimen.

that the main benefit of cyclosporine was the better graft survival up to 5 years and the chance to stay free of steroids.[2]

CONCLUSIONS

From this study, we can conclude that long-term results of conventional therapy (steroid plus azathioprine) without induction is effective for living donor kidney transplants and showed better graft function than cyclosporine-based protocol. Chronic allograft nephropathy was significantly higher in the cyclosporine group, possibly due to the risk of cyclosporine nephrotoxicity, glomerular disease recurrence, and higher frequency of hypertensive patients. Nowadays, it is possible to achieve excellent calcinurin inhibitor-free regimen after introduction of induction therapy and utilization of newer maintenance immunosuppressive agents such as mycophenolate mofitel and sirolimus.

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CONFLICT OF INTEREST

None declared.

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