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RESEARCH ARTICLE

HISTOPATHOLOGICAL SIGNS AND THEIR RELATIONSHIPS WITH SURVIVAL IN CHRONIC ALLOGRAFT NEPHROPATHY

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ABSTRACT

Aim: To investigate possible factors contributing to chronic allograft dysfunction (CAD), efficacy of histopathological evaluation in recipients with chronic allograft nephropathy (CAN), and determinative role of histopathological signs in graft survival.

Methods: Data of the recipients who underwent renal transplantation (n=270) 10 years in our department were evaluated. The recipients were evaluated in 3 groups as normal allograft function (NAF) group (n=154) and CAD (n=64) and CAN (n=52) groups.

Results: Regarding the allograft survival, the NAF group had the best allograft survival. The frequency of hepatitis C virus (HCV) positivity was significantly lower in the NAF group. Although not significant, the frequency of HCV differed between the CAN patients with and without glomerulopathy (34% and 23.5%, respectively). According to the 3-year allograft survival, graft loss was observed to be earlier in the patients with transplant glomerulopathy, without a significant difference. The frequency of proteinuria was higher in the CAD and CAN groups than in the NAF group and proteinuria was appeared firstly in the CAN group and then in the CAD and NAF groups. Fibrointimal thickening was present in 17 patients in the CAN group and the graft survival was observed to decrease with an increase in the fibrointimal thickening. In the CAD group, 30 patients had mild fibrosis and 20 had moderate/severe fibrosis. Tubulointerstitial fibrosis was more severe in the patients who received cyclosporine A.

Conclusions: CAN after renal transplantation is frequently encountered and the risk of developing CAN increases in the long term after transplantation.

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INTRODUCTION

Renal transplantation is the most appropriate therapeutic option for patients with end-stage renal insufficiency. Chronic allograft dysfunction (CAD) or chronic allograft nephropathy (CAN) is the leading cause of allograft loss. The present study aimed to investigate possible factors contributing to CAD, efficacy of histopathological evaluation in recipients with CAN, and determinative role of histopathological signs in graft survival.

MATERIALS AND METHODS

The present study evaluated 341 recipients who underwent renal transplantation from living (n=274) or deceased donors (n=67) between June 1, 1986 and December 31, 2003 in the General Surgery Department of Istanbul University Cerrahpasa Medical Faculty. Despite having the diagnosis of CAD, recipients who were younger than 15 years of age, died or lost the graft within the initial months after transplantation, did not attend the follow-up visits within the first year after transplantation, and did not complete the 1-year follow-up as of December 2004 were excluded from this study. Accordingly, 286 recipients were included in the present study. Data were analyzed using the Statistical Package for the Social Sciences (version 10.0; SPSS Inc., Chicago, IL, USA). A p value <0.05 was considered statistically significant.

RESULTS

The present study included 286 recipients (76 females and 192 males). The mean age of the recipients at the time of transplantation was 30±9 years (range, 15-61 years). The mean age of donors was 47.9±11 years and the number of female donors was higher (Female/Male: 131/85).

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Table 1. General characteristics of the study groups

	NAF Group n=154	CAD Group n=64	CAN group n=52	p
Recipients				
Age, years	31±10	27.9±9	28.9±9.6	0.047
Female/Male	42/112	24/40	11/41	0.129
Duration of hemodialysis, months	19	19	19	-
Frequency of HCV	39 (26)	26 (40)	16 (31)	0.012
Donors				
Living/Deceased	118/34	53/11	43/9	0.553
Age	46.5±12	49.4±10	50±11	0.118
Female/Male	82/70	37/27	29/23	0.869

Data are presented as mean±standart deviation, median, number/number or number (%), where appropriate.
NAF, normal allograft function; CAD, chronic allograft dysfunction; CAN, chronic allograft nephropathy; HCV, hepatitis C virus.

Table 2. Immunological data of the study groups

	NAF Group n=154	CAD Group n=64	CAN group n=52	p
HLA mismatch	2.3±1	2±1	2.3±0.8	**0.110**
Initial immunosuppressive therapy				
AZA+Steroid	30 (19.5)	31 (48.4)	3 (5.8)	-
AZA+Steroid+CsA	76 (49.4)	26 (40.6)	39 (75.0)	-
AZA+Steroid+TAC	24 (15.6)	1 (1.6)	5 (9.6)	-
MMF+Steroid+CsA	12 (7.8)	2 (3.1)	3 (5.8)	-
MMF+Steroid+TAC	10 (6.5)	4 (6.3)	2 (3.8)	-
Acute rejection	50 (32.5)	27 (42.2)	20 (38.5)	**0.267**

Data are presented as mean±standart deviation or number (%), where appropriate.
NAF, normal allograft function; CAD, chronic allograft dysfunction; CAN, chronic allograft nephropathy; HLA, human leukocyte antigen; AZA, azathioprine; CsA, cyclosporine A; TAC, tacrolimus; MMF, mycophenolate mofetil.

Table 3. Follow-up characteristics of the study groups after transplantation

	NAF Group n=154	CAD Group n=64	CAN group n=52	p
Creatinine level (mg/dL)				
At the 6th month	1.2±0.3	1.4±0.5	1.4±0.4	0.004
At the 1st year	1.3±0.3	1.6±0.6	1.5±0.5	<0.001
Uric acid level at the 1st year (mg/dL)	1.4±0.2	1.5±0.25	1.55±0.2	0.288
Hypertension	99 (64.3)	51 (79.7)	46 (88.5)	0.001
Hyperlipidemia	55 (35.7)	23 (35.9)	28 (53.8)	0.125
Post-transplant diabetes	21 (13.6)	4 (6.3)	3 (5.8)	0.267
Proteinuria	21 (13.6)	33 (51.6)	39 (75.0)	<0.001

Data are presented as mean±standart deviation or number (%), where appropriate.
NAF, normal allograft function; CAD, chronic allograft dysfunction; CAN, chronic allograft nephropathy.

Table 4. Comparison of the allograft survival among the study groups

	NAF Group n=154	CAD Group n=64	CAN group n=52	p
Survival (%)				
1-year	94	89	98	
3-year	88	71	88.5	
5-year	83	56.7	75.5	
7-year	80	43.4	58.5	
10-year	80	31.6	37	**<0.001**

NAF, normal allograft function; CAD, chronic allograft dysfunction; CAN, chronic allograft nephropathy.

The recipients were grouped as follows: 1) normal allograft function (NAF) group (n=154) which included the recipients without a permanent impairment in allograft functions during the follow-up period, 2) CAD group (n=64) which included the recipients in whom CAD was determined but could not be explained histopathologically by biopsy, 3) CAN group (n=52) which included the recipients in whom CAD was detected and histopathological condition was identified by biopsy. The comparison of general characteristics of these groups is demonstrated in Table 1. The NAF group were slightly older than the CAD group (31±8.8 years vs. 27.9±9.6 years, p=0.05). There was no difference between the three groups in terms characteristics (age and gender) of the recipients and donors. The median duration of hemodialysis was 19 months for all groups. The frequency of hepatitis C virus (HCV) positivity was the lowest in the NAF group (p=0.012). The immunological data of the study groups are presented in Table 2. Approximately half (53%) of the patients receiving dual chemotherapy developed CAD and biopsy could be performed in three recipients. Considering CAD and CAN groups together, impairments in allograft functions were detected in 70 (44.3%) of the patients receiving cyclosporine A (CsA)-based therapy and in 12 (26%) of the patients receiving tacrolimus (TAC)-based therapy (p=0.001). The follow-up characteristics of the study groups after transplantation are presented in Table 3. There was a significant difference among the 3 groups in terms of creatinine levels at the 6th month and 1st year. The creatinine levels were significantly lower in the NAF group than in the CAD and CAN groups at the 6th month (p=0.029 and p=0.016, respectively) and 1st year (p<0.001 and p=0.004, respectively), whereas no difference was determined between the CAD and CAN groups in terms of creatinine levels at the 6th month (p=0.983) and 1st year (p=0.244). The frequencies of hypertension and proteinuria were higher in the CAD and CAN groups than in the NAF group. No significant difference was determined among the three groups regarding the frequencies of hyperlipidemia and post-transplantation diabetes mellitus.
When the time of appearance of proteinuria was evaluated, it was observed that proteinuria appeared earliest in the CAN group (46.6±23 months) and then in the CAD group (47.6±29 months); there was no significant difference between the CAN and CAD groups. Proteinuria appeared later in the NAF group (83±57 months) and the NAF group differed significantly from the other two groups regarding proteinuria (p=0.016 and p=0.047, respectively). Although the rate of recipients with proteinuria was low in the NAF group, the amount of proteinuria was not different between the NAF and other groups. When the allograft survival was compared among the groups (Table 4), the NAF group had the best graft survival. It was observed that allografts were rapidly lost after 7 years in the CAN group and after 5 years in the CAD group.

When the histopathological findings in the CAN group were evaluated, in addition to the signs of CAN, relapse or de novo glomerulonephritis signs were observed in 11 recipients. While there was no glomerulosclerosis in 12 biopsy materials, more than 50% of the glomeruli were sclerotic in 4 biopsy materials. The age of donors was higher than that of the recipients with CAN in whom glomerulosclerosis was more frequent. With respect to the relationship between the use of calcineurin inhibitor and sclerotic glomerulus, biopsy of the CAN group showed glomerulosclerosis in 57% of the recipients receiving CsA and in 50% of the recipients receiving TAC. Evaluation of the effect of sclerotic glomerulus on allograft survival revealed no effect on the 3-year survival.

Assessment of the post-biopsy creatinine elevation in the patients with glomerulosclerosis demonstrated that creatinine elevation after 6 months was more rapid in patients with more than 30% sclerosis in their glomeruli. Transplant glomerulopathy, one of the signs of CAN, was detected in 17 (38.7%) patients and the lesion was considered severe in 2 of them. There was a difference, although statistically not significant, in the frequency of HCV between the CAN patients with and without glomerulopathy (34% and 23.5%, respectively, p=0.520). According to the 3-year allograft survival, graft loss was observed to be earlier in the patients with transplant glomerulopathy; however, the difference was not significant. There was no significant difference in the 24-h amount of proteinuria between the patients with and without transplant glomerulopathy (1.9±1 and 1.47±0.9, respectively; p=0.219). Tubulitis signs, likely consistent with acute rejection, were detected in 4 (9%) biopsy samples. Tubulitis, not suggestive of acute rejection, was detected in 65.1% of the cases. While there was no tubulointerstitial fibrosis in only 2 (2.2%) patients, 30 patients had mild fibrosis and 20 had moderate/severe fibrosis. Tubulointerstitial fibrosis was more severe in the patients who received CsA.

Evaluation of the allograft survival in terms of tubulointerstitial fibrosis, the 3-year survival was poor in those with moderate/severe fibrosis. The serum creatinine level showed no remarkable change in the patients with no/mild tubular atrophy; however, a progressive elevation was observed in the patients with moderate/severe tubular atrophy. Hyaline arteriopathy was observed in 20 recipients. Hyaline arteriopathy had no effect on survival. Creatinine elevation was not different between those with mild and moderate/severe hyaline arteriopathies after biopsy. Fibrointimal thickening was observed in 17 patients and the correlation between fibrous intimal thickening and allograft survival revealed that graft survival was decreased with an increase in the fibrointimal thickening. Hypertension was not observed in 5 recipients, whereas 32 (61%) recipients had controlled blood pressure and 15 (29%) recipients had uncontrolled blood pressure despite drug use. Evaluation of the correlation of hypertension with glomerulopathy in renal biopsy, tubulointerstitial fibrosis, hyaline arteriopathy, and fibrointimal thickening demonstrated that fibrointimal thickening was lower in the group with well-controlled hypertension (p=0.041). No correlation was detected between other histopathological changes and hypertension. When the changes in the immunosuppressive drugs were evaluated, it was observed that azathioprine (AZA) was replaced with mycophenolate mofetil (MMF) in 34 recipients with CAN and cyclosporine A was replaced with TAC in 6 recipients with CAN. Changing the immunosuppressive drug after biopsy favorably affected allograft survival in the recipients with CAN. The rates of 3-year survival were 65% in the recipients in whom drug was changed and 93% in the recipients in whom drug was not changed (p=0.002).

Among the groups with similar serum creatinine levels at the time of biopsy, creatinine level was lower in the group for which drug change was not observed; however, the difference was not statistically significant. Of the recipients, 37 received angiotensin converting enzyme inhibitor (ACEI) or angiotensin receptor blocker (ARB). The 3-year survival was slightly better in those received ACEI and/or ARB (89% vs. 82%); however, the difference was not statistically significant (p=0.498).

DISCUSSION

Chronic rejection is the leading entity that causes graft failure within the first 10 years of post-transplantation period. In general, CAN is the second leading factor causing graft loss following various reasons (such as cardiac reasons, infection) that lead to mortality in patients with functioning kidney. Many studies have found the prevalence of graft failure to be 25-50% in the first 10 years. Hariharan *et al*. (2000) evaluated kidney transplantations performed in the USA between 1988 and 1996 and reported that the rate of 1-year graft survival increased to 93.9% from 88.8% for living donor grafts and to 87.7% from 75.7% for deceased donor grafts. According to the data from the Turkish Nephrology Society, chronic rejection was the leading cause of graft loss by 45.7% in 2003 (Erek *et al*., 2003). A study from Tel Aviv showed that 1-, 2-, and 3-year survival rates were lower in the patients receiving renal transplants from deceased donors among the transplant groups with similar mean ages (Chkhotua *et al*., 2003). In the same study, allograft survival was reported to be better in the groups receiving renal transplants from living donors, either relative or not, than in the group receiving renal transplants from deceased donors. In the present study, the frequency of HCV positivity was higher in the group with allograft dysfunction. A study from Ireland reported similar results (Giblin *et al*., 2004) and indicated that hepatitis C seropositivity before transplantation unfavorably influenced post-transplant allograft survival in the long term. Neither living nor cadaver transplantation is performed in our transplantation unit if at least one DR locus match was not defined and there were 4 or more HLA mismatches. Therefore, there was no significant difference in the degree of HLA mismatch between the study groups (p=0.110). In the initial years of renal transplantation in Turkey, the drugs available in the markets were being used; thus, only dual therapy (AZA+Steroid) could be used in renal transplantations performed between 1986 and 1990. Comparison of the recipients receiving dual and triple therapies revealed that allograft dysfunction was more common in the recipients receiving dual therapy. This might either be due to subacute rejection related to suboptimal immunosuppression or result from longer follow-up period in these recipients than in the other two immunosuppressive therapy groups. In our clinic, CsA was included in therapy protocols after 1990 (modified form [Neoral®] after 1996). Evaluation of the recipients with CAN and CAD together, allograft dysfunction was detected in 70 (44.3%) of 158 patients receiving CsA therapy and in 12 (26%) of 46 patients receiving TAC therapy (p=0.001).

A prospective study on cyclosporine-induced nephrotoxicity revealed that a median of 3 years led to the development of CsA-related chronic irreversible histopathological changes (Nankivell *et al*., 2004). In the present study, creatinine levels at the 6th month and 1st year after the transplantation significantly differed among the 3 groups (p=0.004 and p<0.001, respectively). Hariharan *et al*. (2002) reported that the long-term survival was excellent in the recipients of whom the 1st year creatinine level was lower than 1.5mg/dL and the Delta creatinine was lower than 0.3 mg/dL. They suggested that effective treatment of acute rejection episodes and a decrease in their incidence were closely related to the increase in the 1st year creatinine level and that this indirectly affected the allograft survival (Hariharan *et al*., 2002). Proteinuria was more common in the recipients with CAN and CAD than in the NAF (p<0.001). In a study investigating the effect of

proteinuria on the long-term renal allograft survival, the relative risk for graft failure was 2.33 ($p<0.0001$) in the group with a proteinuria level of 0.5-1g/day and 3.46 ($p<0.0001$) in the group with a proteinuria level of >1g/day; significant outcomes were also obtained in terms of patient mortality (a relative risk of 2.05 for those with a proteinuria level of 0.5-1 g/day, $p=0.0002$ and a relative risk of 2.3 for those with a proteinuria level of >1 g/day, $p<0.0001$) (Fernández-Fresnedo *et al*., 2004). In the present study, allograft survival was observed to be best in the NAF group. The reason for better allograft survival in the CAN group than in the CAD group might be due to the change in immunosuppressive therapy that was performed based on the transplant biopsy. In our series, the mean age of donors was significantly higher in the group with sclerotic glomerulus ratio of >30% (43±13 years vs. 54.3±10 years; $p=0.019$). However, evaluation of the effect of post-biopsy glomerulosclerosis on renal functions revealed that creatinine elevation was significant in the group with higher number of sclerotic glomerulus at the 24th month ($p=0.008$). In another study, it was detected that double contours in the glomerular capillaries, development of glomerulosclerosis, and chronic vascular changes (such as fibrointimal thickness) was observed in the later stages of CAN (Nankivell *et al*., 2003). In the present study, evaluation of the relationship between tubular atrophy and renal functions revealed that renal functions were progressively impaired in patients with moderate/severe tubular atrophy. In a study in which the use of CsA and TAC was investigated, the rate of allograft interstitial fibrosis was reported to be higher with modified form of CsA (Neoral®) (Lemström *et al*., 1995). In the literature, the presence of fibrointimal thickening has been observed to cause a substantial decrease in the long-term allograft survival (Freese *et al*., 2001). In the present study, it was observed that the presence of fibrointimal thickening reduced the 3-year survival ($p=0.028$).

A study performed on rats reported a remarkable intimal hyperplasia due to hypertension (Schindler *et al*., 2003). In the present study, fibrointimal thickening was higher in the group with uncontrolled hypertension, which is in line with this experimental study. The rates of 3-year survival were 65% in the group in which immunosuppressive therapy was not changed and 93% in the group in which immunosuppressive therapy was changed ($p=0.002$). New immunosuppressive drugs being not paid by the social insurance was among the causes of not switching the treatment. Many studies have been performed on the outcomes of switching the immunosuppressive therapy. In a retrospective study, 4-year CAD-free allograft survival rates was evaluated in patients who underwent renal transplantation between 1994 and 1997 and received TAC and CsA therapy. The CAF-free survival rate was determined as 91.4% and 92.4% for tacrolimus and cyclosporine microemulsion therapies, respectively, and as 87.6% for conventional CsA and it was reported that this outcome was independent of MMF and other relevant variables (Meier-Kriesche and Kaplan, 2002).

Contribution of the Authors

A.A.O designed the study, analyzed the data and wrote the manuscript. M.A contributed to the data analysis and writing of the manuscript.

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