The Renal Histopathological Findings in Patients with Renal Allograft Dysfunction: A Retrospective Single Center Study

Mana Mohammadi Afrakoti,[1] Amir Ahmad Nassiri,[2]
Monir Sadat Hakemi,[3] Arya Afroughe,[3] Mohammad Reza Ganji[3]

[1]Non-communicable Diseases Research Center, Alborz University of Medical Sciences, Karaj, Iran
[2]Nephrology Department, Imam Hossein Hospital, Shahid Beheshti University of Medical Sciences Tehran, Iran
[3]Nephrology Department, Shariati Hospital, Tehran University of Medical Sciences, Tehran, Iran

Keywords. kidney transplantation, kidney biopsy, chronic allograft dysfunction, IFTA

Introduction. Despite many advances in the development of knowledge and application of new immunosuppressive medications over the past two decades, the improvement has only been seen in the short-term outcome of kidney transplantation while the long-term survival of kidney transplantation has not significantly improved. Allograft kidney biopsy may help to determine the causes of allograft dysfunction which may change the treatment strategy.
Methods. In this retrospective study, kidney transplant recipients who underwent kidney biopsy in Shariati hospital during the years 2004 to 2015, at least three months after the kidney transplantation, were included for evaluation. Chi-square, ANOVA, post-hoc LSD, and T-test were used for data analysis.
Results. A total number of 525 renal transplant biopsies were performed; 300 of them had complete medical records. The reported pathologies consisted of acute T-Cell mediated rejection (TCMR) (17%), interstitial fibrosis and tubular atrophy/chronic allograft nephropathy (IFTA/CAN) (15%), calcineurin inhibitor (CNI) nephrotoxicity (12.8%), borderline changes (10.3%), glomerulonephritis (GN) (8.9%), antibody mediated rejection (ABMR) (6.7%), transplant glomerulopathy (TG) (5.3%), normal (8.4%), and other pathologies (15.6%). C4d was positive in 19.9% of the biopsies. The pathology category had a significant correlation with allograft function ($P < .001$), but it had no significant relationship with age and gender of the recipient, donor and donor source ($P > .05$). Moreover, in about 50% of cases, treatment interventions were based on pathological results, which were effective in 77% of cases. The two-year graft and patient survival after kidney biopsy were 89% and 98%, respectively.
Conclusion. Acute TCMR, IFTA/CAN, CNI nephrotoxicity were the most common causes of allograft dysfunction based on the transplanted kidney biopsy. In addition, pathologic reports were helpful for proper treatment.

IJKD 2023;17:168-73
www.ijkd.org
DOI: 10.52547/ijkd.7256

Original Paper

INTRODUCTION

Kidney transplantation represents the best replacement therapy for most patients with end-stage kidney disease (ESKD).[1] Over last three decades, the short and medium-term outcome of kidney transplantation has improved significantly, mostly due to improvement in surgical techniques, advances in immunosuppressive therapy and better

clinical care for transplant recipients. However, this tendency to progress is not parallel with long-term graft survival, and the rate of improvement of late allograft survival has remained modest. [2,3,4]

There is a wide spectrum of etiologies that explain graft damage. In recent years, several large-scale studies have been conducted to investigate and determine the causes of graft loss in kidney transplant recipients.[5,6] Based on recent studies, there have been changes in the prevalence and causes of graft failure. Thus, the graft lost due to acute rejection or premature death, are now considered as important as other damaging mechanisms.[6,7] Chronic allograft nephropathy (CAN) is the leading cause of late graft failure after kidney transplantation. The incidence of CAN varies and could occur in 23% of the recipients after five years of transplantation and in as high as 90% of them after ten years.[5,6] The histopathologic signs of CAN include progressive tubular atrophy and interstitial fibrosis as well as vasculopathy and glomerulopathy. From the clinical point of view, CAN is characterized by marked and progressive decline in graft function, three months after transplantation, often in combination with proteinuria, generally in the non-nephrotic range and hypertension.[6] As the histopathologic signs of CAN are nonspecific, the 1997 Banff classification renamed it as interstitial fibrosis and tubular atrophy (IFTA) without evidence of any specific etiology.[7] The pathogenesis of CAN is complex and incompletely understood, and results from the accumulation of immune (direct antigen presentation, previous episodes of acute cellular and acute humoral rejection) and nonimmune (drug toxicity, donor disease, recurrent disease and infections) insults to the kidney. [6,8] It is important to understand how an allograft dysfunction occurs. To shed light on this matter, Nankivell *et al.* conducted a study and analyzed 120 biopsy samples of kidney allografts from patients who received kidney and pancreas transplants simultaneously.[5] Based on the results of this study, they were able to describe two phases of allograft damage: The first phase or the initial fibrogenic phase resulted in acute allograft dysfunction due to reperfusion injury, the second phase or the late phase was characterized by arterial hyaline fibrosis present in about 95% of biopsies within 10 years following transplantation.[5]

After accurately determining the etiology of CAN, it is essential to make a precise decision for treatment plans, including changing the type and dose of immunosuppressive drugs to increase allograft survival. It should be noted that despite improving immunosuppressive protocols in renal transplantation, CAN still remains a major impediment to long-term graft survival.[6]

In this regard, our study was conducted to evaluate the causes of kidney transplant dysfunction based on kidney biopsy results over a 10-year period, and to determine patient and graft survival and related factors following kidney biopsy.

MATERIALS AND METHODS

Study Design and Participants

This research was a retrospective observational cohort study conducted in Shariati hospital. We reviewed the records of all patients who received kidney transplantation at the renal transplantation ward of Shariati hospital affiliated to Tehran University of Medical Sciences between March 20th, 2004, and March 20th, 2015. The inclusion criteria for patients were: 1) having kidney transplant surgery in Shariati hospital during the above-mentioned period, 2) having allograft biopsy, 3) having passed at least three months from their transplantation, and 4) having a serum creatinine level of less than 5 mg/dL. The written informed consent was obtained from all the study participants prior to using their medical history for this research. The study code of ethics was 903-90-10-18 from Tehran University of Medical Sciences. In this research, the causes of kidney dysfunction was identified on the basis of kidney biopsy results. For prognostic evaluation, the medical records of patients who could be traced were carefully assessed, and the course of glomerular filtration rate (GFR) changes was monitored for at least two years following the first biopsy of the transplanted kidney. We extracted demographic and clinical information from the medical records at three, six, twelve, and 24-months following kidney biopsy. An expert pathologist observed and reviewed all biopsy results. The total number of biopsy samples was 525, of which 300 had complete medical records.

Definitions

The histopathological entities detected in this study are reported according to the Banff 2007;

Acute T-Cell Mediated Rejection (TCMR),[8] IFTA/CAN,[8] calcineurin inhibitor (CNI) nephrotoxicity,[8,9] borderline pathology,[8] Antibody-Mediated Rejection (ABMR),[8,10] Transplant glomerulopathy (TG),[11,12] which were previously considered as a unique glomerular duplication of the glomerular basement membrane,[11] and is now defined as a histological feature of chronic ABMR caused by repeated episodes of endothelial activation, injury and repair leading to pathological abnormalities including double contouring or multi-layering of the peritubular capillary (PTC) basement membrane.[10,11] Glomerulonephritis (GN) was defined as recurrent if the cause of ESKD was GN, and de novo GN if the patient had no history of GN in the past.

Statistical Analysis

The data were entered into IBM SPSS statistics (version: 18) and were analyzed by using chi-square, ANOVA, post-hos LSD and T-test.

Table 1. Baseline Characteristics of the Participants

Recipient Characteristics	Value
Age (mean ± SD), y	33.66 ± 12.6
Gender, Male (%)	58.2
First Kidney Transplant (%)	98
Donor Type (%)	
LURD	73.7
LRD	9
Cadaver	17.3
Donor Gender, Male (%)	74.7
Donor Age (mean ± SD), y	31.8 ± 7.3
Causes of ESRD (%)	300 (100)
Diabetes Milletus	27 (9)
Hypertension	42 (14)
GN	80 (26.3)
ADPKD	22 (7.3)
Reflux Nephropathy	23 (7.7)
Hereditary Nephritis	12 (4)
Kidney Stones	10 (3.1)
Pregnancy	3 (0.6)
Unknown	81 (27)
Dialysis Vintage Median (min/max), y	2.4 y (0.13 y)

RESULTS

The study included 525 renal transplant biopsies. Out of them, 306 (58%) were males and 219 (42%) were females. The mean recipient age was 33.7 years (Min, max: 7, 72 years). The mean duration of follow up was 4.7 years (Min, max: 1, 18 years). Living unrelated, living related, and cadaveric donors were the sources of donors in 221, 27, and 52 of the 300 recipients who had complete follow-up, respectively. The mean age of donors was 31.8 years (Min, max: 20, 70). Allograft biopsy was done according to clinical indications (increased serum creatinine and / or proteinuria) and for surveillance of the transplanted kidney in 87 and 13% of the cases, respectively. The kidney biopsy was performed just once in 246 of the patients, which represents 82% of the total. The time between kidney transplantation and allograft biopsy ranged from a minimum of 3 months to a maximum of 17 years with median of 3.5 years. Characteristics of the patients are listed in Table 1.

Our histopathologic findings consisted of acute TCMR in 89 cases (17%), IFTA/CAN in 79 cases (15%), CNI nephrotoxicity in 67 cases (12.8%), borderline in 54 cases (10.3%), GN in 47 cases (8.9%), ABMR in 35 cases (6.7%), TG in 28 cases (5.3%), normal histology in 44 cases (8.4%), and other not specified changes in 82 cases (15.6%). There were 37 cases of concurrent CNI toxicity and other not specific changes, and also 12 cases of simultaneous ABMR and TCMR. Among 525 pathologies, C4d staining was done in 312, of which 62 were C4d positive (19.9%) and 250 were C4d negative (80.1%).

GN was diagnosed in 47(8.9%) patients out of which 15 (32%) cases were determined to have recurrent GN based on former medical history. Due to the lack of access to prior medical information, we were unable to classify the remaining cases as de novo or recurrent. Of 15 recurrent cases, 5 patients had Ig-A nephropathy (33.3%), 5 patients had FSGS (33.3%), 4 patients had MPGN (26.7%), and one had (6.7%) membranous nephropathy. Histopathologic results are reported in Table 2.

In this study, the GFR (calculated by the MDRD method) was measured and compared at 3, 6, 12, and 24-months intervals after allograft biopsy and the last GFR was measured at the end of study. The last GFR was more than 60 cc/min, 30 to 60 cc/min and less than 30 cc/min, in 67%, 7.4% and 33% of the patients; respectively. One-way analysis of variance (ANOVA) and LSD post-hoc tests revealed the highest relationship between IFTA/CAN, ABMR, and GN ($P < .001$) and acute TCMR ($P < .05$) with last calculated GFR.

At the end of the study, 93% of patients were alive and twenty percent of those who were alive underwent dialysis. In 66% of the patients who

Table 2. Histopathological Findings

Pathology	Frequency (%)
Total	525 (100)
Normal	44 (8.4)
Acute TCMR	89 (17.0)
ABMR	35 (6.7)
Borderline	54 (10.3)
TCMR/ABMR	12 (2.3)
CNI Nephrotoxicity	67 (12.8)
ATN	23 (4.4)
GN	47 (8.9)
Recurrent	15 (31.9)
IgA nephropathy	5 (33.3)
FSGS	5 (33.3)
MPGN	4 (26.7)
MGN	1 (6.7)
IFTA/CAN	79 (15)
TG	28 (5.3)
DG	7 (1.3)
HUS/TTP	10 (1.9)
Pyelonephritis	12 (2.3)
Interstitial Nephritis	7 (1.3)
Diabetic Nephropathy	2 (0.4)
Viral Infection	9 (1.7)
BKVAN	4 (44.4)
C4d Staining	312 (59.4)
Positive	62 (19.9)

died, renal allograft was still functional. Causes of death were severe infection and sepsis (57%), myocardial infarction (7%) and unknown in 36% of the patients. The 2-year patient and allograft survival after kidney biopsy was 98% and 89%, respectively. There was a significant correlation between histopathologic features of the allografts and patients' survival ($P < .001$). All 30 individuals with normal pathology survived without the need for dialysis. The most common documented pathologies among those who died were ABMR (29.4%), Acute TCMR (17.6%), and IFTA/CAN (17.6%). According to the findings of kidney transplant biopsies, 50.7% of patients received proper treatment which was effective in 77% of them. There was significant a correlation between allograft biopsy findings and time period after kidney transplantation ($P < .05$). Histopathological findings based on the duration between kidney biopsy and transplantation are shown in Table 3.

In this study, there was no association between age, gender of the recipient and donor, donor source and allograft function ($P > .05$).

Assessment of the post-transplant medical complications showed that hypertension was the most common complication observed in 96 (32%) patients. Hyperlipidemia was the second common complication seen in 52(17%) patients. The other medical complications included new onset diabetes mellitus after transplantation (NODAT) in 16 patients, chronic hepatitis C in 4 patients, skin cancer in 2 patients and hematological malignancy in 2 patients.

DISCUSSION

Chronic Allograft Injury (CAI) is a multifactorial pathologic process that leads to glomerulosclerosis, interstitial fibrosis, and tubular atrophy.[14] IFTA is a final common finding in CAI, but it cannot determine the primary cause of kidney transplant injury.[15] The majority of kidney transplant biopsies in this study were performed as a result of elevated serum creatinine and the results highlight the importance of performing a kidney transplant biopsy for correct diagnosis, appropriate therapeutic intervention, and enhancement of allograft function. Data analysis revealed that Acute TCMR, IFTA/CAN and CNI nephrotoxicity were the most common causes of CAI.

In a study, McDonald *et al.* investigated the relationship between acute T cell mediated rejection

Table 3. Histopathological Findings on the Basis of the Duration Between Allograft Biopsy and Kidney Transplantation

Pathology	Time from Biopsy to Transplantation		
	< 1 year 124 Cases (41.3%)	1 to 4 years 83 Cases (27.7%)	> 4 years 93 Cases (31%)
Normal	12.1	0	14
Borderline	12.1	10.8	14
TCMR	13.7	15.7	9.7
ABMR	8.9	2.4	10.8
GN	5.6	8	14
IFTA/CAN	10.5	16.9	16.9
CNI nephrotoxicity	13.7	13.7	16.3
Others	23.4	32.5	4.3

and kidney transplant outcome. They studied 4,325 kidney transplant patients between 1997 and 2004 and found that acute rejection was associated with an increased risk of graft loss, especially if it occurred late after transplantation along with the evidence of vascular rejection.[16]

Halloran *et al.* have remarked that patients with late onset allograft dysfunction usually have a new disease, such as ABMR or recurrent GN, beginning with an injury-response and ending up to pathological changes such as tubular atrophy and interstitial fibrosis.[17] However, as shown in the study by Rush *et al.*, the importance of fibrosis is that it does not necessarily indicate a poor prognosis for all allografts, at least in the short term. [18]

Chronic ABMR is characterized by the following histological findings; C4d deposits in peri tubular capillaries (PTCs), transplant glomerulopathy, PTC membrane multilayering, intimal fibrous thickening and IFTA.[19] According to the Banff Criteria, a positive C4d in PTC is essential for diagnosis of ABMR which indicates microcirculatory damage. However, Banff 2007 classification introduces a new category as suspicious for ABMR, which is defined as evidence of antibody-dependent tissue damage, in addition to either a positive anti-HLA antibody and a negative C4d, or positive C4d in PTC in the absence of alloantibody.[20] It is worth mentioning that in our study, ABMR rate was relatively lower than other similar studies, which could be due to the fact that C4d test was performed halfway through this study, and unfortunately, we were not able to measure donor specific antibody (DSA) in our center.

It has been shown by Terasaki *et al.* that the presence of DSA can cause 5% graft loss per year, which implies that after 4 years, 20% of grafts would have been lost regardless of whether the transplant had been from an alive donor or not.[21] In our study, CNI nephrotoxicity had significant correlation with allograft dysfunction. However, in recent studies, the impact of chronic CNI nephrotoxicity in the development of CAI has remained controversial.[22]

In this research, BK virus was evaluated in kidney biopsy specimens; it was uncommon (1.7%) and had no correlation with allograft dysfunction ($P > .05$). However, BK virus might affect allograft survival. In a study by Hogan *et al.*, BK virus incidence post kidney transplantation was reported to be 52 to 56% and also it had a major role in allograft failure and loss.[23] The relevance of early detection of BK-related nephropathy and its effect on allograft function was recently highlighted in a study by Sharma *et al.*[24]

Hypertension was the most common medical complication after kidney transplantation which occurred in 32% of our patients. In a large-scale study by Opelz *et al.* on 29,751 patients, an increase in systolic and diastolic blood pressure levels after transplantation was shown and it was associated with an increased risk of allograft failure, [25] which was also shown in other studies.[26,27]

Hyperlipidemia was the second most common medical complication after transplantation and was seen in 17% of patients. According to Agarwal *et al.*, CAI has been associated to hypertriglyceridemia and hypercholesterolemia in kidney transplant recipients.[28]

CONCLUSION

In conclusion, given the heterogeneity of causes of CAI and the absence of effective therapies, emphasis should be placed on prevention. The best course of action could be to reduce the risk of acute rejection by choosing proper immunosuppressive treatments. Moreover, early diagnosis and management of cellular and antibody mediated rejection could be helpful and improve allograft survival. Prompt screening for CMV and BK virus infections is also essential. Hyperglycemia, hypertension and hyperlipidemia should be precisely controlled.

REFERENCES

1. Jeon HJ, Bae HJ, Ham YR, et al. Outcomes of end-stage renal disease patients on the waiting list for deceased donor kidney transplantation: A single-center study. Kidney Res Clin Pract. 2019; 38(1):116-123.
2. Meier-Kriesche HU, Schold JD, Srinivas TR, Kaplan B. Lack of improvement in renal allograft survival despite a marked decrease in acute rejection rates over the most recent era. Am J Transplant. 2004; 4(3):378-83.
3. Li C, Yang CW. The pathogenesis and treatment of chronic allograft nephropathy. Nat Rev Nephrol. 2009; 5(9):513-19.
4. Ganji MR, Haririan A. Chronic Allograft Dysfunction. Iran J Kidney Dis. 2012; 6(2):88-93.
5. Nankivell BJ, Borrows RJ, Fung CL-S, O'Connell PJ, Allen RD, Chapman JR. The natural history of chronic allograft nephropathy. N Engl J of Med. 2003; 349(24):2326-33.
6. El-Zoghby ZM, Stegall MD, Lager D, et al. Identifying specific causes of kidney allograft loss. Am J Transplant.

2009; 9(3):527-35.

7. Racusen LC, Solez K, Colvin RB, et al. The Banff 97 working classification of renal allograft pathology. Kidney Int. 1999; 55(2):713–23.
8. Soleza. K, Colvinb RB, Racusenc LC, et al. Banff 07 Classification of Renal Allograft Pathology: Updates and Future Directions. Am J Transplant.2008; 8: 753–60.
9. Naesens M, Kuypers DRJ, Sarwal M. Calcineurin Inhibitor Nephrotoxicity. Clin J Am Soc Nephrol. 2009; 4(2):481-508.
10. Colvin RB. Antibody-mediated renal allograft rejection: diagnosis and pathogenesis. J Am Soc Nephrol 2007; 18(4):1046-56.
11. Roufosse C, Simmonds N, Clahsen-van Groningen M, Haas M, Henriksen KJ, Horsfield C, et al. A 2018 Reference Guide to the Banff Classification of Renal Allograft Pathology. Transplantation. 2018; 102(11):1795-1814.
12. Gloor J, Sethi S, Stegall MD, et al. Transplant glomerulopathy: subclinical incidence and association with alloantibody. Am J Transplant. 2007; 7(9):2124-32.
13. Sis B, Campbell P, Mueller T, et al. Transplant glomerulopathy, late antibody-mediated rejection and the ABCD tetrad in kidney allograft biopsies for cause. Am J Transplant. 2007; 7(7):1743-52.
14. Parajuli S, Aziz F, Garg N, et al. Histopathological characteristics and causes of kidney graft failure in the current era of immunosuppression. World J Transplant. 2019; 9(6): 123-133.
15. Langewisch E and Mannon RB. Chronic Allograft Injury. Clin J Am Soc Nephrol. 2021; 16:1723-29.
16. McDonald S, Russ G, Campbell S, Chadban S. Kidney transplant rejection in Australia and New Zealand: relationships between rejection and graft outcome. Am J transplant. 2007; 7(5):1201-8.
17. Halloran PF, Reeve JP, Pereira AB, Hidalgo LG, Famulski KS. Antibody-mediated rejection, T cell– mediated rejection, and the injury-repair response: new insights from the Genome Canada studies of kidney transplant biopsies. Kidney Int. 2014; 85(2):258-64.
18. Rush DN, Cockfield SM, Nickerson PW, Arlen DJ, Boucher A, Busque S, et al. Factors associated with progression of interstitial fibrosis in renal transplant patients receiving tacrolimus and mycophenolate mofetil. Transplantation. 2009; 88(7):897-903.
19. Halloran P, De Freitas D, Einecke G, et al. An integrated view of molecular changes, histopathology and outcomes in kidney transplants. Am J Transplant. 2010; 10(10):2223-30.
20. Solez K, Colvin R, Racusen LC, et al. Banff 07 classification of renal allograft pathology: updates and future directions. Am J Transplant. 2008; 8(4):753-60.
21. Terasaki P, Ozawa M, Castro R. Four-year follow-up of a prospective trial of HLA and MICA antibodies on kidney graft survival. Am J Transplant. 2007; 7(2):408-15.
22. Issa N, Kukla A, Ibrahim HN. Calcineurin Inhibitor Nephrotoxicity: A Review and Perspective of the Evidence. Am J Nephrol. 2013; 37:602-12.
23. Hogan TF, Borden EC, McBain J, Padgett B, Walker D. Human polyomavirus infections with JC virus and BK virus in renal transplant patients. Ann Intern Med. 1980; 92(3):373-8.
24. Sharma R, Zachariah M. BK Virus Nephropathy: Prevalence, Impact and Management Strategies, Int J Nephrol Renovasc Dis. 2020; 13:187-192
25. Opelz G, Wujciak T, Ritz E. Association of chronic kidney graft failure with recipient blood pressure. Kidney Int. 1998; 53(1):217-22.
26. Campistol JM, Romero R, Paul J, Gutiérrez-Dalmau A. Epidemiology of arterial hypertension in renal transplant patients: Changes over the last decade. Nephrol Dial Transplant. 2004; 19(3): iii62–iii66.
27. Alexandrou ME, Ferro CJ, Boletis I, Papagianni A, Sarafidis P. Hypertension in kidney transplant recipients, World J Transplant. 2022; 12(8): 211-222.
28. Agarwal A, Ramesh Prasad GV. Post-transplant dyslipidemia: Mechanisms, diagnosis and management. World J Transplant. 2016; 6(1): 125-134.

Correspondence to:
Monir Sadat Hakemi, MD
Associate Professor of Internal Medicine and Nephrology, Nephrology Department, Shariati Hospital, Tehran University of Medical Sciences, Postal Code: 1411713135, Tehran, Iran
Tel: 0098 21 8490 2469
E-mail: mhakemi@tums.ac.ir

Received December 2022
Revised February 2023
Accepted March 2023