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Histopathologic patterns of nonrejection injury in renal allograft biopsies and their clinical characteristics; a single centre south Indian study

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ABSTRACT

Background: Graft dysfunction (GD) is the major complication of renal transplantation, and may result in graft loss. The major causes of GD are immunological rejection and non-rejection injury (NRI), which have different prognostic and therapeutic connotations. Meticulous renal allograft biopsy (RAB) evaluation and its correlation with clinico-laboratory features are crucial for timely identification of the varied NRI.

Objectives: To evaluate the clinico-laboratory characteristics and histopathologic features of NRI in "clinically indicated" RABs in our institution.

Patients and Methods: This was a prospective study conducted over a period of five years on renal transplant recipients who underwent "clinically indicated" RAB for GD.

Results: A total of 192 biopsies were evaluated which showed NRI, rejection and NRI with concurrent rejection in 57.3%, 26.6% and 3.6% cases respectively. The NRI category, with or without concurrent rejection, comprised of acute tubular injury (ATI) (44%), calcineurin inhibitor induced (CNI) toxicity (19.7%), infections (12.8%), recurrent glomerulonephritis (GN) (7.7%), de novo GN (1.7%), chronic interstitial nephritis (9.4%), thrombotic microangiopathy (2.6%) and renal vein thrombosis (1.7%). Mean patient age was 34.9 years with male: female ratio of 8:1.

Conclusion: Timely differentiation between rejection and NRI is indispensable for improved allograft survival. Acute tubular injury is the major NRI causing delayed graft function (DGF), and is commonly associated with deceased donor renal transplantation. The blood concentration of CNI does not correlate with the extent of renal damage. Acute tubular injury and CNI toxicity are the major NRI, in the first six months post-transplantation and after six months post-transplantation, respectively.

Original Article

Implication for health policy/practice/research/medical education:
Our study shows the heterogeneity in histological spectrum of NRI, occurring in the renal allograft. Timely differentiation between immunological rejection and NRI is indispensable for improved allograft survival. In our study acute tubular injury (ATI) and calcineurin inhibitor-induced (CNI) toxicity were the major causes of NRI and respectively need to be differentiated from active antibody mediated rejection and transplant glomerulopathy. Our study provides clinic-pathological information that may be useful for clinical practice and investigation. The study may help in understanding the demographics and pathology of NRI as a cause of graft dysfunction (GD).

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Introduction

End-stage renal disease (ESRD) is a rapidly increasing global health problem that is associated with high morbidity and mortality (1). In India, the crude-and age-adjusted ESRD incidence rates are 151 and 232/million populations, respectively (1-4). Renal transplantation is the treatment of choice as it leads to longer survival and enables superior quality of life (1,4,5). However the major

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complication of renal transplantation is graft dysfunction (GD) which, if not promptly treated, may culminate in graft loss. The major causes of GD are immunological rejection and non-rejection injury (NRI), which have different prognostic and therapeutic connotations (5-7). Meticulous renal allograft biopsy (RAB) evaluation is crucial for accurate and timely identification and differentiation of rejection from the varied NRI, many of which pose a clinical and diagnostic dilemma (5-7). Further the causes of NRI vary between studies and there is a relative paucity of information on the histopathologic patterns of GD.

Objectives

The aim of this study was to evaluate the clinico-laboratory characteristics and histolopathologic features of NRI in "clinically indicated" RABs in our centre, which is a tertiary care hospital in south India.

Patients and Methods

Study design

This was a prospective study conducted in the department of pathology in collaboration with department of nephrology, M. S. Ramaiah Medical College and hospitals, Bangalore, over a duration of five years. All renal transplant recipients who underwent "clinically indicated" RAB for GD from August 2014 to July 2019 were consecutively included. The RAB's were processed, as per standard protocol, for light microscopy, C4d immunohistochemistry (IHC) and direct immunofluorescence microscopy.

For light microscopic evaluation, sections of 2 to 3 µm thickness were cut from formalin-fixed paraffin-embedded tissue blocks, and stained by hematoxylin and eosin. Gomori's trichrome, periodic acid Schiff and Jones silver methenamine staining was performed when required. DIF study was conducted on 3 to 4 µm thick frozen sections; using fluorescein isothiocyanate conjugated polyclonal rabbit anti-human immunoglobulin (Ig) IgG, IgM, IgA, kappa, lambda, C1q and C3 antibodies. The C4d IHC was done using "Dako REAL™ EnVision™ Detection system" with rabbit anti-human C4d polyclonal antibody (Master Diagnostica, MAD-000672QD-R). Donor specific antibody level (DSA) was performed by the bead based immunoassay (Luminex) method, when and where ever required, at the time of biopsy or GD or at times according to the clinicians approach and discretion,

serum tacrolimus levels were measured by chemiluminescent microparticle immunoassay method on the EDTA blood sample.

Clinical data including patient's age, gender, allograft age, basic renal disease, donor type, immunosuppression, and laboratory investigations like serum creatinine, blood urea nitrogen levels and serum tacrolimus levels were collected from the patient's case file.

The final diagnosis was made for each case after analyzing and correlating the clinical data and results of light microscopy, DIF study and C4d IHC. Histological categories were classified as per Banff'17 modified update diagnostic categories for RAB's into six categories; normal (category-1), antibody mediated rejection (ABMR) (category-2), borderline T-cell mediated rejection (category-3), T-cell-mediated rejection (TCMR) (category-4), interstitial fibrosis and tubular atrophy (category-5) and NRI (category-6) (8,9). All the transplant patients were under standard immunosuppression protocol, comprised of tacrolimus (0.05 mg/kg/d), prednisolone (10-20 mg/d) and/ or mycofenolate sodium (360 mg, three or four times a day).

Ethical issues

The research followed the tenets of the Declaration of Helsinki. Informed consent was obtained from all patients and the research was approved by ethical committee of Ramaiah Medical College, Bangalore, India (Ref#MSRMC/EC/2014).

Statistical analysis

All continuous data were expressed as mean and standard deviation and qualitative variables as frequencies and percentages. The comparison of mean serum creatinine, blood urea nitrogen levels and serum tacrolimus levels between the different causes of NRI was carried using analysis of variation (ANOVA) test. The comparison of difference in proportion of transplantation type and the different causes of NRI were carried out using chi-square test. SPSS version 18.0 was used for statistical analysis. Additionally, $P < 0.05$ was considered as significant.

Results

A total of 192 consecutive biopsies obtained from 173 patients, over a duration of 5 years were included in the study. The transplant recipient's age ranged from 19 years to 63 years with a mean age of 35.4 years and male; female ratio of 8.6:1. The mean age was 34.9 years for males (age range 19- 63 years) and 41.9 years for females (age range 25- 56 years).

The histological findings in renal allograft biopsies (categorized as according to the Banff 2017 update) are depicted in Table 1. NRI was found in 57.3% (110/192), rejection in 26.6% (51/192) and NRI with concurrent T cell mediated rejection or antibody mediated rejection in 3.6% (7/192) of the biopsies (Table 1).

The different histological entities in the NRI category with or without concurrent rejection, and their clinical characteristics are given in Tables 2 and 3, the commonest being acute tubular injury (ATI) (44.4%) followed by

Table 1. Histological findings in renal allograft biopsies (categorized according to Banff'2017 update)

Banff diagnostic category	Number of cases	Percentage
Normal (Category-1)	4	2.1
Antibody mediated rejection (Category-2)	33	17.2
Borderline changes suspicious for T-cell mediated rejection (category-3)	7	3.6
T-cell mediated rejection (Category-4)	11	5.7
Interstitial fibrosis and tubular atrophy (Category-5)	15	7.8
Non-rejection injury (Category-6)	110	57.3
Antibody mediated rejection with concurrent T-cell mediated rejection (Categories 2+4)	5	2.6
Non-rejection injury with concurrent T-cell mediated rejection (Categories 4+6)	5	2.6
Non-rejection injury with concurrent Antibody mediated rejection (Categories 4+6)	2	1
Total	192	100

Table 2. Histologic spectrum of non-rejection injury and their clinical and demographic characteristics

Histological diagnosis	No. of cases (%)	Mean age (y)	Sex			Type of transplantation		
			Males	Females	M:F	LRRT	LNRRT	DDRT
ATI	52 (44.4)	36.7	47	5	9.4:1	32	6	14
CNI toxicity	23 (19.7)	33.9	18	5	3.6:1	17	3	3
Infections	15 (12.8)	32.5	13	2	6.5:1	11	2	2
Recurrent GN	9 (7.7)	40.9	8	1	8:1	6	2	1
De novo GN	2 (1.7)	29	2	0	-	1	1	-
CIN	11 (9.4)	38.4	11	0	-	5	4	2
TMA	3 (2.6)	36.5	3	0	-	3	-	-
Renal vein thrombosis	2 (1.7)	32	2	0	-	2	-	-
Total	117	34.9	104	13	8:1	77	18	22

ATI, Acute ischemic tubular injury; CNI Toxicity, Calcineurin inhibitor (tacrolimus) toxicity; CIN, chronic interstitial nephritis; DDRT, Deceased donor renal transplantation; GN, glomerulonephritis; LNRRT, Live non-related renal transplantation; LRRT, Live related renal transplantation; TMA, Idiopathic thrombotic microangiopathy.

Table 3. Clinical characteristics of patients with non rejection injury

Histological diagnosis	Mean serum creatinine (mg/dL); (range)	Mean Blood urea nitrogen levels (mg/dL); (range)	Mean serum tacrolimus levels (ng/mL); (range)
ATI	2.43; (1.12 to 6.6)	34.6; (8 to 93.4)	5.46; (1.1 to 10.8)
CNI Toxicity	2.48; (1.3 to 7.2)	36.1; (7.9 to 122.4)	9.78; (3.2 to 18.2)
Infections	3.75; (1.27 to 16)	44.49; (11.2 to 163.2)	5.21; (3.1 to 11.3)
Recurrent GN	1.8; (1.05 to 2.5)	19.07; (13.5 to 32.3)	5.89; (3.2 to 9.1)
De novo GN	2; (1.8 to 2.2)	38.85; (32.9 to 44.8)	4.05; (3.5 to 4.6)
CIN	1.81; (1.36 to 3.2)	24.6; (11.3 to 57.6)	5.75; (3.8 to 8.7)
TMA	10.2; (2.3 to 18.1)	84.25; (24 to 144.5)	4.25; (4.1 to 4.4)
Renal vein thrombosis	6.93; (7.3 to 6.55)	76.15; (108.8 to 43.5)	7.05; (4.6 to 9.5)
P value	0.040	0.036	0.003

ATI, acute ischemic tubular injury; CNI toxicity, calcineurin inhibitor (tacrolimus) toxicity; CIN, Chronic interstitial nephritis; GN, glomerulonephritis.

calcineurin inhibitor induced toxicity (CNI toxicity) (19.7%) and infections (12.8%). There was a statistically significant difference in the mean values of serum creatinine, blood urea nitrogen and serum tacrolimus levels between the different causes of NRI (Table 3). The serum tacrolimus levels were higher in CNI toxicity, compared to other histological entities, and this association was statistically significant (P = 0.003). Table 4 depicts the association between the type of renal transplantation and the different causes of NRI. Even though ATI (63.6%; 14/22) was the major cause of NRI in patients who had undergone deceased donor renal transplantation (DDRT), this association was not statistically significant (P = 0.659).

Table 4. Comparison between the type of renal transplantation and the histological entities

Type of transplantation	ATI	CNI toxicity	Infections	Recurrent GN	De novo GN	CIN	TMA	Renal vein thrombosis	*P* value
LRRT (n=77)	41.5%	22.1%	14.2%	7.8%	1.3%	6.5%	3.9%	2.6%	0.503
LNRRT (n=18)	33.3%	16.7%	11.1%	11.1%	5.6%	22.2%	0	0	0.376
DDRT (n=22)	63.6%	13.6%	9.1%	4.5%	0	9.1%	0	0	0.659
Total	52	23	15	9	2	11	3	2	

ATI, acute ischemic tubular injury; CNI toxicity, calcineurin inhibitor (tacrolimus) toxicity; CIN, Chronic interstitial nephritis; GN, glomerulonephritis; DDRT, Deceased donor renal transplantation; LNRRT, Live non-related renal transplantation; LRRT, Live related renal transplantation; TMA, Idiopathic thrombotic microangiopathy.

Table 5. Indications for renal allograft biopsy

Histological diagnosis	Indication						Total
	Creeping/ elevated serum creatinine levels	Clinical suspicion of rejection	Active urinary sediments	Oliguria	Delayed graft function	Unexplained proteinuria	
ATI	16	18	0	9	9	0	52
CNI toxicity	10	9	0	1	0	3	23
Infections	9	5	1	0	0	0	15
Recurrent GN	1	1	5	0	0	2	9
De novo GN	0	0	0	0	0	2	2
CIN	5	4	1	0	0	1	11
TMA	1			1	1		3
Renal vein thrombosis	1	0	0	1	0	0	2
Total	43	37	7	12	10	8	117

ATI, acute ischemic tubular injury; CNI toxicity, calcineurin inhibitor (tacrolimus) toxicity; CIN, Chronic interstitial nephritis; GN, glomerulonephritis; TMA, Idiopathic thrombotic microangiopathy.

The clinical indications for RAB are given in Table 5. Creeping/elevated serum creatinine level (36.8%) was the major indication. The major NRI detected in RAB's conducted for oliguria and anuria was ATI and for active urinary sediment was recurrent GN.

The histologic findings corresponding to the timing of biopsy are depicted in Table 6.

Acute ischemic tubular injury

Biopsies with ATI showed flattening of the tubular epithelium, loss/attenuation of brush border and non-isometric cytoplasmic vacuolation. Tubules, in some of the biopsies, also showed luminal cellular debris comprised of desquamated epithelial cells and few degenerating inflammatory cells (Figure 1A). All these cases were negative for peritubular capillary C4d deposits and DSA. The majority (51.9%) of the cases were diagnosed within 1st week post transplantation and 27% of the cases had undergone deceased donor renal transplantation "DDRT". Ninety percent and 75% of the cases biopsied for delayed graft function (DGF) and oliguria, respectively, showed ATN. These patients were treated with hemodialysis and their renal functions were closely monitored.

Calcineurin inhibitor (tacrolimus) toxicity

The histologic alterations, in biopsies with CNI Toxicity, ranged from patchy isometric vacuolization of proximal tubular epithelial cytoplasm (17.4%; 4/23), hyaline arteriolopathy (nodular to circumferential hyaline thickening of the vessel wall) (82.6%.; 19/23), toxic glomerulopathy (segmental duplication/ thickening of the glomerular capillary walls) (28.1%; 6/23) and patchy to stripped interstitial fibrosis (52.2%; 12/23) (Figure 2A and B). In addition, vascular smooth muscle cell vacuolization (4.3%; 1/23), arteriolar mucoid intimal thickening (8.6%; 2/23), segmental tuft sclerosis (13%; 3/23), periglomerular fibrosis (30.4%; 7/23) and intratubular dystrophic calcifications (8.6%; 2/23) and tubular atrophy (52.2%; 12/23) were also evident.
Eleven (47.5%) cases were associated with high blood tacrolimus levels (therapeutic range; 10- 12 ng/mL in 1st month, 6-10 ng/mL in 2-3 months and 5-8 ng/mL). Two and one of the cases respectively showed concurrent T-cell mediated rejection and antibody mediated rejection. Around 76% of these patients responded to minimization of tacrolimus dosage or replacement by sirolimus. Follow up data of the remaining cases was unavailable.

Infections

Infections detected in RAB comprised of seven cases of BK virus nephropathy (BKVN) (Figure 3A), four cases of acute pyelonephritis, two cases of tuberculosis (Figure 3.

Table 6. Histologic diagnosis with the time of biopsy

Histologic diagnosis	Within 1st week	1st Week–3rd month	>3rd month–6th month	>6th Month–1 year	>1 Year	Total
ATI	27	15	5	4	1	52
CNI tooxicity	0	4	3	2	14	23
Infections	0	1	2	3	9	15
Recurrent GN	0	0	0	0	9	9
De novo GN	0	0	0	0	2	2
CIN	0	3	1	2	5	11
TMA	0	2	1	0	0	3
Renal vein thrombosis	2	0	0	0	0	2
Total	29	25	12	11	40	117

ATI, acute ischemic tubular injury; CNI toxicity, calcineurin inhibitor (tacrolimus) toxicity; CIN, Chronic interstitial nephritis; GN, glomerulonephritis; TMA, Idiopathic thrombotic microangiopathy.

B), one case of cytomegalovirus (CMV) infection and one case of cryptococcosis with concurrent CMV infection. The cases of BKVN showed intranuclear inclusions in the tubular epithelial cells along with variable interstitial mononuclear inflammation and tubulitis. All the cases showed immunohistochemical positivity for SV40 antigen. Two cases and one case of BKVN showed concurrent T-cell mediated rejection and antibody mediated rejection respectively. The cases of acute pyelonephritis exhibited abundant neutrophilic infiltrates in tubules and interstitium, with micro-abscess formation. Two of the latter cases responded well to antimicrobial therapy. Both the cases of tuberculosis exhibited caseating granulomatous inflammation and both improved on antitubercular therapy.

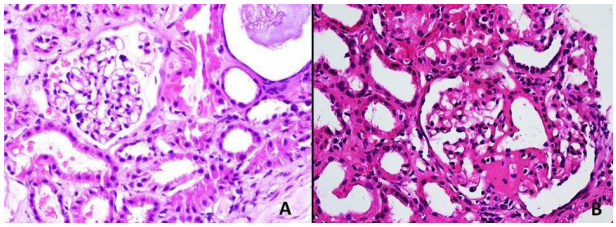

Figure 1. (A) Acute ischemic tubular Injury. Tubules show degenerative changes characterized by fraying of the apical cytoplasm, dilation and simplification of the lining epithelium. **(B)** *De novo* focal segmental glomerulosclerosis. A glomerulus shows segmental obliteration of tuft architecture with hyalinosis, sclerosis and synechiae formation

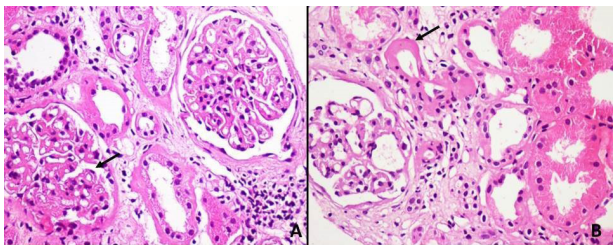

Figure 2. Calcineurin inhibitor induced toxicity. **(A)** toxic glomerulopathy with fairly open capillary lumina lined by thickened membranes and have a focal double contoured appearance (arrow). **(B)** hyaline arteriolopathy with arterioles showing nodular to circumferential arteriolar hyalinosis (arrow).

Recurrent GN and De novo GN

There were 9 cases of recurrent GN comprised of four cases of IgA nephropathy (IgAN) (Figure 4. A and B), two cases of focal segmental glomerulosclerosis (FSGS), two cases of diabetic nephropathy (DN) and one case of membranous GN (MGN). These diseases recurred 14 to 22 months post transplantation. The two cases de novo GN comprised of one case each of post-transplant DN and FSGS (Figure 1B). The former was detected 44 months and latter at 18 months post-transplantation. The cases of IgAN showed mesangial hypercellularity and increased mesangial matrix with predominantly mesangial IgA deposits. FSGS cases showed focal and segmental sclerosis of the glomeruli with IgM and C3 deposits in sclerotic areas. Cases of MGN showed diffuse global uniform thickening of glomerular capillary basement membranes with granular membranous deposits of IgG and C3.

Chronic interstitial nephritis (CIN)

CIN was present in 11 cases, which showed patchy lymphocyte predominant chronic interstitial inflammation with mild tubulitis and tubular injury. In addition, five

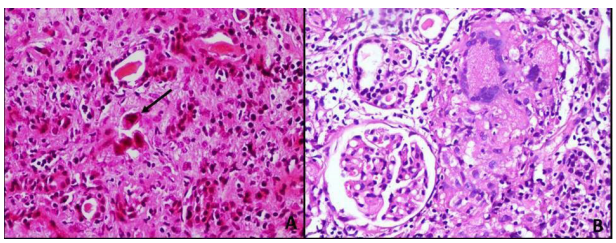

Figure 3. Infections. **(A)** BK viral nephropathy, tubular basement membrane denudation with detached tubular epithelial cells exhibiting viral cytopathic effect (arrow). **(B)** tuberculosis, interstitium with epithelioid granuloma.

of these cases showed interstitial infiltrates of eosinophils. None of these cases showed features of rejection.

Thrombotic microangiopathy (TMA) and renal vein thrombosis

The three cases of idiopathic TMA showed intra-glomerular fibrin thrombi in a focal and segmental pattern (Figure 5A). The two cases of renal vein thrombosis were diagnosed on the 2nd and 3rd day post-transplantation, respectively. Doppler ultrasound showed diastolic reversal of flow in renal artery. The RAB showed oedema, congestion and loss of endothelium with vascular thrombosis and necrotic glomeruli and tubules (Figure 5B). Subsequent graft nephrectomy showed thrombus in renal vein.

Discussion

ATI represents the main cause of DGF and must be differentiated from other causes of DGF i.e., active antibody mediated rejection (hyperacute and accelerated), urinary tract obstruction, renal vascular thrombosis/ atheroemboli, volume depletion and fluid collections (urinoma, perinephric hematoma and lymphocele) (10,11). Literature review reveals that nearly 90% of acute renal failure, in the first week post-transplant is due to ATI. Factors associated with higher frequency of ATI include renal ischemia due to donor hypoperfusion, prolonged cold and warm ischemia times and administration of nephrotoxic drugs. Similar to our study, Philip et al and Aryal et al found that ATI comprises the major group of NRI (6,12). Generally, one is conservative in performing invasive studies in DGF. However as RAB remains the only definitive procedure to differentiate from rejection, we usually perform a RAB in our centre. Grafts with ATI generally recover between 1 to 90 days and require nothing more than dialysis support; our experience also has been similar (10).

CNI Toxicity formed the second largest group, in the present study (19.7%) which is similar to studies by Philip et al (16%) and Aryal et al (28.5%) (6,12). However, in studies by Severova et al and Devadass et al (41.3%), it was the predominant cause of NRI (13,14). The pathogenesis of CNI toxicity is multi-factorial and involves enhanced rennin secretion, relative nitric oxide deficiency, superoxide and peroxynitrite induced injury and loss of vascular endothelial growth factor support to the microvasculature (5). The blood levels of CNIs does not correlate well with the extent of renal damage, further structural nephrotoxicity can occur even when the blood drug concentrations are within therapeutic range (5). In our study, drug levels were in therapeutic range, in 52.5% of cases, which bears striking similarity to observations by Philip et al (52.6%) (12). Similar

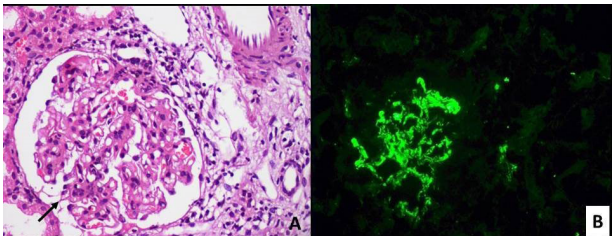

Figure 4. Recurrent Ig A nephropathy. **(A)** a glomerulus showing increase in mesangial matrix and cellularity (arrow). **(B)** direct immunofluorescence; showing granular mesangial deposits of IgA (+++).

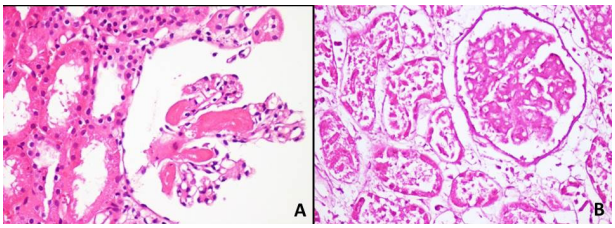

Figure 5. (A) thrombotic microangiopathy: Glomerulus shows fibrin thrombi in the lumen of the capillaries at the hilar region. **(B)** renal vein thrombosis, renal parenchyma with coagulative necrosis of the glomerulus and tubules.

to the studies of Philip et al and Severova et al, hyaline arteriolopathy was the most significant histological finding (12,13). CNI induced hyaline arteriolopathy should be differentiated from hypertension, diabetes mellitus and pre-existing donor disease. Clinical history and findings in zero-hour implantation help in differentiation. Further hypertensive arteriolosclerosis exhibits dominant sub-endothelial arteriolar hyaline deposits (in contrast to nodular medial deposits seen with CNI toxicity) and concurrent arterial intimal fibroelastosis. Hyaline arteriolopathy is characteristically associated with toxic glomerulopathy (5). Similarly all the six cases of toxic glomerulopathy, in the current study, showed concurrent arteriolopathy. CNI induced toxic glomerulopathy should be differentiated from transplant glomerulopathy. Unlike toxic glomerulopathy, transplant glomerulopathy shows positivity for peritubular capillary C4d deposits and DSA, concurrent microvascular inflammation, chronic allograft arteriopathy and transplant capillaropathy and absence of significant hyaline arteriolopathy.

In synchrony with Philip et al (10.9%), infections comprised the third largest group (12.8%) in our study (12). Similar to Devdass et al (80%) and Philip et al (69%), BKVN was the commonest infection detected (46.7%; 7/15), in our study. Infrequently, concurrent BKVN and acute rejection can occur, the diagnosis of which can be challenging (15). In our study, a high proportion of BKVN cases with concurrent acute rejection (42.9%; 3/7) were detected. In these cases along with the viral intranuclear inclusions and immunohistochemical positivity for SV40

antigen, indicators of acute rejection like endarteritis, prominent interstitial mononuclear inflammation with lymphocytic tubulitis, C4d deposits in peritubular capillaries and DSA were present. Acute pyelonephritis was the next commonest infection (26.6%). These cases should be differentiated from acute antibody mediated rejection, which can also show interstitial neutrophils and neutrophilic microvascular inflammation. However in rejection, abundant neutrophilic infiltration and micro-abscess are uncommon and peritubular C4d deposits will be present along with circulating DSA (6,16). In the study by Aryal et al acute pyelonephritis was the commonest infection detected in RAB (75%) (6). CMV is another common pathogen in renal transplant recipients, causing symptomatic infection in the first two to three months post transplantation (5). Co-infection of CMV with other fungal or viral organisms can occur (17). In the present study both the cases CMV infection were detected within three months post transplant and one of the cases showed co-infection with cryptococcus. The frequency of renal allograft tuberculosis in our study was 13.3% (2/15) which is lower than that found by Philip et al (23.1%; 3/13) (12). Unlike Philip et al, where two cases showed concurrent T-cell mediated rejection, none of our cases were associated with rejection (12).

Recurrent GN is diagnosed when RAB shows the same GN/ disease process that led to ESRD in the patient while, de novo GN is diagnosed when the primary GN/ disease process is different to the disease seen in RAB (18). The prevalence of recurrent GN varies between 3 and 15% (19). The diseases that are known to recur in allografts are MGN, FSGS, IgAN, Lupus nephritis, membranoproliferative GN, anti-GBM disease and pauci-immune crescentic GN (5,19) In the current study the commonest recurrent GN was IgAN followed by FSGS and DN. Philip et al reported one case of recurrent GN, which was recurrent IgAN (12). Aryal et al, Devadass et al and Puntamekar et al did not observe any recurrent GN (6,14,20). Different studies give disparate clinical presentations of recurrent GN ranging from asymptomatic urinary abnormalities to rapidly progressive GN (21). The major presentation in our study was active urinary sediment.

In the present study two cases of de novo DN were identified. Studies have shown frequency of *de novo* GN, ranging from 0.6% to 2.5% with FSGS being the commonest (12,14). Around 17.2% of the NRI cases observed by Devadass et al was de novo GN which comprised of crescentic GN, collapsing glomerulopathy, FSGS and Ooxalosis (14). Aryal et al and Puntamekar et al did not observe any de novo GN (6, 20).

This heterogeneity in the frequency and histologic spectrum of recurrent and *de novo* GN could be due to race and genetic variability, environmental factors, methods of case identification, varying follow up periods and differing biopsy practices between centres.

CIN comprised 9.4% of NRI in our study, unlike other studies where CIN was not observed. These were probably cases of drug induced/allergic tubulointerstitial nephritis. If the tubulointerstitial inflammation is accompanied by significant lymphocytic tubulitis, endarteritis, arterial fibrinoid change, microvascular inflammation or peritubular capillary C4d deposits, then a diagnosis of rejection has to be considered. Even though eosinophilic infiltrates, preponderance of inflammation at corticomedullary junction and presence of ill-formed non-necrotizing granulomas suggest drug induced interstitial nephritis, sometimes it is difficult to differentiate from rejection purely on morphological grounds (5). At these times, it is better to initiate anti-rejection therapy with steroids, that also helps to reduce the interstitial inflammation (5).

Idiopathic TMA, as a cause of NRI, was present in 2.6% of the cases. These cases have to be differentiated from active antibody mediated rejection, which would show microvascular inflammation, peritubular C4d deposits and circulating DSA (5). Besides, CNI induced TMA would have shown toxic tubulopathy, hyaline arteriolopathy and probably elevated blood tacrolimus levels (5). Various other studies have identified TMA (due to causes other than rejection) in RAB, these cases were associated with BKVN, CMV infection, CNI toxicity, hemolytic uremic syndrome and connective tissue disorders or were idiopathic (12).

Renal vein thrombosis occurs due to surgical technical problems (including trauma to vessels during procurement or dissection and difficult anastomosis) and hypercoaguable state (21). As these cases may show neutrophilc glomerular and peritubular capillaritis, they should be differentiated from active antibody mediated rejection which would show peritubular C4d deposits and circulating DSA (5).

In our study the major NRI causing GD in the 1st week post transplantation was ATI followed by renal vein thrombosis. The commonest NRI causing GD between 1st week and six months post-transplantation was ATI followed by CNI toxicity. After six months post-transplantation CNI toxicity was the commonest cause followed by infections and recurrent GN. Similarly in the studies conducted by Devadass et al and Aryal et al ATI was the commonest cause of NRI in the first six months post transplantation and CNI toxicity was the major NRI causing GD after six months post-transplantation (6,14).

Conclusion

Meticulous evaluation of RAB is indispensable to

differentiate between rejection, NRI and NRI with superimposed rejection. Accurate diagnosis of various NRI especially ATI, CNI toxicity, infections, recurrent and *de novo* GN and TMA is of great significance for clinical management and hence for improved allograft survival. ATI, CNI toxicity and infections account for the majority of NRI, which largely corresponds with other Indian studies. The major NRI detected in RAB conducted for oliguria and DGF is ATI and for active urinary sediment is recurrent GN. ATI is commonly associated with DDRT. The blood concentration of CNI does not correlate with the extent of renal damage. BKVN is the commonest infection detected in RAB. IgAN is the major recurrent GN followed by FSGS and DN. ATI and CNI toxicity continue to be the major NRI, in the first six months post-transplant and after six months post-transplant, respectively, as evidenced from previous Indian studies.

Limitations of the study

One of the limitations of the study was the small sample size in some of the subgroups of NRI. Further it was not possible to retrieve some of the clinical details like degree of HLA match, 24-hour urinary protein levels and urine analysis findings for some of the cases hence these parameters were excluded from the study.

Authors' contribution

CW was the principal investigator of the study. VM, GP, SS, GM and RK were included in preparing the concept and design. VMV and ME revisited the manuscript and critically evaluated the intellectual contents. All authors participated in preparing the final draft of the manuscript, revised the manuscript and critically evaluated the intellectual contents. All authors have read and approved the content of the manuscript and confirmed the accuracy or integrity of any part of the work.

Conflicts of interest

The authors declare that they have no competing interests.

Ethical considerations

Ethical issues (including plagiarism, data fabrication, double publication) have been completely observed by the authors.

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