SPECTRUM OF RENAL ALLOGRAFT BIOPSY FINDINGS IN RENAL TRANSPLANT PATIENTS AT A TERTIARY CARE CENTER IN RAWALPINDI, PAKISTAN

Khurram Mansoor, Muhammad Osama, Muhammad Ishaque*, Sohail Sabir, Hafeez ud Din*, Muhammad Asif*, Haroon Sabir**

Armed Forces Institute of Urology/National University of Medical Sciences (NUMS) Rawalpindi Pakistan, *Armed Forces Institute of Pathology/National University of Medical Sciences (NUMS) Rawalpindi Pakistan, **Pakistan Naval Ship Shifa Karachi Pakistan

ABSTRACT

Objective: To evaluate outcome of diagnostic kidney biopsy in patients with renal allograft dysfunction at a tertiary care hospital.

Study Design: Retrospective observational study.

Place and Duration of Study: Armed Forces Institute of Urology, Rawalpindi, from Jan 2014 to Jan 2020.

Methodology: A consolidate registry review was carried to formulate this study. The registry data exists at our center containing information about the graft dysfunction (manifesting as proteinuria, deranged urea and creatinine or urine sediment abnormalities) and other major indications which warrant probing with biopsy. The histopathological diagnosis of these biopsies is confirmed from the nephro-pathology registry before finalization of diagnosis.

Results: A total of 94 diagnostic kidney biopsies were performed in patients with graft dysfunction. Out of 94 biopsies, 80 (85.1%) patients were male while 14 (14.9%) were female patients. The most frequent single cause for graft dysfunction was Cell Mediated Rejection (n 12, 24.5%) followed by Interstitial Fibrosis and Tubular Atrophy/Acute Tubular Injury. The most common cause among the glomerulonephritis was Membranoproliferative Glomerulonephritis (n 3, 6.1%) followed by others. The most common cause for mixed pathology remainedcell mediated rejection with Interstitial fibrosis and tubular atrophy (n 8, 17.8%).

Conclusion: Cell mediated rejection is thecommonest pathology responsible for renal allograft dysfunction both as a single lesion as well as part of mixed pathology.

Keywords: Allograft dysfunction, Cell-mediated rejection, Renal biopsy.

INTRODUCTION

Iversen and brun and alwall were the first clinicians who performed percutaneous renal biopsies in 1950s[1,2]. In their procedure, patients were seated in upright position and the procedure achieved >40% of tissue diagnosis. Kark and Muehrcke in 1954 modified the technique with a different set of needles and with patients in a prone position. As a result, 96% tissue diagnosis with no major complications were noted with the later technique.

Renal biopsy remains the cornerstone of investigations in renal allograft dysfunction, both in terms of diagnosis and management. Dysfunction of a kidney transplant often requires histological sampling by percutaneous ultrasound-guided core needle biopsy. Although the transplant kidney biopsy is more specialized than native kidney biopsy, the indications and complications are less well understood than the native kidney biopsy[3].

The concept of protocol transplant biopsies has also evolved over time. Although the risks and benefits of this procedure have long been debated but the sole benefit of achieving diagnosis in cases of subclinical rejection is of paramount importance in all such cases[4]. Further, favoring a proactive approach for doing diagnostic kidney biopsies in patients with renal allograft dysfunction is the fact that the estimated risk of serious complications in transplant kidneys is comparatively less than the native kidneys[5,6]. Further, in literature special importance has been laid on doing pre-emptive kidney biopsy in cadaveric donors. A very large sample sized study from Hungary have reported significant benefit in diagnosing problems like acute tubular necrosis, arteriosclerosis and chronic tubulointerstitial nephritis etc, in these kidneys on zero hour (just before the transplant surgery)[7].

Data suggests that the introduction of renal allograft biopsy has altered the diagnosis in 27-46% of patients and management in 38-83%, even after the first year of transplant[8]. The discovery of C4d staining was a milestone in the diagnosis and management of renal allograft rejection[9]. The advanced techniques of tissue typing and cross matching are not easily and widely available in Pakistan and are also a financial burden for a majority of transplant candidates and their families. Under such circumstances where these

Correspondence: Dr Khurram Mansoor, Consultant Nephrologist, Armed Forces Institute of Urology, Rawalpindi Pakistan
Received: 13 May 2020; revised received: 01 Jul 2020; accepted: 07 Jul 2020

tests are sometimes done with older and cost effective techniques the rejections could be picked up with early and sometimes pre-emptive biopsies. It is a recognized fact that graft survival rates and their causes vary in different transplant centers of Pakistan. This has actually acted as an impetus for conducting our study and to work out if the spectrum of causes in our institute is any different from others.

METHODOLOGY

This was a retrospective study conducted at Armed Forces Institute of Urology (ERC/ IRB no. Uro-Adm-Trg-1/IRB/2020/103), Rawalpindi, Pakistan. We assessed and interpreted the registry data of our institute dating from January 2014 to January 2020. Non-probability consecutive sampling technique was used (WHO sample size calculator used with confidence interval 95%, margin of error 5%, population frequency adding acute and chronic rejection 34%) to enroll 94 biopsy specimens after satisfying inclusion criteria (patients of either gender who underwent renal biopsy for graft dysfunction) while those having inade-quate specimen were excluded.

AFIU is one of the largest renal transplant centers in Northern Pakistan. Only first degree live-related renal transplants have been done in this center after seeking permission from federal Human Organ Transplant Authority. The transplanted patients if at all face graft dysfunction, undergo an early kidney biopsy. The transplant registry at our center contains clinical information about the patients who have graft dysfunction in detail. The histopathological diagnosis of these biopsies is obtained from nephro-pathology registry maintained in histopathology department of Armed Forces Institute of Pathology. The final diagnosis of graft dysfunction thus encompasses both the clinical and pathological parameters of our patients.

In this study, all patients first gave informed and written consent for undergoing the same standard operating procedure for doing diagnostic transplant kidney biopsy. Two cores from the renal graft were taken with Monopty (trucut) biopsy needle (18G) under real time ultrasound guidance. Light microscopy for histopathological assessment was done. All biopsies were reported by the same group of histopathology consultants (three in number). All patients were observed for 12 hours post-biopsy. Each patient underwent 2 consecutive urine routine examinations and two complete blood count evaluations 12 hours apart. They were closely watched for post-biopsy hematuria and any change in vital signs. Major post-biopsy complications if any were recorded for future consultation.

The data gathered, was analyzed by descriptive statistics such as mean and standard deviation (SD) for continuous variables and frequencies and percentages for qualitative variables. SPSS version 20 was used for statistical analysis.

RESULTS

The study was conducted from January 2014 to January 2020 and the number of renal transplant biopsies done over the above mentioned study period were 94. All included biopsies were adequate. Out of 94 biopsies, 80 (85.1%) biopsies were from male patients while 14 (14.9%) from female patients, as shown in figure.

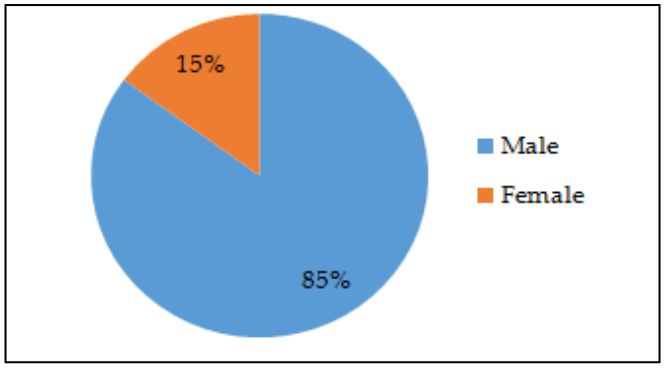

Figure: Pie-chart showing age distribution of all cases.

The mean age of all patients who underwent biopsies was 36.72 ± 11.47 years with range of 15-60 years. The mean duration of biopsies from the date of transplant was 28.65 ± 39.81 months (range: 1-72 months). Around 50% of the biopsies were done within 24 months of transplant and out of these, 25% were done within first 7 months. The rest (50%) of the biopsies were done 24 months post-transplant and out of these, 25% biopsies were done after 48 months of transplantation.

Out of 94 transplant biopsies, 49 (52.1%) had single cause for graft dysfunction while 45 (47.9%) had multiple causes for transplant dysfunction.

Among singular causes, the most common cause of renal transplant dysfunction was cell-mediated rejection (CMR) (n 12, 24.5%), followed by Acute Tubular Injury (ATI) and Interstitial Fibrosis and Tubular Atrophy (IFTA) (n 8, 16.3%). Membranoproliferative Glomerulonephritis (MPGN) was the most common form of glomerulonephritis (GN) in the category of single lesions (n3, 6.1%). Three out of five patients with

glomerulonephritis were found to have Membranoproliferative Glomerulonephritis, while Focal and Segmental Glomerulosclerosis (FSGS) and Membranous Glomerulonephritis (MN)were found in a single patient each. The single causes of allograft lesions are shown in table-I.

Table-I: Single pathological lesion on graft biopsies in patients with graft dysfunction (n=94).

Cause	n (%)
Cell mediated rejection	12 (24.5%)
Anti-body mediated rejection	3 (6.1%)
Glomerulonephritis	5 (10.2%)
Interstitial fibrosis and tubular atrophy	8 (16.3%)
Calcineurin inhibitor toxicity	2 (4.1%)
Acute tubular injury	10 (20.4%)
Borderline rejection	4 (8.2%)
Renal vein thrombosis	2 (4.1%)
Minor changes	2 (4.1%)
BK virus nephropathy	1 (2.0%)

A total of 43 (47.9%) biopsies showed more than one pathological lesion on microscopy. A breakdown of these combination of lesions is shown in table-II. The commonest combination was CMR and IFTA.

with renal allograft dysfunction in Pakistan. The largest so far conducted Pakistani study, was published in 2012 by the Sindh Institute of Urology and Transplant by Kazi *et al*[10]. In terms of gender distribution, age and even histopathological lesions, their results are quite close to our study.

This study focuses on various causes of renal transplant dysfunction as detected on renal allograft biopsies in our center. All transplants done in our center were first degree relatives and ABO compatible. The male to female ratio was 4:1, which is almost the same as reported by Kazi *et al* (3:1) and even in other developing countries, including studies from neighboring countries like Nepal (by Aryal *et al* in 2012) and India (by Puntambekar *et al* in 2017 and Patil *et al* in 2018)[10-13]. In our study, the mean age of patients at the time of biopsy was 36.72 ± 11.47 years which is quite close to what Kazi *et al* found (35.7 ± 10.5)[10]. In developed countries, renal allograft rejection is higher in older aged donors[14]. Whereas, in majority of such studies, a younger recipient age group is associated with favorable outcome in terms of rejection[14-16]. Not only the donor and recipient age, but many other factors have also been implicated in various studies lea-

Table-II: Distribution of multiple pathological lesions on graft biopsies in patients with graft dysfunction (n=94).

Cause	n (%)
Antibody mediated rejection + Calcineurin inhibitors toxicity	1 (2.2%)
Antibody mediated rejection + Glomerulonephritis	1 (2.2%)
Antibody mediated rejection + Interstitial fibrosis and tubular atrophy	5 (11.1%)
Antibody mediated rejection + Interstitial fibrosis and tubular atrophy + Acute tubular injury	1 (2.2%)
Antibody mediated rejection + Interstitial fibrosis and tubular atrophy + CNI toxicity	1 (2.2%)
Antibody mediated rejection + Cell mediated rejection + Interstitial fibrosis and tubular atrophy	1 (2.2%)
Antibody mediated rejection + Interstitial fibrosis and tubular atrophy + Glomerulonephritis	1 (2.2%)
Borderline rejection + CNI toxicity + Interstitial fibrosis and tubular atrophy	1 (2.2%)
Borderline rejection + Interstitial fibrosis and tubular atrophy	1 (2.2%)
Cell mediated rejection + Antibody mediated rejection	1 (2.2%)
Cell mediated rejection + Calcineurin inhibitor toxicity	1 (2.2%)
Cell mediated rejection + Calcineurin inhibitor toxicity + Interstitial fibrosis and tubular atrophy	3 (6.7%)
Cell mediated rejection + Interstitial fibrosis and tubular atrophy	8 (17.8%)
Cell mediated rejection + Interstitial fibrosis and tubular atrophy + Acute tubular injury	1 (2.2%)
Cell mediated rejection + Interstitial fibrosis and tubular atrophy + Glomerulonephritis	1 (2.2%)
Glomerulonephritis + Acute tubular injury	1 (2.2%)
Glomerulonephritis + Interstitial fibrosis and tubular atrophy	6 (13.3%)
Glomerulonephritis + Interstitial fibrosis and tubular atrophy + Calcineurin inhibitor toxicity	1 (2.2%)
Interstitial fibrosis and tubular atrophy + Acute tubular injury	2 (4.4%)
Interstitial fibrosis and tubular atrophy + Pyelonephritis	1 (4.4%)
Pigment cast nephropathy with interstitial nephritis	1 (2.2%)
Polyoma virus nephropathy + Interstitial fibrosis and tubular atrophy	2 (4.4%)
Transplant glomerulopathy + Interstitial fibrosis and tubular atrophy	3 (6.7%)

DISCUSSION

There was very little data available on the spectrum of renal allograft biopsy findings in patients ding to graft loss in patients with renal allografts[17].

In our study, cell mediated rejection was the most common cause of renal allograft dysfunction followed

by acute tubular injury and interstitial fibrosis and tubular atrophy respectively. The same pattern of distribution has been identified by Kazi *et al* as well but with slight variation as detailed below[10].

Around 50% of the episodes of graft dysfunction occurred within 24 months post-transplant, among which, 25% occurred within 7 months of transplantation, while 25% episodesoccurred after 48 months.

Despite advancements in immune suppression and patient friendly protocols, the incidence of acute rejections still persists in renal transplant population. We have found acute rejections to be the commonest cause of graft dysfunction in our patients. Cell mediated rejection was present in 24.5% of causes. It was the most common pathology (both as single as well as part of mixed pathology). It was followed by Borderline rejection which was evident in 8.2% of the cases. Regarding cell mediated rejection, it is worth mentioning that it has even remained as the commonest cause in mixed pathology lesions in graft dysfunction as well. Antibody mediated rejection accounted for 6.1% of all the cases. The overall incidence of rejections in our study was only slightly higher than the previously published Pakistani study (24% including all types of acute rejections)[10]. One explanation for this would be the presence of delayed graft dysfunction in some of our patients. Although they did not meet classical definition of delayed graft dysfunction i.e requiring dialysis in first week post-surgery rather they were slow to regain normal renal function otherwise. We are aware of the fact that there is a direct relationship between anyform of delayed graft dysfunction and rejection[18]. This factor was not catered in the last published study so it is difficult to comment from their perspective[10]. Rate of classical delayed graft function (patients requiring dialysis in first week after surgery) in our study was about 2% which matches with data from other centers permitting only live donor transplants[16].

Most of our patients were inducted with Basiliximab in our institute. As per protocol at our center, all our transplant candidates falling in intermediate and low risk group are inducted with Basiliximab. Around 90% of our transplant recipients are usually form these two groups and thus receive Basiliximab. Rest of the patients, (from the high risk group) were inducted with Anti thymocyte globulinas a depleting agent. Although it has a protective effect on rejections but the main side effect of Anti thymocyte globulin is predisposition to various infections, which should be kept in mind in post-transplant period for ensuring adequate renal function. This situation further aggravates once these patients fail to comply with the follow up plan in the clinics and many times harbor sub-clinical infections.

Immunosuppression monitoring is widely available for calcineurin inhibitors, while no such monitoring is available for anti-proliferative drugs. Some physicians consider the number of cases of rejection as an indicator of adequacy of immunosuppression. The actual contribution of immunosuppression to renal transplant dysfunction is hard to estimate as the factor of infection secondary to immunosuppression is also a likely cause for graft dysfunction. That is why Kidney Disease Improving Global Outcome advocate the use of depleting agents for high-risk population only[19]. The rate of graft rejection varies in different parts of the world due to center-specific transplant protocols.

After rejections, second most common cause of transplant dysfunction was Acute tubular injury, which accounted for 20.4% (vs 24% by Kazi *et al*)[10]. This was followed by Interstitial fibrosis and tubular atrophy (16.3%) and Calcineurin inhibitor toxicity (4.1%). Whereas the calcineurin inhibitor toxicity as reported by Kazi *et al* was 11%[10]. Since different tubular injuries mentioned above form a spectrum of pathology which often has overlapping features, therefore this disparity in distribution could be a difference of interpretation as well[20].

Glomerulonephritis accounted for 10.2% cases. Membranoproliferative glomerulonephritis, was the most common type (6%). It was difficult to differentiate between recurrent and de novo GN as native kidney disease is not known in most of the cases in our setup. Pigment cast nephropathy as a cause of Acute tubular injury was found in one of the biopsies. BK virus nephropathy constituted the least common isolated cause of graft dysfunction, i.e., 2%.

When the biopsy findings discussed above were compared with the initial clinical presentation, we found that asymptomatic rise of serum creatinine was the most common patient presentation.

The limitations of our study include a retrospective study design, single center-based study and lacking the use of newer method of tissue staining for C4d, making it difficult to compare our study findings with older studies[21]. Likewise, the new and emerging biopsy assessment and interpretation techniques including molecular analysis techniques can also prove to be beneficial in confirming difficult diagnoses[22].

Despite the above, our study gives an excellent overview of biopsy-proven causes of graft dysfunction in our transplant population.

CONCLUSION

The gold standard investigation for the diagnosis of causes of renal allograft dysfunction is renal allograft biopsy. Asymptomatic rise of serum creatinine was the most common initial presentation of graft dysfunction. Cell mediated rejection remained the most common pathology both in single as well as mixed etiology for graft dysfunction in live related renal transplant patients.

CONFLICT OF INTEREST

This study has no conflict of interest to be declared by any author.

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