Original Research Article

Clinical significance of protocol allograft renal biopsy in a tertiary care centre in India.

Abstract

Background: The assessment of biopsy findings at one month post-kidney transplant is crucial in determining the success of the procedure and identifying potential complications. **Aim**: This study aimed to investigate the association between biopsy findings at one month and donor status, as well as HLA DR(Human Leukocyte Antigen – DR isotype) mismatch, in kidney transplant recipients. **Methods**: A total of 30 kidney transplant recipients were included in the study. Biopsy findings at one month were categorized as either normal or indicative of rejection. The association between biopsy findings and donor status (cadaveric vs. live donor) and HLA DR mismatch (present vs. absent) was analyzed using the Fisher exact test. **Results**: Among patients who received a cadaveric donor kidney, all four (100%) exhibited normal biopsy findings at one month, with no cases of rejection observed. In contrast, among recipients of a live donor kidney, 23 (88.5%) had normal biopsy findings, while 3 (11.5%) showed signs of rejection. Regarding HLA DR Mismatch, among patients without HLA DR mismatch, 26 (96.3%) had normal biopsy findings at one month, with only 1 case (3.7%) showing rejection. Conversely, among patients with HLA DR mismatch, only 1 (33.3%) had normal biopsy findings, while 2 cases (66.7%) exhibited rejection.**Conclusion**: Our findings suggest that HLA DR mismatch may be associated with an increased risk of biopsy findings indicative of rejection at one month post-kidney transplant. However, no significant association was observed between donor status and biopsy findings.

Keywords

Graft dysfunction, renal transplantation, renal allograft protocol biopsy.

Introduction

The short- and long-term survival rates following renal transplantation have improved. Allograft failure, however, continues to be a major problem. [1]For better results, it is essential to identify the causes of graft dysfunction as soon as possible. The gold standard for identifying the root cause of renal allograft failure is renal biopsy. [2] In the past, biopsies were carried out in response to clinical symptoms or abnormal lab findings. Protocol biopsies, however, can find early indications of chronic allograft nephropathy and subclinical acute rejection. These findings support the development of customised immunosuppressive regimens. [3, 4, 5]

Aims and Objectives:

Primary objective: Determine the utilization of protocol biopsy in renal transplant patients for early detection of renal histological abnormalities.

Secondary objective: Assessment of incidence of subclinical rejections and causes of allograft abnormality in renal transplant recipients and its association with recipient characteristics like age, gender, basic disease, donor characteristics like age, sex, glomerular filtration rate, relationship, HLA mismatch, induction therapy, type of maintenance immunosuppression, drug level of the calcineurin inhibitor used.

Methodology:

The study protocol was approved by the Institute Ethics Committee,Jaipur,India. All patients who received either a cadaveric or live donor kidney transplant over 6 months (July 2022 to Dec 2022) and had stable graft function at one month were eligible for inclusion in the study. Patients were informed about the study before kidney transplantation. All those who met the inclusion criteria and give consent to participate wereenrolled; patients were not excluded on any other grounds (Flowchart 1).

Ethical consideration:Institutes ethic committee, Jaipur, India.

Human participation protection: Study was conducted after informed consent as a part of therapeutic measures. Confidentiality of the information was maintained.

Study design: Prospective cohort study

Study period: July 2022 to Dec 2022.

Inclusion criteria:

All patients who received either a cadaveric or live donor kidney transplant over 6 months (July 2022 to Dec 2022) and had stable graft function at one month were eligible for inclusion in the study.

Procedure

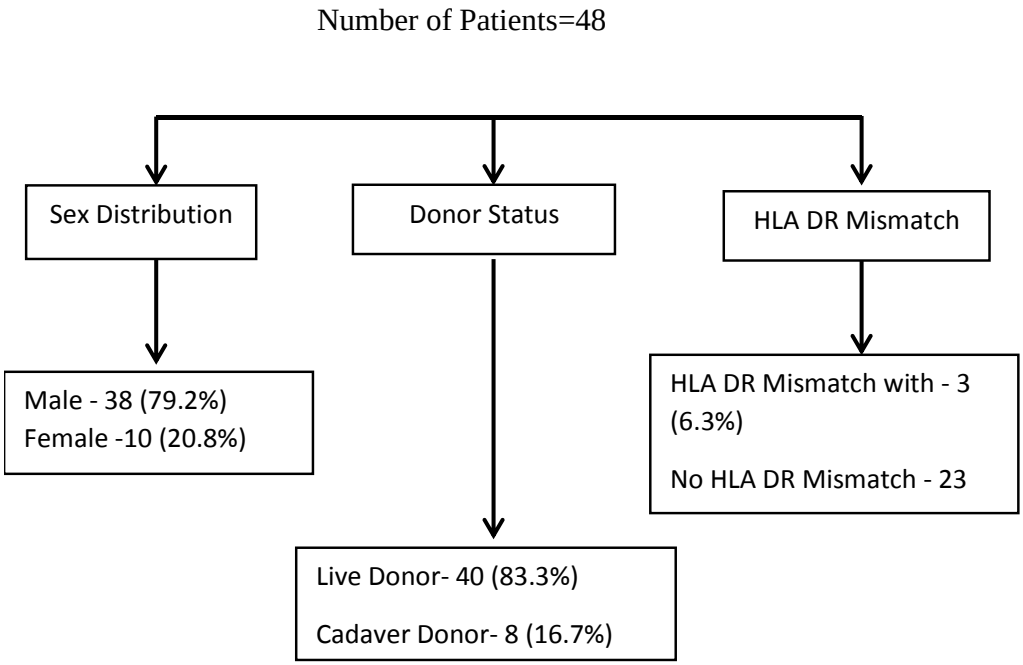
Biopsies were performed at the end of 1 month after transplantation. Patients were also undergone biopsies whenever clinically indicated. The major trigger for such biopsies were acute graft dysfunction as defined by a persistent increase of serum creatinine by >15% from baseline after excluding anatomical and surgical causes. The primary study endpoint wasa 6-month serum creatinine value and urine examination for proteinuria.

The choice of immunosuppressive agents for induction was decided on a case-to-case basis after an assessment of immunological risk. For maintenance immunosuppression, all patients received a standard triple-drugregime consisting of a CNI (tacrolimus/cyclosporine), antimetabolite (mycophenolate mofetil), and prednisolone. Tacrolimus dose was adjusted to keep the trough level at 7–10 ng/ml for the first three months, and 3-7 ng/ml for subsequent months according to standard guidelines. Cyclosporine dose was adjusted to keep the two-hour post-dose cyclosporine (C2) level at 800-1000 ng/ml for the first three months, and 400-600 ng/ml for subsequent months.

All post-transplant biopsies were done under ultrasound guidance using spring-loaded 18-gauge biopsy gun under aseptic conditions and local anaesthesia with 2% lignocaine. Two cores wereobtained. The standard procedure of renal biopsy was followed. Biopsies wereanalysed by light microscopy and immunofluorescence by experienced pathologists and scored using Banff's schema. Electron microscopy analysis was done if required. C4d staining was also performed.

CNI (tacrolimus/cyclosporine) drug level was monitored in 1st monthas a routine practice and whenever clinically indicated.

All histologically proven (as classified by Banff schema) acute rejection (AR) episodes (clinical and subclinical) were treated according to standard operative practice therapy. Patients with borderline rejection (BL) changes received any specific treatment, except CNI dose adjustment to maintain blood levels in the upper range of target values, and werefollowed up. Patients with evidence of CNI toxicity on protocol biopsies were undergone 25–50% reduction in CNI dose in one step and then further modification as per CNI level. The diagnosis of BKV nephropathy was confirmed by immunohistochemistry using stain SV40.



Flowchart 1: Demographic Profile of Patients.

Statistical analysis

The SPSS statistical package was used for data analysis. Student's *t*-test and Fisher exact test were used to compare continuous and categorical data, respectively. A p-value of less than 0.05 was considered statistically significant. Multiple linear regression analysis was used to measure the effect of donor age, HLA mismatches, mycophenolate mofetil (MMF) use and protocol biopsy on outcome measures.

Results

Table 1- Association of Biopsy finding at one month with donor status and HLA DR Mismatch using Fisher exact test

Variable		Biopsy finding at 1 month		Total	*p*-value
		Normal	Rejection		
Donor Status	Cadaver	4(100)	0	4(100)	1.000 (S)
	Live	23(88.5)	3(11.5)	26(100)	
HLA DR Mismatch (Live related)	No	22(95.6)	1(4.4)	23(100)	0.020 (S)
	Yes	1(33.3)	2(66.7)	3(100)	

The mean age of all the patients was 34.63±10.61 years. Out of 48 cases, most of the patients (79.2%, 38/48) were male, and the rest 10(20.8%) were female. The majority of them (83.3%, 40/48) have received from a live person, and the rest eight (16.7%) received from a cadaver. Out of eight patients who received from a cadaver, one (12.5%) died, two (25%) patients needed biopsy before one month and two (25%) were loss to follow-up and rest four (50%) cases had normal biopsy findings at one month. Out of 48 patients, two (4.2%) patients died, two (4.2%) patients had a loss to follow-up, and one (2.1%) had a nephrectomy. Around one-third (30.2%, 13/43) of patients had indicated biopsy before one month. Out of 30 patients

who had a biopsy at one month, most of them (90%,27/30) patients had normal biopsy findings at the end of one month while one patient had ABMR, one had TCMR 1A, and the rest one had TCMR 2A Banff. The p-value for the association between donor status and biopsy findings at one month was found to be 1.000, indicating no significant association. (Table1).

Among patients without HLA DR mismatch, 22 (95.6%) had normal biopsy findings at one month, with only 1 case (4.4%) showing rejection. In contrast, among patients with HLA DR Mismatch, only 1 (33.3%) had normal biopsy findings, while 2 cases (66.7%) showed rejection (Table1).The p-value for the association between HLA DR mismatch and biopsy findings at one month was found to be 0.020, indicating a statistically significant association.

Discussion

This preliminary analysis of our early protocol biopsy experiences aimed to evaluate the presence of subclinical rejection in kidney transplant recipients with stable or improved renal function. Although these patients had no clinical indication for renal allograft biopsy, we discovered that over 11.5% of them exhibited signs of subclinical rejection, ranging from Banff 1A to Banff 2A. These findings align with previous studies by Rush et al., which suggested that subclinical acute tubulitis is frequently present but often goes unrecognized. [7,8, 9] Thus, it is possible that we underestimated the actual incidence of acute rejection in our patient population.

Interestingly, our study revealed a slight preponderance of rejection in patients with HLA-DR mismatch, with two live donor recipients displaying subclinical rejection at one month on protocol biopsy. Choi et al. also reported a similar association between HLA-DR mismatch and the incidence of subclinical rejection.[2] Moreover, the incidence of subclinical rejection was found to be dose-dependent on the number of HLA-DR mismatches, as demonstrated by

Rush et al.[10]Patients without HLA-DR mismatches had a 20% incidence of subclinical rejection, while those with one and two HLA-DR mismatches had incidence rates of 30% and 63%, respectively.

Our findings are consistent with the study conducted by Fu MS et al., which emphasized the importance of performing protocol biopsies in transplant recipients with stable renal function to detect various unsuspected lesions, including subclinical rejection, chronicity, calcineurin inhibitor (CNI) toxicity, recurrent primary disease, de novo glomerulopathy, BK virus nephropathy, and asymptomatic urinary tract infections. [11]Early detection of these conditions through protocol biopsies can potentially lead to improved long-term graft survival. Additionally, the study by Kumar et al. demonstrated the safety and utility of protocol biopsies performed at 3 months after transplantation using real-time ultrasound guidance for early identification of subclinical histological abnormalities.[12]

Our preliminary analysis of early protocol biopsies has revealed a significant proportion of patients displaying signs of subclinical rejection, indicating that the actual incidence of acute rejection may have been underestimated in our patient population. The observed preponderance of rejection in patients with HLA-DR mismatch supports previous findings linking HLA-DR mismatch and the occurrence of subclinical rejection. These results emphasize the importance of recognizing and appropriately managing subclinical rejection in kidney transplant recipients, particularly in those with HLA-DR mismatch and unrelated donor status.

Conclusion

The protocol biopsy of renal transplant recipients is a valuable tool for monitoring graft function and detecting early signs of rejection. Early detection and treatment of rejection can

improve graft survival and patient outcomes. The histological examination of renal tissue samples obtained from the protocol biopsy can provide valuable information about graft function and the presence of rejection, which can guide immunosuppressive therapy and improve patient outcomes. The protocol biopsy is a safe and minimally invasive procedure that should be considered as part of the routine management of renal transplant recipients.

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