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Original Research Article

Clinical Significance of Protocol Allograft Renal Biopsy in a Tertiary Care Centre in Pakistan

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Abstract: ***Background:*** Protocol allograft renal biopsy is performed at scheduled intervals in clinically stable transplant recipients to detect subclinical graft pathology. Its routine use remains debated, particularly in resource-limited settings. Objective: To assess the clinical significance of protocol allograft renal biopsy in detecting subclinical graft pathology and influencing management in renal transplant recipients. ***Methods:*** This was a hospital-based prospective observational study conducted at Faisalabad Medical University from June 2024 to June 2025, including 190 renal transplant recipients who underwent protocol allograft renal biopsy. ***Results***: The mean age was 36.8 ± 10.9 years, and mean serum creatinine at biopsy was 1.34 ± 0.28 mg/dL. Normal histology was observed in 38.9% of cases, while subclinical rejection was detected in 32.6%. Patients with rejection had higher creatinine (1.42 ± 0.31 mg/dL) and lower eGFR (59.6 ± 13.2 mL/min) compared to those with normal biopsy findings. Management was modified in 48.4% of patients, including steroid therapy and immunosuppressive adjustment, leading to significant improvement in serum creatinine in treated groups. ***Conclusion***: Protocol renal allograft biopsy detects clinically silent graft pathology in a substantial proportion of transplant recipients and significantly influences therapeutic decisions. Its implementation may contribute to early intervention and improved graft preservation in tertiary care transplant centers.

Keywords: Renal transplantation, Protocol biopsy, Subclinical rejection, Banff classification, Allograft function

INTRODUCTION

End-stage renal disease is an illness that is best treated by renal transplantation as it offers better survival and quality of life than maintenance dialysis [1]. Even with the current development of surgical practice and the use of immunosuppressive medications, chronic allograft dysfunction is still a major cause of late graft loss [2]. Long-term graft failure has a significant contribution of the immune-mediated injury, toxicity of calcineurin inhibitors, recurrent primary disease, and progressive interstitial fibrosis [3]. Notably, such pathological changes can also occur in patients whose serum

creatinine is stable and whose graft functioning seems to be intact [4]. Protocol allograft renal biopsy is a planned biopsy that is conducted at specified intervals to all patients with transplant whose condition is stable clinically [5]. As opposed to indication biopsies, which is conducted in reaction to increasing creatinine or suspect clinical indicators of rejection, protocol biopsies is expected to identify subclinical pathological alterations prior to apparent clinical aggravation [6]. A significant proportion of stable transplant recipients have been reported to develop subclinical acute rejection during the first-year post-transplant period and is linked to poor long-term graft outcomes unless treated [7]. Early histological diagnosis helps to initiate therapeutic intervention and maximization of immunosuppressive treatment in time [8].

Besides the detection of rejection, protocol biopsies are also able to identify interstitial fibrosis and tubular atrophy, transplant glomerulopathy, antibody-mediated rejection, and drug-induced nephrotoxicity [9]. These results usually follow biochemical abnormalities and might not be anticipated solely from therapeutic drug monitoring [10]. Research has recommended that management changes can be made according to protocol biopsy results and that this can help increase graft survival and limit chronic injury progression [11]. Nevertheless, the operation is not risk-free and can cause bleeding and arteriovenous fistula, and its cost-effectiveness is debatable [12]. In low- and middle-income nations, such as Pakistan, the system lacks resources and local data, making the routine use of protocol biopsy even more difficult [13]. The immunological risk profile, donor characteristics, and infection burden can also affect histopathological patterns in transplant recipients [14]. It is thus critical to assess the diagnostic output and the clinical implications of the protocol biopsy in these settings [15]. Whereas certain centers suggest a universal protocol biopsy as a means of improving long-term graft preservation [16], others suggest a selective strategy founded on risk stratification.

Objective

To assess the clinical significance of protocol allograft renal biopsy in detecting subclinical graft pathology and influencing management in renal transplant recipients.

Methodology

This was a hospital-based prospective observational study conducted at Faisalabad Medical University from June 2024 to June 2025, including 190 renal transplant recipients who underwent protocol allograft renal biopsy.

Inclusion Criteria

- Adult renal transplant recipients aged ≥18 years with stable graft function undergoing scheduled protocol biopsy at predefined post-transplant intervals (e.g., 3 months, 6 months, or 12 months).
- Patients with stable serum creatinine levels and no clinical suspicion of acute rejection at the time of biopsy.
- Patients who provided informed written consent.

Exclusion Criteria

- Patients undergoing indication biopsy due to graft dysfunction or rising serum creatinine.
- Patients with contraindications to renal biopsy such as uncorrected coagulopathy or uncontrolled hypertension.
- Patients with incomplete clinical or histopathological data.

Data Collection

Baseline demographic and transplant-related data were recorded, including age, gender, primary renal disease, donor type (living related, living unrelated, or deceased donor), time since transplantation, immunosuppressive regimen, and serum creatinine levels at the time of biopsy. Percutaneous renal allograft biopsy was done using ultrasound-guided procedures in a standardized method. Biopsy samples were stained using the light microscopy, immunofluorescence and in some cases electron microscopy. Histopathological analysis was performed according to the Banff classification criteria. Results were classified as normal histology, focal changes, acute cellular rejection, antibody-mediated rejection, interstitial fibrosis

and tubular atrophy (IFTA), calcineurin inhibitor toxicity and recurrent/de novo glomerular disease. The decisions to modify the immunosuppressive therapy, to start the anti-rejection management, change the dosage or to do nothing with the management were recorded as a result of the biopsy clinical management decisions. Short-term outcomes such as change in serum creatinine and graft function were followed in the patients.

Statistical Analysis

Data were entered into Microsoft Excel and analyzed using SPSS version 26. Continuous variables were expressed as mean ± standard deviation (SD), while categorical variables were presented as frequency and percentage. Associations between histopathological findings and demographic or clinical variables were analyzed using the chi-square test. A p-value of <0.05 was considered statistically significant.

RESULTS

The cohort included 190 renal transplant recipients with a mean age of 36.8 ± 10.9 years, predominantly male (64.2%). Mean serum creatinine was 1.34 ± 0.28 mg/dL and mean eGFR was 64.7 ± 12.4 mL/min, indicating stable graft function at biopsy. Most patients had living related donors (62.1%) and were on tacrolimus-based therapy (81.1%) with a mean trough level of 6.5 ± 1.5 ng/mL.

Table 1. Baseline Demographic, Clinical, and Transplant Characteristics Stratified by Gender (N = 190)

Variable	Total (N=190)	Male (n=122)	Female (n=68)	p-value
Age (years), Mean ± SD	36.8 ± 10.9	37.2 ± 11.1	36.1 ± 10.5	0.523
Age 18–30 years	58 (30.5%)	36 (29.5%)	22 (32.4%)	0.679
Age 31–45 years	84 (44.2%)	56 (45.9%)	28 (41.2%)	0.548
Age >45 years	48 (25.3%)	30 (24.6%)	18 (26.5%)	0.781
Serum Creatinine (mg/dL), Mean ± SD	1.34 ± 0.28	1.36 ± 0.29	1.31 ± 0.26	0.214
eGFR (mL/min/1.73m²), Mean ± SD	64.7 ± 12.4	63.9 ± 12.7	66.1 ± 11.8	0.268
Time Since Transplant (months), Mean ± SD	8.6 ± 3.1	8.9 ± 3.3	8.1 ± 2.8	0.091
Living Related Donor	118 (62.1%)	74 (60.7%)	44 (64.7%)	0.591
Living Unrelated Donor	52 (27.4%)	36 (29.5%)	16 (23.5%)	0.371
Deceased Donor	20 (10.5%)	12 (9.8%)	8 (11.8%)	0.672
Tacrolimus-Based Regimen	154 (81.1%)	98 (80.3%)	56 (82.4%)	0.728
Tacrolimus Trough Level (ng/mL), Mean ± SD	6.5 ± 1.5	6.4 ± 1.6	6.6 ± 1.4	0.417
Triple Therapy (Tacrolimus + MMF + Steroid)	168 (88.4%)	108 (88.5%)	60 (88.2%)	0.954
Induction with Basiliximab	96 (50.5%)	60 (49.2%)	36 (52.9%)	0.629
Induction with ATG	54 (28.4%)	36 (29.5%)	18 (26.5%)	0.666
No Induction Therapy	40 (21.1%)	26 (21.3%)	14 (20.6%)	0.908

Normal histology was observed in 38.9% of biopsies, while subclinical rejection was detected in 32.6%. Patients with normal histology had lower creatinine (1.29 ± 0.25 mg/dL) and higher eGFR (67.2 ± 11.9 mL/min) compared to rejection categories such as acute cellular rejection IA (creatinine 1.43 ± 0.30 mg/dL; eGFR 58.7 ± 13.1 mL/min) and active antibody-mediated rejection (creatinine 1.58 ± 0.38 mg/dL; eGFR 52.4 ± 15.3 mL/min). Tacrolimus levels were generally lower in rejection groups (5.4–5.8 ng/mL).

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Table 2. Histopathological Findings with Clinical Correlation (Banff Classification) (N = 190)

Histopathological Category	n (%)	Mean Creatinine (mg/dL) ± SD	Mean eGFR (mL/min) ± SD	Mean Tacrolimus Level (ng/mL) ± SD	C4d Positive n (%)
Normal Histology	74 (38.9%)	1.29 ± 0.25	67.2 ± 11.9	6.8 ± 1.4	2 (2.7%)
Borderline Changes	28 (14.7%)	1.37 ± 0.28	61.4 ± 12.7	6.1 ± 1.5	4 (14.3%)
Acute Cellular Rejection IA	16 (8.4%)	1.43 ± 0.30	58.7 ± 13.1	5.8 ± 1.6	3 (18.8%)
Acute Cellular Rejection IB	8 (4.2%)	1.49 ± 0.34	55.6 ± 14.2	5.6 ± 1.7	2 (25.0%)
Active ABMR	6 (3.2%)	1.58 ± 0.38	52.4 ± 15.3	5.4 ± 1.8	6 (100%)
Chronic Active ABMR	4 (2.1%)	1.63 ± 0.42	49.8 ± 16.7	5.2 ± 1.9	4 (100%)
IFTA Grade I	20 (10.5%)	1.36 ± 0.27	60.9 ± 11.4	6.3 ± 1.3	1 (5.0%)
IFTA Grade II	12 (6.3%)	1.44 ± 0.31	56.3 ± 12.6	6.0 ± 1.4	1 (8.3%)
Calcineurin Inhibitor Toxicity	14 (7.4%)	1.41 ± 0.29	58.2 ± 10.9	7.9 ± 1.2	0 (0%)
Transplant Glomerulopathy	6 (3.2%)	1.55 ± 0.36	53.7 ± 13.8	6.2 ± 1.5	3 (50.0%)
Recurrent/De Novo GN	8 (4.2%)	1.47 ± 0.33	55.8 ± 12.1	6.4 ± 1.4	1 (12.5%)

Comparison between normal and subclinical rejection groups showed significantly higher serum creatinine in the rejection group (1.42 ± 0.31 vs. 1.29 ± 0.25 mg/dL; p = 0.004) and lower eGFR (59.6 ± 13.2 vs. 67.2 ± 11.9 mL/min; p = 0.002). Time since transplant was longer in the rejection group (9.4 ± 3.4 vs. 7.9 ± 2.8 months; p = 0.006), and tacrolimus levels were lower (5.9 ± 1.6 vs. 6.8 ± 1.4 ng/mL; p = 0.001).

Table 3. Comparison of Clinical and Laboratory Parameters Between Normal and Subclinical Rejection Groups

Variable	Normal Biopsy (n=74) Mean ± SD	Subclinical Rejection (n=62) Mean ± SD	p-value
Age (years)	35.9 ± 10.2	38.4 ± 11.1	0.184
Time Since Transplant (months)	7.9 ± 2.8	9.4 ± 3.4	0.006
Serum Creatinine (mg/dL)	1.29 ± 0.25	1.42 ± 0.31	0.004
eGFR (mL/min/1.73m²)	67.2 ± 11.9	59.6 ± 13.2	0.002
Tacrolimus Trough Level (ng/mL)	6.8 ± 1.4	5.9 ± 1.6	0.001
Male Gender	46 (62.2%)	42 (67.7%)	0.481
Living Related Donor	50 (67.6%)	36 (58.1%)	0.233

Protocol biopsy influenced clinical management in 48.4% of patients. Steroid pulse therapy reduced mean creatinine from 1.46 ± 0.33 to 1.28 ± 0.27 mg/dL (p = 0.003), while immunosuppressive dose adjustment improved creatinine from 1.39 ± 0.29 to 1.26 ± 0.25 mg/dL (p = 0.008). Switching calcineurin inhibitors reduced creatinine from 1.52 ± 0.36 to 1.33 ± 0.28 mg/dL (p = 0.021).

Table 4. Impact of Protocol Biopsy on Clinical Management and Short-Term Outcomes

Management Change	n (%)	Mean Creatinine Before (mg/dL)	Mean Creatinine After 3 Months (mg/dL)	p-value
No Change in Therapy	98 (51.6%)	1.31 ± 0.24	1.30 ± 0.22	0.418
Steroid Pulse Therapy	34 (17.9%)	1.46 ± 0.33	1.28 ± 0.27	0.003
Immunosuppressive Dose Adjustment	40 (21.1%)	1.39 ± 0.29	1.26 ± 0.25	0.008
Switch of Calcineurin Inhibitor	12 (6.3%)	1.52 ± 0.36	1.33 ± 0.28	0.021
Treatment for Antibody-Mediated Rejection	6 (3.1%)	1.61 ± 0.40	1.37 ± 0.34	0.017

DISCUSSION

The present paper demonstrates that protocol allograft renal biopsy may play a major role in clinical practice, even in cases of transplant recipients with a stable graft. The result was even though the mean serum creatinine at the biopsy point was relatively normal (1.34 ± 0.28 mg/dL) and eGFR was preserved (64.7 + 12.4 mL/min), more than half of the patients have some histopathological abnormalities. It is worth noticing that subclinical rejection has been detected in 32.6% of recipients, which demonstrates that biochemical stability is not a reliable tool to rule out undergoing graft injury. The rates of subclinical rejection, described in this study, are similar to the previous study findings, which discussed the rates of the subclinical rejection between 2035%, which strengthens the necessity of protocol biopsy in early detection [17]. Serum creatinine (1.42 ± 0.31 mg/dL) and eGFR (59.6 ± 13.2 mL/min) were higher in the patients having subclinical rejection as opposed to the patients having normal histology (1.29 ± 0.25 mg/dL and 67.2 ± 11.9 mL/min). Also, tacrolimus trough levels were smaller in cases of rejection (5.9 ± 1.6 ng/mL vs. 6.8 ± 1.4 ng/mL) and may possibly indicate under-immunosuppression. Past studies have also indicated that decreased levels of calcineurin inhibitors would be linked to high chances of subclinical rejection, which would confirm the careful monitoring of therapeutic drugs [18][19]. Notably, the findings of protocol biopsies resulted in managerial modifications in 48.4 percent of patients. Such interventions as steroid pulse therapy and immunosuppressive adjustments led to the significant short-term serum creatinine improvement, e.g. 1.46 0.33 to 1.28 0.27 mg/dL after steroid treatment. Also, previous studies have established that the treatment of subclinical rejection identified on protocol biopsy as early as possible enhances the graft functionalities and can decrease the development of chronic allograft dysfunction [20]. Moreover, persistent alterations including interstitial fibrosis and tubular atrophy and premature antibody-mediated rejection were identified despite constant clinical values. Past studies have highlighted that early detection of these alterations enables prompt adjustment of immunosuppressive treatment and thereby optimizes the chances of enhancing the long-term survival of the graft [21]. In general, the results are consistent with the existing studies in favor of using protocol allograft biopsy as a useful method to provide evidence of occult graft pathology, support personalized treatment, and achieve positive outcomes of transplantation, especially in the context of resource scarcity when early graft preservation is an essential factor.

Conclusion

It is concluded that protocol allograft renal biopsy has substantial clinical significance in renal transplant recipients with stable graft function. Despite near-normal serum creatinine levels, a considerable proportion of patients demonstrated subclinical rejection and early chronic changes detectable only on histopathology. Nearly half of the biopsies led to modifications in immunosuppressive therapy, resulting in measurable short-term improvement in graft function. These findings support the role of protocol biopsy as a valuable tool for early detection of occult graft injury and optimization of post-transplant management in tertiary care settings.

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