# CHARACTERISTICS OF PROTEINURIA AND ITS RELATIONSHIP WITH THE PRESENCE OF ANTI-HLA ANTIBODIES AFTER TRANSPLANTATION IN PATIENTS WITH RENAL ALLOGRAFT DYSFUNCTION DURING THE FIRST 6 MONTHS OF FOLLOW-UP

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## Abstract

***Objectives:*** To investigate the characteristics of proteinuria and its association with the presence of anti-HLA antibodies following kidney transplantation in patients with renal allograft dysfunction during the first 6 months of follow-up at Military Hospital 103. ***Methods:*** A prospective, descriptive, longitudinal study was conducted on 51 patients who underwent kidney transplantation from living donors and exhibited renal allograft dysfunction during the first 6 months post-transplantation, from June 2019 to January 2021. ***Results:*** Proteinuria occurred in 33.3% of patients within the first 6 months, with the highest incidence observed at the end of the first month (19.6%). Post-transplant panel-reactive antibody (PRA) positivity was found in 78.4% of patients. Among these, 17 out of 51 patients (33.3%) tested positive for donor-specific anti-HLA antibodies (HLA-DSA). The incidence of proteinuria, both overall and at specific time points (the 1st month and 6th month), was significantly higher in the HLA-DSA (+) group compared to the HLA-DSA (–) group ($p < 0.05$). However, no significant association was observed between proteinuria and the presence of anti-HLA-DP or anti-HLA-DQ antibodies. The presence of HLA-DSA post-transplantation was strongly associated with the development of proteinuria (OR = 30.36; $p < 0.01$). ***Conclusion:*** Proteinuria was observed in 33.3% of patients during the first 6 months after kidney transplantation. The presence of HLA-DSA was significantly associated with the occurrence of proteinuria post-transplantation (OR = 30.36; $p < 0.01$).

**Keywords:** Proteinuria; Anti-HLA antibody; Panel-reactive antibody; Kidney transplantation.

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## INTRODUCTION

Proteinuria is a sign of renal injury and a predictor of the course of most kidney diseases. Proteinuria itself may play a role in the progression of renal disease by promoting the development of fibrogenesis and glomerulosclerosis. Furthermore, proteinuria in the general population has been shown to be associated with morbidity and mortality [1]. The prevalence of proteinuria after kidney transplantation ranged from 7.5% to 45%, depending on its definition; most previous studies measured proteinuria between 1 month and 1 year after kidney transplantation. Early postoperative proteinuria is a valuable biomarker to predict early renal outcomes after kidney transplantation [2]. Prognostic value of proteinuria and eGFR for graft and patient survival was comparable, and these two variables remain significant risk factors even in a multivariate model that takes into consideration the most important clinical variables (donor age, rejection, delayed graft function, and cytomegalovirus, etc.) [3]. Late-onset proteinuria after renal transplantation has been universally associated with poor allograft outcomes. However, the significance of early low-grade post-transplant proteinuria remains uncertain [4]. In this study, our objective is: *To investigate the characteristics of proteinuria and its association with the appearance of PRA after transplantation in patients with renal allograft dysfunction during the first 6 months of follow-up at Military Hospital 103.*

## MATERIALS AND METHODS

### 1. Subjects

Including 51 patients with indications for kidney transplantation, who received kidneys from living donors, renal allograft dysfunction (including delayed graft function (DGF) and reduced kidney function (RKF)) in the first 6 months, and post-transplant follow-up at Military Hospital 103.

- DGF: Defined as the need for dialysis support during the first week after transplantation or a decrease in creatinine of < 25% within the first 24h after surgery.

- RKF: Patients were evaluated as having RFK after transplantation when the serum creatinine level at one post-transplant study time point increased by ≥ 25% compared with the patient's baseline creatinine level.

* *Inclusion criteria:* Patients who received a kidney transplant at Military Hospital 103 from a living donor (with or without the same bloodline); periodically and fully monitored for 6 months after the transplant at Military Hospital 103; patients with renal allograft dysfunction after transplantation, including DGF and RKF, during the

6-month follow-up; consent to participate in the study.

* *Exclusion criteria:* Patients who were not followed up as planned; found to have malignancies during follow-up; suffering from severe acute diseases; had surgery-related changes in kidney function immediately after transplantation: Renal artery stenosis, ureteral stenosis, renal artery occlusion, etc.; did not consent to participate in the study.

* *The study period:* From June 2019 to January 2021.

**2. Methods**

* *Study design:* A prospective, descriptive, longitudinal study.

* *Sample selection and sample size calculation:* Choose a convenient sample. 51 patients met the inclusion criteria.

* *Research objectives:*

Post-transplant data (postoperative period): Record the status of delayed kidney transplant function and increased creatinine after transplantation.

Data during 6 months of post-transplant follow-up was collected: Patients were re-examined, and data were collected at the following time points: 1 month, 3 months, and 6 months; blood test: Glucose, urea, creatinine, GOT, GPT, CRP, drug concentration (Tacrolimus), urinary protein; HC, HST, BC, TC; eGFR at follow-up times according to the CKD-EPI 2009 formula.

Post-transplant anti-HLA antibody data (PRA): PRA testing (anti-HLA antibody testing) was performed in the same way and technique both before and after transplantation; test interpretation: The specific PRA (+) percentage was shown in each class (class I and class II); anti-HLA antibodies and MFI fluorescence intensity were identified corresponding to each antibody; HLA-DSA was determined, comparing with the corresponding donor HLA test (transplant profile data).

Limitations of HLA-DSA (+) identification: HLA of recipients and donors before transplantation was only identified at 6 points: HLA-A, HLA-B, HLA-DRB1, so some antibodies appearing in kidney transplant patients, such as HLA-DRB3, HLA-DP, HLA-DQ, had not been examined in donors; therefore, it is not possible to determine whether it is HLA-DSA or not.

* *Data processing:* Using IBM SPSS 22.0 software.

**3. Ethics**

The study was approved by the Research Ethics Committee of the Vietnam Military Medical University (Decision No. 1231/QD-HVQY dated 01/11/2017). Military Hospital 103, Vietnam Military Medical University granted permission for the use and publication of the research data. The authors declare to have no conflicts of interest in this study.

## RESULTS

### 1. Some general characteristics of the study group

**Table 1.** Some general characteristics of the study group (n = 51).

| Characteristics | | Value |
|---|---|---|
| Age (year) | | 38.49 ± 11.10 |
| Sex | Male | 39 (76.5%) |
| | Female | 12 (23.5%) |
| Causes of CKD | Chronic glomerulonephritis | 45 (88.2%) |
| | Other causes | 6 (11.8%) |
| Mean BMI (kg/m$^2$) | | 20.75 ± 2.83 |
| eGRR of donated kidney (mL/min) | | 51.19 |
| CNIs | Tacrolimus | 47 (92.2%) |
| | Neoral | 4 (7.8%) |

*(CKD: Chronic kidney disease; BMI: Body mass index; GFR: Glomerular filtration rate; CNIs: Calcineurin inhibitors)*

The mean age of the study population was 38 years; male patients comprised the majority (76.5%). Chronic glomerulonephritis was the leading cause of end-stage chronic kidney disease, observed in 88.2% of cases. The mean body mass index (BMI) was 20.75 ± 2.83 kg/m². The mean glomerular filtration rate (GFR) of the donated kidneys was 51.19 mL/min/1.73m². Among the calcineurin inhibitors (CNIs) used as maintenance immunosuppressive therapy, Tacrolimus was the preferred agent, prescribed in 92.2% of patients.

**Table 2.** The rate of patients with renal allograft dysfunction at different follow-up time points within 6 months after transplantation (n = 51).

| Time points | Number (n) | Percentage (%) |
|---|---|---|
| The first 7 days after transplantation | 20 | 39.2 |
| The first month | 18 | 35.3 |
| The 2nd and 3rd month | 8 | 15.7 |
| The 4th, 5th, and 6th month | 5 | 9.8 |

Among 51 patients with renal allograft dysfunction after transplantation, 20 patients (39.2%) experienced early impairment of kidney function within the first

week. The highest rate of renal allograft dysfunction during the follow-up period was observed in the first month post-transplantation (35.3%).

**Table 3.** Characteristics of proteinuria at different time points during the first 6 months of follow-up (n = 51).

| Proteinuria | Positive, n (%) | Negative, n (%) |
|---|---|---|
| The end of the 1st month | 10 (19.6) | 41 (80.4) |
| The 3rd month | 2 (3.9) | 49 (96.1) |
| The 6th month | 5 (9.8) | 46 (90.2) |
| Proteinuria after transplantation | 17 (33.3) | 34 (66.7) |

The incidence of proteinuria among the study cohort varied across follow-up time points. The highest incidence was recorded at the end of the 1st month post-transplantation (19.6%), whereas a markedly lower rate was observed at the 3rd month (3.9%). Overall, the cumulative incidence of proteinuria during the 6th month follow-up period was 33.3%.

**Table 4.** Characteristics of PRA after transplantation (n = 51).

| Characteristics of PRA | | Number (n) | Percentage (%) |
|---|---|---|---|
| PRA (–) | | 11 | 21.6 |
| PRA (+) | Class I (HLA-A, B) | 3 | 5.9 |
| | Class II (HLA-DR, DP, DQ) | 19 | 37.3 |
| | Class I + Class II | 18 | 35.3 |
| | HLA - DSA (+) | 17 | 33.3 |
| | Mean % PRA (median/quartile) | 11,5 (6 - 24) | |

PRA-negative (PRA (–)) patients accounted for 21.6%, while PRA-positive (PRA (+)) patients made up 78.4% of the study population. Among those with PRA (+) after transplantation, 17 patients (33.3%) were found to be positive for HLA-DSA (HLA-DSA+).

**2. The association between the occurrence of proteinuria and anti-HLA antibodies after transplantation in patients with renal allograft dysfunction during the first 6 months of follow-up.**

**Table 5.** The association between proteinuria and HLA-DSA at follow-up times (n = 51).

| Proteinuria | | HLA-DSA (+) (n = 17) | HLA-DSA (–) (n = 34) | p |
|---|---|---|---|---|
| The end of the 1st month | Negative | 10 (58.8) | 31 (91.2) | < 0.05[a] |
| | Positive | 7 (41.2) | 3 (8.8) | |
| The 3rd month | Negative | 16 (94.1) | 33 (97.1) | > 0.05[a] |
| | Positive | 1 (5.9) | 1 (2.9) | |
| The 6th month | Negative | 13 (76.5) | 33 (97.1) | < 0.05[a] |
| | Positive | 4 (23.5) | 1 (2.9) | |
| Proteinuria after transplantation | Yes | 11 (64.7) | 6 (17.6) | < 0.001[b] |
| | No | 6 (35.3) | 28 (82.4) | |

*([a]Fisher's exact test; [b]Chi-square test)*

The incidence of post-transplant proteinuria and proteinuria during follow-up was higher in the HLA-DSA (+) group compared to the HLA-DSA (–) group. The difference was statistically significant ($p < 0.05$) at the end of the first month and 6 months post-transplantation.

**Table 6.** The association between the occurrence of proteinuria and HLA-DP, DQ at follow-up time points (n = 51).

| Proteinuria | | HLA-DP, DQ (+) (n = 17) | HLA-DP, DQ (–) (n = 34) | p |
|---|---|---|---|---|
| 1st month | Negative | 15 (88.2) | 26 (76.5) | > 0.05[a] |
| | Positive | 2 (11.8) | 8 (23.5) | |
| 3rd month | Negative | 17 (100) | 32 (94.1) | > 0.05[a] |
| | Positive | 0 (0) | 2 (5.9) | |
| 6th month | Negative | 16 (94.1) | 30 (88.2) | > 0.05[a] |
| | Positive | 1 (5.9) | 4 (11.8) | |
| Proteinuria after transplantation | Yes | 3 (17.6) | 13 (38.2) | > 0.05[b] |
| | No | 14 (82.4) | 21 (61.8) | |

*([a]Fisher's exact test; [b]Chi-square test)*

There was no significant association between the presence of HLA-DQ or HLA-DP antibodies and the occurrence of proteinuria at any follow-up time point.

**Table 7.** Logistic regression of factors associated with the occurrence of proteinuria after transplantation (n = 51).

| Factors | OR | 95%CI | p |
|---|---|---|---|
| Age of donor | 0.98 | 0.87 - 1.10 | > 0.05[a] |
| BMI of recipients (before transplant) | 1.22 | 0.87 - 1.71 | > 0.05[a] |
| Hypertension (after transplant) | 1.15 | 0.03 - 39.65 | > 0.05[a] |
| PRA (+) (after transplant) | 0.25 | 0.01 - 4.90 | > 0.05[a] |
| HLA-DSA (+) (after transplant) | 30.36 | 2.85 - 323.91 | < 0.01[a] |
| Tacrolimus < 5.0 ng/mL | 0.94 | 0.08 - 10.99 | > 0.05[a] |

*([a]Multivariate Logistic Regression)*

HLA-DSA positivity after transplantation was significantly associated with the occurrence of proteinuria. Specifically, patients in the HLA-DSA (+) group had a 30-fold higher risk of developing proteinuria compared to those without HLA-DSA (OR = 30.36; p < 0.01).

No significant association was found between kidney donor age, pre-transplant recipient BMI, post-transplant hypertension, PRA positivity after transplantation, or Tacrolimus trough concentration < 5.0 ng/mL, and the occurrence of post-transplant proteinuria (p > 0.05).

## DISCUSSION

The rate of patients with renal allograft dysfunction during the 6-month follow-up period after kidney transplantation is summarized in table 2. Specifically, 20 patients (39.2%) experienced early renal function decline within the first week post-transplantation. The highest incidence of reduced renal function during follow-up was recorded in the first month, affecting 35.3% of patients. After that point, the rate gradually declined over time.

According to table 3, the occurrence of proteinuria in transplant recipients at different follow-up intervals varied. The highest rate was observed at the end of the first month after transplantation, with 19.6% of patients

developing proteinuria. This rate dropped to 3.9% by the third month, but increased again to 33.3% at the six-month follow-up. These findings are consistent with the results of Junseok J et al. (2022), the prevalence of proteinuria after kidney transplantation ranges from 7.5% to 45%, depending on the definition used and the timing of assessment, typically between 1 month and 1year post-transplantation [2].

The results of the PRA assessment after transplantation (*Table 4*) showed that PRA (–) patients accounted for 21.6% (11/51 patients), while PRA (+) patients comprised 78.4% (40/51 patients). Among the PRA (+) group, 33.3% (17/51 patients) were also positive for HLA-DSA (PRA+/HLA-DSA+), whereas 45.1% (23/51 patients) were PRA+ but HLA-DSA negative (PRA+/HLA-DSA–). In some PRA (+) patients, additional anti-HLA-DP and anti-HLA-DQ antibodies were detected post-transplantation that were not present in the serum before transplantation. However, it remains unclear whether these antibodies are donor-specific, as no pre-transplant HLA-DP and HLA-DQ typing was performed on the donors. Currently, in Vietnam, HLA testing for kidney transplantation is limited to 6 loci: HLA-A, HLA-B, and HLA-DRB1. Globally, several studies have investigated the impact of HLA-DQ and HLA-DP antibodies on graft outcomes in transplantation.

When analyzing the relationship between the occurrence of proteinuria and HLA-DSA positivity (*Table 5*), it was found that the incidence of proteinuria after transplantation, both overall and at specific time points (the first and sixth months), was significantly higher in the HLA-DSA (+) group compared to the HLA-DSA (–) group ($p < 0.05$). In the assessment of HLA-DSA after transplantation in patients with RKF during the first 6 months, the appearance of anti-HLA-DP and HLA-DQ antibodies was observed. However, since pre-transplant typing for HLA-DP and HLA-DQ was not performed on donors, it is unclear whether these antibodies are donor-specific. These antibodies may represent true HLA-DSA or could be antibodies present in the recipient's serum at undetectable levels at the time of transplantation or that developed later, possibly following blood transfusions during the transplantation process. The threshold for detecting antibodies by mean fluorescence intensity (MFI) varies between laboratories, commonly set at MFI < 500 or MFI < 1000. Based on this, the relationship between the

appearance of HLA-DP and HLA-DQ antibodies and proteinuria after transplantation in patients with reduced renal function within 6 months was investigated. No significant association was found between proteinuria and the presence of HLA-DP or HLA-DQ antibodies (*Table 6*). This finding aligns with the study by Rusai K et al. (2016), which reported that pediatric patients who were HLA-DSA (+) had a significantly higher incidence of proteinuria compared to the HLA-DSA (–) group ($p < 0.05$) [6]. Additionally, no significant associations were observed between post-transplant proteinuria and donor age, recipient BMI before transplantation, post-transplant hypertension, PRA positivity after transplantation, or Tacrolimus trough concentrations < 5.0 ng/mL ($p > 0.05$).

Currently, alongside routine monitoring of post-transplant proteinuria, screening for donor-specific antibodies in stable kidney transplant recipients is standard practice in several transplant centers worldwide. The presence of donor-specific antibodies increases the risk of graft loss, and early intervention may improve outcomes. However, the benefits of reducing graft loss must be balanced against the increased mortality risk associated with intensified immunosuppressive treatment [7].

## CONCLUSION

The overall incidence of proteinuria was 33.3%, with the highest occurrence observed at the end of the first month (19.6%). The rate of proteinuria after transplantation at different time points (first and sixth months) was significantly higher in the HLA-DSA (+) group compared to the HLA-DSA (–) group ($p < 0.05$). Moreover, HLA-DSA positivity after transplantation was strongly associated with the development of proteinuria (OR = 30.36; $p < 0.01$).

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