Proteinuria after kidney transplantation - prevalence and risk factors

INTRODUCTION

Proteinuria (PTN) after kidney transplantation has been recognized as a risk factor for the progression of chronic allograft nephropathy and cardiovascular disease.1

Post-transplant proteinuria is usually defined as urinary protein excretion greater than 300-500 mg/24h for at least 3-6 months.2-4 Other authors consider this proteinuria value to be above 1000 mg/24h.5 The incidence of post-Tx proteinuria in epidemiological studies have ranged between 10 and 45%.2,4

The etiology of post-transplant proteinuria is multifactorial,4 and we can mention chronic allograft nephropathy, transplant glomerulopathy, repeated or recurrent glomerulonephritis, chronic pyelonephritis, nephrotoxicity by calcineurin inhibitors and diabetic nephropathy. High molecular weight Proteinuria is often seen after transplantation, but it tends to be brief and transient, reflecting the degree of preoperative ischemic damage to the graft. There may still be proteinuria from the native kidneys or even from the immunosuppressive therapy, especially related to the use of Sirolimus.6,7,8

Microalbuminuria or high molecular weight proteinuria is associated with a higher incidence of hypertension, acute rejection, graft dysfunction and long-term mortality, being

ABSTRACT

Introduction: Proteinuria after kidney transplantation (Tx) has variable incidence and is associated with cardiovascular risk and graft survival. Objective: To evaluate the prevalence of proteinuria after kidney Tx and its associated factors. Methods: The prevalence of PTN was evaluated according to definition ≥ 500 mg/24 hours. Patients were divided into 3 groups: group A, < 500 mg, B, 500-1000 mg and C, > 1000 mg. We tested the association between PTN and: age/gender of the donor and recipient, type of donor, delayed graft function, acute rejection, hypertension and creatinine. The variables with a p value < 0.20 in the bivariate analysis were included in a multivariate logistic regression analysis. Results: 173 recipients were evaluated, mean age 39 years, 57.2% male and 60.7% deceased donor. The prevalence of PTN after kidney Tx was 24.3%. The distribution of patients according to PTN was 75.7% for group A, 15.6% for group B and 8.7% for group C. The following factors were associated with higher risk of PTN: male recipients, living donor and hypertension. Creatinine at month 12 months post-Tx was higher among patients with proteinuria. 60% of patients with PTN ≥ 500 mg/24 hours were treated with ACEI/ARB. Conclusion: The prevalence of PTN after kidney Tx varied between 24.3%, according to the definition used. The male gender of the recipient, living donor and hypertension were associated with the occurrence of PTN after kidney Tx. Blockade of the renin-angiotensin system must be prescribed to more patients.

Keywords: kidney transplantation; prevalence; proteinuria; risk factors.
reported in large studies as a single prognostic factor for both of the latter. \(^3\), \(^9\), \(^10\)

In patients with post-transplant proteinuria, treatment with inhibitors of the renin-angiotensin-aldosterone system (RAAS) may reduce proteinuria, but there is no evidence from randomized studies that this strategy results in improved graft survival or better patient survival. \(^4\)

The present study examined the prevalence and management of post-Tx PTN in recipients from a single center and the impact of proteinuria in renal function after 36 months of Tx, as well as the factors associated with it. Post-Tx PTN carries an increased mortality in the short and long term post-transplant period, the etiology and variable prevalence of this condition as well as the uncertainty in handling this proteinuria with the RAAS blockage in transplant patients, makes it mandatory to investigate the occurrence of post-renal Tx PTN and the factors associated with it.

**METHODS**

The study population consisted of recipients submitted to renal Tx in recipients from a single center in Fortaleza-Brazil, from January 2005 to December 2008, and included patients older than 18 years, with at least 12 months of follow-up post-Tx. The project was submitted to and approved by the Research Ethics Committee of the UniChristus University Center, under number 021/2011.

The study was a retrospective cohort. The evaluation was carried out by reviewing records of post-renal Tx outpatients.

The following variables were investigated: age and gender of donor and recipient; primary renal disease; type of donor (living or deceased); post-renal Tx hypertension (defined as the presence of systolic blood pressure \(\geq 140\) mmHg and/or diastolic blood pressure \(\geq 90\) mm Hg and/or use of antihypertensive drugs post-Tx); delayed graft function (defined as the need for dialysis during the first week after transplantation) and acute rejection proven by renal biopsy.

Post-renal Tx PTN was defined as greater than or equal to 500 mg/24 hours. In order to assess the prevalence of PTN, the patients were divided into 3 groups. In group A, the patients had PTN less than 500 mg/24 hours; those in group B from 500 mg-1000 mg/24 hours, and Group C Patients with PTN greater than 1000 mg/24 hours.

Proteinuria was also evaluated at different times post-Tx: less than 12 months (period 1) between 12-36 weeks (period 2); between 36-60 months (period 3) and 60 weeks (period 4). Within these same intervals we recorded: serum creatinine, weight and the use of antihypertensive agents. Creatinine and weight in different periods were used to calculate creatinine clearance by the Crockroft-Gault formula. \(^11\)

According to the protocol that guides recipient follow up after renal transplantation in the hospital under study, the 24-hour proteinuria should be evaluated annually.

The use of angiotensin converting enzyme inhibitors (ACEi) or angiotensin receptor blockers (ARB) was recorded.

Continuous variables were expressed as mean ± standard deviation and categorical variables were expressed as a percentage. The variables with normal distribution were compared using the Student \(t\)-test and for those with abnormal distribution we used the Mann-Whitney test. The chi-square and Fisher’s exact tests were used to test the difference between the proportion of variables in patients with and without proteinuria at different periods of study. The variables that reached a \(p\)-value \(< 0.20\) in the bivariate analysis were included in a multivariate logistic regression model to identify factors associated with a higher chance of post-transplant PTN \(\geq 500\) mg/24 hours. A \(p\) value less than 5% was considered statistically significant.

**RESULTS**

290 kidney transplants were performed during the study period, including 173 recipients (59.6%). The reasons for excluding 117 recipients were: younger than 18 years (\(n = 17\)), missing the
24-hour proteinuria during follow-up (n = 18) and incomplete or missing records (n = 82).

Demographic and laboratory characteristics of the patients studied are depicted on Table 1.

The primary renal disease was undetermined in 54 patients; hypertensive nephrosclerosis in 43; 34 for primary glomerular diseases; familiar nephritis in 8 and diabetic nephropathy in 6, among others.

The distribution of patients according to PTN range throughout the follow-up period was 75.7% for group A (< 500 mg/24 h), 15.6% for group B (500-1000 mg/24 h) and 8.7% for group C (> 1000 mg/24 h).

As defined in the study (PTN ≥ 500 mg/24 hours), high PTN prevalence was 24.3% (n = 42). If we considered PTN ≥ 300mg/24 hours over the whole study, we would find a prevalence of 37.6% (n = 65).

An important finding is that, despite the recommendation of performing an annual 24 hour-PTN analysis in the hospital under study, there was a significant percentage of patients who did not undergo PTN in all periods of the study. The best-rated period was between 12-36 months, when only 18.5% did not have a PTN measured. In the other periods, between 55-65% of patients did not undergo PTN assessment.

An association between the recipient’s gender and the presence of PTN ≥ 500 mg/24 h was detected (males: 30.3% versus females 16.2% p = 0.033). In the group of recipients from living donors, 32.4% had post-Tx PTN ≥ 500 mg/24 hours, versus 19% in the group of deceased donors (p = 0.046). There was no association between PTN > 500 mg/24 hours and donor gender (p = 0.924) or the presence of delayed graft function (DGF) (p = 0.659). Among the patients who presented episodes of acute rejection, PTN ≥ 500 mg/24 hours was diagnosed in 31.9% of cases versus 21.4% among those without acute rejection (p = 0.152). In the group of patients who underwent thymoglobulin induction, 18.7% had post-Tx PTN ≥ 500 mg/24 hours, compared to 30.5% in the group that did not use thymoglobulin (p = 0.071). Post-Tx hypertension was associated with PTN ≥ 500 mg/24 hours (27% in the group with hypertension versus 4.8% in the group without hypertension; p = 0.028).

Creatinine at 12 months was higher in patients with PTN ≥ 500 mg/24 hours (1.53 ± 0.61 mg/dL versus 1.26 ± 0.62 mg/dl; p = 0.001). Creatinine clearance at 36 months was not statistically different in patients with and without PTN ≥ 500 mg/24 hours 62.97 ± 22.17 versus 66.85 ± 22.19; p = 0.345).

By assessing the groups according to proteinuria, we found that in groups A, B and C, 44.4%, 62% and 53.3% of the patients used ACEI/ARB, respectively (p = 0.23) (Figure 1).

We included in the multivariate logistic regression analysis the variables with a p significance < 0.20 in the bivariate analysis. The following variables were associated with higher likelihoods of having a PTN ≥ 500 mg/24 hours after transplantation: male recipient (OR = 2.25, CI 1.36 to 4.87; p = 0.004), living donor (OR = 2.21, CI 1.06 to 4.57; p = 0.034) and post-Tx hypertension (OR = 7.46, CI 1.02 to 58.4, p = 0.051) (Table 2).

**DISCUSSION**

Proteinuria is an important marker for the risk of cardiovascular death and general death for the renal transplant recipient and it is associated with lower survival both for the graft and the recipient.3,5 The incidence of proteinuria has increased in recent years, which may be due to choice of older donors and recipients, with more cardiovascular and metabolic risk than before.12

The prevalence of post-renal Tx PTN described in the literature has been variable, mainly because of different definitions used and different post-Tx periods evaluated. Sancho et al.,3 evaluated 375 renal Tx recipients and reported a prevalence of 20.2%, with PTN greater than 500 mg/24 hours. Fernandez-Fresneda et al.5 found that at 12 months post-renal Tx in a cohort of 3,365 patients, 15.3% of the recipients had PTN greater than 500 mg/24 hours and 7.5% above 1,000 mg/24 hours.
### Table 1: Demographics and Laboratory Characteristics of the Study Population

<table>
<thead>
<tr>
<th>Variable</th>
<th>Recipient age</th>
<th>Recipient gender</th>
<th>Donor age</th>
<th>Donor gender</th>
<th>Donor type</th>
<th>DGF</th>
<th>Diabetes mellitus</th>
<th>Hypertension</th>
<th>Use of ACEI/ARB</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>39.3 ± 12.7 years</td>
<td>Male: 57%</td>
<td>32.3 ± 12.1 years</td>
<td>Male: 58%</td>
<td>RLD: 29.5%</td>
<td>35.3%</td>
<td>22.5%</td>
<td>87.8%</td>
<td>50.3%</td>
</tr>
</tbody>
</table>

#### Table 2: Multivariate Analysis of Factors Associated with Post-Transplant Proteinuria

<table>
<thead>
<tr>
<th></th>
<th>p</th>
<th>OR</th>
<th>95% CI for EXP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male vs. Female recipient</td>
<td>0.04</td>
<td>2.256</td>
<td>1.036 4.869</td>
</tr>
<tr>
<td>Live donor versus Deceased</td>
<td>0.03</td>
<td>2.205</td>
<td>1.064 4.572</td>
</tr>
<tr>
<td>AH versus No AH</td>
<td>0.05</td>
<td>7.464</td>
<td>1.014 58.451</td>
</tr>
</tbody>
</table>

Amer et al., investigated 613 patients at 12 months post-Tx and considering a PTN greater than 150 mg/24 hours, found a prevalence of 45%. Changing the PTN cutoff point to 500 mg/24 hours, in the same study the prevalence was only 15.3%. Roodnat et al., when evaluating 722 patients for one year, found 31% with PTN above 200 mg/24 hours.

Ibis et al., in a study with 130 patients during 12 months of follow-up, found a prevalence of 34.3%, considering a PTN above 300 mg/24 hours. Halimi et al., found that 20.4% of the subjects of a cohort of 484 patients had PTN...
greater than 1000 mg/24 hours and 35.2% had PTN above 500 mg/24 hours.

In our study we used a PTN value greater than or equal to 500 mg/24 hours, we found a prevalence of 24.3% of PTN. When the proteinuria cut-off point used was greater than or equal to 300 mg/24 hours, the prevalence was 37.6% - data corroborated by the literature. In Brazil, we do not know of previously published studies that assessed the prevalence of proteinuria after renal Tx. The distribution of patients according to the degree of PTN was similar to the study by Souqiyyeh et al., with 340 patients, which identified 61.5% of patients with 24-hour PTN < 300 mg, 27.1% between 300-1,000 mg and 11.5% above 1,000 mg/24 hours.

The highest percentage of proteinuria not performed was observed in the first 12 months (period 1) and longer than 60 months (period 4), i.e. around 65% of patients. Between 12-36 months post-Tx, only 18.5% of patients did not have the results from the 24h PTN assessment. So in other periods (periods 1 and 4), the prevalence of proteinuria was probably underestimated. In all periods studied, patients with PTN above 1000 mg/24 hours, represented the lowest percentage of patients (between 7.5-10%).

The recipient being male was associated with a greater likelihood of PTN in this study. Amer et al. also found that male recipients had higher levels of proteinuria. Conversely, Sancho et al. found a higher likelihood of female recipients developing PTN in a bivariate analysis. However, in the multivariate analysis of the aforementioned study, being a female recipient was not significantly associated with PTN, suggesting that female patients had other risk factors that contributed to their poor outcome, probably for receiving kidneys from female donors and older donors compared to male recipients - two situations in which the nephron mass may have been reduced compared to the ideal donor and could contribute to the increased risk of proteinuria and graft loss. Donor and recipient gender affect the long-term survival rates, especially for elderly donors.

Regarding delayed graft function, with a prevalence of 35% in this study, there was no association with increased risk of post-Tx PTN. Previous studies found DGF and long warm and cold ischemia times associated with an increased risk of PTN.

In the present study, patients with post-Tx hypertension had a higher prevalence of PTN compared to normotensive patients (p = 0.017), and we found post-Tx hypertension in 87.8% of patients. Hypertension affects more than 90% of renal Tx recipients. The literature has pointed to an increased risk of hypertension in the early stages of renal Tx, in the group of patients with PTN > 0.5g/day. Fernandez-Fresnedo et al. also found high blood pressure as a condition associated with post-Tx proteinuria. In other studies, hypertension was an important predictor of PTN. The reduction of renal mass caused by hypertension may contribute to proteinuria. Brenner et al. proposed the hypothesis that a deficit in the number of glomeruli predisposes the recipient to proteinuria, hypertension and progressive kidney disease. In experimental models, it has been shown that the reduced nephron mass is a determinant of proteinuria and long term survival and that hypertension was associated with more severe glomerular hypertensive proteinuria.

In this study, acute rejection was associated with greater proteinuria level only in the bivariate analysis. In a collaborative study in Spain with 3,365 patients undergoing transplant until 1998, episodes of acute rejection were associated with higher proteinuria rates, with similar results in other previous studies. On the other hand, Sancho et al. showed no association between acute rejection episodes and the presence of proteinuria, just like Shin et al. in their series. The authors explain that they included patients receiving immunosuppression, including the use of mycophenolate mofetil and tacrolimus, as well as induction therapy for those deemed to be of greatest immunological risk, which might have prevented more severe rejection and reduced the risk of PTN. In this study, using more modern

Donor and recipient gender affect the long-term survival rates, especially for elderly donors.
and intense immunosuppression, acute rejection was not associated with higher likelihood of developing post-transplant PTN as well as the use of thymoglobulin in the multivariate analysis.

In our study, patients with proteinuria greater than or equal to 500 mg/24 hours showed a renal function at 12 months (measured by creatinine) significantly lower when compared to patients with proteinuria of less than 500 mg/24 hours. This comes to reinforce the importance of the need for measures to control proteinuria in these patients. Sancho et al.³ reported a 5-year graft survival of 69% in patients with proteinuria versus 93% in those without proteinuria, with no difference in patient survival in both groups. Proteinuria is an indicator of renal injury and a risk factor for subsequent decline in renal function in most kidney diseases. Significant proteinuria or nephrotic proteinuria were risk factors for graft loss in the long term.²⁷ Massy et al.²⁹ found that proteinuria greater than 500 mg/day was associated with lower graft survival. According to Fernandez-Fresnedo et al.⁵ proteinuria expressed as a continuous variable was a risk factor for graft failure. Djamali et al.³⁰ reported poor graft survival associated with PTN above 200 mg in 24 hours and according to Amer et al.⁶ even a low level PTN (less than 500 mg) was related to poor graft survival.

There was no difference in the frequency of ACEI/ARB use in patients with and without PTN in this study. This treatment was instituted in only 60% of patients with PTN greater than or equal to 500 mg/24 hours. Blockade of the renin-angiotensin systems causes a decrease in systolic pressure, in intraglomerular capillary pressure, proteinuria and cardiovascular events.³¹ Moreover, this blockage can reduce the development of interstitial fibrosis and tubular atrophy induced by calcineurin inhibitors,³² cause or exacerbate reduction in glomerular filtration rate,³³ exacerbate hyperkalemia, which is a common finding in delayed graft function, and also an adverse event with calcineurin inhibitors, and lower the hematocrit in 5-10%.³⁴ Therefore, it is prudent to wait 3-6 months after the Tx to start an ACE inhibitor or an aldosterone receptor blocker when indicated.

The data is currently available in the literature for the general population suggesting that protein excretion in urine above 1000 mg/day identifies patients who may benefit from therapy with angiotensin converting enzyme inhibitors (ACEI), angiotensin receptor blockers (ARB), while other authors suggest the limit of 500 mg/day.³⁵,³⁶ These drugs can slow the progression of renal disease. According to US guidelines from the Kidney/Dialysis Outcome Quality Initiative (K/DOQI), physicians should aim for a reduction in proteinuria to less than 500-1000 mg/day, in order to slow the progression of CKD by its effects on glomerular hemodynamics (reducing intraglomerular pressure), and changes in podocyte behavior, in view of the toxic effects of proteinuria at a mesangial level, directly proximal tubular toxicity and inflammatory state induction at a tubule-interstitial level.³⁷

Considering the literature recommendations above, we note that not all recipients with PTN above 500 mg/24 hours used RAAS inhibitors, which can potentially contribute to poor graft survival. It is possible that the use of ACEI/ARB in only 60% of patients with PTN above 500 mg/24 hours, is due to the fact that only 5.1 to 10% of patients had PTN above 1000 mg/24 hours in different periods of the study.

In a retrospective cohort study of 2,031 renal transplant recipients in Austria, the authors found a significant improvement in patient survival and overall graft survival in 10 years in recipients who used an ACE inhibitor or an ARB. However, they found no effect on graft survival censored at death (p = 0.57), suggesting that the benefit may be due to a reduction in mortality, instead of preserving graft function.³⁸ On the other hand, there was no significant effect of RAAS inhibitors in patient and graft survival in kidney Tx among 17,209 recipients evaluated by Opelz et al.³⁹ In this study, graft survival was more closely associated with the control of systolic blood pressure at 1 year, regardless of ACEI or ARB that was used. This finding suggests that
blood pressure to be achieved is more important than the antihypertensive agent used to reach the target BP. Therefore, RAAS blockade will reduce proteinuria, but the effects of long-term patient and graft survival remain unknown.40,41

Proteinuria reduction is the target to be achieved in patients with chronic kidney disease under conservative treatment, in kidney transplant patients and even in patients with microalbuminuria (incipient proteinuria), aimed at nephroprotection.14,41,42 Therefore, antiproteinuria measures need to be adopted with greater promptness in renal Tx recipients, in order to obtain better results in terms of function and survival of renal grafts.

In general, renal transplant units recommend the use of annual 24-hour proteinuria assessments or the protein/creatinine ratio in a spot urine sample in the long-term monitoring of renal Tx recipients. Despite this recommendation, in this study we noticed that a considerable number of patients did not undergo this test in the different post-Tx time intervals, and 18 patients were excluded for not having any results of 24-hour PTN during the whole period of follow-up. This can be justified due to difficulties in collecting the 24-hour sample by patients residing in the countryside of the state, the fact that the 24-hour collection is a laborious examination, not well accepted by the patient, or the failure to request the test by the attending physician in transplant year dates.

The gold standard for quantifying proteinuria is the 24-hour urine sample, since protein excretion follows a circadian rhythm. However, the test is uncomfortable and errors in sample collection are frequent, which may negatively influence its interpretation. In cases of difficulty in collecting urine for 24 hours, the albumin/creatinine ratio or protein/creatinine ratio in an isolated urine sample is a good alternative to 24h proteinuria.43

Because this study was retrospective and the data collection was based on chart review, the biggest limitation and risk was to obtain incomplete data, especially regarding the extent of proteinuria, which may be certainly underestimated in its prevalence and association with the variables studied. Another limitation of this study is that it did not include in the analysis some immunological risk variables as the panel reactivity to anti-HLA antibodies (PRA) and the presence of antidonor antibodies (DSA). These factors indicate a greater immunological risk and it has been associated with chronic antibody-mediated rejection, worse graft function and proteinuria.44

Our study did no aim to evaluate the etiology of proteinuria by renal biopsies. Further studies with assessment of renal histology are needed to understand the causes and consequences of PTN as well as the evaluation of the immune factors mentioned above that have not been studied.

It is imperative to stress that the examination of 24-hour proteinuria should be systematically requested by nephrologists and be performed by patients annually. Guidelines from the Kidney Disease Improving Global Outcomes (KDIGO) in the care of renal transplant recipients suggest the measurement of urinary protein excretion within 1 month of transplantation, then every three months in the first year and then annually.45

CONCLUSION

The prevalence of PTN after renal transplant varied according to the definitions used in the study population. The male recipient, transplantation from a living donor and the presence of post-Tx hypertension were associated with a higher likelihood of developing post-renal Tx PTN. Renal function at 12 months (measured by creatinine) was significantly lower in patients with post-transplant PTN.

There was no difference in the frequency of ACEI/ARB use in patients with and without PTN, and this treatment was instituted in 60% of patients with PTN greater than or equal to 500 mg/24 hours.

In conclusion, proteinuria should be assessed in all recipients after renal transplantation, since, in addition to being a marker of kidney disease, it has also been considered a risk factor for the
progression of chronic allograft nephropathy, and cardiovascular disease.

REFERENCES


