



Predictive Value of Interleukin 2 and Interleukin 8 on Early Rejection in Living Related Kidney Transplant Recipients

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ABSTRACT

Introduction. Early diagnosis of rejection in kidney transplant (KTx) recipients is of paramount importance for long-term graft survival. Cytokines play an important role in rejection via activating T cells. Neutrophil accumulation in the graft indicates cell-mediated rejection. Cellular infiltration is mediated through chemoattractant factors. The aim of this study was to investigate the relationship between graft function and serum levels of interleukin 2 (IL-2) and interleukin 8 (IL-8) in KTx.

Method. Sixty-five patients undergoing KTx were enrolled in the study. Serum samples of IL-2 and IL-8 were collected the day before the operation, on postoperative days 1 and 7 day, and during the first and third month after the onset of rejection. The enzyme-linked immunosorbent assay method was used to determine the IL-2 and IL-8 values.

Results. A total of 9 (13.8%) patients had rejection documented on biopsy samples. Fifty-six patients had stable graft function (SGF). IL-2 and IL-8 values before KTx of both the rejected and SGF patients were not statistically different. Univariate analysis revealed that IL-2 and IL-8 were correlated with rejection ($P = .046$, $P = .015$). IL-8 levels were higher in the rejection group compared to the SGF group on the seventh day and first month postoperatively ($P = .023$, $P = .038$). The rejection group maintained higher levels of IL-8 for 11 days (range: 7–30) compared to the SGF group ($P = .002$) and the IL-8 levels correlated with serum creatinine levels ($r = 0.621$, $P = .001$). IL-2 levels were higher in the rejection group on days 1 and 7 compared to the SGF group ($P = .042$, $P = .031$). IL-2 and IL-8 levels were correlated with low eGFR in the third month in the rejection group ($r = 0.421$, $P = .037$; $r = 0.518$, $P = .008$).

Conclusion. Determining the cytokine levels in the early post-KTx period may be helpful in tailoring immunosuppressive regimens in patients with a risk of rejection.

KIDNEY transplantation (KTx) is considered the best treatment of end-stage renal disease. Acute rejection (AR) is one of the most important causes of allograft dysfunction and may lead to early and long-term graft failure in KTx recipients despite rigorous anti-rejection therapy [1]. Factors such as being a younger recipient or an older donor, donor-recipient HLA mismatch, pre-transplant anti-HLA antibodies, panel-reactive antibodies, and the adequacy of baseline immunosuppression

are the major predictors of acute rejection [2]. Transplant renal biopsy is the gold standard for diagnosing AR and guiding appropriate therapy; however, many centers do not

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Table 1. Demographic Data of the Renal Transplant Recipients

	Patients (N = 65)	Rejection (n = 9)	Stable Graft Function (n = 56)	P
Age (mean ± SD)	36.78 ± 12.94	34.67 ± 6.83	37.13 ± 13.68	.601
Female/male, n (%)	25 (38.5)/40 (61.5)	3 (33.3)/6 (66.7)	22 (39.3)/34 (60.7)	.733
Mean follow-up ± SD (months)	26 ± 9	26.87 ± 8.9	25.06 ± 10.5	.766
First transplantation/second transplantation, n (%)	54 (83.1)/11 (16.9)	7 (77.8)/2 (22.2)	47 (83.9)/9 (16.1)	.648
Cold ischemia time (min)	59.62 ± 21.37	51.25 ± 20.79	60.84 ± 21.37	.239
Warm ischemia time (min)	6.23 ± 2.52	5.75 ± 2.31	6.35 ± 2.56	.569
Primary kidney disease, n (%)				
Chronic glomerulonephritis	27 (41.5)	5 (55.6)	22 (39.3)	.214
Tubulo-interstitial nephritis	9 (13.8)	3 (33.3)	6 (10.7)	
Unknown	18 (27.7)	0	18 (32.1)	
Primary nephrosclerosis	4 (6.2)	0	4 (7.1)	
Amyloidosis	1 (1.5)	0	1 (1.8)	
Diabetic nephropathy	6 (9.2)	1 (11.1)	5 (8.9)	

Abbreviation: SD, standard deviation.

routinely consider graft biopsy during the early stages of renal dysfunction [3]. A non-invasive test would facilitate the diagnosis of AR.

Interleukin 8 (IL-8) is a chemoattractant factor produced by mononuclear cells and causes migration of cells such as neutrophils to the injury site. The activation of mononuclear cells increases IL-8 production [4]. Moreover, serum IL-8 levels have been associated with renal allograft function [5]. Interleukin 2 (IL-2) is a pro-inflammatory molecule that plays a role in the induction and effector phases of many immune and inflammatory responses [6]. Both serum IL-2 and IL-8 levels may allow early prediction of allograft injury [7,8]. We investigated whether serum levels of IL-2 and IL-8 had diagnostic value in allograft dysfunction.

MATERIALS AND METHODS

Study Population and Procedures

This prospective cohort study analyzed patients undergoing living related KTx between 2013 and 2017. After ethical committee approval and obtaining informed consent, a total of 65 patients (61.5% male; mean age 36.78 ± 12.94 years) were included. Patients with a follow-up period of less than 3 months, positive complement-dependent cytotoxic cross-match, or flow cytometry were excluded from the study. KTx recipients were on a standard

triple-drug immunosuppressive regimen, consisting of calcineurin inhibitors (tacrolimus) with either mycophenolate mofetil or mycophenolate sodium and a steroid (prednisone).

Study Endpoints

The primary outcome was biopsy-proven rejection during follow-up in KTx recipients.

Collection of the Samples

The blood samples were collected before and after KTx on the first day, seventh day, first month and third month following the procedure and at the time of rejection. The samples were centrifuged at 2600 g for 10 min and stored until analysis day at -80°C. Standard enzyme-linked immunosorbent assay (Invitrogen Diagnostics, Life Technologies, Carlsbad, Calif, United States) was used to detect IL-2 and IL-8 levels.

Clinical variables, including rejection episodes and stable graft function (SGF), were also monitored. A rejection episode was defined based on clinical or biopsy findings according to the Banff criteria [9]. Clinical rejection was identified by increased creatinine levels in the absence of infection, obstruction, or evidence of drug toxicity. AR episodes were treated with a high daily dose of intravenous methylprednisolone (500 mg each dose) for 3 days; in refractory cases, with antithymocyte globulin (2.5–3 mg/kg/day) was administered for 10–12 days.

Table 2. The Characteristics of Patients in Both Rejection and Stable Graft Function Group

	Patients (N = 65)	Rejection (n = 9)	Stable Graft Function (n = 56)	P
Pre-transplant immunologic status				
Pre-transplant anti-HLA antibody status positive/negative, n (%)	15 (21.3)/50 (78.7)	2/7 (22.2/77.8)	13/43 (23.2/76.8)	.948
HLA mismatches	2.12 ± 0.46	2.30 ± 0.48	2.09 ± 0.45	.189
Induction immunosuppressive therapy, n (%)				
Antithymocyte globulin	3 (4.6)	0	3 (5.4)	.135
Simulect (Novartis, Basel, Switzerland)	12 (18.5)	2 (22.2)	10 (17.9)	
Antithymocyte globulin + Simulect	1 (1.5)	1 (11.1)	0	
IVIg + plasmapheresis	1 (2.7)	0	1 (1.8)	
Maintenance immunosuppressive regimen				
Tacrolimus+MMF+prednisone/tacrolimus+MYF+prednisone, n (%)	47/18 (72.3/27.7)	7/2 (77.8/22.2)	40/16 (71.4/28.6)	.693
Post-transplant HLA antibody status				
Positive/negative, n (%)	11/54 (16.9/83.1)	2/7 (22.2/77.8)	9/47 (16.1/83.9)	.609

Abbreviations: IVIG, intravenous immunoglobulin; MMF, mycophenolate mofetil; MYF, mycophenolate sodium.

Table 3. Serum Creatinine, IL-2, and IL-8 Levels

Parameter	Time	Stable Graft		P
		Function (n = 56)	Rejection (n = 9)	
Creatinine (mg/dL)	Pre-KTx	7.64 ± 1.92	6.77 ± 1.78	>.05
	Day 1	1.91 ± 1.07	2.06 ± 0.79	>.05
	Day 7	1.57 ± 0.34	2.71 ± 1.86	.044
	Month 1	1.27 ± 0.39	1.90 ± 2.01	.028
IL-2 (ng/mL)	Pre-KTx	1.20 ± 0.34	1.21 ± 0.37	>.05
	Day 1	6.9 ± 1.1	7.2 ± 2.7	>.05
	Day 7	12.8 ± 3.9	16.2 ± 2.1	.042
	Month 1	10.4 ± 3.7	19.4 ± 4.8	.031
IL-8 (ng/mL)	Pre-KTx	10.1 ± 3.4	15.2 ± 3.8	>.05
	Day 1	11.3 ± 6.6	12.1 ± 2.8	>.05
	Day 7	48.21 ± 10.57	51.27 ± 9.78	>.05
	Month 1	52.74 ± 33.58	56.16 ± 15.32	>.05
	Day 7	79.84 ± 18.54	190.21 ± 35.98	.023
	Month 1	86.74 ± 18.74	137.32 ± 15.55	.038
	Month 3	85.74 ± 15.14	95.87 ± 21.47	>.05

Abbreviations: IL-2, interleukin 2; IL-8, interleukin 8; KTx, kidney transplantation.

Statistical Analysis

Analyses were performed with SPSS 21.0 (IBM, Armonk, NY, United States). Data were given as mean ± standard deviation and the means were compared using the Student's *t*-test. One-way analysis of variance (post hoc) tests were used to analyze the groups. Fisher's exact test was used for categorical values and a multivariate regression analysis was performed for demographic variables. Correlations were calculated using Spearman's test and *P* values < .05 were considered statistically significant.

RESULTS

A total of 65 patients who underwent living related-donor KTx were included in the study. Demographic and clinical characteristics of KTx recipients are summarized in Tables 1 and 2.

All patients were classified according to graft function. Serum creatinine levels were higher in the rejection than the SGF group at the seventh day and first month (*P* = .044 and *P* = .028 respectively). No significant differences between

Table 4. Univariate Regression Analysis of Rejection Risk Factors

Factors	Adjusted OR	Confidence Interval (%)	P
Age	1.67	2.60–4.67	.397
Sex	1.29	2.29–5.21	.734
Warm ischemia	1.06	2.75–3.84	.102
Cold ischemia	0.98	1.18–4.31	.777
Anti HLA antibody	1.11	2.26–4.78	.308
HLA mismatch	1.08	0.65–4.31	.094
Immunosuppressive regimen	0.71	0.29–2.89	.694
Need for dialysis	1.16	0.84–5.47	.135
IL-2	3.14	1.98–6.78	.046
IL-8	5.38	1.54–9.01	.015

Abbreviations: IL-2, interleukin 2; IL-8, interleukin 8; OR, odds ratio.

other periods were observed (Table 3). There was no relationship between age, warm and cold ischemia time, or number of HLA mismatches with allograft outcomes for each group on univariate analysis (*P* > .05). Nevertheless, IL-2 and IL-8 were predictive for AR (*P* = .046 and *P* = .015, respectively) (Table 4). The positive and negative predictive values for IL-2 and IL-8 before and after KTx were assessed by receiver operating characteristic analysis and are shown in Table 5.

Serum levels of IL-2 were higher on the first and seventh days following KTx in the AR group (*P* = .042 and *P* = .031, respectively). Meanwhile, IL-8 levels were higher on the seventh day and first month in the AR group (*P* = .023 and *P* = .038, respectively). The serum IL-8 levels remained high for an average of 11 days (range: 7–30) in the AR group compared to the SGF group (*P* = .002). The IL-8 levels correlated with serum creatinine levels (*r* = 0.621, *P* = .001) and the serum IL-2 and IL-8 levels were correlated with low eGFR on the third month in the AR group (*r* = 0.421, *P* = .037; *r* = 0.518, *P* = .008).

DISCUSSION

AR following KTx is still a major clinical problem affecting both patient and graft survival. Most ARs occur within few weeks after KTx [10]. Active and immediate evaluation of changes in graft function is of utmost importance in KTx recipients, especially during the early period. Even though early AR episodes may be predicted by markers and prevented with therapeutic interventions, they remain a major risk factor for subsequent graft dysfunction [11].

Of the renal allograft rejection cases, 66.7% occurred within the first month and 83% within 3 months after KTx. Early rejection episodes, predicted by urinary or serum markers, may be prevented with appropriate immunosuppressive regimens [12]. Researchers have reported that increased serum concentrations of IL-8 on day 1 was negatively correlated with creatinine clearance early after KTx [13]. Kwon et al examined the IL-8 content in urine sampled on day 0 after renal transplantation and observed that patients with high IL-8 concentrations showed characteristics of acute renal failure [14]. Other researchers found serum IL-8 to be negatively correlated with eGFR [15].

This study has demonstrated that serum IL-8 levels correlate with allograft rejection in the early post-KTx period. We studied the association of IL-8 levels in serum with kidney allograft survival. In this study, we analyzed 65 KTx recipients with a mean 26 months of follow-up. Nine patients had biopsy-proven rejection; we found that of IL-8 levels significantly increased in the serum of patients in the rejection group on the seventh day and in the first month.

Trailin et al evaluated serum and urinary IL-2 and demonstrated that higher urinary levels of IL-2 in the late post-KTx period is associated with declining kidney allograft function and with lower eGFR at baseline and during the first and the second year of follow-up [15]. They also

Table 5. Predictive Value of IL-2 and IL-8 in Rejection

Period	Predictors, ng/mL	AUC (95% CI)	PPV	NPV	Sensitivity	Specificity	P
Pre-KTx	IL-2	0.716 (0.685-0.883)	0.48	0.53	0.71	0.62	.684
Day 1	IL-2	0.901 (0.725-0.985)	0.64	0.78	0.83	0.74	.041
Day 7	IL-2	0.881 (0.638-0.901)	0.59	0.81	0.85	0.78	.028
Month 1	IL-2	0.824 (0.624-0.892)	0.41	0.57	0.73	0.72	.248
Month 3	IL-2	0.604 (0.538-0.714)	0.39	0.46	0.69	0.65	.864
Pre-KTx	IL-8	0.685 (0.536-0.735)	0.39	0.61	0.68	0.61	.725
Day 1	IL-8	0.797 (0.723-0.894)	0.59	0.72	0.73	0.69	.542
Day 7	IL-8	0.764 (0.547-0.935)	0.68	0.77	0.88	0.37	.038
Month 1	IL-8	0.706 (0.647-0.876)	0.53	0.75	0.81	0.77	.046
Month 3	IL-8	0.607 (0.538-0.839)	0.51	0.64	0.72	0.74	.367

Abbreviations: AUC, area under the curve; CI, confidence interval; IL-2, interleukin 2; IL-8, interleukin 8; KTx, kidney transplantation; NPV, negative predictive value; PPV, positive predictive value.

assumed that urinary IL-2 was superior to serum IL-2 [15]. Jin et al found IL-2 expression to be significantly higher in the AR group compared to the SGF group [16]. Other researchers found intracellular expression of IL-2 in AR patients [16]. However, we found serum IL-2 levels significantly increased in AR patients on the first and seventh days. We also found that both serum IL-2 and IL-8 levels were correlated with low eGFR in the third month in the AR group.

In this study, we have seen that clinical variables such as sex, age, HLA compatibility, anti-HLA antibody, and immunosuppressive regimen have no significant effects on rejection. However, Ding et al examined whether clinical variables such as panel reactive antibody levels had significant effects on acute rejection and found that a panel reactive antibody level >10% had significant influence on acute rejection [17].

This study has some drawbacks that merit mentioning. First, we have evaluated only serum levels of IL-2 and IL-6, not urinary levels. Second, this was a single-center study carried out on a small group of Caucasian patients and limited to 26 months of follow-up. Therefore, the conclusions should be replicated in a longer study with a larger cohort representative of the general population of KTx recipients.

CONCLUSION

Our study shows that determining the cytokine levels in the early post-KTx period may be helpful in tailoring immunosuppressive regimens in patients with a risk of rejection. Measuring IL-2 and IL-8 in the serum may be a surrogate marker of the immunosuppressive status of the KTx recipients.

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