

## Serum & urinary interleukin-2 levels as predictors in acute renal allograft rejection

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**Background & objectives:** In spite of potent immunosuppression, acute rejection continues to be the single largest cause of graft dysfunction after renal transplantation. Renal allograft biopsy, though invasive, continues to be the reference standard, though certain clinical and biochemical parameters are helpful in assessment of these patients. Acute renal allograft rejection is mediated by T lymphocytes, which express increased number of interleukin-2 receptors (IL-2R). The soluble component of IL-2R in serum and urine may be useful in detecting early graft rejection. This study assesses the possibility of using serum and urinary IL-2R estimation in early prediction and diagnosis of acute renal allograft rejection.

**Methods:** Sequential estimation of serum and urinary IL-2R levels along with serum creatinine values were assessed in 23 live related renal allograft recipients. The age of renal allograft recipients was  $35 \pm 8.3$  yr, with male : female ratio of 22 : 1. Samples were collected pre-transplant (day 0) and post-transplant upto 30 days and the patients were followed for 6 months after transplantation. Eight recipients experienced graft dysfunction and graft biopsies were evaluated.

**Results:** Serum and urinary IL-2R patterns along with serum creatinine levels were correlated with the occurrence of graft rejection on histology. Eight recipients experienced acute graft rejection after transplantation and 15 had stable graft function. Serum IL-2R levels at various periods after transplantation were found to be significantly ( $P < 0.05$ ) elevated in graft recipients experiencing acute rejection as compared to the non-rejection group. The rise in urinary IL-2R levels in some of the rejection group recipients, was not statistically significant.

**Interpretation & conclusion:** Sequential serum and urinary IL-2R assay may serve as a predictor for early graft dysfunction. Study with larger sample size and for longer duration is required to further validate the results.

**Key words** Interleukin-2 receptors - renal allograft rejection - T cell dysfunction - transplantation

Renal transplantation offers a definitive therapeutic modality for patients with end stage renal disease; however, 50 to 70 per cent of these patients suffer graft dysfunction after transplantation<sup>1</sup>. Despite potent immunosuppression, acute rejection continues to be the single largest cause of graft dysfunction in majority of patients. Cyclosporine toxicity, acute tubular necrosis (ATN) and infections

may also contribute to the causation of graft dysfunction in some of these patients<sup>1</sup>. Apart from clinical indicators of the graft dysfunction such as increased body weight, decreased urine output, graft tenderness, raised serum creatinine and diethylene triamine penta acetic acid (DTPA) scan, the renal allograft biopsy continues to be the reference standard. Being invasive, the procedure is associated

with potential risk. Fine needle aspirates have a lower risk, but being of limited usefulness, have not been widely applied.

Acute renal allograft rejection is mediated by T lymphocytes. T cells expressing cell surface interleukin-2 receptors (IL-2R) were found in the kidneys of renal allograft recipients<sup>2</sup>. On antigenic challenge, T cells in the graft express increased number of IL-2R. The component of the receptor released in soluble form as a consequence of activation is termed as soluble IL-2R (sIL-2R)<sup>3,4</sup>. The soluble IL-2R retains immuno-reactivity and ability to bind with IL-2, but it is about 55kD smaller than the membrane bound form<sup>5</sup>. The IL-2R is of considerable diagnostic and therapeutic interest in clinical transplantation. Monoclonal antibodies to IL-2R inhibit T cell proliferation<sup>6</sup> and prevent rejection of renal allografts<sup>7</sup>. The immunosuppressive potential of cyclosporine is critically related to its capacity to impair the production of IL-2. Initial clinical studies indicate that the soluble form of IL-2R is released in the serum and urine of renal allograft recipients experiencing acute rejection<sup>8,9</sup>, which can be measured directly by utilizing antibody against IL-2R<sup>10</sup>.

The development of a reliable, non-invasive and economical diagnostic test for graft rejection has so far been an elusive goal. The present study was undertaken to assess the possibility of using serum and urinary IL-2R estimation in early prediction and diagnosis of acute renal allograft rejection.

### Material & Methods

The study was performed on 40 consecutive live related renal allograft recipients during January - June 1999 at Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow, a tertiary care hospital in north India. Post-transplant, all patients received triple drug immunosuppression consisting of prednisolone, azathioprine and cyclosporine. Sequential serum (5 ml) and urinary (10 ml) samples were collected pretransplant and on post-transplant days 1, 5, 10 and 30 in 23 patients; in the remaining 17 patients, sequential samples could not be collected hence excluded from the study. Serum and urinary samples were aliquoted

and snap frozen at  $-40^{\circ}$  C for estimation of soluble human IL-2R using enzyme immunoassay kit (Immunotech, France). The results were expressed in pM; normal reference range of serum IL-2R was  $70\pm 45$ pM (Immunotech, France).

All renal allograft recipients were followed for a period of 6 months after transplantation. Episode(s) of graft dysfunction, if any, were recorded; 8 of 23 experienced graft dysfunction during this period and graft biopsies were received for evaluation in all of them. Serum and urinary IL-2R patterns were correlated with the occurrence of graft rejection on histology (Table II). Serial serum creatinine estimation by alkaline picrate method<sup>11</sup> using Jaffe's principle, was also performed up to 6 months post-transplant in all cases.

The study protocol was approved by the ethics committee of the institute. Data were analyzed using student's t-test for significance and Kaplan Meier survival curve using log rank test.

### Results & Discussion

The age of renal allograft recipients ranged from 18 to 48 yr, ( $35\pm 8.3$ , mean $\pm$ SD yr), 22 of them were males and one was female. Eight renal allograft recipients experienced acute graft dysfunction within 3 to 16 days after transplantation whereas 15 had stable graft function with no evidence of acute rejection, infection or cyclosporine toxicity during 6 months post-transplantation. Histological evaluation of renal allograft biopsies obtained from 8 patients experiencing acute graft dysfunction revealed acute rejection (5 had acute cellular rejection and 3 had acute vascular rejection).

The patients experiencing acute renal allograft rejection showed higher concentration of serum IL-2R at various periods after transplantation as compared to the non-rejection group and the rise was statistically significant ( $P < 0.05$ , Table I). Though, urinary IL-2R levels were relatively higher at various periods after transplantation in patients experiencing rejection compared to non-rejection group, the difference was not statistically significant (Table I).

**Table I.** Serum and urinary IL-2R levels in renal allograft recipients

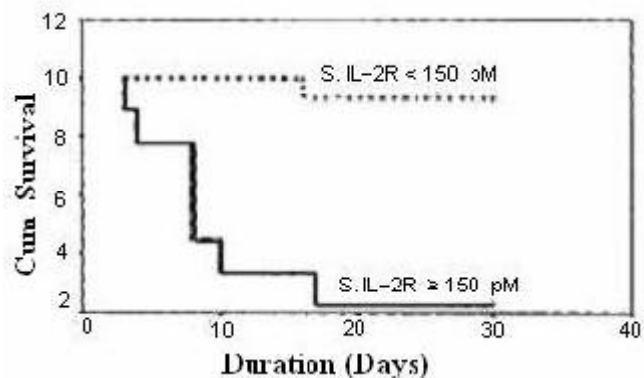
Day post-transplant	Serum IL-2R (pM)		Urinary IL-2R (pM)	
	Rejection group (n=8)	Non rejection group (n=15)	Rejection group (n=8)	Non rejection group (n=15)
0 (pre-transplant)	180.9±51.0*	126±41.2	33.3 ± 7.5	22.1±19.3
1	172.0±36.6*	95.4±52.3	28.3 ± 15.0	12.2±18.0
5	148.5±59.0*	76.7±34.5	48.3 ± 42.0	34.7±51.4
10	182.5±26.5*	68.4±47.8	21.0±19.2	19.2±10.1
30	159.3±90.1	56.5±40.5	13.6±4.2	13.1±18.6

\*  $P < 0.05$  compared to non rejection group

Survival curves were constructed with random pre-rejection IL-2R levels (cut-off value of  $\geq 150$  pM of serum IL-2R was taken as high value). There was more chance for the graft rejection with serum IL-2R level of  $\geq 150$  pM during pre-rejection period (Figure).

Serum creatinine estimations performed at various periods after transplantation in the patients revealed that immediate pre-transplant (day 0) serum creatinine levels in those enjoying stable graft function were 1.6 to 6.6 mg/dl (mean±SD = 4.89±1.96) whereas, those experiencing acute graft rejection after transplantation had creatinine levels of 5.2 to 9.2 mg/dl (mean±SD=7.15±1.40) which were significantly higher ( $P < 0.05$ ) as compared to non-rejection group (Table II).

Post transplant changes in serum and urinary IL-2R in patients experiencing graft rejection have earlier



**Fig.** Kaplan Meier survival curve showing graft survival in patients with pre-rejection serum IL-2R  $\geq 150$  pM.

been reported by Colvin<sup>8,12</sup>, however, urinary IL-2R levels were much variable. The rise in serum IL-2R is not specific to transplant rejection as raised serum IL-2R levels may be encountered in other conditions associated with T cell activation such as early stages of HIV, seropositivity<sup>13</sup>, IL-2 therapy<sup>14</sup>, post-transplant viral infections<sup>15,16</sup> and autoimmune diseases<sup>17</sup>. In our study none of the patients had any other cause of graft dysfunction during the first 6 months such as infections or cyclosporine toxicity and none of them received IL-2R therapy for graft rejection. Serum levels of IL-2R may be elevated due to poor clearance of IL-2R by the kidney graft<sup>18</sup>. Further studies need to be done to study this aspect.

**Table II.** Serum creatinine (mg/dl) in renal allograft recipients

Day post-transplant	Rejection group (n=8)	Non-rejection group (n=15)
0 (pre-transplant)	7.15±1.40*	4.89±1.96
1	2.88±0.90	2.34±1.04
5	2.60±1.34	1.57±0.45
10	2.88±1.62	1.46±0.24
30	1.15±0.23	1.16±0.24
45	1.20±0.26	1.07±0.17
60	1.17±0.41	1.06±0.15
90	0.95±0.21	1.10±0.21
120	1.37±0.52	0.97±0.32
180	1.30±0.24	1.13±0.18

\*  $P < 0.05$  compared to non-rejection group

The present observations indicate that serum IL-2R may be a diagnostic and prognostic indicator in patients experiencing acute renal allograft rejection, however, the findings need further validation by studying large number of patients for a longer period before making any recommendations.

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