Evaluation of serum interleukin 6 and tumour necrosis factor alpha levels, and their association with various non-immunological parameters in renal transplant recipients

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INTRODUCTION
Renal transplant rejection involves both immunological and non-immunological factors. The objective of the present study was to investigate the association between immunological factors, such as serum interleukin 6 (IL-6) and tumour necrosis factor alpha (TNF-α), and non-immunological parameters, such as age, serum creatinine (Scr), creatinine clearance (CrCl) and dyslipidaemia, in renal transplant recipients (RTRs).

METHODS
This study included 90 RTRs and 90 healthy controls. Biochemical parameters, including serum IL-6 and TNF-α, were estimated using standard protocols. CrCl was calculated using the Cockcroft-Gault equation, and the type of rejection was confirmed on biopsy. Student’s t-test and univariate and multivariate analyses were performed using the Statistical Package for the Social Sciences for Windows version 15.

RESULTS
The mean levels of serum IL-6 and TNF-α were significantly higher in RTRs than in the control group (p < 0.001). These parameters were also found to be significantly different between the transplant rejection (TR) and transplant stable (TS) groups (p < 0.001). CrCl was significantly decreased in the TR group when compared to the TS group (p < 0.001). The two cytokines, IL-6 and TNF-α, correlated significantly with all metabolic parameters, such as Scr, CrCl and dyslipidaemia. Multiple regression analysis showed that TNF-α and CrCl were the strongest predictors of IL-6.

CONCLUSION
We conclude that immunological factors, as well as non-immunological factors such as CrCl, Scr and dyslipidaemia, play important roles in the pathogenesis of graft rejection and renal graft dysfunction.

Keywords: creatinine clearance, interleukin 6, renal transplant recipients, total cholesterol, tumour necrosis factor alpha
In a three-year period from January 2008 to December 2010, a total of 180 subjects were enrolled in this study. The study included 90 RTRs (mean age 32.33 ± 8.55 years) and 90 unrelated healthy controls (mean age 30.40 ± 8.04 years). The RTRs were recruited from the inpatient and outpatient services at the Department of Nephrology, Institute of Medical Sciences, Banaras Hindu University, Varanasi, India, and the unrelated healthy controls were age- and sex-matched staff/employees from our institute. Among the 90 RTRs, 50 were transplant stable (TS) with a mean age of 31.52 ± 9.48 years, and 40 experienced transplant rejection (TR) and had a mean age of 33.35 ± 7.35 years. The TR group included 26 patients with AR and 14 with CAI. All RTRs were maintained on triple immunosuppressive therapy with cyclosporine A, azathioprine and prednisone. The mean time period from the day of transplantation to the day of transplant rejection was 20.15 ± 8.80 (range 6–36) months. AR was defined based on a decline in renal function for at least three months after transplantation, and was confirmed on biopsy. CAI was defined based on clinical criteria, elevation of SCr, reduction in urine output and response to antirejection therapy, and confirmed on biopsy. CAI was defined based on a decline in renal function for at least three months after transplantation, and was confirmed on biopsy (features of tubular atrophy, interstitial fibrosis, arteriosclerosis and glomerulosclerosis were observed). Patients with CAI due to post-transplant hypertension, recurrent disease and drug toxicity were excluded from the study. Informed consent was obtained from all enrolled patients and the study was approved by the ethical committee of the institute. This study adhered to the principles of the Declaration of Helsinki.

METHODS

RESULTS

Age, taken as a demographic parameter, showed no significant difference among the various groups (Table I). In the RTRs, the metabolic parameters SCr and CrCl were significantly raised and lowered, respectively, when compared to the control group. Although the SCr of the TS group was within normal limits, it was still slightly higher than that of the control group. SCr was highest in the TR group, with significantly higher values in patients with CAI than those with AR. CrCl was significantly lowered in the RTRs when compared to the control group. Although CrCl in the TS and control groups did not show significant variation, the variation seen was greater than that in the TR group. Within the TR group, CrCl was significantly decreased in patients with CAI when compared to those with AR.

The level of the two cytokines (i.e. serum IL-6 and serum TNF-α) evaluated in this study were higher in the RTRs than the control group. The levels of both serum IL-6 and serum TNF-α correlated with clinical and histological findings. SCr, total cholesterol (TC), high-density lipoprotein (HDL) and low-density lipoprotein (LDL) levels were assayed using standard methodology. CrCl was calculated using the Cockcroft-Gault equation. All biopsies were reviewed by a renal pathologist, and rejections that were confirmed on biopsy were classified according to the Banff ’05 criteria.

Data was reported as mean ± standard deviation. Statistical analysis was performed using the Statistical Package for the Social Sciences for Windows version 15 (SPSS Inc, Chicago, IL, USA). Student’s t-test was used to compare the means of different groups and ascertain significance. Univariate analysis was used to study the relationship between the variables. Multiple regression analysis was used to determine the independent factors affecting dependent variables. A p-value of < 0.05 was considered statistically significant. 

Table I. Demographics and clinical characteristics of patients.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Control group (n = 90)</th>
<th>Transplant stable (n = 50)</th>
<th>Renal transplant recipients (n = 90)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>30.40 ± 8.04</td>
<td>31.52 ± 9.48</td>
<td>33.35 ± 7.35</td>
</tr>
<tr>
<td>SCr (mg/dL)</td>
<td>0.95 ± 0.17</td>
<td>1.16 ± 0.21*</td>
<td>2.12 ± 0.68*</td>
</tr>
<tr>
<td>CrCl (ml/min/1.73 m²)</td>
<td>85.00 ± 16.09</td>
<td>79.37 ± 14.33</td>
<td>45.76 ± 13.06*</td>
</tr>
<tr>
<td>Cytokine (mg/mL)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum IL-6</td>
<td>7.51 ± 2.63</td>
<td>8.96 ± 3.96</td>
<td>47.80 ± 20.10*</td>
</tr>
<tr>
<td>Serum TNF-α</td>
<td>6.62 ± 2.57</td>
<td>13.24 ± 3.33*</td>
<td>47.65 ± 26.85*</td>
</tr>
<tr>
<td>Lipid profile (mg/dL)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TC</td>
<td>167.11 ± 30.07</td>
<td>135.20 ± 14.26*</td>
<td>161.85 ± 28.76*</td>
</tr>
<tr>
<td>HDL</td>
<td>46.37 ± 7.37</td>
<td>43.56 ± 8.89</td>
<td>37.65 ± 5.32*</td>
</tr>
<tr>
<td>LDL</td>
<td>103.76 ± 25.60</td>
<td>75.48 ± 12.15*</td>
<td>99.75 ± 26.70*</td>
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<td></td>
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</tbody>
</table>

Data is presented as mean ± standard deviation.

*p < 0.001 vs. control group. †p < 0.001 vs. TS group. §p < 0.01 vs. TS group. §p-values for AR group vs. CAI group.

CrCl: creatinine clearance; HDL: high-density lipoprotein; IL-6: interleukin 6; LDL: low-density lipoprotein; NS: not significant; SCr: serum creatinine; SD: standard deviation; TC: total cholesterol; TNF-α: tumour necrosis factor alpha.
TNF-α were significantly elevated in patients from the TR group when compared to those from the TS and control groups. However, when the cytokine levels were compared between the TS and control groups, only serum TNF-α was significantly elevated in the TS group. When the difference between the cytokine levels of patients with AR and those with CAI were analysed, both IL-6 and TNF-α were found to be higher in patients with CAI (Table I).

The lipid profile parameters evaluated in our study were TC, HDL and LDL. The levels of all these parameters were lower in the RTRs than in the control group. Similar trends were observed when the RTRs were split into TS and TR groups and the respective group’s lipid profile parameters were compared with the control group. When compared to the TS and control groups, the TR group had a significantly lower HDL level, while both TC and LDL levels were within normal limits. There was a greater reduction in HDL level in patients with CAI than in those with AR, although the difference was not significant (Table I).

In the RTRs, univariate analysis showed that serum IL-6 and TNF-α levels correlated significantly with metabolic parameters and dyslipidaemia (Table II). Multiple linear regression analysis was performed using all significant factors identified on univariate analysis so as to account for the influence of any confounding factors. The model predicted 87% ($R^2 = 0.93$) of variance for serum IL-6 on the basis of these factors ($p < 0.001$). Serum TNF-α ($\beta = 0.587$, $p = 0.001$) and CrCl ($\beta = 0.308$, $p = 0.03$) were the strongest predictors of serum IL-6, while the other variables were not (Table III).

**DISCUSSION**

Renal allograft rejection involves a network of cytokines, growth factors and co-stimulatory molecules. Several cytokines play an important role in the activation of immune responses. Our study showed a significant increase in the level of cytokines in RTRs, with both serum IL-6 and TNF-α levels being significantly higher than that in the control group. In our study, the mean serum IL-6 level in the control group was $7.51 \pm 2.63$ pg/mL. Similarly, studies by Waiser et al.\(^{[20]}\) and Kamimura et al.\(^{[21]}\) found slightly lower levels of IL-6 in controls, with reported mean values of 3.5 ± 1.1 pg/mL and 0.24 ± 0.05 pg/mL, respectively. We also found that the elevation of serum TNF-α was significantly higher in both the TS and TR groups. This finding is consistent with other studies, which reported significantly lower serum TNF-α levels in controls than in transplant recipients experiencing organ rejection.\(^{[8,10,22]}\)

We postulate that injury to the endothelium might trigger the release of IL-6 in RTRs. A German study reported that when compared to controls, both the levels of serum IL-6 in patients undergoing episodes of rejection and the levels of IL-6 in biopsy tissues of kidneys undergoing rejection were elevated.\(^{[19]}\) This increase in IL-6 could be the result of increased activation of nuclear factor-kappa B, which is an inducible transplantation factor essential for the activation of several important inflammatory cytokine genes such as the IL-6 and IL-8 genes.\(^{[23,24]}\)

Lipid abnormalities are very common in patients undergoing rejection, and hyperlipidaemia or dyslipidaemia is an important non-immunological risk factor for the rejection of grafts. High cholesterol, high LDL and elevated triglycerides have been reported in RTRs, before and after transplant.\(^{[25,26]}\) The elevation of cytokines and lipids indicates that RTRs are not only characterised by persistent immune alloactivation, but also by lipid metabolism disorders. In our study, the serum IL-6, TNF-α, TC and LDL levels of patients with CAI were significantly higher than those in patients with AR.

Lipid levels are modulated by various mechanisms.\(^{[29]}\) Reports suggest that oxidised LDL may lead to increased production of proinflammatory cytokines and chemokines by macrophages and the endothelium.\(^{[30,31]}\) A study by Güllestad et al. on the role of IL-6 in heart allograft CAD reported an increase in IL-6 and LDL in patients with transplant CAD.\(^{[22]}\) In that study, the authors also showed that

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**Table II. Correlation between immunological and non-immunological factors in renal transplant recipients.**

<table>
<thead>
<tr>
<th>Non-immunological factor</th>
<th>Serum IL-6</th>
<th>Serum TNF-α</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>$0.23$</td>
<td>$0.21$</td>
</tr>
<tr>
<td>Scr</td>
<td>$0.89$</td>
<td>$0.92$</td>
</tr>
<tr>
<td>CrCl</td>
<td>$-0.83$</td>
<td>$-0.77$</td>
</tr>
<tr>
<td>TC</td>
<td>$0.65$</td>
<td>$0.61$</td>
</tr>
<tr>
<td>HDL</td>
<td>$-0.35$</td>
<td>$-0.39$</td>
</tr>
<tr>
<td>LDL</td>
<td>$0.65$</td>
<td>$0.60$</td>
</tr>
</tbody>
</table>

*p-value* $< 0.05$ was considered statistically significant.
CrCl: creatinine clearance; HDL: high-density lipoprotein; IL-6: interleukin 6; LDL: low-density lipoprotein; r: correlation coefficient; Scr: serum creatinine; TC: total cholesterol; TNF-α: tumour necrosis factor alpha.

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**Table III. Multiple regression analysis for predictors of serum creatinine in renal transplant recipients.**

<table>
<thead>
<tr>
<th>Non-immunological factor</th>
<th>Unstandardised coefficients</th>
<th>Standardised coefficient $\beta$</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>$0.204$</td>
<td>$0.177$</td>
<td>$0.073$</td>
</tr>
<tr>
<td>TNF-α</td>
<td>$0.561$</td>
<td>$0.157$</td>
<td>$0.587$</td>
</tr>
<tr>
<td>Scr</td>
<td>$0.887$</td>
<td>$7.954$</td>
<td>$0.025$</td>
</tr>
<tr>
<td>CrCl</td>
<td>$-0.337$</td>
<td>$0.151$</td>
<td>$0.308$</td>
</tr>
<tr>
<td>TC</td>
<td>$0.118$</td>
<td>$0.085$</td>
<td>$0.126$</td>
</tr>
<tr>
<td>HDL</td>
<td>$0.152$</td>
<td>$0.215$</td>
<td>$0.051$</td>
</tr>
</tbody>
</table>

Note: Age, serum interleukin 6, glomerular filtration rate, total cholesterol, high-density lipoprotein and low-density lipoprotein were taken as independent variables. Low-density lipoprotein reached tolerance limit zero.
CrCl: creatinine clearance; HDL: high-density lipoprotein; Scr: serum creatinine; SE: standard error; TC: total cholesterol; TNF-α: tumour necrosis factor alpha.
heart transplant patients with coronary dysfunction leading to heart allograft rejection have lower HDL levels, and higher TC and LDL levels than controls and patients with stable allografts. These findings are similar to that of our study on RTRs. We also found lower HDL levels, and higher TC and LDL levels in the TR group when compared to the TS group. Gullesstad et al further reported an inverse correlation between HDL level and the levels of TNF-α and IL-6 in their cohort of patients with or without allograft rejection, which is similar to what was observed in our patients with or without transplant rejection.

A complex interrelation exists between cytokines and lipids. Studies have suggested that IL-6 may cause an increase in circulating lipid levels, probably through a decrease in peripheral lipoprotein lipase. In our study, HDL levels showed negative significant correlation with IL-6 and TNF-α, and were higher in the control and TS groups than in the TR group. During graft rejection, endothelium activation leads to the expression of adhesion molecules such as intercellular adhesion molecule 1, vascular cell adhesion molecule 1 and E-selectin. HDL disrupts the sphingosine kinase signalling pathway, resulting in the inhibition of the endothelium, which protects against the formation of atherosclerotic plaques in the blood vessels of the graft.

Endothelium-derived cytokines play an important role in several diseases, including atherosclerosis, graft rejection, asthma, vasculitis and sepsis. Chen et al suggested that TNF-α might be a potent regulator of lipid metabolism. Kario et al showed that a close relationship between high lipoprotein levels and IL-6 is present in haemodialysis patients. IL-6 is responsible for increased hepatic production of fatty acids, triglycerides and cholesterol. Our finding that hypercholesterolemia is an important risk factor for renal graft rejection is in agreement with the study by Swan. The elevation of serum IL-6 and TNF-α levels in RTRs therefore appears to be associated with dyslipidaemia. In our study, we obtained a negative significant correlation between IL-6 and TNF-α levels, and CrCl, thus indicating that these cytokines are associated with declining renal function. Similar results were also reported by other studies on patients with renal transplant rejection and chronic renal failure.

In conclusion, immunological factors, as well as non-immunological factors such as SCr, CrCl and dyslipidaemia, play important roles in renal graft dysfunction and the pathogenesis of graft rejection.

REFERENCES