Original Article

Evaluation of Pre transplant T-Cell activation status by soluble CD 30 determination

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Abstract

Objective: To evaluate the utility of serum CD30 (sCD30) levels as predictor of early acute graft rejection in live related renal transplant programme.

Methods: This prospective study included 50 consecutive renal transplant recipients who received their first live related renal allograft at the Sindh Institute of Urology and Transplantation (SIUT) between October 2006 and March 2007. Blood samples were obtained one day before transplantation and on the third and fourteenth post-transplant days. Blood samples were also obtained from 50, age and sex matched healthy control individuals. Levels of serum sCD30 were measured by Enzyme Linked Immunosorbent Assay (ELISA).

Result: Donor-recipient blood group matching was identical in all patients. Pre-transplant lymphocyte crossmatch for T and B cells was negative, and panel reactive antibodies (PRA) were 0% for all recipients. The mean age of recipients was 31.6± 10.23 years (range 5 to 55 years), while mean donor age was 32.74± 8.48 years (range 21-50 years). Eleven (22%) recipients and donors were HLA identical while remaining (78%) were one haplotype match. Average serum sCD30 pre-transplant levels (37.8±4.97U/ml) were significantly higher than those of healthy individual's mean value of 8.48± 4.97 U/ml, (P = 0.001). Eight (16%) patients developed acute rejection episode during this follow up period. Rejections were described and classified according to BANFF 97 classification.

Conclusion: In this small single center study the serum levels of sCD30 did not show any significant difference between rejection and non rejection group in our transplant population (JPMA 59:212; 2009).

Introduction

Kidney transplantation world wide is an established therapy of choice for end stage renal failure. Over the last few decades, improvements in surgical techniques, availability of better diagnostic and therapeutic tools for infections and immune monitoring have resulted in overall improved outcome in renal transplantation. Despite these advances, acute rejection remains a major limitation to successful outcome of kidney transplantation.[1] Low rates of rejection obtained in kidney transplantation can be credited primarily to the availability of more effective immunosuppressive agents and treatment regimens.[2] However, effective immunosupression often results in increased incidence of viral and other complications such as polyoma virus infections, cytomegalovirus (CMV) associated complications, and post transplant lymphoproliferative disorders.[3,4] Hence pre and post transplant risk estimation in kidney transplantation is important for selection of appropriate treatment strategies.

More potent immunosuppressive agents may be used in patients with higher risk of rejection. HLA matching and pre-transplant antibody status are well established parameters predicting increased risk of early rejections as well as long term graft survival.[5-7] Graft biopsy though considered the 'gold standard' has its own limitations of invasiveness and related complications.[8] Non invasive tools for diagnosis of rejection and prediction of outcome from rejection have been described with urinary mRNA levels of Granzyme B, Perforin and FOXP3.[9-11]

More recently higher levels of soluble CD30 (sCD30) in serum have been reported as a biomarker for prediction of early graft rejection.[12,13] The CD30 molecule, a member of the tumour necrosis factor receptor superfamily, is a 120 kD membrane glycoprotein recognized originally on Reed-Sternberg cells of Hodgkin's lymphoma.[14] CD30 is mainly expressed on activated T cells that secrete Th2 type cytokines. After activation of CD30 + T cells, sCD30 is released into the circulation.

The accuracy of pre-transplant panel reactive antibodies (PRA) and serum levels of sCD30 in predicting early (<6 months) acute rejection in living and deceased donor kidney transplant has been studied[15,16] where sCD30 serum levels have been shown to be more accurate predictor of early post transplant acute rejection.[15,16] A multi-center study reported a significant association between high

sCD30 levels and increased graft rejections.[17] The aim of the present study was to evaluate the usefulness of serum sCD30 as a predictor of acute graft rejection in a live related donor renal transplant programme.

Patients and Methods

Blood samples were taken from 50 consecutive transplants recipients. Individuals undergoing a second transplant, recipients who developed post transplant CMV infection and those who received mono/ or polyclonal antibody induction were excluded from the study. Samples were also obtained from 50 age and sex matched healthy individuals (normal controls).

Lymphocyte crossmatch and PRA of patients were determined by microlymphocytotoxicity assay. HLA Class I typing was done using 120 well trays from Collaborative Transplant Study (CTS) Heidelberg Germany. Molecular typing of Class II HLA DR was done using sequence specific primers (SSP) from CTS Heidelberg Germany.

Maintenance immunosuppressive regimen was standard triple therapy which consisted of a calcineurin inhibitor (CsA microemulsion) combined with prednisone and azathioprine. Blood samples were obtained one day before transplant and on the 3rd and 14th post transplant days. Serum was separated within three hours of blood collection and stored at -80ºC until testing. Human sCD30 instant ELISA kits were obtained from Bender Medsystem (Vienna Austria) and used according to the manufacturer's instructions.

All recipients were followed closely during the first six months post transplant in the transplant out patient clinic at SIUT. Any rise in serum creatinine was investigated with CyA levels, ultra sound and colour Doppler imaging, viral markers (CMV and Polyoma) in selected cases, and graft biopsies. Acute rejections were reported according to updated Banff's 97 classification.[18] C4d staining was done on unfixed, frozen sections using monoclonal mouse anti-human C4d antibody (Quidel, San Diego CA USA) by indirect immunofluorescence according to previously established methodology.[19]

Comparisons between groups were done using Chi square statistics for categorical variables and two sided t tests for continuous variables. Continuous data are reported as mean ± SD, whereas skewed data are reported as median. Statistical significance was identified by a two-sided P value of less than 0.05. Statistical package for the social sciences (SPSS Version 10.) was used for all analysis.

The study protocol was approved by the ethical review committee of SIUT.

Results

Fifty renal transplant recipients, who received renal allograft from live related donors between October 2006 and March 2007, were followed for six months after transplant. Recipient and donor demographics are given in Table 1. Donor-recipient blood group matching was identical in all patients. Pretransplant lymphocyte crossmatch for T and B cells were negative, and PRA were negative (0%) in all recipients. All donor-recipient pairs were at least one haplotype match, with one haplotype 3 antigen match in 21 (42%) patients, one haplotype 4-antigenmatch in 9 (18%), one haplotype 5-antigen match in 9 (18%) and one haplotype 6-antigen match in 2 (4%). Nine (18%) patients were HLA identical.

Serum sCD30 levels measured prior to transplant and on the third and 14th days post transplant are given in

Table 1: Demographics of transplant recipients and donors.

Recipients	**n=50**
Sex	
Male	38
Female	12
M:F Ratio	3:1
Mean Age (in years)	31.6+10.23
Range (in years)	5 to 55
Donors	**n=50**
Sex	
Male	34
Female	16
M:F Ratio	2:1
Mean Age (in years)	32.74+8.48
Range (in years)	21 to 50

Table 2. No significant difference in serum sCD30 levels was found in recipients developing rejection or no rejection. There were also no significant differences in duration of haemodialysis before transplant in the two groups (Table 2).

Average sCD30 level of transplant recipients before transplantation was 37.8±25.2 U/ml which was much higher than those of the healthy individuals (8.48± 4.97 U/ml, P = 0.001).

Eight (16%) out of 50 patients developed acute rejections during this follow up period. Rejections were described and classified according to BANFF 97 classification.[18] Table 3 shows details of patients developing acute rejection.

Table 2: Serum sCD30 (U/ml) levels in renal transplant recipients.

	Renal Allograft Recipients withoutRejection	Renal Allograft Recipients with Acute Rejection	*P* value
Pre transplant	37.00+26.25	42.00+19.83	0.618
Day 3 post transplantation	20.92+11.07	28.75+15.63	0.591
Day 14 post transplantation	22.04+21.25	37.75+40.89	0.095
Duration of haemodialysis (in months)	9.05+10.34	7.37+6.18	0.111

Discussion

The identification of pre and post transplant risk factors associated with an increased incidence of allograft rejection is of immense importance for the successful implementation of individually tailored immunosupression. Until recently the only established pre-transplant immunologic parameters that provided useful clinical prognostic information, regarding incidence of post transplant acute rejections were HLA matching and PRA before transplant.[6,15,16] Immunosuppressive drugs especially induction protocols are now used to overcome HLA disparities to prevent acute rejection. However a major drawback with PRA is that patients with negative assays or low levels cannot be allocated to the high or low risk group and estimation of immunologic risk is not possible. For this reason novel markers are needed for proper monitoring of post transplant risk. Recently several studies have suggested that elevated pre and post transplant serum levels of the sCD30 molecule might be predictive of an increased incidence of acute rejection and worse allograft prognosis.[15-17,20] All these studies showed high pre-transplant levels of sCD30 in patients waiting for transplant. However not all showed an association of high levels with acute rejection, while others showed association with grade of acute rejection.[21]

Our study shows that serum sCD30 levels are significantly higher in allograft recipients waiting for transplantation than in healthy controls. This is in accordance with a number of previous studies.[13,15,22] Based on published results, pre transplant sCD30 serum levels higher than 100 U/ml have been classified as a risk factor for the survival of kidney allograft.[20] Furthermore using multivariant analysis Pelzl et al[17] showed that risk of graft rejection in two years follow-up was significant only in patients with sCD30 in the range 400-800 U/ml and >800 U/ml and not the group where sCD30 was<400 U/ml. In the present study the mean serum sCD30 levels in pre transplant recipients were 36.95 ± 26.28 and 42.25 ± 19.46 U/ml, with and without rejection respectively. These levels are considerably lower than those reported in other studies[17,20] and none of our patients had serum sCD30 level greater than the cut off value of 100U/ml and there was no statistically significant difference between the serum sCD30 levels between the two groups of renal allograft recipients post transplantation. The reason(s) for these low pre-transplant serum sCD30 levels are not clear but might reflect an immunodeficiency state, as PRA was 0% in majority. This is further collaborated with low haemodialysis duration prior to transplant as dialysis duration enhances T cell responses and predisposes to sensitization.[17,23] Duration of haemodialysis in our patients is shorter as compared to other studies.[21] Furthermore significant time dependent fluctuations of serum sCD30 levels have been seen in patients waiting for renal transplants.[21] We were unable to demonstrate any significant difference in levels of sCD30 on day 3 and 14 of post transplant among patients who developed acute rejection from those who did not. Also serum levels of sCD30 did not show significant correlation with different grades of rejection. This is in contrast to the study findings by Rajakarrar et al,[12] that demonstrated an association between pre transplant serum sCD30 levels and the grade of rejection. Significantly higher levels of serum sCD30 were seen in cases with antibody-mediated rejection based on C4d staining, compared to those with cell-mediated rejection. They advocated that sCD30 could be a tool to predict antibody-mediated rejection and enable immunosuppressive regimens to be individualized. In our

Table 3: Immunological profiles of patients with acute rejection.

Pt	HLA match	XM	PRA %	Time of rejection	BANFF category	sCD30 pre-tx	sCD30 day3	sCD30 day 14
1	1 H	Negative	0	Day 5	IA	48	32	32
2	1 H	Negative	0	Day 4	IA	80	64	20
3	1 H	Negative	0	Day 5	IIA	32	24	24
4	1 H	Negative	0	Day 7	IA.	48	24	24
5	1 H	Negative	0	Day 17	IIA	24	24	20
6	1 H	Negative	0	Day 6	IA	52	16	36
7	1 H	Negative	0	Day 23	IB	36	32	40
8	>1H (5Ag)	Negative	0	Day 10	IIA	18	14	06

1H: one haplotype; 5Ag: 5 antigen; HLA: human leukocyte antigen; XM: crossmatch; PRA: panel reactive antibodies; Pre-Tx: pretransplant.

series, all rejections were of cell mediated type and only three cases had mild intimal arteritis. Antibody medicated rejection was not seen in our transplant patients. This is perhaps due to the fact that majority of our transplants were live related with a minimum of 3 antigen match with one at HLA DR locus and 0% PRA. These findings partly explain the observed lack of correlation between sCD30 and acute rejections in our cohort of patients.

In conclusion, the findings of our study demonstrate that serum sCD30 levels in our transplant patient population indicate low levels of sensitization supported by 0% PRA in all recipients and good HLA match giving lower grade of rejections by BANFF criteria.[18] Thus sCD30 levels do not predict the risk of rejection in our renal transplant recipients specially the fact all were first transplants. However, this is a small and single centre study with short duration of follow-up. Further follow-up of these patients is necessary to look at graft survival. Analysis of sCD30 in transplants should be evaluated where sCD30 may play a more significant role in predicting acute rejections and long term graft survival.

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