

Evaluation of Anti- HLA Class I Antibodies in Chronic Rejection of Kidney Transplantation

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ABSTRACT

Studies have shown that patients who do not produce donor specific and / or panel reactive anti-HLA antibodies have a longer graft survival. The purpose of this study was to evaluate the posttransplant humoral immune response towards HLA-class I antigens and the measurement of the serum creatinine levels which are used in monitoring posttransplant function of kidney.

Serum samples from 132 renal transplant recipients were screened for preformed anti-HLA class I panel reactive antibodies (PRA) by means of microlymphocytotoxicity assay.

The results revealed the presence of PRA in 26 (19.7%) out of 132 transplanted patients. Graft function was evaluated by measurement of serum creatinine levels which revealed the mean of 1.75 mg/dl (SD: 1.08). Because of clinical significance of presence of different PRA amounts (>10%, > 20% and >50% of panel reactivity) in patients, correlation with kidney function status was analyzed. The obtained data highlighted a higher presence of serum creatinine levels in PRA-positive patients compared to negative patients (P<0.01).

These results (and further studies for class II, ...) can be used to implement new therapeutic strategies to curtail post transplant alloantibodies production and better allografts survival.

Keywords: Anti-HLA Antibodies, Kidney Transplantation, Rejection

INTRODUCTION

Improvements in immunosuppressive therapy have greatly reduced acute rejection (ARj) episodes, ensuring better short-term graft outcome, but have not modified long-term survival in renal transplantation.¹ Chronic rejection represents the major threat to long-term survival of organ allografts.² Chronic rejection which is responsible for the majority of late allograft failure is defined as progressive functional

deterioration of the transplanted tissue, occurring months or years after engraftment. It is associated with vascular obliteration and other structural changes that lead to gradual fibrosis of graft.³ It is now well accepted that chronic rejection (CRj) can be induced by both immune and / or non immune mechanisms. The rate of chronic kidney loss is lower in recipients who were well matched with their donor for HLA antigens than in poorly matched patients.² This suggests that late transplant failure is caused by the patient's immune responses against the disparate HLA antigens of the graft. Recipients with antibodies to non-donor HLA antigens which may be detected by any method are at increased risk of graft failure.⁴ In

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Anti-HLA Antibodies in Rejection of Transplanted Kidney

addition, antibodies eluted from the grafts demonstrated both specific anti-donor and non specific activity as well as cross reacting anti-HLA activity.⁵ These alloantibodies, have generally been induced by previous transplantation, pregnancies or multiple blood transfusions. Two different classes of anti HLA antibodies can occur in sera of kidney graft recipients: antibodies directed against HLA-class I antigens, expressed on nucleated cells and platelets and antibodies against HLA class II antigens, expressed on B lymphocytes, activated T lymphocytes and cells of the macrophage lineage including endothelial.⁶ Measurement of the serum creatinine levels is a simple, inexpensive diagnostic test that lies at the core of early post transplantation management. Large elevations in plasma creatinine concentration (ie, greater than the 25% from base line) almost always indicated a significant, potentially graft-endangering event.⁷ Although extensively studied, the individual contribution of anti HLA class I and class II antibodies to kidney graft rejection remains an unsolved issue.⁶ The purpose of this study was to determine if the presence of panel reactive antibodies in the patient's sera correlated with the measurement of serum creatinine level which is used in monitoring post transplant function of kidney allografts.

MATERIALS AND METHODS

Patients

Sera from 132 patients (68 men and 64 women) who had received transplants at Hashemi Nejad Hospital, Tehran between February 2002 and January 2003 were obtained. Sera were collected at least 6 months after transplantation at the time of hospital readmission, regardless of the cause. No exclusion criterion was made for any reason. The mean of patient's graft age was 5.57 years (SD: 4.08) range from 1 to 15 years. The sera were transported frozen to the study center in Immunogenetics laboratory of Immunology Department of Tehran University of Medical Sciences.

Panel Reactive Antibodies (PRA)

Panel reactive antibodies determination in the complement-dependent cytotoxicity (CDC) assay were performed using lymphocyte cells from 30 unrelated persons in the presence of rabbit complement to screen preformed cytotoxic anti HLA

antibodies. Complement dependent lymphocytotoxic test detects HLA class I-specific antibodies. To determine whether HLA class II specific antibodies are present, it is necessary to screen against B lymphocytes and this is less commonly undertaken.⁸ Measurement of the serum creatinine levels was performed at the time of sampling.

Statistical Analysis

Statistical analysis was done by parametric statistical methods: one way ANOVA, t-test and Means \pm SD. P-values less than 0.05 were considered significant.

RESULTS

The presence of panel reactive antibodies (PRA) in post transplant sera was analysed in patients who received transplants against random panel of T cells. The results of cytotoxic screening on samples collected revealed that 106 persons (80.3% of patients) revealed no panel reactivity and 26 persons (19.7% of patients) showed panel reactivity (ranged from 3.3% to 96.6%) (Table 1).

Graft function was evaluated by measurement of serum creatinine levels: The mean of measurement of serum creatinine levels was 1.75 mg/dl ranged from 0.7 mg/dl to 8mg/dl (SD: 1.08) (Table 2). Because of clinical significance of presence of different PRA concentrations (i.e. 10%, 20%, and 50% of panel reactivity) in patients, correlation with kidney function status was analyzed.

Table 1. Frequency of patients versus panel reactive antibodies using Microlymphocytotoxicity test.

PRA	Number of patients	Percentage (%)#
0.0	106	80.3
3.3	15	11.4
6.6	3	2.3
13.3	1	0.8
16.6	1	0.8
23.3	#1	0.8
63.3	1	0.8
80.0	#1	0.8
83.3	1	0.8
86.6	1	0.8
96.6	1	0.8
Total	132	100

Table 2. Frequency of serum creatinine in four different groups of patients.

Creatinine # (mg/dl)	Number of patients	Percentage (%)
<1.5	59	44.7
1.5 - 2.0	40	30.3
2.0 - 2.5	21	15.9
> 2.5	12	9.1
#Total	132	100

The obtained data highlighted a higher presence of serum creatinine levels in PRA-positive patients compared to negative patients ($P < 0.01$) (Table 3 and Figures 1,2,3).

Comparison of the means and standard deviations of patient's serum creatinine in three PRA-

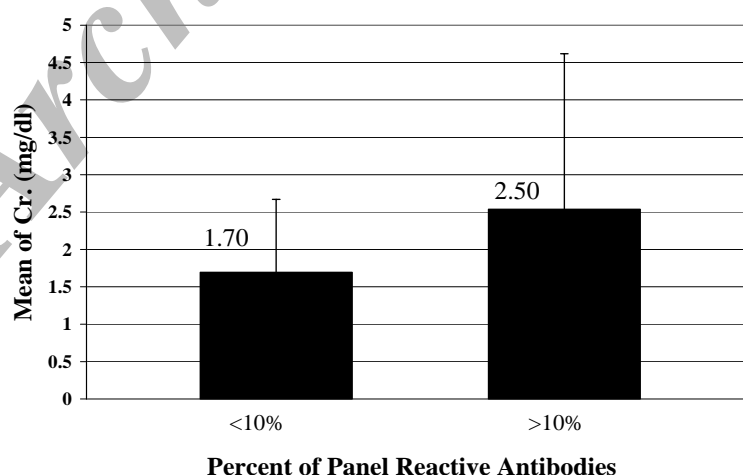
positive groups (>10%, >20% and >50% of panel reactivity) revealed no significant correlation.

DISCUSSION

Barr et al,⁹ showed significant association between development of anti-HLA antibodies and that of chronic rejection. Martin et al,¹⁰ demonstrated that Donor specific antibodies fixed onto kidney transplant can be detected by flow cytometry in at least 70.6% of recipients experiencing chronic allograft nephropathy. Piazza et al,¹ reported that flow cytometry cross match (FCXM) positive patients (24.6% of patients) had higher serum creatinine (2.5 ± 1.3 mg/dl vs. 1.7 ± 0.5 mg/dl) and reported the presence of anti HLA class I antibodies in 85% of fcxm positive patients.

Table 3. Mean and SD of patients versus presence of different panel reactive antibodies (PRA).

	PRA	Number of patients	Mean PRA	SD	P-value
PRA>10%: Positive	PRA (-)	124#	1.70	0.98	P<0.01
	PRA (+)#	8	2.54	2.08	
PRA>20%: Positive	PRA (-)	126	1.69	0.97	P<0.01
	PRA (+)#	6	2.75	2.42	
PRA>50%: positive	PRA (-)	127	1.70#	0.97	#P<0.01
	PRA (+)#	5	2.68	2.68	

**Figure 1. Mean and SD of Creatinine (Cr.) versus percentage of PRA (>10% of panel reactivity = positive PRA)**

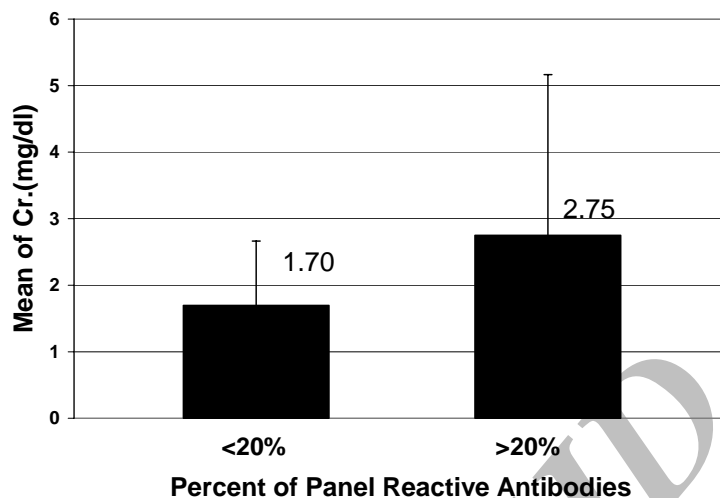


Figure 2. Mean and SD of Creatinine (Cr.) versus percentage of PRA (>20% of panel reactivity = positive PRA)

Aragao et al,¹¹ reported a high incidence of anti HLA class I IgG antibodies (60%) during chronic allograft nephropathy. Pelletier et al,¹² by studying kidney or kidney-pancreas recipients reported that 18% of them had MHC reactive antibodies. The majority of these patients produce alloantibodies directed at MHC class II only (68%). According to the published data, which suggest a high incidence of MHC class I reactive alloantibodies, the authors

postulated two possible explanations for this result: 1: There may be a high frequency of early graft loss in recipients who develop anti HLA class I antibodies in association with acute rejection 2: alloantibodies production may spread from MHC class I to class II alloantibodies with time, especially if graft MHC class II expression is increased during episodes of graft inflammation such as when it occurs during episodes of acute rejection.

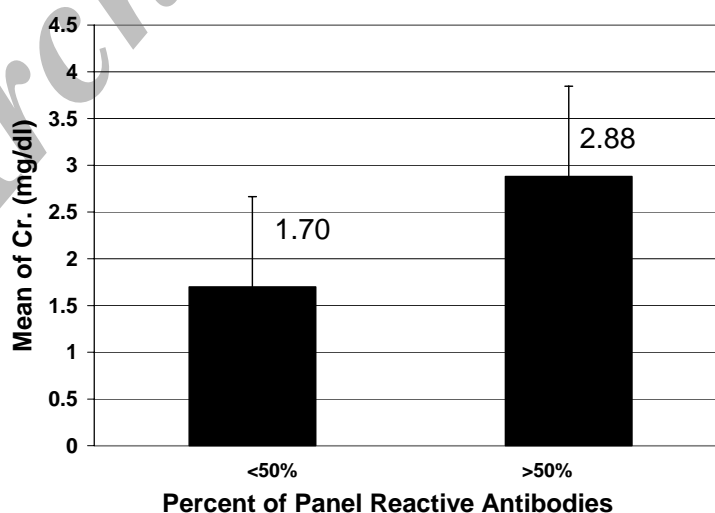


Figure 3. Mean and SD of Creatinine (Cr.) versus percentage of PRA (>50% of panel reactivity = positive PRA)

Our study supports the finding of other centers of the detrimental role to the kidney graft played by anti HLA antibodies. According to the analyzed results of this study and demonstration of significant correlation that was seen between production of anti HLA class I antibody production and serum creatinine, the presence of alloantibody production constitutes a negative prognostic event and a risk factor for medium and long-term graft survivals. In conclusion, diagnostic tests such as microlymphocytotoxicity, may serve as a basis for the improvement of therapeutic management and consequently help to increase the rate of graft survival after kidney transplantation.

Early detection of antibody production may be useful in identifying those patients who need a kidney biopsy and in developing appropriate immunosuppressive protocol to curtail post transplant alloantibody production.⁴ However it is only recently that effective control of antibody production has been demonstrated using new immunosuppressive drug combinations (e.g. tacrolimus and mycophenolate mofetil); plasmapheresis combined with intense immunosuppression; high-dose IVIG and monoclonal antibodies to B cells (notably anti-CD20 or rituximab).^{13,14} In addition, the induction of mixed hematopoietic chimerism in transplant recipients has the potential to induce both T cell and B cell tolerance. In the future strategies aiming at achieving mixed chimerism in the clinical setting may allow us to overcome the problem of allosensitization.⁵

REFERENCES

1. Piazza A, Poggi E, Borrelli L, Servetti S, Monaco PI, Bunomo O, et al. Impact of donor-specific antibodies on chronic rejection occurrence and graft loss in renal transplantation. *Transplantation* 2001; 27,71(8):1106-12.
2. Suci-Foca N, Reed E, Dagati VD, Ho E, Cohen D, Benvenisty A, et al. Soluble HLA antigens, anti-HLA antibodies, and antiidiotypic antibodies in the circulation of renal transplant recipients. *Transplantation* 1991; 51(3):593-601.
3. Harris PE, Bian H, Reed E. Induction of high affinity fibroblast growth factor receptor expression and proliferation in human endothelial cells by anti-HLA antibodies. *J Immunol* 1997; 1,159(11):5697-704.
4. Reinsmoen NL, Nelson K, Zeevi A. Anti-HLA antibody analysis and crossmatching in heart and lung transplantation. *Transpl Immunol* 2004; 13(1):63-71.
5. Baid S, Saidman S, Tolkoff-Rubin N, Williams WW, Delmonico FL, Cosimi AB, et al: Managing the highly sensitized transplant recipient and B cell tolerance. *Curr Opin Immunol* 2001; 13(5):577-81.
6. Susal C, Opelz G. Kidney graft failure and presensitization against HLA class I and class II antigens. *Transplantation* 2002; 27,73(8):1269-73.
7. Denovitch GM. Hand book of kidney transplantation. USA: Walters Kluwer Company, 2001:177-8.
8. Harmer AW, Heads AJ, Vaughan R W. Detection of HLA class I – and class II-specific antibodies by flow cytometry and PRA-STAT screening in renal transplant recipients. *Transplantation* 1997; 63(12):1828-1832.
9. Barr ML, Cohen DJ, Bevenisty AI, Hardy M, Reemtsma K, Rose EA, et al. Effect of anti-HLA antibodies on the long term survival of heart and kidney allografts. *Transplant Proc* 1993; 25(1 Pt 1):262-4.
10. Martin L, Guignier F, Mousson C, Rageot D, Justrabo E, Rife G. Detection of donor-specific anti-HLA antibodies with flow cytometry in eluates and sera from renal transplant recipients with chronic allograft nephropathy. *Transplantation* 2003; 27,76(2):395-400.
11. Aragao E, Moura LA, Pacheco-Silva A. Monitoring anti-HLA Class I IgG antibodies in renal transplant recipients. *Transplant Proc* 2004; 36(4):836-7.
12. Pelletier RP, Hennessy PK, Adams PW, VanBuskirk AM, Ferguson RM, Orosz CG. Clinical significance of MHC-reactive alloantibodies that develop after kidney or kidney –pancreas transplantation. *Am J Transplant* 2002; 2(2):134-41.
13. Theruvath TP, Saidman SL, Mauiyyedi S, Delmonico FL, Williams WW, Tolkoff-Rubin N, et al. Control of antidonor antibody production with tacrolimus and mycophenolate mofetil in renal allograft recipients with chronic rejection. *Transplantation* 2001; 15,72(1):77-83.
14. Michaels PJ, Fishbeine MC, Colvin RB. Humoral rejection of human organ transplants. *Springer Semin Immunopathol* 2003; 25(2):119-40.