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The Effect of Desensitization on Sequel of Anti-MICA Antibody on Renal Graft Function in Live Related Renal Transplant – A Case Control Study

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ABSTRACT

Introduction: Renal transplantation is distinctive remedial measure of choice in cases of end stage renal disease, but the beneficiary with pretransplant HLA and non HLA antibodies present a unique challenge to the transplant community. However over the decades several desensitization protocols have been deployed which have allowed successful transplantation in HLA antibodies positive patients, but the data is quite limited with respect to the non HLA antibodies like anti-MICA antibody that to in Indian scenario. Our study focused on assessing the overall outcome for anti-MICA antibody on renal graft function.

Material and Methods: Prospective case control study was donewhere outcome of 50 anti-MICA antibody positive renal transplant recipients (Group I) was compared with 50 anti-MICA antibody negative renal transplant recipients (Group II).Group I recipients underwent a desensitization protocol using combination of Rituximab, PLEX and IV Ig.

Results: Mean serum creatinine levels were higher at all the intervals in Group I over six month observation period but there was no significant divergence notedamongst both the groups. We observed that history of blood transfusion and longer waiting period on dialysis poses a significant risk of sensitization to MICA antigen. Rejection rate, graft function and patient survival after six months of follow up, post transplantation were comparable between both the groups.

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Conclusion: This beneficial effect can be attributed to the desensitization of anti-MICA antibody positive cases prior to the transplant. We opine,a combination of Rituximab, PLEX + IV Ig is reasonable choice to keep check on acute rejection without significantly enduring to the risk of opportunistic and other severe infections in such sensitized patients.

Keywords:renal transplant, anti MICA antibody, desensitization protocol, renal graft function

INTRODUCTION:

Renal transplantation is one of the best remedial measurefor cases of end stage renal disease (ESRD), promoting a restoration of near normal health and providing the lengthening life expectancy. Despite renal transplant rejection being firmlycorelated with human leucocyte antigen (HLA) antibodies,[1,2] 11-20% of patients without HLA antibodies develop chronic allograft dysfunction.[3]Additionally, hyperacute rejection can occur in the absence of HLA antibodies, embroiling the role of other non-HLA alloantigen in renal graft dysfunction,[4,5]one such antigen is major histocompatibility complex class I related chain A (MICA).[6]Basic exploratory studies with limited number of patients have denoted that MICA antibodies detected after transplantation might havecorrelation with deranged performance of kidney allograft. A scrutiny of eluatesfrom renalenduring immunologic rejection has proposed that MICA antibodies may have a vital role in the pathogenesis of kidney allograft rejection.[7-11]

Improvement in immunosuppressive therapy, focused to curb the effect of T-cell mediated immune responses on the graft have improvedoverall graft survival and diminished acute rejection.[12] However, rejection due to antibody-mediated graft damage arising from B-cell responses to mismatched HLA remains a concern.

Several desensitization protocols have been deployed over the decades which have allowed successful transplantation in HLA antibodies positive patients, renal graft outcome are now acceptable, patients survival and quality of life have improved but the data is quite limited with respect to non HLA antibodies like anti-MICA antibodies specially in Indian scenario.

Mostof the studies done on anti-MICA antibodies in renal transplant were retrospective one in which no desensitization protocol was used to reduce the detrimental effect of the antibodies on renal allograft.[13,14] These studies showed reduced graft survival in recipients with preformed anti-MICA antibodies. As per now most of clinical work on anti-MICA antibody have been executed on deceased donors and very constraineddata is there for live related transplants. Aim of the present study was to find out the role of MICA antibodies in patients who have been planned for the live related renal transplantation on graft function.

MATERIAL AND METHODS

Study population:100 renal transplant cases were divided into:-

Group I - 50 anti-MICA antibodypositive renal transplant patients (Case)

Group II - 50 anti-MICA antibody negative transplant patients (Control)

Study period: Study was carried after approval from institutional ethics committee from March 2016 to March 2018.

Study design: A prospective case control study in which the case and control were followed for six months after renal transplant.

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Sample size: Calculated by using the formula stated by Charan and Biswas.[15]

$n = 4pq/d^2$

$n = 4 \times 0.47 \times 0.53/ (0.11 \times 0.11) = 82$

Where n is required sample size, p = prevalence of cause, q = 1-p, d = precision

Taking 80% power, 5% significance level with 0.11 precision, the calculated sample size was 82. We enrolled 100 patients.

Inclusion criteria:

All the recipients with a negative complement dependent cytotoxicity (CDC) crossmatch and negative antibodies to donor specific Class I and Class II HLA ascertained by Luminexcrossmatch were included in the study. Analysis of anti-MICA antibody was done using Luminex platform by SAB assay (Lifecodes LSATM MIC, Immucor, USA).

Exclusion criteria:

1) CDC cross match positive patients
2) DSA (donor specific antibodies) positive patients
3) ABO incompatible transplant patients
4) Second time transplant patients
5) Patients who had undergone another solid organ graft in addition to renal transplant (kidney with pancreas or kidney with liver etc.)
6) All recipients who were HIV, HCV or HBsAg positive

It was prospective study; however for information regarding native kidney disease, history of dialysis duration, blood transfusion, pregnancy and post transplant follow up, data was collected when patient visited OPD or had got admitted for any reason.

Desensitization protocol

The prospective anti MICA antibody positive renal transplant recipients (Group I - Case) underwent therapeutic desensitization as per our institute's protocol:

- Inj Rituximab 375mg/m^2 – IV infusion – seven days before transplant
- Removal of antibodies by plasmapheresis (PLEX) – twosessions (one session / day), started two days prior to transplant
- IV Ig – 100mg / kg in post PLEX

No desensitization was used for anti MICA antibody negative patients (Group II - Control).

Immunosuppresion was carried out using standard dose of Calcineurin inhibitors (CNIs) (as per trough levels),MycophenolateMofetil (MMF) and steroids in both the groups. All the study subjects got prophylaxis against Cytomegalovirus (CMV) and Pneumocystis pneumonia.

Post transplant follow up

All the subjects were persuaded at weekly intervals for the first month, fortnightly for next three months, monthly for next three months. On every follow-up visit, the complete blood counts, renal and liver function tests, urine routine & microscopy were done and other tests like electrocardiogram & ultrasound Doppler studies were performed as and when required.

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All cases of rejection were biopsy proven and diagnosed as per the Banff classification. They were dealt as per standard anti-rejection treatment. Humoralgraft rejections cases were treated with the combination of Rituximab, PLEX and IVIg; Cellular rejections were treated with Methyl prednisolone +/- ATG.

Outcome of transplant was assessed and data was collected and analyzed in terms of:-

1. History of blood transfusion
2. Pretransplant dialysis duration
3. Graft function (serum creatinine at various intervals)
4. Incidence of rejection (biopsy proven)
5. Incidence of major infectious complications
6. Incidence of New onset diabetes after transplant (NODAT) in previously non-diabetic individuals
7. Mortality and cause of death

Statistical analysis

Results of the study were contemplated in frequencies, percentages and mean $\pm$SD.Chi-square testwas appliedfor comparison of categorical / dichotomous variables and unpaired t-test to compare continuous variables between Group Iand Group II. Relative risk (RR) with its 95% confidence interval was calculated to find out the strength of association. The p-value < 0.05 was considered significant. Analysis was done with the help ofnSPSS 16.0 version.

RESULTS

Mean age of the patients in anti-MICA antibody positive group was 45.06$\pm$ 10.77 years and 39.62$\pm$13.24 years in negative group. Gender distribution comprised of 71% males and 29% females. Diabetic nephropathy (DN) was the most common basic kidney disease (BKD) amongstgroup I (38%) and chronic glomerulonephritis was most common BKD in group II (32%). Chronic interstitial nephritis (30%) and DN (30%) were the second most common BKD in anti-MICA antibody positive and negative groups respectively as displayed in table 1. There was significant difference in timeline of pre-transplant dialysis between both the groups. History of blood transfusion was positive in 34% of group I and 14% in group II; this difference is statistically significant. Mean serum creatininelevels in our study were higher at all the intervals in anti-MICA antibody positive cases over six month observation period butno significant difference ($p>0.05$) in serum creatininereadings between group I and group II were found at all the intervals of six month follow up as shown in figure 1. No statisticallysignificant difference was seen in overall incidence of infections and NODAT for first six months after transplantation in both the groups as shown in table2 and 3. Also the groups were comparable in premise of rejection and mortality ($p>0.05$) as shown in table2.

DISCUSSION

In our study,outcome of fifty anti-MICA antibody positive renal transplant recipients were compared with fifty anti-MICA antibody negative renal transplant recipients. All the patients included underwent a living donor renal transplant as there is very low incidence of cadaveric renal transplant at the centre. Anti-MICA antibody positive cases underwent a desensitization protocol using combination of Rituximab, PLEX and IV Ig.

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The mean age of the patients in anti-MICA antibody positive group was 45.06 years and 39.62 years in negative group analogous to Indian work carried out by Bharat V Shah et al. in which the mean age was 46 years.[16] The average age in both the groups is lower than that seen in western population.[17]SubcontinentalESRDcases are younger as compared to their western equivalents. The median age of patients entering ESRD programs is 44 years in Indian subcontinent as compared to 52-63 years in developed countries.[18]Lag in detection and failure to institute strategies that postpone progression of renal failure lays the path for ESRD at a younger age.[19] Moreover, as all our recipients underwent a living donor renal transplant with donor being their relative, they received kidney transplant at relatively younger age, whereas in western world most of patients undergoing renal transplant received kidney from cadaveric source hence their wait time might be longer.

In our study, anti-MICA sensitization was more often in males (71%) than in females (29%), similar to observation from other series which depreciates the contingency that pretransplantation anti-MICA antibodies could be associated with pregnancy.[20,21]

In our study, diabetes constituted major cause of ESRD in anti-MICA antibody positive group and second most common cause in control group similar to study conducted by Suresh Chandra Dash et al.[22] The mean duration of pre-transplant dialysis was 8.32 month and 5.2 month in anti-MICA antibody positive and negative cases respectively which is statistically significant. In a study by Lemy Anne et al. in 59 anti-MICA antibody positive cases, the mean duration of dialysis was 40 month which is much higher than our study. This higher duration might be due to longer waiting period as most of the western countries transplants are cadaveric.[23] We observed that protracted waiting time on dialysis is associated with higher endangerment of sensitization to MICA antigen. This may be due to more incidence of anemia or exposure to other unknown factors on dialysis which predispose to formation of these antibodies.

History of blood transfusion was positive in 34% group I and 14% in group II which is statically significant and similar to study done by Sanchez Zapardiel E et al.[20]Lemy Anne et al also demarcated that blood transfusion was a self-reliant risk factor for evolution of anti-MICA antibody.[23]This fact thus suggests that blood transfusion may be important sensitizing event in the development of anti-MICA antibodies. Hence it is inferred that blood transfusion poses a significant risk for dialysis patients in becoming sensitized to MICA antigens and can limit or delay future chances of successful transplantation.

Incidence of biopsy proven rejection was 4% in either group. Balwani Manish et al. found increased acute rejection rate in pretransplant anti-MICA antibody positive patients compared to comparison group, however rate of chronic rejection was indistinguishable in both the groups.[14] This was a retrospective study with mean follow up period of 6.5 years and no desensitization done in anti-MICA antibody positive group which may be the cause of higher acute rejection rate as compared to our study.

Incidence of infection was comparable in both groups similar to that of Kahwaji J et al, in which Rituximab in conjugation with PLEX and IVIg did not appear to increase the risk of infectious complications in living donor recipients.[24]The cumulative incidence of NODAT in our study was 7% which was within the reported range in randomized controlled trials (4 to 25%).[25]Tacrolimus clearly has more incidence of NODAT when compared to other CNIs. In our study both the groups received Tacrolimus based maintenance

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immunosuppressionhence this could be the possible explanation for the similar incidence of NODAT in both the groups. Both the study groups had two deaths each which is not statistically significant.

CONCLUSION

It's been established that the presence of anti-MICA antibodies detected in organ transplant recipients either pre- or post-transplantation can culminate in acute rejection, chronic allograft dysfunction or reduced graft survival. However, most of the studies done on anti-MICA antibodies in renal transplant were retrospective studies in which no desensitization protocol was used to reduce the detrimental effect of these antibodies on renal allograft.Most of these studies showed reduced graft endurance in recipients with preformed anti-MICA antibodies. In our study rejection rate, graft function and patient survival after six months of follow up post transplantation were comparable between both the groups. This beneficial effect can be attributed to the desensitization protocol given prior to transplantation in anti-MICA antibody positive cases. However long term persuasion will be needed to decide the utility of desensitization of these patients in respect to prevention of acute rejection, prevention of acute & chronic graft dysfunction and the long term graft survival.

A combination of Rituximab, PLEX + IVIg, in our opinion suffices as reasonable choice to keep check on acute rejection without as a matter of course for increasing the risk of opportunistic and other serious infections in such sensitized patients. We also observed that history of blood transfusion and longer waiting period on dialysis are associated with higher risk of sensitization to MICA antigen; hence blood transfusion in all these patients should be avoided as far as possible.

Table 1. Comparison of baseline characteristics of Group I and GroupII

Parameters	**Anti MICA antibodies positive (n=50)**		**Anti MICA antibodies negative (n=50)**		**p-value[1]**
	No.	**%**	**No.**	**%**	
Age in years					
<40	16	32.0	26	52.0	0.12
40-50	16	32.0	12	24.0	
>50	18	36.0	12	24.0	
Gender					
Male	39	78.0	32	64.0	0.12
Female	11	22.0	18	36.0	
Comorbidity					
Hypertension	29	58.0	26	52.0	0.54
Diabetes mellitus	18	36.0	15	30.0	0.52
Basic kidney diseases					
Autosomal dominant polycystic kidney	2	4.0	3	6.0	

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disease					
Alport syndrome	0	0.0	1	2.0	
Chronic glomerulonephritis	9	18.0	16	32.0	
Chronic interstitial nephritis	15	30.0	13	26.0	
Diabetic nehpropathy	19	38.0	15	30.0	
HTN nephrosclerosis	1	2.0	2	4.0	
IgA nephropathy	3	6.0	0	0.0	
Systemic lupus erythematous	1	2.0	0	0.0	

[1]-Chi square test

Figure 1. Comparison of serum creatinine between the groups across the time periods

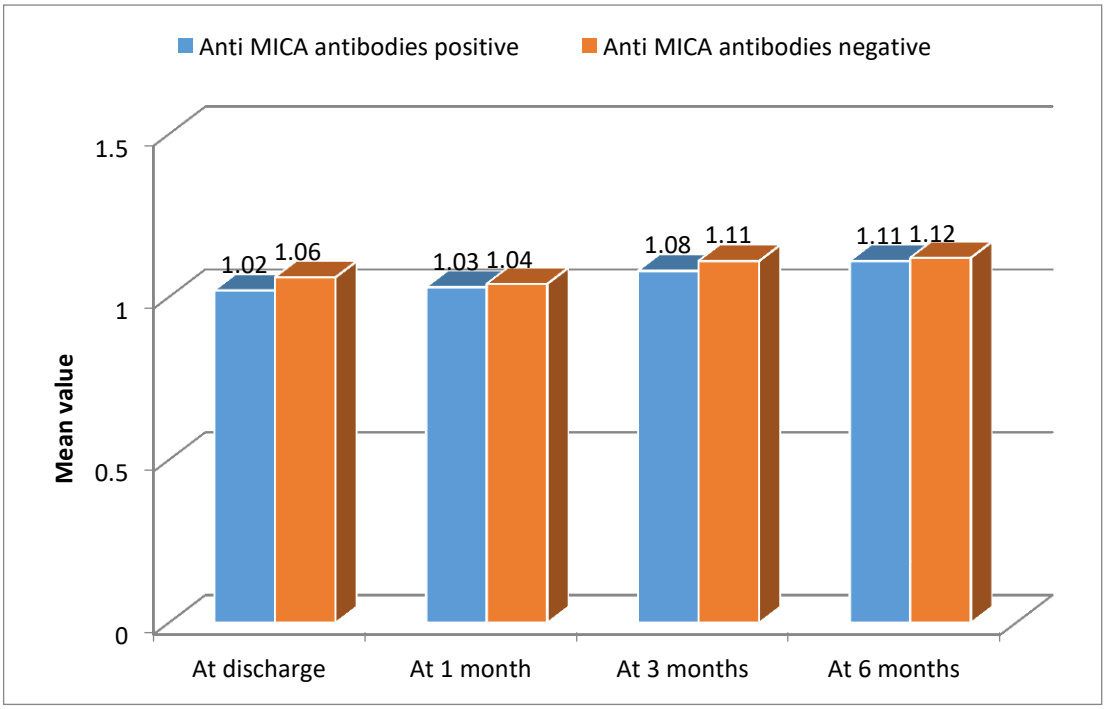

Table 2. Comparison of pretransplant dialysis, history of blood transfusion, incidence of NODAT, incidence of rejection & mortality between Group I and Group II

Parameters	**Anti MICA antibodies positive (n=50)**		**Anti MICA antibodies negative (n=50)**		**p-value[1]**
	No.	**%**	**No.**	**%**	
Pre-transplant dialysis duration in months					
0-6	21	42.0	39	78.0	0.001*
7-12	22	44.0	8	16.0	
>12	7	14.0	3	6.0	
History of blood transfusion					

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Yes	17	34.0	7	14.0	0.01*
No	33	66.0	43	86.0	
Incidence of NODAT					
Present	2	4.0	2	4.0	0.79
Absent	48	96.0	48	96.0	
Incidence of rejection					
Present	2	4.0	2	4.0	1.00
Absent	48	96.0	48	96.0	
Mortality					
Death	2	4.0	2	4.0	1.00
Alive	48	96.0	48	96.0	

[1]-Chi square test, *-significant

Table 3: Comparison of incidence of infection betweenGroup I and Group II

Type of Infection	**Anti MICA antibodies positive (n=50)**		**Anti MICAantibodies negative (n=50)**		**RR (95%CI)**	**p- value[1]**
	No.	**%**	**No.**	**%**		
BK virus						
Present	1	2.0	3	6.0	0.49 (0.08-2.70)	0.30
Absent	49	98.0	47	94.0	1.00 (Ref.)	
Herpes Zoster						
Present	2	4.0	1	2.0	1.34 (0.59-3.07)	0.55
Absent	48	96.0	49	98.0	1.00 (Ref.)	
CMV						
Present	3	6.0	1	2.0	1.53 (0.83-2.79)	0.30
Absent	47	94.0	49	98.0	1.00 (Ref.)	
UTI						
Present	6	12.0	4	8.0	1.22 (0.70-2.12)	0.50
Absent	44	88.0	46	92.0	1.00 (Ref.)	
Chest Infection						
Present	2	4.0	2	4.0	1.00 (0.36-2.71)	1.00
Absent	48	96.0	48	96.0	1.00 (Ref.)	
Tuberculosis					1.00 (Ref.)	

Present	1	2.0	0	0.0	2.02 (0.65-2.46)	0.31
Absent	49	98.0	50	100.0	1.00 (Ref.)	
Fungal infection					1.00 (Ref.)	
Present	1	2.0	0	0.0	2.02 (0.65-2.46)	0.31
Absent	49	98.0	50	100.0	1.00 (Ref.)	

[1]Chi-square test, RR-Relative risk, CI-Confidence interval

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