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A RANDOMIZED CONTROLLED TRIAL OF LOW DOSE ANTI-THYMOCYTE GLOBULIN PLUS RITUXIMAB VERSUS CONVENTIONAL DOSE OF ANTI -THYMOCYTE GLOBULIN ALONE AS INDUCTION AGENT IN HIGH-RISK RENAL TRANSPLANT RECIPIENTS.

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ABSTRACT **INTRODUCTION:** The role of B cell depleting agents in induction protocols is controversial. Aim of this study is to compare the efficacy and safety of combination of low- dose rituximab plus anti-thymocyte globulin as induction agent compared to conventional dose of anti-thymocyte globulin alone in high-risk renal allograft transplant recipients**MATERIALS AND METHODS:** A total of 60 high-risk renal transplant recipients, with two or more risk factors for rejection such as zero or one HLA antigen match, multiparous women, multiple blood transfusions, second transplant, cross match positive status by Luminex technology but negative by CDC technique were included in the study Group 1 patients received Inj. Anti-thymocyte globulin on day '0' and day '4' to a total cumulative dose of 3mg/kg body weight. Group 2 patients received 200mg of rituximab at least seven days before surgery and 1.5mg/kg anti-thymocyte globulin on the day of surgery. Follow up period varied between one to four years.**RESULTS:** Group 2 patients have significantly lesser number of rejection episodes (p= 0.049, 0.02 at end of 1st, 2nd years), better patient survival (86.7%, 80%, 76.7% in group 1; 90%, 86.7%, 86.7% in group 2 at the end of 1st, 2nd, 3rd years), better graft survival (86.7%, 76.7%,73.4% in group 1, 90%, 86.7%, 83.4% in group 2), even though not of statistical significance**CONCLUSIONS:** Combination of low-dose rituximab plus low-dose anti-thymocyte globulin is non-inferior, safe and cost effective compared to conventional dose of anti-thymocyte globulin alone in high-risk renal transplant recipients.

KEYWORDS : Rituximab, anti-thymocyte globulin, rejection, infection, graft loss.

INTRODUCTION

Induction therapy aims at reducing the risk of rejection and improving graft outcomes in renal allograft recipients. Current induction protocols are predominantly T cell directed with anti -thymocyte globulin being major game player in high-risk transplant recipients. Even though anti-thymocyte globulin is pan depleting, its major effect is on T cells with effect on B cells is seen at higher doses. This increases the risk of infection and is expensive for the patients in a country like India.

In solid organ transplant recipients, B cells are classically known for their role in the production of alloreactive antibodies and formation of plasma cells which destroy graft. Recent evidence has expanded the role of B cell in transplant immunology as antigen presenting cells to naïve T cells which can trigger rejection.[1] The role of B cells as antigen presenting cells is elucidated by Ng et al in B cell deficient mouse model (μMT mice) which received skin transplantation[2]. These mice demonstrated similar number of IFNγ producing CD4+ and CD8+ T cells compared to wild controls in early phase but in later phase μMT mice showed a smaller number of IFNγ + cells which indicates that memory T cell development is dependent on B cells[2]. Sarwal and his colleagues demonstrated that B cell infiltrates in renal biopsies with cellular rejection are associated with steroid resistance and poor graft survival[3]. Several other studies have also shown CD20+ B cell clusters in T cell mediated rejection without associated antibody mediated rejection[4,5]. Hence, the role of B cells is much beyond antibody mediated rejection.

Rituximab, a humanized murine CD20 antibody which depletes circulating CD20 cells is shown to be beneficial in desensitization protocols, ABO incompatible transplants and in the treatment of antibody mediated rejection. However, its role as induction agent is controversial in view of conflicting results in various studies.

With this background, we have conducted a randomized controlled trial in high-risk renal transplant recipients to assess the efficacy and cost effectiveness of a combination of low dose rituximab plus low dose anti-thymocyte globulin with conventional dose of anti-thymocyte globulin alone as induction agent.

MATERIALS AND METHODS

This is a randomized controlled trial conducted in high-risk transplant recipients between January 2016 and January 2019. Patients recruited in this period are followed up for a minimum period of one year and a maximum of four years. Institutional ethics committee clearance was obtained.

INCLUSION CRITERIA:

1) 0 OR 1 HLA Match.
2) Previous cross match positive status.
3) Cross match positive by luminex technology and negative by cytotoxicity (CDC) technique.
4) Women with multiple pregnancies.
5) Previous renal transplant.
6) Multiple blood transfusions.

Patients with a minimum of two risk factors were included in the study.

EXCLUSION CRITERIA:

Patients who underwent desensitization protocol in the past.

STUDY PROTOCOL:

A total of 60 patients were recruited in the study. These patients were divided into two groups.

Group 1 patients received anti thymocyte globulin at a dose of 1.5mg/kg body weight on day of surgery and on day 4 to a cumulative dose of 3mg/kg body weight.

Group 2 patients received inj. Rituximab 200mg one week prior to the surgery and inj. anti-thymocyte globulin 1.5mg/kg body weight single dose on the day of surgery.

Patients in both arms were started on triple immunosuppression with steroids (1mg/kg body weight), tacrolimus (0.1mg/kg body weight) and mycophenolate mofetil(1.5gm/day) on pre-operative day 4 and continued to post operative period.

All the patients were followed with renal function tests and complete blood pictures. Renal biopsies are performed whenever there is deterioration in renal function. Record is made of biopsy proven rejections, infections, graft function and mortality at the end of first, second, third and fourth year.

Primary outcome was biopsy proven rejection episodes and secondary outcomes were infections, graft function, graft loss and death at the end of first, second, third and fourth years. Data analysis was done and

results were expressed in terms of percentages, mean, median and p value. Chi square test and test for proportions was done to assess the significance. A p value of less than or equal to 0.05 is considered significant

RESULTS:

Out of 60 patients, 30 patients at random were assigned to each group. There is no significant difference in the baseline characteristics in both groups. Mean age in group 1 is 34.7±9.1 and 35.5±8.1 in group 2 (p=0.31). Females are slightly more in group 2 compared to group 1(p=0.047). Mean number of risk factors in group 1 is 2.2±0.4, and 2.3±0.4 in group 2 (p=0.6). There is no significant difference in diabetes and hypertension in both groups (p=0.8 and 0.74 respectively).

At the end of one year:

Table 1 shows results at the end of one year.

Parameter	Group 1 (mean ± SD)	Group 2 (mean ± SD)	P value
Rejection episodes	0.4± 0.5	0.16± 0.46	0.049
Infection episodes	0.8±1.2	0.43± 0.62	0.4
S. creatinine	1.3±0.23	1.0±0.17	0.01

At the end of first year patients who received rituximab plus anti thymocyte globulin have a smaller number of rejection episodes(p=0.049) and lower baseline serum creatinine(p=0.01) levels with no difference in the number of infection episodes(p=0.4) between two groups.

At the end of second year:

Table2: Results at the end of second year

Parameter	Group 1 (mean ±SD)	Group 2 (mean ±SD)	P value
Rejection episodes	0.29±0.46	0.04±0.2	0.02
Infection episodes	1.57±0.96	1.09±0.25	0.04
S.Creatinine	1.16±0.98	0.37±0.49	0.01

At the end of 2nd year, the number of rejection episodes(p=0.02), infection episodes(p=0.04) and baseline creatinine levels(p=0.01) are less in patients who received rituximab plus anti thymocyte globulin compared to patients who received anti thymocyte globulin.

At the end of 3rd year:

Table 3: Patient survival data

Patient survival	Group 1	Group 2	P value
1st year	86.7%	90%	0.15
2nd year	80%	86.7%	0.47
3rd year	76.7%	86.7%	0.98

A total of 24 patients completed 3rd year follow up. Of these 16 are from group 1 and 8 patients are from group 2. Two patients from group 1(12.5%) developed rejection whereas none in group 2 developed rejection. However, this does not reach statistical significance(p=0.3). There was no significant difference in the number of infection episodes between two groups(p=0.2). The baseline serum creatinine levels are less in group 2 patients compared to group 1(p=0.02).

At the end of 4th year:

Table 4: Graft survival data in both groups

Graft survival	Group 1	Group 2	P value
1st year	86.7%	90%	0.15
2nd year	76.7%	86.7%	0.98
3rd year	73.4%	83.4%	0.87

Only 11 patients completed 4th year. Of these four patients are from group 2. None of the patients in both groups developed rejection episodes. In view of COVID pandemic we were unable to collect data of some patients. As the sample size is very small statistical analysis is not performed.

GRAFT SURVIVAL AND PATIENT SURVIVAL:

During the follow up period 7 patients from group 1 and 3 patients from group 3 died. However, this doesn't reach statistical significance(p=0.1). One patient from each group died with coronary artery disease and one patient from group 2 died in Road traffic accident.

Excluding deaths, one patient in each group lost graft due to BK virus and 1 patient in group 1 lost their graft due to resistant rejection and returned to dialysis.

Table 3 showing patient survival data in both groups

Table 4: Graft survival in both groups

Patient survival rates in group 1 at the end of 1st, 2nd, 3rd years are 86.7%, 80% and 76.7%. The corresponding survival rates in group 2 are 90%, 86.7% and 86.7% respectively(p=0.15, 0.47, 0.98 respectively)

Graft survival rates in group 1 at the end of 1st, 2nd ,3rd years are 86.7%, 76.7%and 73.4% respectively. The corresponding graft survival rates in group 2 are 90%, 86.7% and 83.4% respectively (p=0.15, p= 0.98, p=0.87 respectively). The lack of statistical significance could be because of small sample size.

It has been observed that the rejection episodes occurring in group 2 patients are steroid sensitive and were easily treatable compared to rejections in group 1 patients. Only one antibody mediated rejection occurred in group 2 patients which responded to steroids.

COST ANALYSIS:

The average cost for induction therapy in patients who received anti thymocyte globulin alone is one lakh Indian rupees compared to sixty thousand Indian rupees in patients who received both rituximab and anti-thymocyte globulin

DISCUSSION:

B cells play a key role in graft outcomes following renal transplantation. They can have a negative impact on the transplant outcomes by producing donor specific antibodies, forming memory B cells and by acting as antigen presenting cells to T cells. This has triggered interest in B cell depleting agents as induction agents in renal transplantation. Of the B cell depleting agents Rituximab has been evaluated as induction agent with controversial results in various trials.

A systematic review of studies on rituximab by Philip S. Macklin et al reported that rituximab induction is not associated with patient or graft survival benefits nor a reduction in the acute rejection episodes [9]. In another meta-analysis by Wisit Cheungpasitporn et al which included four randomised controlled trials with 480 renal transplant recipients, there were no statistical differences in the risks of infection, graft loss and mortality at 3–6 months after transplantation with pool RRs of 1.02 (95% CI 0.85–1.21), 0.55 (95% CI 0.21– 1.48) and 0.58 (95% CI 0.17–1.99), respectively.

In a retrospective analysis Vivek patak et al [6] studied 1152 kidney transplantations conducted from July 2005 to October 2017. Induction protocol included inj. Thymoglobulin 1.5mg/kg body weight for the first two days of postoperative period, Inj. Rituximab 200mg preoperatively in low immunological risk patients. Steroids are withdrawn on post-operative day 5. The patient survival rates at 1,5 and 12 years were 97.7%, 94.8% and 92.4% respectively. And the corresponding graft survival in this study was 97.2%, 94.8% and 86.1% respectively[6]. This study showed that thymoglobulin plus rituximab can facilitate steroid free regimens in patients with low immunological risk. The results of this study are in correlation with our study. Our study has shown that patients who received thymoglobulin plus rituximab has lower creatinine levels, lesser number of rejection episodes compared to patients who received thymoglobulin alone. There was no difference in the infection risk between two groups. These patients also have better patient and graft survival even though it has not reached statistical significance which could be because of small sample size.

In another study, MWF van den Hoogen[7] evaluated the safety and efficacy of rituximab (375mg/m^2) as induction agent compared to the placebo. This study found that there was no significant difference in the biopsy proven acute rejection compared to placebo in patients with low immunological risk (p=0.24) but found to be beneficial in patients with high immunological risk(p=0.004). Treatment with rituximab was found to be safe in this study.

In another retrospective study, Tomita Y[8] et al retrospectively analysed the safety of rituximab induction in non-sensitised ABO compatible live renal transplant recipients. They found that biopsy proven acute rejections(p=0.04) and denovo production of donor specific antibodies(p=0.005) with no significant difference in the patient and graft survival (p=0.45 and 0.11 respectively).

In our study, patient survival rates in group 1 at the end of 1st, 2nd, 3rd years are 86.7%, 80% and 76.7%. The corresponding survival rates in group 2 are 90%, 86.7% and 86.7% respectively. Patients in group 2 have significantly lesser number of biopsy proven rejection episodes compared to group 1 at the end of 1st and 2nd years (p=0.049, 0.02 respectively). Patients in group 2 have lower baseline serum creatinine levels compared to group 1 at the end of 1st, 2nd ,3rd years (p= 0.01, 0.01. 0.02 respectively). There is no significant difference in the infection episodes in both groups at the end of 1st and 3rd years (p= 0.4, p=0.2 respectively). However, during 2nd year, thymoglobulin group patients developed more infections(p=0.049).

Graft survival rates in group 1 at the end of 1st, 2nd ,3rd years are 86.7%, 76.7%and 73.4% respectively. The corresponding graft survival rates in group 2 are 90%, 86.7% and 83.4% respectively. Excluding patients who died with infectious complications and cardiac issues, one patient from each group lost graft due to BK virus and one patient from group 1 lost graft with resistant rejection.

Studies by Vivek patak etal, Tomita Y et al document positive benefit of rituximab induction in renal transplant recipients in terms of rejection episodes without significant adverse effects. Study by Vivek patak et al showed patient survival and graft survival benefit whereas study by Tomita Y didn't show patient and graft survival benefit. However, none of the above studies are randomised, prospective controlled trials comparing rituximab with conventional induction therapies. Our study demonstrated that induction with low-dose rituximab plus low -dose anti thymocyte globulin is superior to conventional doses of anti-thymocyte globulin alone in high-risk renal allograft transplant recipients in terms of rejection episodes, patient and graft survival. There was no significant difference in the infection episodes between two groups. The average cost of the combination therapy is also significantly less compared to the treatment cost of conventional thymoglobulin alone.

CONCLUSION:

Induction with combined low-dose thymoglobulin plus rituximab is non-inferior to conventional dose of anti-thymocyte globulin alone. Patients who received combined therapy has lesser rejection episodes which is of statistical significance, better patient and graft survival even though it has not reached statistical significance probably due to low sample size. The episodes of rejection in patients with combination therapy responded to steroids easily compared to anti-thymocyte globulin alone. Given the cost effectiveness with no change in adverse effects and non-inferiority, combined induction therapy can be considered in high-risk renal transplant recipients.

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