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Long-Term Assessment of Immune Tolerance State in Steroid-Free Kidney Transplant Recipients

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Abstract

Immune tolerance state amog kidney transplant recipients is associated with better outcome and could be achieved using immunosuppressive medication. However, these medications had long term side effects. Achieving immune tolerance using minimal immunosuppression is the future perspective.Our study included 50 steroid avoidance kidney transplant recipients who received kidney transplantation 5 years ago. The used induction therapy is basiliximab. CD4, CD8 and regulatory t cells expression was assessed using flow cytometry technique.The study included 50 kidney transplant recipients with male predominance (31) and mean age 24.78±10.4. ForteenKTrs (28%) had acute rejection episodes which were managed by methyl-prednisilone (10 mg/kg) for 5 days. None of KTrs who experienced rejection episodes needed more anti-rejection therapy. There was inverse correlation between regulatory t cells and expression of both CD4 and CD8. However, the correlation between regulatory t-cells and CD8 was of statistical significance. A state of immune tolerance reflected by good graft function was occurred successfully 5 years after transplantation among steroid avoidance kidney transplant recipients.

Keywords: Kidney transplantation, immune tolerance, regulatory t cells

Introduction

The current adjustment of immunosuppression medications among kidney transplant recipients is based on target levels with higher targets in patients with high immunologic risks and low levels in low immunologic risk patients (1).Corticosteroids are a cornerstone of immunosuppressive therapy in KTX despite their side effects and morbidity. More than 95% of transplant recipients are treated with CS as a usual component of clinical immunosuppressive regimens. They are effective in reducing the incidence of acute rejection but are an important cause of morbidity and probably mortality (2).Moreover, they have adverse effects on cardiovascular risk factors such as hypertension, hyperglycaemia, or hyperlipidaemia, deleterious effects on bone metabolism, and may contribute to an increased risk of infection (3).Steroid sparing with discontinuation within the first few weeks after transplant has gained widespread acceptance, with more than 25% of patients being discharged after transplant off steroid therapy (4).There are no studies of different times in the first year to determine when CS therapy can be discontinued safely. In some centers, steroid therapy discontinuation is accomplished up to 6 months posttransplant. Late discontinuation of steroid therapy (ie, more than 1 year post-transplant) is no longer recommended. Steroid therapy withdrawal should be performed only when there is close frequent monitoring of the patient (5).Tolerance in renal transplantation is an exceptional finding. Approximately 100 cases of tolerance in renal

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transplantation have been reported to date, mainly in patients who were not compliant with their immunosuppressive regimens or in individuals who had previously received a bone marrow transplant for hematological disorders (6).At the present time, in looking for tolerance in renal transplantation, physicians in clinical practice have implemented protocols and surgical procedures in which tolerance was the planned objective before the transplant.The ability of the immune system to differentiate self from nonself is critical in determining the immune response to antigens expressed on transplanted tissue. The immune system responds to the antigens through the interaction of T cells with the MHC (7). According to animal model studies, acceptance/tolerance of an allograft is an active process often determined by the presence of regulatory T cells (Regulatory T-cells). Therefore, numerous studies have focused on ways of either expanding endogenously occurring Regulatory T-cells or using exogenously expanded Regulatory T-cellss to achieve acceptance of the allograft while avoiding the complications of long-term immunosuppression (8).It cannot be assumed that tolerance induced by Regulatory T-cells is totally stable and one of the real challenges will be to develop a means of monitoring the immune response. Some studies have labeled expanded Regulatory T-cells with deuterium to monitor the adoptively transferred cells by flow cytometry (9).Maintenance therapies vary by type of organ, institutional preference, and organ recipient demographics. A multimodal approach is commonly employed to prevent rejection by blocking immune responses through several pathways. Commonly used immunosuppressive drugs include CNIs, mammalian Target of Rapamycin (mTOR) inhibitors, corticosteroids, mycophenolate preparations, CTLA4-Ig, and anti-CD20 mAb (10). Herein, we will evaluate the expression of regulatory t cells among steroid avoidance kidney transplant recipients and its relation to t helper cells and t cytotoxic cells expression.

Topic

Expression of regulatory T cells in steroid-avoidance kidney transplant recipients which mean immune tolerance and low risk for rejection gives more assurance and make it safer to use steroid-avoidance protocols and encourage reducing more immunosuppressive drugs.

Patients and Methods

This cross-sectional study was held in Urology and Nephrology Center, Egypt in the duration between 1/2020 and 1/2021. The study included 50steroid avoidance kidney transplant recipients who received kidney transplantation 5 years ago. The used induction therapy is basiliximab.Maintenance immunosuppression: (Tac+MMF)The patient received 10mg/kg pulse steroid at day -1, 0 and 1 then 5mg/kg at day 2 then 100 mg/day till tacrolimus trough level was above 5 ng/dL. Then, steroids were stopped abruptly. All patients were followed up twice weekly during the 1st month, once weekly during the 2nd, month, every 2 weeks during the 3rd month then monthly for the rest of the 1st year. After that, follow-up visits were decreased gradually to become every 3 months. Patients were followed-up by serum creatinine and estimated glomerular filtration rate (eGFR), sodium, potassium, fasting blood sugar, total cholesterol, uric acid, complete blood picture, tacrolimus trough level and urinalysis.

*Assessment of regulatory T cell expression***:** Separated lymphocytes from 10 ml blood sample will be used for detection of CD4, CD25, CD127, CD8 by flowcytometr technique flow cytometry technique.

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Data Analysis

All data were tabulated in SPSS program V. 21. Descriptive analysis was used. Q-q plot analysis was used to assess data distribution. Spearman correlation test was used to determine correlation between non parametric continuous data. Level of significance was ≤0.05

Results

The study included 50 kidney transplant recipients with male predominance (31) and mean age 24.78±10.4. They received kidney transplantation from living related donors mainly with female predominance (31KTrs) and mean age 38.5±10.3. The most prevalent medical problem was hypertension (22KTrs). Most of them received pre-transplant hemodialysis (43KTrs). Kidney transplantation was mainly between blood group compatible donors and recipients. Most of them were mismatched in 2 HLA class I alleles and 1 HLA class II alleles. Two patients had ani-HLA antibodies (donor non-specific) before transplantation. Delayed graft function occurred in 4 patients and mean ischemia time was 49.34±11.86 minutes. Total received steroid dose in the 1st 3 months after transplantation was 2.9±1.4 gm (table 1).

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Table (1): Baseline and operational data

	Steroid-free group (50 KTrs) No. (%)
Recipient sex (male)	31 (62%)
Recipient age (years) mean±Sd	24.78±10.4
Donor sex (male)	19 (38%)
Donor age (years) mean±Sd	38.5±10.3
Consanguinity (related)	44 (88%)
Pre-transplant hypertension	22 (44%)
Pre-transplant blood transfusion	7 (14%)
Pre-transplant erythropoietin use	20 (40%)
Pre-transplant dialysis	43 (86%)
Degree of HLA I mismatch:	
0 mismtach	2 (4%)
1 mismatch	9 (18%)
2 mismatch	33 (66%)
3 mismatch	4 (8%)
4mismatch	2 (4%)
Degree of HLA II mismatch:	
0 mismatch	8 (16%)
1 mismatch	42 (84%)
Anti-HLA antibodies (positive)	2 (4%)
Blood group matching:	
Same	40 (80%)
Different compatible	10 (20%)
Ischemia time (minutes) mean±Sd	49.34±11.86
Time to dieresis:	
Immediate	46 (92%)
delayed	4 (8%)
Total dose of steroids in the 1st 3 months (gm) mean±Sd	2.9±1.4

Post-transplant surgical complications were rare among the studied group. Post-transplant hypertension was the main medical complication (22 KTrs). Other complications had smaller incidence rate. ForteenKTrs (28%) had acute rejection episodes which were managed by methyl-prednisilone (10 mg/kg) for 5 days. None of KTrs who experienced rejection episodes needed more anti-rejection therapy. Acute rejection episodes per patient were only 1. Rejection was biopsy-proven. Biopsy was either protocol biopsy (4 KTrs) or event-based biopsy (46 KTrs) (table 2).

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Table (2): Post-transplant complications

	Steroid-free group (50 KTrs) No. (%)
Bleeding	2 (4%)
Wound dehiscence	0
Lymphocele	0
Post-transplant hypertension	23 (46%)
Post-transplant diabetes	5 (10%)
Bacterial infection	5 (10%)
CMV infection	1 (2%)
Hepatic impairment	3 (6%)
Gastrointestinal troubles	1 (2%)
Malignancy	0
Acute tubular necrosis	3 (6%)
Acute cellular rejection	14 (28%)
Chronic rejection	0

All patients were alive with good function graft at time of inclusion in the study. Serial creatinine over 5 years follow-up was illustrated in table 3.

Table (3): Follow-up serum creatinine over 5 years

s. creatinine (mg/dl)	**Steroid-free group (50 KTrs) mean±Sd**
S. cr after 1 year	0.97±0.4
S. cr after 2 year	0.99±0.28
S. cr after 3 year	1.01±0.28
S. cr after 4 year	1.03±0.29
S. cr after 5 year	1.06±0.29
Creatinine clearance at last follow-up (ml/min)	94.2±47.8

Mean CD4+ expression was 30.7±14.8 and CD8+ expression was 24.2±7.2. Median regulatory t-cells expression was 1.95 (0.1, 11.9) (table 4).

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Table (4): immune tolerance components among both groups

	Steroid-free group (50 KTrs)
Total lymphocytic No./µL	2.5±0.7
CD4 (%)	30.7±14.8
CD8 (%)	24.2±7.2
Regulatory T-cells (%) Median (min, max)	1.95 (0.1, 11.9)

There was inverse correlation between regulatory t cells and expression of both CD4 and CD8. However, the correlation between regulatory t-cells and CD8 was of statistical significance ***(p value: 0.0001)*** **(table 5).**

Table (5): Correlations between regulatory T cells and CD4, CD8

	Spearman correlation (r^2)	**P value**
Regulatory T-cells: CD4	-0.209	0.144
Regulatory T-cells: CD8	-0.529	***0.0001***

Discussion

Many side effects are associated with the use of corticosteroids. This was the drive for many trials to withdraw corticosteroids at certain time point post renal transplantation (11). Steroid-avoidance immunosuppressive regimens have been used widely in different centers worldwide. The results are promising and support the safety and efficacy of these regimens even if the patient is at high risk (12).Immunologic tolerance achievement could be the rescue to improve the overall patient and graft survival among steroid-avoidance immunosuppressive kidney transplant recipients (13). Our study included 50 steroid avoidance kidney transplant recipients who received kidney transplantation 5 years ago. The used induction therapy is basiliximab.CD4, CD8 and regulatory t cells expression was assessed using flow cytometry technique.In our study, Living related donation is the role in our center. Taber et al., had variable percent of deceased donors (14). We reported steroid-free group were mismatched in 2 alleles only along with Ahmad et al., (15). On the other hand, Gaber et al., reported more mismatched alleles in steroid-free groups (16).*Hruba et al.,* found higher rejection rate among steroid free group (17). *Cantarovich et al.*, also reported higher rejection episodes among steroid-free group with larger sample size (18).Various mechanisms have been proposed to explain suppressive function of Treg on naive or effector T cells. These include interactions between stimulatory (IL-2 and CD28) and inhibitory (glucocorticoid-induced tumor necrosis factor receptor family-related protein (GITR) and cytotoxic T lymphocyte-associated antigen 4 (CTLA-4)) signals, stimulation of dendritic cells via CD80/CD86 molecules, and cell–cell inhibition of effector cells by membrane-bound and soluble factors (e.g. transforming growth factor-β (TGF-β) and IL-10) (19). Valloton et al., reported lower levels of regulatory t cells among kidney transplant recipients when compared to healthy population (20). Level of regulatory t-cells after kidney transplantation is affected by types of immunosuppressive medications.Camirand&Riella, reported that steroids have minimal or no effect on expression of CD4+CD25^{high}CD127^{low} regulatory t-cells (21). Steroids by its effect on IL2 may decrease the trigger to develop CD4+CD25+CD127- regulatory t cells. Louis et

al., also reported no significant effect of steroids on regulatory t-cells expression at any time after kidney transplantation with the use of ATG as induction therapy (22). On the other hand, Furukawa et al., stated that corticosteroids likely benefit Treg prevalence and activity. Additionally, steroids may also create a favorable immune environment for Treg through modulation of local cytokine expression (23).

Conclusion

A state of immune tolerance reflected by good graft function was occurred successfully 5 years after transplantation among steroid avoidance kidney transplant recipients. Regulatory t-cells were expressed sufficiently to down regulate CD8+ cells expression.

Limitations and future studies

Our study has some limitations as being retrospective, lack of randomization, and lack of comparative control group. Future plan is to measure serial regulatory t cells and try to withdraw immunosuppressive medications.

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