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Evaluation of renal transplantation recipients those who do not use steroid with panel reactive antibody and donor specific antibody

Evaluación de receptores de trasplante renal que no usan esteroides con panel de anticuerpos reactivos y anticuerpos específicos del donante

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ABSTRACT

Background: Steroids are the mainstream drugs of immunosuppressive regimen in renal transplantation. They are successfully used on induction, maintenance and rejection treatment. Due to complications caused by steroids, treatments are switched to immunosuppressive agents. Graft dysfunction risk caused by reduced total immunosuppression disturbs clinicians very often. We documented the differences among patients by means of clinical presentation and PRA/DSA levels between patients who are using steroids and patients that were prescribed for steroid-free regimen. **Methods:** 82 individuals who did not use steroid and 52 patients on steroid treatment were included with similar rates of age, sex, primary renal disease, dialysis type, posttransplant follow-up duration and donor type. Pre and posttransplant PRA, DSA levels, posttransplant and current graft function and comorbidities were evaluated. **Results:** Individuals who do not use steroids were found to have a lower posttransplant creatinine level and glomerular filtration rate (GFR) compared to steroid users. Posttransplant and current spot urinary protein/creatinine rates were also lower in the steroid-free group. However DM, BKVN and induction therapy rates were higher in the steroid-free group. PRA and DSA levels were similar in both groups. On the other hand, posttransplant PRA-I levels were significantly higher in those with less steroid use time. **Conclusions:** Although steroid free regimens usually worry the clinicians, they can be preferred in patients with low immunological risk for rejection to avoid its side effects such as uncontrolled diabetes, obesity, musculoskeletal problems and cataracts.

KEYWORDS: renal transplantation; corticosteroids; panel reactive antibody; donor specific antibody

RESUMEN

Antecedentes: Los esteroides son los principales fármacos del régimen inmunosupresor en el trasplante renal. Se utilizan con éxito en tratamientos de inducción, mantenimiento y

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rechazo. Debido a las complicaciones causadas por los esteroides, los tratamientos se cambian a agentes inmunosupresores. El riesgo de disfunción del injerto causado por la reducción de la inmunosupresión total perturba a los médicos con mucha frecuencia. Documentamos la diferencia entre los pacientes por medio de la presentación clínica y los niveles de PRA/DSA en aquellos que utilizan esteroides y a los que se les prescribió un regimen sin esteroides. **Material y métodos:** Se incluyeron 82 individuos que no usaban esteroides y 52 pacientes en tratamiento con esteroides con tasas similares de edad, sexo, enfermedad renal primaria, tipo de diálisis, duración del seguimiento postrasplante y tipo de donante. Se evaluaron la ARP pre y postrasplante, los niveles de DSA, la función y comorbilidades postrasplante y actual del injerto. **Resultados:** Se encontró que las personas que no usan esteroides tienen un nivel de creatinina postrasplante y una tasa de filtración glomerular (TFG) más bajas en comparación con los usuarios de esteroides. Las tasas de proteína/creatinina urinarias postrasplante y puntuales actuales también fueron más bajas en el grupo sin esteroides. Sin embargo, las tasas de DM, BKVN y terapia de inducción fueron más altas en el grupo sin esteroides. Los niveles de PRA y DSA fueron similares en ambos grupos. Por otro lado, los niveles de PRA-I postrasplante fueron significativamente más altos en aquellos con menos tiempo de uso de esteroides. **Conclusiones:** Aunque los regimenes libres de esteroides suelen preocupar a los clínicos, pueden ser preferidos en pacientes con bajo riesgo inmunológico de rechazo para evitar sus efectos secundarios, como diabetes no controlada, obesidad, problemas musculoesqueléticos y cataratas.

PALABRAS CLAVE: trasplante renal; corticosteroides; panel de anticuerpos reactivos; anticuerpos específicos del donante

INTRODUCTION

Avoidance or early cessation of steroids in kidney transplantation is supported by recent guidelines, however later cessation of steroids was not supported recently.[1] Steroid-free regimens have been tried to avoid steroids' adverse effects such as diabetes mellitus, hypertension, hyperlipidemia, avascular necrosis and osteopenia; nevertheless, an increase is observed in acute rejection rates.[2-3] On the first few days of posttransplantation, acute rejection incidence was found lower in steroid-free group, on the other hand there are many restrictions due to design of the clinical trials.[4-5] In renal transplantation, acute rejection incidence is higher in those steroid-free immunosuppressive regimens despite the improvement of steroid-free therapies.

In addition, long-term graft survival is not known in patients with acute rejection in a steroid-free immunosuppressive regimen.[6] Our knowledge for whether a steroid-free immunosuppressive regimen will become the first line therapy is insufficient. That is why, only selected population can be preferred for this.[7] In an updated metaanalysis, discontinuation of the steroid after kidney transplantation significantly increased the risk of acute rejection, there was no difference in patient mortality or graft loss up to five years after transplantation, so prospective long-term studies are recommended.[8]

In chronic graft dysfunction, contribution of antibody mediated rejection (AMR) is highly accepted. AMR is triggered by humoral immunity that is mediated by several antibodies, especially donor specific HLAs. These antibodies cause serious problems in renal transplantation and donor specific antibodies (DSA) that occur after renal engraftment cause acute rejection.[9] Several clinical trials showed that the presence of DSAs are related with poor graft function.[10-12] Our aim in this study is to emphasize the importance of preventing unnecessary immunosuppression with immunological monitoring. At the same time, it is to increase the graft survival by intervening early in the treatment in the patient who needs it.

METHODS

We evaluated PRA and DSA levels of adult individuals with functioning grafts (25 ml/min/1,73 m^2 or more) in our transplantation unit that has been working since 1994. Individuals with inadequate information were excluded. We included 82 steroid free individuals and 52 steroid user individuals. By the way, we noted individuals' primary kidney diseases, dialysis

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type, donor type, duration of transplantation, comorbidities such as diabetes mellitus (DM), hypertension (HT) or cardiovascular disease (CVD), DGF ratios, induction therapy, duration of steroid use, cessation of steroid and its etiology. Pretransplant PRA and posttransplant PRA and DSA levels which had been used for immunological monitorization were also noted with pre and posttransplant current graft function. Steroid cessation time, etiology of avoidance and duration of steroid use before cessation were also noted in steroid free group. Acute rejection or BK virus nephropathy history were noted if present, in both groups.

SSO method

Sequence-specific oligonucleotides method was performed according to the manufacturer's instructions (Lifecodes HLA SSO Typing Kit Immucor, USA). For the first amplification step, 16 µl of mix containing master mix, H_2O, and Taq polymerase was added on four microliters of DNA (15-200 ng) in an Eppendorf tube (200 µl). The total volume of 20 µl samples was placed in the thermal cycle and the program was run. For the second hybridization step, the probe mix was warmed at 56° C for seven minutes. The probe mix was sonicated and vortexed before use. Then, 15 µl of probe mix was added on five microliters of amplicon in 96 well plates, and the samples were placed in the thermal cycler and the hybridization program was run for 20 minutes. During this run, the Luminex fluoro analyzer instrument was prepared for the analysis. When the hybridization program ended at 56° C, 170 µl diluted Streptavidin were added on the samples in the wells, and the wells were placed in the Luminex instrument. The results were analyzed by MatchIt Software Program.

Panel reactive antibody method

Lifecodes LifeScreen Class I and II ID Kits (Immucorgamma, USA) were used for Class I and Class II identification, respectively. After the 96 well plates were moisturized, wash buffer, patient/control sera and HLA Class I or II ID beads were added into the wells. The plate was incubated at room temperature for 30 minutes in the dark. After incubation the wells were washed with 200 µl buffer for three times. Then, conjugate was prepared in appropriate concentration and added into the wells. After incubation at room temperature for 30 minutes in the dark, 150 µl wash buffers were added into the wells. The plate was gently mixed in the Luminex Fluoroanalyzer instrument, and the results were analyzed by MatchIt Software program.

Statistical analysis

Statistical analyzes were performed using IBM® SPSS® 25 (NY, USA) software. The suitability of variables to normal distribution is examined using analytical methods (Kolmogorov-Smirnov / Shapiro-Wilk tests). Descriptive statistics were done by giving the mean ± standard deviation, median and IQR, minimum-maximum value. In categorical variables, frequency and percentage values were given and Pearson's or Fisher's Exact Chi Square test were used for comparison of categorical variables. In comparison of independent groups between continuous variables, t-test was used for variables that conform to normal distribution, and Mann-Whitney U test was used for non-normal distribution. In comparison of more than two groups, Kruskal-Wallis test was used and after post hoc

Bonferroni correction was used. $p<0.05$ was considered significant.

RESULTS

Age, sex, number of transplantation, mismatch, current GFR, acute rejection, posttransplant malignancy, HT presence were similar in both groups. Clinical, biochemical and immunological analysis features of the study groups were shown in **Table 1**. Posttransplant creatinine (mg/dl), actual creatinine (mg/dl), posttransplant eGFR (ml/min per/1.73 m^2), posttransplant spot urine protein/creatinine, actual spot urine protein/creatinine, DM, BKVN presence and induction therapy were significantly different. Immunological analysis showed no marked difference.

Steroid free individuals' initial therapy was CNI based regimen and 61% of individuals received induction therapy. After cessation of steroid, most patients had continued CNI based regimen. 95% of individuals under steroid treatment were stopped after one year

Table 1. Clinical, biochemical and immunological analysis features of the study groups

Continuous Variable	**Steroid-free group (n=82) mean ± SD**	**Control group (n=52) mean ± SD**	**P†**
Age (year)	46.0 ± 9.5	46.4 ± 12.1	0.448
Tx number	1.05 ± 0.2	1.02 ± 0.1	0.381
Mismatch	3.45 ± 0.7	3.56 ± 0.6	0.494
Posttransplant creatinine(mg/dl)	0.79 ± 0.4	1.30 ± 0.3	***<0.001***
Actual creatinine(mg/dl)	0.99 ± 0.6	1.44 ± 0.5	***<0.001***
Posttransplant eGFR (ml/min per/1.73 m^2)	68.8 ± 12	63 ± 12.8	***<0.001***
Actual eGFR (ml/min per/1.73 m^2)	58.8 ± 19	57 ± 17	0.428
Posttransplant spot urine protein/creatinine	0.1 ± 0.2	0.14 ± 0.3	***<0.001***
Actual spot urine protein/creatinine	0.11 ± 0.4	0.29 ± 1.0	***<0.001***
Categorical Variables	**n (%)**	**n (%)**	**p***
Gender(F/M)	32/50	21/31	0.875
Presence of DM	14(17.1)	2(3.8)	***0.021***
Presence of HT	45(54.9)	31(59.6)	0.590
Post-transplant BKVN	4(10)	0(0)	***0.020***
Induction Therapy	50(56,3)	44(35,7)	***0.010***
Acute rejection	6(7.3)	7(13.5)	0.242
Posttransplant malignancy	0(0)	1(1.9)	0.388
Pretransplant PRA positivities	4(28.6)	10(32.3)	1.000
Pretransplant PRA I positivities	2(14.3)	7(22.6)	0.698
Pretransplant PRA II positivities	3(21.4)	7(22.6)	1.000
Posttransplant PRA positivities	19(25.7)	13(27.1)	0.863
Posttransplant PRA I positivities	6(8.1)	7(14.9)	0.240
Posttransplant PRA II positivities	16(94.1)	8(100)	0.484
Posttransplant PRA-A positivities	3(4.1)	3(6.3)	0.679
Posttransplant PRA-B positivities	4(5.4)	5(10.4)	0.314
Posttransplant PRA-DR positivities	3(4.1)	1(2.1)	1.000
Posttransplant PRA-DQ positivities	14(18.9)	9(18.8)	0.981
Posttransplant DSA positivities	10(13.5)	6(12.5)	0.208
Posttransplant non-DSA positivities	18(24.3)	10(20.8)	0.198

† Independent t-test was used and *Pearson's or Fisher's Exact Chi-square *p<0.05 was considered significant*

of transplantation. Etiology of avoidance was not specified but most common causes were osteoporosis, avascular necrosis, uncontrolled DM and HT.

Transplant time and steroid use duration were compared with other parameters in **Table 2**. Transplant time and steroid use duration were significantly different in those who had received induction therapy. It was observed that those who received induction therapy had shorter transplant and steroid use times. Posttransplant PRA I positivity was found to be more significant for those with shorter steroid use duration.

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Table 2. Tx time, steroid usage time compare with other parameters

Groups	Subgroups	Tx Time(month) Mean±SD	Tx Time(month) Median (IQR) Min-Max	p-Value	Steroid Usage Time(month) Mean±SD	Steroid Usage Time(month) Median (IQR) Min-Max	p-Value
Gender							
	Female (n=32)	13.9 ± 5.2	13.0 (6.0) 4-24	0.480†	4.8 ± 3.1	4.0 (4.0) 1-15	0.191
	Male (n=50)	13.1 ± 4.8	13.5 (5.0) 3-24		5.7 ± 3.2	5.0 (5.0) 2-12	
DM							
	No (n=68)	13.4 ± 5.0	13.5 (6.0) 3-24	0.858	5.5 ± 3.2	5.0 (6.0) 1-15	0.590
	Yes (n=14)	13.9 ± 5.0	13.0 (7.0) 6-24		5.0 ± 3.1	3.5 (4.0) 2-12	
CAD							
	No (n=76)	13.3 ± 4.7	13.0 (6.0) 3-24	0.391	5.4 ± 3.2	5.0 (5.0) 1-15	0.411
	Yes (n=6)	15.5 ± 7.6	14.5 (14.0) 4-24		4.8 ± 3.0	4.5 (5.0) 2-10	
HT							
	No (n=37)	13.2 ± 5.1	13.0 (7.0) 3-24	0.877	5.5 ± 3.5	5.0 (6.0) 1-15	0.663
	Yes (n=45)	13.6 ± 4.9	13.0 (6.0) 3-24		5.3 ± 2.9	5.0 (4.0) 2-12	
Donor Type							
	Cadavere (n=28)	12.1 ± 4.4	12.0 (5.0) 3-24	0.076	4.7 ± 2.8	4.0 (4.0) 2-12	0.569
	Live (n=54)	14.2 ± 5.1	14.0 (8.0) 4-24		5.7 ± 3.3	5.0 (5.0) 1-15	
DGF							
	No (n=72)	13.7 ± 5.0	14.0 (6.0) 3-24	0.198	5.3 ± 3.1	5.0 (4.0) 1-15	0.106
	Yes (n=10)	11.4 ± 4.2	11.5 (5.0) 3-17		5.8 ± 3.6	5.0 (6.0) 2-12	
İnduction Theraphy *							
	No (n=32)	17.6 ± 3.9	16.5 (6.0) 9-24	***<0.001***	6.8 ± 3.4	11.8 (6.0) 1-15	***<0.001***
	ATG (n=16)	10.6 ± 3.8	11.0 (5.0) 3-17		5.3 ± 2.9	5.0 (3.0) 2-12	
	IL-2 ANT (n=34)	10.9 ± 3.4	12.0 (5.0) 3-18		4.2 ± 2.6	3.0 (4.0) 1-10	
Pre Tx PRA							
	Negative (n=10)	7.1 ± 2.5	7.0 (5.0) 3-10	1.000	3.2 ± 1.1	3.0 (2.0) 2-5	0.943
	Positive (n=4)	6.8 ± 2.5	8.0 (4.0) 3-8		2.8 ± 1.7	2.5 (3.0) 1-5	
Pre Tx PRA I							
	Negative (n=12)	7.3 ± 2.3	7.5 (4.0) 3-10	0.457	3.2 ± 1.3	3.0 (3.0) 1-5	0.517
	Positive (n=2)	5.5 ± 3.5	5.5 (-) 3-8		2.5 ± 0.7	2.5 (-) 2-3	
Pre Tx PRA II							
	Negative (n=11)	7.2 ± 2.4	7.0 (4.0) 3-10	0.751	3.2 ± 1.1	3.0 (2.0) 2-5	0.813
	Positive (n=3)	6.3 ± 2.9	8.0 (-) 3-8		2.7 ± 2.1	2.0 (-) 1-5	
Post Tx PRA							
	Negative (n=55)	13.4 ± 5.0	13.0 (6.0) 4-24	0.799	5.5 ± 3.1	5.0 (5.0) 1-15	0.624
	Positive (n=19)	13.0 ± 5.3	14.0 (6.0) 3-23		5.1 ± 3.4	5.0 (5.0) 2-12	
Post Tx PRA I							
	Negative (n=68)	13.7 ± 4.9	14.0 (6.0) 3-24	0.055	5.5 ± 3.2	5.0 (5.0) 1-15	***0.037***
	Positive (n=6)	9.0 ± 5.0	9.0 (11.0) 3-15		4.7 ± 3.1	4.0 (5.0) 2-10	
Post Tx PRA II							
	Negative (n=58)	13.4 ± 5.0	13.0 (6.0) 4-24	0.864	5.5 ± 3.1	5.0 (4.0) 1-15	0.673
	Positive (n=16)	12.9 ± 5.6	14.0 (6.0) 3-23		5.1 ± 3.6	3.5 (6.0) 2-12	
Post Tx DSA							
	No (n=64)	13.3 ± 5.3	13.0 (6.0) 3-24	0.757	5.5 ± 3.2	5.0 (5.0) 1-15	0.396
	Yes (n=10)	13.5 ± 3.1	14.0 (4.0) 8-18		4.8 ± 3.2	4.0 (5.0) 2-10	
Post Tx NDSA							
	No (n=56)	13.5 ± 5.0	13.0 (6.0) 4-24	0.924	5.5 ± 3.1	5.0 (5.0) 1-15	0.889
	Yes (n=18)	12.8 ± 5.5	14.0 (7.0) 3-23		5.3 ± 3.4	5.5 (6.0) 2-12	

Mann-Whitney U test and † t-test were used and p<0.05 was considered significant.

* Kruskal-Wallis test was used and after post hoc Bonferroni correction was used

DISCUSSION

DSAs that occur against renal graft causes antibody mediated rejection (AMR) which is a reason for graft dysfunction.[13] It has become essential to detect antibodies. Solid-phase immunity analysis, especially LUMINEX®, is more sensitive than previous complement dependent lymphocytotoxicity (CDC) analysis, that is why it is recommended for high risk patients.[14] We monitorized these parameters periodically and if needed, we perform pathological analysis for direct treatment.

Clinicians should be careful in case of development of AMR as it once happens, no efficient treatment is available. Posttransplant DSA occurrence risk is determined by the intensity of immunosuppressive therapy and patients' compliance. Thus, immunological tests may be helpful in the follow-up for those patients who have quitted steroid therapy.

Our experiment revealed that spot urine/ creatinine ratio and serum creatinine levels are lower in contrast to higher eGFR. This may be related to the selection of patients with low immunological risk. This also may be the sign that we defined patients' risk accurately.

Patients' history of DM, occurence of new onset diabetes mellitus after transplantation (NODAT) or poorly controlled DM under steroid therapy might cause switching therapy to steroid-free regimens. A clinical trial reported that switching therapy to steroid-free regimen was related to lower risk of NODAT occurrence in 3 years of follow-up.[15] This is possible if there is an early cessation of steroid.

In our work, we found that the suspension of steroids was done too late. Also the fact that BKVN was detected more in this group may be the reason why the clinicians decided to use reduced doses of immunosuppressive agents. The high induction therapy rates of the steroid-free group may have led the clinician to cease steroids more comfortably. The fact that those with less steroid use in the steroid-free group are among those who received more induction therapy supports this view. In a clinical trial it was reported that duration of steroid use is shorter in the group who had taken effective induction therapy, and this was related to lower steroid side effects and better graft function.[16]

This study is a retrospective study and tried to compare a steroid-free group with a control group. The steroid was discontinued for two reasons. In the first place, when a steroid related side effect was present and second, when the patient had poor kidney function. Therefore, the results we have obtained may seem in favor of steroids. For this reason, it is stated that following this study in the long term, conducting a randomised controlled trial will give much better results.

This might be a warning for clinicians that posttransplant PRA I levels are higher in the group that used steroids for a shorter time, but it must be supported by other parameters and more clinical trials. In a clinical trial it was reported that steroid might be quitted early and showed that it could potentially be useful in elderly patients as well as sensitized recipients with PRA ≤60%, regardless of the degree of HLA sensitivity. However, it seems to be beneficial to continue steroids in young and highly sensitized patients.[17]

In this study, choice of the steroid-free regimen was determined by the clinician according to each patient's condition, and the patients' discontinuation was one year after the transplant. Despite this, acute rejection rates were not found to be higher in contrast to literature.

Complement based DSAs/ IgG subgroups might be more useful in immunological monitorization.[18,19] Immunologic responses are important factors for renal transplantation and anti-HLA antibodies may affect long term graft function.[20] That's why more innovative approaches should be performed to prevent critical sensitization, occurence of anti-HLA antibodies, posttransplant non immunologic complications and optimal treatment of chronic active ABMR.[21]

CONCLUSIONS

Randomized controlled trials are needed to assess the reliability of steroid-free regimens. Until then, it would be safer to choose steroid-free regimens only in a selected group of patients.

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