Pre-transplant angiotensin II type 1 receptor antibodies: a risk factor for decreased kidney graft function in the early post-transplant period?

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ABSTRACT

Angiotensin II type 1 receptor antibodies (AT1Rab) are associated to a significantly lower graft survival and a higher risk of acute rejection after kidney transplantation. This study aimed to evaluate graft function and BPAR during the 1st year post-transplant (PT) in adult kidney transplant recipients (KTR), between 03/2009 and 08/2012. Pre-KT sera were screened for AT1Rab (ELISA) and HLA-DSA (Luminex). Three groups were analyzed: AT1Rab only (n = 13); HLA-DSA only (n = 8); and no AT1Rab or HLA-DSA (n = 90). No differences were observed in clinical characteristics across groups. A higher percentage of BPAR was observed in the AT1Rab positive group, but this difference was not significant. KTR with AT1Rab had a lower mean eGFR (20 mL/min/1.73m²) when compared to KTR with no Abs at 12 months. The significant difference in eGFR was observed since the 1st month PT. Multivariate analysis showed 4 factors independently and significantly associated with eGFR at 12mos PT: BPAR (-18.7, 95%, CI -28.2 to -9.26, p<0.001), AT1Rab (-10.51, CI -20.9 to -0.095, p = 0.048), donor age (-0.42, CI -0.75 to -0.103 p = 0.010), and recipient age (-0.36, CI -0.67 to -0.048, p = 0.024). In this study AT1Rab in pre-transplant sera from KTR, was an independent and significant risk factor contributing to a lower eGFR 12 months PT. This finding deserves to be confirmed in a larger KTR population.

Anticuerpos contra el receptor 1 de angiotensina II pretrasplante: ¿un factor de riesgo para la disminución de la función del injerto en el periodo postrasplante temprano?

RESUMEN

Los anticuerpos dirigidos contra el receptor 1 de angiotensina II (anti-AT1,R) han sido asociados a una supervivencia significativamente menor del injerto y a un mayor riesgo de rechazo agudo en receptores de trasplante renal. El objetivo de este estudio fue evaluar la función renal y los episodios de rechazo agudo confirmados por biopsia (RACB) durante el primer año de evolución postrasplante (PT) en receptores de trasplante renal (RTR) adultos, efectuados entre 03/2009 y 08/2012. El suero pretrasplante fue analizado para detectar la presencia de anti-AT1,R (ELISA) y para anticuerpos HLA donante específico (ADÉ-HLA) (Luminex). Se conformaron tres grupos para el análisis: presencia exclusiva de anti-AT1,R (n = 13); presencia exclusiva de ADÉ-HLA (n = 8); y ausencia de anti-AT1,R y de ADÉ-HLA (n = 90). No se observaron diferencias en las características clínicas entre los grupos. Un porcentaje mayor, no significativo, de RACB fue observado en el grupo anti-AT1,R positivo. Los RTR con anti-AT1,R tuvieron una menor tasa de filtrado glomerular estimado (TFGe) promedio (20 mL/min/1.73m²) a 12 meses PT cuando se compararon con los RTR sin anticuerpos. Esta diferencia significativa en la TFGe se observó desde el primer mes PT. El análisis multivariado mostró cuatro factores independientes y significativamente asociados con TFGe a 12 meses PT: RACB (-18.7, 95% IC -28.2 a -9.26, p < 0.001), anti-AT1,R (-10.51, IC -20.9 a -0.095, p = 0.048), edad del donante (-0.42, IC -0.75 a -0.103, p = 0.010) y edad del receptor (-0.36, IC -0.67 a -0.048, p = 0.024). En este estudio la presencia de anti-AT1,R en suero pretrasplante de RTR fue un...
**Key words.** AT₁R antibodies. Kidney transplant recipients. Graft function.

**INTRODUCTION**

In recent years, the production of antibodies (Abs) against non-HLA related antigens has been described in renal transplant recipients; they can either be alloantibodies or autoantibodies. Some of the Abs whose implications on renal graft survival are beginning to be understood, are angiotensin type I receptor (AT₁R) antibodies (AT₁Rab).

The gene for AT₁R is on chromosome 3 and via 4 exons, it encodes a G protein coupled to a receptor with seven transmembrane domains. This receptor is responsible for most angiotensin II-mediated physiological cardiovascular responses, including blood pressure regulation and fluid and electrolyte balance.

Anti-AT₁Rab recognize a structure constituted by the second extracellular loop and act as receptor agonists fostering vascular inflammatory processes. AT₁Rab may mediate graft vasculopathy by endothelial activation. AT₁Rab have been shown to have direct effects on endothelial cells as well as smooth muscle cells by inducing Erk 1/2 mediated signal transduction. These Abs can also activate transcription factor activator protein-1 (AP-1) downstream from Erk 1/2, increase the binding activity of nuclear factor-κB (NF-κB) transcription factor as well as the expression of NF-κB pro-inflammatory target genes for chemokines such as MCP-1 and RANTES.

A relationship has been established between the presence of AT₁Rab and hypertension, cardiac hypertrophy and renal fibrosis; these all result from excessive activity of the angiotensin II-AT₁R complex and lead to increased cardiovascular morbidity and mortality.

There are multiple pathways by which AT₁Rab may develop, including both autoreactive and alloreactive responses. Autoantibodies can arise as a result of molecular mimicry and cross-reactivity. Inflammatory events might lead to de novo expression of autoantigens, proteins that become accessible after injury or as a result of the transplant process itself. AT₁Rab were initially described in women with pre-eclampsia and malignant hypertension.

**Due to the receptor’s protein nature,** it is polymorphic and some of its variants have been linked to an increased response to angiotensin II and therefore, to renal and cardiovascular disease. The most studied polymorphism is A1166C that, when present in the donor, leads to an increased risk of reactivity with the recipient’s antibodies since this polymorphism is associated to an increased expression of the AT₁R. Patients may also develop Abs to AT₁R polymorphisms through the usual mechanisms of allosensitization (pregnancy, transfusions and transplants) as well as upon immunosuppressant therapy withdrawal or non-adherence to the treatment.

Although AT₁Rab have been shown to belong to the IgG1 and IgG3 sub-classes that fix complement, some reports have underscored the low frequency of C4d positivity in biopsies from patients with these Abs and graft rejection; this suggests a different pathogenesis to that due to the effect of donor specific HLA Abs (DSA). In this regard, It has been suggested that genes regulated by AT₁R triggered transcription factors and not complement-dependent cytotoxicity to act as the effector pathway leading to vascular injury.

The association of AT₁Rab (pretransplant or of de novo synthesis) with acute rejection has been well documented, particularly in humoral responses; it has been described as a severe vascular rejection, resistant to therapy even in transplants of grafts obtained from identical HLA relatives. A relationship has been established between the presence of AT₁Rab and hypertension, cardiac hypertrophy and renal fibrosis; these all result from excessive activity of the angiotensin II-AT₁R complex and lead to increased cardiovascular morbidity and mortality.

There are multiple pathways by which AT₁Rab may develop, including both autoreactive and alloreactive responses. Autoantibodies can arise as a result of molecular mimicry and cross-reactivity. Inflammatory events might lead to de novo expression of autoantigens, proteins that become accessible after injury or as a result of the transplant process itself. AT₁Rab were initially described in women with pre-eclampsia and malignant hypertension.

**The purpose of this study was to evaluate the renal graft’s function and the acute rejections events documented by biopsy, during the first year post transplant.**

**Palabras clave.** Anticuerpos anti-AT₁R. Receptores de trasplante renal. Función del injerto.
renal transplant in patients with AT1Rab before transplant, and compare them to kidney transplant recipients with pre-transplant donor specific HLA Abs (DSA) only, and patients with neither anti-AT1R Abs nor HLA-DSA before transplant.

MATERIAL AND METHODS

This is a retrospective, cohort study of living donor renal transplant recipients (RTR) at the Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán (Mexico City), transplanted between March 2009 and August 2012. We systematically store pre-transplant serum samples from the donor and the recipient for future studies in the transplantation laboratory. They are stored at -70 °C until used that in this study, was September 2013. AT1Rab were determined by ELISA (CellTrend GMBH, Luckenwalde, Germany) and titers ≥ 17 IU were considered positive.30,31 HLA-DSA determinations were performed with Luminex Labscreen Single Antigen beads class I and class II (One Lambda, Canoga Park, CA, USA); a MFI ≥ 500 was considered positive. HLA-DSA determination is systematically conducted as part of the patient’s immunological risk evaluation in all renal transplant candidates in the Institute, as well as in the post-transplant period in those with biopsy-documented acute rejection (cellular or humoral) and 12 months after transplant coinciding with the graft’s protocol biopsy. If acute rejection is suspected, defined as a ≥ 25% verified increase in serum creatinine when compared to baseline and in the absence of obvious causes (infection, urinary tract obstruction, dehydration, CNI toxicity), a percutaneous biopsy of the renal graft is performed.

Kidney transplant recipients (KTR) were divided into three groups according to their pre-transplant serological status:

- KTR with positive AT1Rab.
- KTR with positive HLA-DSA, and
- KTR with neither AT1Rab nor HLA-DSA.

Patients with both anti-AT1R and HLA-DSA were excluded.

Demographic variables were obtained from the donor and recipient, their history of pre-transplant cardiovascular disease and sensitization, the risk of CMV, ischemia duration, induction and maintenance immunosuppressive therapy.

The analyzed outcomes included: renal function during the first year after transplant (MDRD equation); incidence of acute rejection documented by graft biopsy; development of de novo HLA-DSA; and development of hypertension in that same time period.

Statistical analysis

Categorical variables are shown as frequencies and proportions whereas continuous variables were analyzed with Kolmogorov-Smirnov test in order to determine their distribution. Those variables with normal distribution are shown as mean ± standard deviation and median with range for those with abnormal distribution. Between-group comparisons were established by χ² or Fisher’s exact test for categorical variables and with ANOVA or Kruskal-Wallis’ test for continuous variables (distribution depending). Multivariate analysis with linear regression was used to determine which variables affected the eGFR one year after transplant. All possible associated factors to an eGFR < 70 mL/min/1.73 m² one year post-transplant, were also analyzed by logistic regression. P < 0.05 was considered statistically significant.

RESULTS

Of 174 renal transplants performed during the study period, 120 kidneys were obtained from a living related donor and of these, 114 had available sera for AT1Rab determination.

According to the Ab determination in pre-transplant sera, 13 patients were included in the AT1Rab group, 8 patients in the DSA group, and 90 patients in the group with neither AT1Rab nor HLA-DSA antibodies. Three patients having both AT1Rab and HLA-DSA were excluded from the analysis.

Demographic characteristics and the relevant variables analyzed are shown in table 1. No differences were observed in donor eGFR, donor/recipient gender/age, recipient body mass index, kidney transplant number, haplotype-matches, warm/cold ischemia duration in all groups, and there were no cases of delayed graft function. Tacrolimus trough levels during the first year were similar in the 3 groups, as well as ACE inhibitor/AT1R blocker use or hypertension development during the first year post-transplant.

There were differences in terms of the induction immunosuppressant therapy used between groups, particularly that of thymoglobulin in patients with no Abs. However, this was due to the fact that 3 patients in that group underwent a second transplant and 4 had a very high %PRA. Regardless, the %PRA was lower in the serologically negative group of pa-
patients (Table 1). During follow-up, all patients were on triple immunosuppressive therapy with tacrolimus, mycophenolate mofetil and steroids.

**Main outcomes**

Table 2 shows renal function evolution for a year after transplant and comparing all three KTR groups. A decreased eGFR one year post-transplant is evident in patients with AT1Rab (51.7, r 43-63.6 mL/min) when compared with KTR and positive HLA-DSA (58.3, r 52.8-72.8 mL/min) and the KTR group with no antibodies (71.5 r 56.3-82.6 mL/min), p = 0.03. Only the difference between AT1Rab and no antibodies group was significant (Bonferroni test: p = 0.049). The eGFR difference between patients with HLA-DSA versus the other two groups was not significant. We must emphasize that from the first month post-transplant, the eGFR was significantly lower in the group of patients with AT1Rab when compared with no antibodies group (p = 0.01). When comparing all three groups in terms of the eGFR delta between the first and twelfth month post-transplant, no statistically significant differences were established (Table 2).

Despite a higher percentage of biopsy-proven acute rejection in the AT1Rab group, there was no significant difference between the groups. We must mention that the acute rejection events in the AT1Rab group, tend to occur at a latter post-transplant time period when compared to those documented in the other two groups.

In terms of de novo DSA appearance and the frequency of hypertension one year after transplant, no difference between groups was detected (Table 2).

Lineal regression multivariate analysis showed four factors independently and significantly associated with this outcome:

- Biopsy proven acute rejection (β = -18.7, 95% CI -28.2 to -9.26; p < 0.001).
- AT1Rab (β = -10.51, CI -20.9 to -0.095; p = 0.048).
- Donor age (β = -0.42, CI -0.75 to -0.013; p = 0.01) and recipient age (β = -0.36, CI -0.67 to -0.048; p = 0.024).

<table>
<thead>
<tr>
<th>KTR characteristics (n = 111)</th>
<th>AT1Rab (n = 13)</th>
<th>HLA DSA (n = 8)</th>
<th>No Abs (n = 90)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female gender recipient</td>
<td>6 (46.15%)</td>
<td>3 (37.5%)</td>
<td>41 (45.56%)</td>
<td>0.75</td>
</tr>
<tr>
<td>Female gender donor</td>
<td>9 (69.2%)</td>
<td>5 (62.5%)</td>
<td>53 (58.8%)</td>
<td>0.76</td>
</tr>
<tr>
<td>Recipient age at KT (years)</td>
<td>26 (25-35)</td>
<td>26 (25-44)</td>
<td>28 (22-40)</td>
<td>0.8</td>
</tr>
<tr>
<td>Donor age at KT (years)</td>
<td>37 (29-51)</td>
<td>29.5 (25-37)</td>
<td>41 (29-45)</td>
<td>0.22</td>
</tr>
<tr>
<td>Second kidney transplant</td>
<td>0</td>
<td>0</td>
<td>3 (3.3%)</td>
<td>0.68</td>
</tr>
<tr>
<td>High risk for CMV infection</td>
<td>0</td>
<td>1 (12.5%)</td>
<td>14 (15.56%)</td>
<td>0.30</td>
</tr>
<tr>
<td>Warm ischemia time (min)</td>
<td>5 (4-6)</td>
<td>3 (3-8)</td>
<td>4 (3-5)</td>
<td>0.10</td>
</tr>
<tr>
<td>Cold ischemia time (hours)</td>
<td>1 (1-2)</td>
<td>1 (1-2)</td>
<td>1 (1-2)</td>
<td>0.75</td>
</tr>
</tbody>
</table>

Induction IS therapy:

<table>
<thead>
<tr>
<th></th>
<th>AT1Rab (n = 13)</th>
<th>HLA DSA (n = 8)</th>
<th>No Abs (n = 90)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Class I % PRA</td>
<td>1.23 (0-7)</td>
<td>18.4 (0-70)</td>
<td>4.35 (0-76)</td>
<td>0.01</td>
</tr>
<tr>
<td>Class II % PRA</td>
<td>5.9 (0-37)</td>
<td>25 (0-83)</td>
<td>2.4 (0-74)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>0 Haplotypes</td>
<td>3 (23%)</td>
<td>4 (50%)</td>
<td>29 (32.5%)</td>
<td>0.57</td>
</tr>
<tr>
<td>1 Haplotype</td>
<td>8 (61.5%)</td>
<td>4 (50%)</td>
<td>48 (53.3%)</td>
<td>0.87</td>
</tr>
<tr>
<td>2 Haplotypes</td>
<td>2 (15.3%)</td>
<td>0</td>
<td>12 (13.3%)</td>
<td>0.48</td>
</tr>
<tr>
<td>Donor pre-N eGFR* (ml/min/1.73 m²)</td>
<td>106 (88-119)</td>
<td>108 (105-119)</td>
<td>108 (94-117)</td>
<td>0.83</td>
</tr>
<tr>
<td>PreTx hypertension</td>
<td>6 (50%)</td>
<td>6 (75%)</td>
<td>72 (80.9%)</td>
<td>0.057</td>
</tr>
<tr>
<td>PreTx use of ACEIs or ARBs</td>
<td>4 (33.3)</td>
<td>4 (50%)</td>
<td>46 (52.27%)</td>
<td>0.49</td>
</tr>
<tr>
<td>BMI pre KT</td>
<td>25.1 (21.6-25.6)</td>
<td>24.05 (22.5-25.3)</td>
<td>23 (21-26.5)</td>
<td>0.92</td>
</tr>
<tr>
<td>BMI at 1st year PT</td>
<td>23.9 (21.6-26)</td>
<td>27 (24.7-29)</td>
<td>23.5 (21-26)</td>
<td>0.46</td>
</tr>
<tr>
<td>Median tacrolimus levels during 1st year (íg/L)</td>
<td>10.3 (9.5-10.8)</td>
<td>10 (9.1-11.14)</td>
<td>9.8 (8.8-10.7)</td>
<td>0.46</td>
</tr>
</tbody>
</table>

Logistic regression multivariate analysis

When analyzing factors relating to an eGFR below 70 mL/min after a one-year post-transplant follow-up, five variables remained significant: biopsy proven acute rejection, OR 6.27 (95%CI 1.18-33.06, p = 0.039), AT 1Rab, OR 6.17 (1.09-34.79, p = 0.035), de novo DSA, OR 4.49 (1.11-18.13, p = 0.035), recipient age, OR 1.04 (1.005 -1.09, p = 0.027) and donor age, OR 1.06 (1.01 - 1.11, p= 0.009).

DISCUSSION

The documented association between AT 1Rab and graft function deterioration in the early post-transplant period is interesting, particularly because the Abs effect on this variable was independent of other possible causes that could explain the decreased glomerular filtration rate observed in the AT 1Rab positive KTR group, even during the first month post-transplant. Although no other study has, to date, reported AT 1Rab as a cause of early graft dysfunction –in the absence of acute rejection–, our results could partly explain their negative impact on graft survival; two very recent studies on AT 1Rab (pre-transplant or de novo) have clearly demonstrated the presence of these Abs representing an independent risk factor.23,24 Giral, et al., screened 599 KTR before transplantation to determine the degree of pre-sensitization against AT 1R as a risk factor for graft survival and acute rejection (AR). They established a threshold of AT 1Rab levels of 10 IU based on the time period to graft failure. AT 1Rab > 10 IU were detected in 283 patients (47.2%) before transplant. KTR who had AT 1Rab > 10 IU had a 2.6-fold higher risk of graft failure, 3 years PT and subsequently (p = 0.0005).23 Moreover, Taniguchi, et al., tested PT AT 1Rab and HLA-DSA in sera from 351 KTR; 134 with BPR and/or lesions constituted the abnormal biopsy group (ABG) and 217 patients, the control group (CG). The ABG’s rate of AT 1Rab was significantly higher than in the CG (18 vs. 6%, p < 0.001), and 79% of ABG patients with positive AT 1Rab (> 15 IU) lost their grafts vs. 0% in the CG. In Tąnguch’s, et al., study, patients with both AT 1Rab and HLA-DSA had lower graft survival than those with HLA-DSA alone (log-rank p = 0.007).24 The synergistic negative effect of AT 1Rab and HLA-DSA on kidney transplants was initially described in a patient with accelerated kidney transplant rejection and hypertensive encephalopathy.28 In our study, a higher threshold for AT 1Rab positivity (≥ 17 IU) was used than that in the description of acute rejection and graft loss;23,24 this could have added strength to the association of AT 1Rab with worse graft function.

As previously mentioned, we determined that renal function deterioration begins during the first...
month post-transplant, as reflected by a decreased eGFR in the AT₁Rab group when compared with the HLA-DSA positive group and that with no antibodies (62.9 mL/min vs. 81.9 mL/min vs. 78.5 mL/min, respectively). However, the renal function deltas at one and twelve months post-transplant, were similar in all three groups. Despite the limited study sample, this finding suggests that the main change in kidney function developed in the early post-transplant period and that the AT₁Rab could be responsible, since all three groups are most similar when comparing all possible variables that could compromise early eGFR between groups (Table 1), including the donors’ eGFR. We must emphasize that the documented acute rejections in the AT₁Rab positive group developed after the eGFR was documented in the first post-transplant trimester. Although this study does not allow the establishment of a causal relationship between positive AT₁Rab and graft dysfunction, the fact that these Abs promote transplant vasculopathy by increasing the effects of angiotensin II has been well-documented; they promote cell growth, apoptosis, fibrosis, extracellular matrix remodeling and inflammation.⁵²⁻⁵⁴ In any case, our findings suggest initial hemodynamic injury mediated by AT₁Rab, although it apparently is not progressive during the first year. This contrasts with the injury mediated by HLA-DSA that tends to progress after initial injury, as documented in various studies. Several pathways have been suggested by which AT₁Rab may develop.³⁻¹⁰,¹⁶ The transplant process itself may lead to increased AT₁R expression, thus exacerbating the effects of antibodies on the receptors of activated vascular cells. Ischemia and reperfusion may also lead to oxidative stress, triggering an alloantigen-mediated immune response.¹⁰,³⁵,³⁶ Others have postulated that the endothelium’s natural balance may be altered, thus increasing its susceptibility to an antibody attack by AT₁Rab and generating a cascade of events leading to vascular rejection. AT₁Rab may amplify local inflammation, increase antigen expression and the production of Th1 cytokines and inflammatory chemokines.¹⁰

In terms of acute rejection episodes, our study found an increased proportion of rejection, both humoral and cellular, in the positive AT₁Rab group although this was not statistically significant. Whether the Abs are directly responsible for injury or are only a marker of the inflammatory milieu leading to the vascular injury and culminating in graft loss, remains to be determined.

It is important to increase our study sample and evaluate our patients by measuring post-transplant AT₁Rab titers in order to determine their behavior over time, as well as follow the patients’ renal function for a longer time period to establish whether the same findings persist.

AT₁Rab may represent a relevant link in the chain of factors compromising renal graft injury. If subsequent research provides similar results, we would expect that the determination of these Abs will be part of the routine evaluation of patients in the pre-transplant period, in the estimation of their immunological risk.

In summary, anti-AT₁R Abs in KTR pre-transplant sera was an independent and significant risk factor for decreased eGFR early in the post-transplant period. This finding deserves to be confirmed in a larger KTR population.

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