Prevalence of antibody-mediated graft rejection in pediatric renal transplant patients. An observational single-center study

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Abstract

Introduction: The main objective in managing kidney transplant recipient patients is to maintain an adequate state of immunosuppression to avoid the presentation of immunological rejection of the graft. Antibody-mediated active rejection is one of the most frequent causes of graft dysfunction in the early posttransplant period, in addition to representing a significant cause of decreased survival; however, in Mexico, there are few reports of this prevalence event, especially concerning the pediatric population.

Methods: This was a retrospective, cross-sectional, descriptive study. All children with a kidney transplant who have been transplanted at the UMAE Hospital de Pediatría CMNO. The list of patients diagnosed with "graft rejection," "humoral rejection," "acute graft rejection," "acute humoral rejection," and "antibody-mediated rejection" from January 2018 to December 2020 was requested in the medical file. Later, with the affiliation number, the electronic files were reviewed. The data necessary for the investigation were emptied and analyzed in a capture sheet.

Results: A total of 103 patients who received a kidney transplant in 3 years were studied; 2 were excluded, leaving 101 patients. Of these, 15 patients presented acute rejection classified by renal graft biopsy, with 1 (6.6%) classified as cellular rejection, 3 (20%) classified as mixed rejection, and 11 (73.3%) classified as antibody-mediated rejection, representing 10.9% of the transplanted patients, of which 8 (72.7%) received a transplant from a related living donor, and 3 (27.3%) received it from a cadaveric donor. An equitable distribution by gender was found; there were 6 (54.5%) male patients and 5 (45.4%) female patients. The age range was from 7 to 19 years, with a mean of 13 years.

Conclusion: The prevalence of antibody-mediated rejection in pediatric patients at the UMAE Hospital de Pediatría CMNO was 10.9%.

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Antibody-mediated rejection is the primary cause of graft dysfunction and loss after renal transplantation. Three categories of antibody-mediated renal function changes are currently described: the presence of the C4d marker without rejection, acute antibody-mediated rejection (AMR), and chronic antibody-mediated rejection (CAMR) [1].

The diagnosis of AMR requires the diagnosis of histological lesions (glomerulitis, peritubular capillaritis, thrombotic microangiopathy, acute tubular necrosis, intimal arteritis), latent evidence of interaction between antibodies and endothelium (peritubular C4d, glomerulitis + capillaritis, endothelial activation) and the presence of donor-specific antibodies [2]. AMR is classified into two phenotypes: early rejection during the first three months after transplantation and late rejection after the first year after transplantation. The first occurs in a patient with a reactive panel of positive antibodies before transplantation, usually C4d positive, and the second with de novo anti-HLA antibodies, usually C4d negative, associated with a lack of pharmacological adherence with worse therapeutic response [3].

RCMA is one of the leading causes of graft loss and is associated with a poor prognosis. The diagnostic feature is the histologic finding of transplant glomerulopathy (TG) [4]. TG is diagnosed in advanced stages by light microscopy due to the appearance of double contours and expansion of the mesangial matrix. The changes precede any clinical manifestation. Electron microscopy shows multilamination of the capillary basement membrane and thickening and duplication of the glomerular basement membrane. From the clinical point of view, it progresses in 2 stages. A subclinical stage without alterations in renal function or proteinuria, whose only finding is TG in the protocol biopsies, and a second clinical-stage, characterized by chronic graft dysfunction, with proteinuria and arterial hypertension [5].

Patients with chronic rejection may associate elements of active damage to the microcirculation mediated by antibodies, known as active chronic rejection, which is defined by the concomitant presence of histological evidence of chronic tissue damage such as TG if there is no chronic microangiopathy; severe basement membrane delamination of peritubular capillaries (on electron microscopy; new-onset arterial intimal fibrosis, ruling out other causes; evidence of antibody interaction (current or recent) given by any of the following: C4d in capillaries peritubular, moderate microcirculatory inflammation, molecular markers (endothelial-associated transcripts), evidence of specific donor antibodies (HLA and non-HLA) [6, 7].

Although a low prevalence of antibody-mediated graft rejection has been reported in nonsensitized patients, it increases significantly in high-risk patients, such as previously sensitized patients, in whom it can reach 10% to 35% [6]. This figure is alarming since antibody-mediated graft rejection is the leading risk factor for renal graft loss in the first year after transplantation, in addition to the fact that all untreated antibody-mediated rejection culminates in renal graft loss.

In Mexico, some data provide the prevalence of this event in the pediatric population. Hence, the objective of this study was to determine the prevalence of antibody-mediated graft rejection in pediatric kidney transplant patients at the UMAE Pediatric Hospital CMNO.

Materials and methods

Study design
The present study is observational, descriptive, and retrospective.

Scenery
The study was carried out in the nephrology department at the High Specialty Medical Unit, Pediatric Hospital, Western National Medical Center, Mexican Institute of Social Security, Guadalajara, Jalisco-Mexico, from January 1, 2017, to January 30, December 2020.

Participants
Pediatric patients diagnosed with stage 1-3-T chronic kidney disease who required hospitalization due to the need for treatment of acute rejection, classified as antibody-mediated, were included. Patients with preformed antibodies were excluded. Cases with incomplete data for analysis, incomplete medical records, or without postadmission followup were eliminated.

Variables
The variables were age, sex, type of transplant, transplant time, blood group, donation source, recipient-donor HLA, induction, induction schedule, immunosuppression schedule, graft biopsy,
cumulative dose of thyroglobulin, and identification of donor-specific HLA antibodies.

**Data sources/measurements**
The source was indirect; the institutional electronic file, the registry of the statistics services, nephrology, and the transplant unit were reviewed.

**Biases**
To avoid possible interviewer, information, and memory biases, the data were guarded at all times by the principal investigator with a guide and records approved in the research protocol. Observation and selection bias was avoided by applying the participant selection criteria. All the clinical and paraclinical variables of the period above were recorded. Two researchers independently analyzed each record in duplicate, and the variables were recorded in the database once their agreement was verified.

**Studio size**
The sample was nonprobabilistic, census type, and all possible cases of the period under study were included.

**Quantitative variables**
Descriptive statistics were used. Scaled results are expressed as the means and standard deviations. Categorical data such as sex are presented as proportions.

**Statistical analysis**
Noninferential statistics are used. The prevalence of AMR was calculated with a confidence interval for a proportion. The statistical package used was SPSS 25.0 (IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp.).

**Results**

**Participants**
A total of 103 patients who received a kidney transplant in 3 years were studied; of these, two were excluded, one for not having a complete clinical file and the other for having preformed antibodies, leaving 101 patients with complete clinical files.

**Baseline characteristics of the study population**
Of the 101 transplanted patients, 60 (59.4%) were male, and 41 (40.6%) were female. The mean age of the patients at the time of transplantation was 12 years. In most transplants, 80 (79.2%) were made from grafts from a living donor, and 21 (20.7%) were made from grafts from a cadaveric donor. During the review period, a total of 15 patients were found who presented with acute rejection classified by renal graft biopsy (histopathology), with 1 (6.7%) classified as cellular rejection, 3 (20%) classified as mixed rejection, and 11 (73.3%) classified as antibody-mediated rejection.

**Antibody-mediated rejection**
The 11 patients with antibody-mediated rejection represent 10.9% of the transplant patients, 8 (72.7%) received a living-related donor transplant, and 3 (27.3%) received a cadaveric transplant. Of the total number of patients cataloged with antibody-mediated rejection, an equal distribution by gender was found since there were 6 (54.5%) patients of the male gender and 5 (45.4%) of the female gender. The age range was from 7 to 19 years, with a mean of 13 years and a mode of 14 years of age. Figure 1 shows the distribution by the age of the population of patients with rejection. The time elapsed from the transplant date to the presentation of the rejection; the shortest period was one month, and the longest was 23 months, with an average presentation time of 11 months, as shown in figure 2.

**Secondary analyses**
The compatibility variable was defined as a dichotomous variable: <3 antigens and ≥3 antigens. Of the 11 patients who pre-
sented antibody-mediated rejection, it was only possible to obtain compatibility data for 8 of them, all of which were living donor transplants; all eight patients shared more than three antigens. As described in more detail, one shared two haplotypes, five shared one haplotype, and two shared only class II antigens. We do not have the HLA of the three patients whose transplant was from a cadaveric donor.

The induction received during kidney transplantation was performed with basiliximab in 10 cases (90.9%) and 1 (9%) with thymoglobulin. The only patient who received induction with thymoglobulin presented rejection 23 months after the kidney transplant.

Regarding the maintenance immunosuppression regimen received by the patients at the time of presentation of rejection, all of them had a triple regimen, having corticosteroids (prednisone) in common; however, there were variations in the use of antimetabolite and calcineurin inhibitors, as presented in Graph 8. There were six patients in the prednisone + tacrolimus + MMF group and four patients in the prednisone + CsA + MMF group, and only one patient had azathioprine as an antimetabolite. The period elapsed from the transplant to the presentation of rejection in the prednisone + CsA + MMF group was from 1 to 23 months; in the prednisone + tacrolimus + MMF group, it was from 1 to 22 months, and in the patient with prednisone + tacrolimus + azathioprine, it was 16 months. There was no significant difference in time to the presentation of rejection (P = 0.66) when comparing the prednisone + CsA + MMF and prednisone + tacrolimus + MMF groups.

Of the total number of patients, 6 had poor adherence to treatment, confirmed by caregivers and the patients themselves; however, even among these patients, the time elapsed from transplantation to rejection was highly variable, with periods as short as six months to 23 months. Among these patients, one received induction with thymoglobulin.

Of these patients, 10 (90.9%) were treated with plasmapheresis in 5 sessions with intravenous immunoglobulin at a dose of 2 g/kg/dose, in addition to the use of an anti-CD20 monoclonal antibody (rituximab) after completing the five plasmapheresis sessions. One of the 11 patients received thymoglobulin in addition to the treatment above. The remaining patient was discharged for an age before being able to receive treatment.

**Discussion**

To carry out this study, the records of patients diagnosed with graft dysfunction, acute rejection, or graft rejection were retrospectively studied.

The prevalence of antibody-mediated graft rejection found in this study was 10.9%, which correlates with the prevalence in adults found in studies in Europe and Latin America, such as those by Lorent [8] and Borroto Diaz [2], where it was reported in 16.2% and 11.23%, respectively. In Mexico, we found no reports of this event in pediatric patients.

![Figure 3](image-url)  
**Figure 3.** Immunosuppression scheme in the group with AMR.

The time elapsed from the date of kidney transplantation to the presentation of rejection was highly variable, occurring from 1 to 23 months later. The induction received in transplant patients was basiliximab-based in a higher percentage; however, although there is no representative sample of patients with thymoglobulin-based induction, it is observed that in these patients, the rejection presentation time was higher. The incidence of biopsy-confirmed acute rejection was significantly higher in the basiliximab induction group. However, other studies have not reported significant differences in graft survival between the two groups [10].

Regarding maintenance immunosuppression received at the time of presentation of rejection, no differences were observed when comparing the groups (PDN + tacrolimus + MMF vs. PDN + CsA + MMF) (P = 0.66); however, nonadherence to the treatment of maintenance was an essential variable in the presentation of rejection. Nonadherence is a significant and independent risk factor for graft loss. For this reason, we consider that surveillance of pediatric patients, especially adolescents, must be extremely close due to the high risk of nonadherence, and in the entire pediatric transplant population, this surveillance must be strict to maintain their levels of immunosuppression within recommended parameters and thus avoid the presentation of rejection mediated by antibodies.

These patients received treatment in several lines, both plasmapheresis and rituximab, and the use of intravenous gamma globulin. In the future, further studies are proposed that could demonstrate the effectiveness of some of these measures.
It was intended to know the compatibility of HLA antigens since, in previous investigations, it has been evaluated and concluded that HLA compatibility influences graft survival and mortality in renal transplantation; for example, an observational study found that incompatibility HLA overall was associated with significantly increased risk of graft failure, including increased mortality [11]. A significant bias in this study is the lack of knowledge of HLA antigen compatibility in the three patients who presented antibody-mediated rejection and received their grafts from a cadaveric donor. However, many organs procured from cadaveric donors come from other states. The sending of samples to carry out studies and the time necessary to have the results available makes it challenging to carry them out, so these patients should be considered high risk regardless of age and should be induced with thymoglobin. The remaining eight patients received their grafts from a living donor, and all shared more than three antigens. It was decided not to exclude patients from cadaveric donors to include this group of patients and make their evolution known, in addition to the fact that, if they had been eliminated, a lower prevalence would have been reported.

Conclusions
Of the 101 transplanted patients, 60 (59.4%) were male, and 41 (40.5%) were female. The prevalence of antibody-mediated rejection was 10.9%. Of the total number of patients cataloged with antibody-mediated rejection, 6 (54.5%) were male, and 5 (45.4%) were female. In patients classified as having antibody-mediated rejection, the age range was 7 to 19 years, with a mean of 13 years and a mode of 14 years of age. The time elapsed from the transplant date to the presentation of the rejection ranged from 1 to 23 months. The time from presentation of rejection in cadaveric donor patients, in whom HLA compatibility is unknown, was highly variable, ranging from 8 to 22 months. There was no statistically significant difference when comparing the induction scheme received and the presence of rejection. The longest presentation time was in two patients who each received basiliximab and timoguline. There was no statistically significant difference in the time of presentation of rejection when comparing the different maintenance immunosuppression regimens received. However, little adherence to treatment was observed as an intervening variable.

Abbreviations
ARM: antibody-mediated acute rejection.
CMO: Western National Medical Center.
CsA: cyclosporine.

DSA: Donor Specific Antibody (Donor Specific Antibody).
DVR: Related Living Donor.
CKD: Chronic Kidney Disease
ROS: reactive oxygen species.
GT: Transplant glomerulopathy
HLA: Human leukocyte antigen (antigen)
Ig: Immunoglobulin
IMSS: Mexican Institute of Social Security.
AKI: Acute Kidney Injury
MMF: mycophenolate mofetil.
PDN: prednisone.
PRA: Antibody Reactive Panel
rATG: rabbit anti-thymocyte globulin (rATG)
PRR: pattern recognition receptor
TLRs: Toll-like receptors (Toll-like receptors)
TNF: Tumor necrosis factor (tumor necrosis factor)
TRIF: Interferon-B inducer of adapters containing tyrosine domains
UMAE: High Specialty Medical Unit.

Supplementary information
Supplementary materials have not been declared.

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Does not apply.

Author contributions
Mónica Verenice Cámara Carrillo: Conceptualization, Data Curation, Formal Analysis, Fundraising, Research, Methodology, Project Management, Resources, Software, Writing—original draft.
Santa Ramírez Godínez: Conceptualization, supervision, validation, visualization, and writing: review and editing.
Juan Carlos Barrera de León: Methodology, validation, supervision, writing: Review and editing.
All authors read and approved the final version of the manuscript.

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Availability of data or materials
The data sets generated and analyzed during the current study are not publicly available due to participant confidentiality but are available from the corresponding author upon reasonable academic request.

Statements
Ethics committee approval and consent to participate
The research ethics committee approved this study of the High Specialty Medical Unit Hospital de Pediatría CMNO, approval number R-2021-1302-077.

Consent to publication
It does not apply when images or photographs of the physical examination or X-rays/tomographies/MRIs of patients are not published.

Conflicts of interest
The authors report having no conflicts of interest.
References


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