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The effect of pregnancy on humoral rejection in patients after vascularized organ transplantation

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ABSTRACT

The aim of the study was to evaluate the effect of pregnancy on the production of donor- and nondonor-specific anti-human leukocyte antigen antibodies (anti-HLA Abs) in organ allograft recipients. The study group included four pregnant kidney (RT) and four liver (LT) transplant recipients. The genotype of HLA class I (A, B) and class II (DR) antigens was assessed. Anti-HLA antibodies class I and II were evaluated between 36 and 40 weeks' gestation. Two different control groups consisted of the following: group I ($n=8$) with nonpregnant RT ($n=6$) and LT recipients ($n=2$), and group II with healthy pregnant women ($n=10$) with anti-HLA Abs detected between 38 and 41 weeks' gestation. The HLA genotype was determined in fathers of the fetuses from the study group and group II controls. Half of group II controls had donor-specific anti-HLA (A, B, and/or DR) Abs, while nondonor-specific anti-HLA Abs were detected in all subjects from that group. Anti-HLA Abs were found in all group II controls. In the study group, anti-HLA Abs were found in only two LT recipients and one RT recipient, but they were not confirmed as donor-specific. Anti-HLA antibodies were not detected in the study group, whereas six out of ten group II controls had anti-HLA Abs against the HLA of the child's father. Pregnancy in vascularized organ recipients does not trigger the mechanism of humoral rejection involving anti-HLA class I and II antibodies with a potentially adverse impact on graft function.

1. Introduction

Organ transplantation triggers various immunological mechanisms resulting in the rejection and graft loss. Immunological reaction of the recipient's immune system, due to HLA incompatibility or immunization, remains one of the major causes of transplant rejection. Recipient HLA antibodies are the most important and the most common factors responsible for transplant rejection in the first or subsequent transplantations. The risk of transplant rejection is much higher in patients with high antibody titers. Female organ recipients of childbearing age are among patients with very special clinical needs. Pregnancy and blood transfusions are believed to be the most common causes of immunization. According to the literature, approximately 30–50% of pregnant women produce anti-HLA antibodies against the antigens of their child's father. (Ayna et al., 2012; van Kampen et al., 2002). The role of those antibodies remains unclear, but numerous attempts have been made to explain the phenomenon. Most authors focus on the immaturity of the fetal immune system and, particularly, the characteristic immunosuppression due to placental function in pregnancy (Guleria and Sayegh 2007). Regardless, the most recent research suggests that the immune system in pregnancy might be activated rather than suppressed, which makes trophoblast implantation possible (Steinborn et al., 2011). Failure to detect fetal antigens may lead to early pregnancy loss (Christiansen et al., 2010; Nielsen et al., 2010). Despite dramatic medical advances over the last few decades, the field of human reproductive immunology is still based on numerous hypothe-

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ses. Countless ethical issues limit the research on reproductive immunology even in a normal pregnancy, let alone in organ recipients treated with immunosuppressive agents after organ transplantation (Cornella et al., 2009).

The aim of our study was to investigate the effect of pregnancy on humoral rejection in patients after organ transplantation.

2. Methods

2.1. Participants

According to the study protocol, eight pregnant kidney (RT) or liver (LT) transplant recipients were deemed eligible for the study and constituted the study group. Two different control groups were recruited: 8 nonpregnant RT or LT recipients (control group I) and ten healthy pregnant women (control group II). All group I controls were patients of the Department of General and Transplant Surgery and the Transplantation Institute of the Medical University of Warsaw. They received transplants from nonrelated donors.

One patient needed a blood transfusion after kidney transplant and four patients after liver transplant. All transfusions were performed during or after the surgery.

Group II controls were patients of the 1st Department of Obstetrics and Gynecology, Medical University of Warsaw. They were enrolled in the study at between 38 and 41 weeks' gestation. Additionally, fathers of the fetuses from both the study group and the control group II were also subjected to HLA evaluation.

The Local Ethics Committee approved of the research protocol and all patients signed informed consent.

2.2. Determination of HLA alleles, anti-HLA antibodies, and PRA test

The genotyping of the alleles of HLA class I (A, B) and II (DR) antigens was performed using polymerase chain reaction using a sequence-specific primer method (carried out as a routine procedure in graft recipients and donors before transplantation). Moreover, the HLA genotype of the above selected HLA types was evaluated in the fathers of the fetuses from the study group and control group II.

The presence of anti-HLA antibodies (anti-HLA Abs) was evaluated at between 36 and 41 weeks' gestation (or earlier, if the birth occurred before 36 weeks' gestation) using Luminex-xMAP Technology in LABScreen Single Antigen Class I Combi assay (One Lambda, Canoga Park, CA, USA) for anti-HLA class I antibodies and in the LABScreen Single Antigen Class II Antibody Detection Test Group 1 (One Lambda) for anti-HLA class II antibodies.

The panel reactive antibody (PRA) test in RT patients was a routine procedure before transplantation. The PRA score represents the proportion of the population in which the anti-HLA antibodies were found. The PRA score was expressed as a percentage.

2.3. Statistics

The statistical significance of the differences between the obtained values and the expected values was determined using Chi-squared statistics (Pearson's Chi-squared test, Fisher's exact test, or Yates' correction for continuity, where appropriate). A p value of <0.05 was considered statistically significant.

3. Results

There were eight nonpregnant women in control group I: six after kidney transplantation (half of them after the second one) and two after liver transplantation (Table 1). Mean patient age in that group was 30.3 years (range: 20–40 years). There was only one liver recipient (patient number 7) who did not have her HLA genotype evaluated (this is not a standard assessment before this particular procedure). There were three possible immunosuppressant therapy plans: mycophenolate mofetil, cyclosporine, prednisolone (two patients after kidney transplant); mycophenolate mofetil, tacrolimus, prednisone (four patients after kidney transplant); and tacrolimus, prednisone (two patients after liver transplant). The causes of end-stage kidney disease in the RT group included chronic pyelonephritis ($n=2$), glomerulonephritis ($n=1$), amyloidosis associated with rheumatoid arthritis ($n=1$), or chronic kidney disease of unknown causes ($n=2$). The cause of liver failure in LT patients was hepatitis C. The degree of alloimmunization was tested in the PRA test directly before the kidney transplant, revealing the following results: for patients 1 and 4–0%, for patients 3 and 6–3%, for patient 2–23%, patient 5–50% (patients 2, 3, 5, and 6 had two kidney transplantations). Time elapsed between the last transplantation and anti-HLA testing ranged from two days (in patients after the second kidney transplantation) to 12 years. Various types of anti-HLA antibodies were detected in the study population (Table 1). Anti-HLA antibodies against donor HLA were detected in half of the patients. Specific antibodies against the HLA of the donor were found in patients 3 and 7.

Control group II included ten healthy pregnant women: five primiparas and five secundiparas (Table 2), tested at between 38 and 41 weeks' gestation. Mean patient age in that group was 32.3 years (range: 27–40 years). Irrespective of parity, class I or class II anti-HLA antibodies were detected in all group II controls (Table 2). These findings were similar to those of control group I. Six (60%) patients had antibodies against the HLA antigens of the fathers of their children.

The study group consisted of four pregnant women after kidney transplant and four after liver transplant aged 19–35 years (mean age: 26 years; Table 3). Time elapsed between transplantation and pregnancy in that group of patients was 15–70 months. Only one patient (patient 5) was in her second pregnancy (the first delivery occurred before organ transplantation) and all the other patients were primiparous. Glomerulonephritis and primary sclerosing cholangitis were the causes of end-stage kidney disease in the RT and the LT patients respectively. The function of the transplanted organ was normal in all patients from the study group. The most common comorbidities in the RT group were recurrent urinary tract infections and hypertension (treated with one antihypertensive drug). In the LT group there were two patients with colitis ulcerosa. All patients received immunosuppressive drugs: tacrolimus ($n=6$), azathioprine ($n=5$) or cyclosporine ($n=2$). Almost all patients were also treated with prednisolone. The only exception was 1 LT patient who received either one or two extra immunosuppressive drugs.

Some patients had obstetric complications during the observation: there were two cases of preeclampsia, one case of hypertension, and four cases of IUGR. The patients with colitis ulcerosa had to have their prednisolone dose adjusted owing to the exacerbation of disease symptoms.

Anti-HLA antibodies detected in the study group are listed in Table 3. No anti-HLA antibodies were found in the RT group before the transplantation (the PRA result was 0%). All of these patients were primiparous, and all had undergone blood transfusions before or after the transplantation. Anti-HLA-B, anti-HLA-DP, anti-HLA-DQ, and anti-HLA-DR antibodies were detected only in one patient. Two out of four LT patients had anti-HLA antibodies. These were confirmed as antibodies that were not against the donor or paternal HLA.

The proportion of study group patients with anti-HLA antibodies was 37.5% and was significantly reduced compared with control groups I or II ($p<0.05$ and $p<0.01$ respectively). The number of