Viral seroprevalence in pediatric kidney transplant recipients

Çocuk böbrek nakli alıcılarında viral seroprevalans

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The known about this topic

Viral infections occurring in pediatric patients who have undergone kidney transplantation may disrupt the function of the transplanted kidney and lead to loss of the patient. Therefore, it is important to know the levels of antibodies against viral infections, which can be prevented by immunization and to vaccinate patients who are found to be negative.

Contribution of the study

In this study, we aimed to determine the prevalence of viral seropositivity in pediatric patients for whom kidney transplantation was planned in our country. Most of these patients are under follow up in different regions and are referred to organ transplantation centers at the stage when kidney transplantation is planned. In this article, we aimed to emphasize the necessity of viral serology screening in the early stage and administering missed vaccines in pediatric patients who develop renal disease for all physicians who evaluate these patients, mainly including pediatricians.

Abstract

Aim: Viral infections commonly affect kidney transplant recipients and may lead to graft failure and death. The aim of this study was to evaluate the antibody seroprevalence and death viral agents in kidney transplant recipients.

Material and Methods: The records of children who underwent kidney transplantation between 2008 and 2018 in Akdeniz University Faculty of Medicine were retrospectively reviewed. Epstein-Barr virus, cytomegalovirus, hepatitis A virus, hepatitis B virus, varicella, measles, rubella and mumps serologies evaluated before transplantation, were recorded. The clinical characteristics of seronegative and seropositive patients were compared, and factors that affected seropositivity were investigated.

Results: The study included 253 children with a mean age of 16.7±6.23 years. The mean age at transplantation was 11.4±5.01 years. The seropositivity rates for vaccine-preventable viral infections varied: hepatitis B 89.7%, hepatitis A 60.5%, measles 78.7%, rubella 88.1%, mumps 61.2%, and varicella 71.9%. Cytomegalovirus seropositivity was 92.1% and Epstein-Barr virus seropositivity was 82.2%. Hepatitis B antibody positivity was 91.8% in patients undergoing hemodialysis, 94.5% in patients undergoing peritoneal dialysis, and 87.6% in patients undergoing transplantation.

Bulgular: Çalışmaya ortalaması yaş 16.7±6.2 yıl ve ortalamada böbrek nakli yapılan 253 çocuk hasta dahil edildi. Aşı ile önlenemeyen viral etkenler için böbrek nakli öncesi seropozitivite oranları sırasıyla hepatit B virüsü %89.7, hepatit A virüsü %60.5, kızamık %78.7, kızamıkçık %68.1, kabakulak %61.2 ve varicella %71.9 bulundu. Sitomegalovirüs seropozitifliği %92.1 olarak bulundu.

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undergoing peritoneal dialysis, and 84.9% in pre-emptive transplantation patients (p=0.037). The mean age at transplantation was higher in patients with seropositivity for both cytomegalovirus and Epstein-Barr virus compared with seronegative patients (p<0.001 for both). The mean age at transplantation and diagnosis of glomerular disease was found to be effective for varicella seropositivity in multivariate regression analysis (OR 0.860, 95% CI: 0.808–0.915, p<0.001 and OR 2.520, 95% CI: 1.321–4.739, p=0.005, respectively).

**Conclusion:** It is important to screen patients with chronic kidney disease in terms of vaccine-preventable diseases to identify risky groups of patients and to immunize these patients before end-stage kidney disease develops.

**Keywords:** Children, kidney transplantation, vaccine, viral serology

**Introduction**

Infectious diseases are one of the important factors that lead to failure and loss of the transplanted kidney in recipients (1). Therefore, it is important to determine patients who carry risk, and to protect and immunize these patients against viral and bacterial infections. Considering that live vaccines cannot be administered in the post-transplant period and inactive vaccines cannot establish sufficient immune response because of immunosuppressive agents used in the post-transplant period, serology screening directed to vaccine-preventable diseases in the pre-transplant period has vital importance (2).

There is a low number of studies evaluating vaccination status and viral seroprevalence in pediatric kidney transplant recipients (1, 3, 4). In a study in which vaccination rates belonging to the pre-transplant period in 51 pediatric patients who underwent kidney transplantation (Tx) between 2008 and 2011 in our country were examined, the measles, rubella and mumps seropositivity rates were found as 72.5%, 64.7%, and 64.7%, respectively (3). In a study conducted in Canada in which 2555 kidney transplant recipients were evaluated, 44% were found to be seronegative for hepatitis B virus (4). Prelog et al. (5) examined antibody titers for measles, rubella, mumps, varicella, hepatitis B, diphtheria and tetanus before Tx in 35 pediatric patients and they observed that only 26% of the patients had protective antibody titers against all agents.

The aim of this study was to determine the present antibody responses to viral infectious agents and the factors that influenced these responses in pediatric patients with end-stage renal disease (ESRD) for whom kidney Tx was planned.

**Material and Methods**

Study group: The records of pediatric patients aged between 0 and 18 years who underwent kidney Tx between March 2008 and December 2018 at Akdeniz University Faculty of Medicine Hospital, were reviewed retrospectively. Patients whose medical records could be obtained in full were included in the study. The patients’ demographic characteristics, kidney donor sources, and the renal replacement therapy performed before Tx, were recorded. Epstein-Barr virus (EBV), cytomegalovirus (CMV), hepatitis A virus (HAV), hepatitis B virus (HBV), varicella, measles, rubella and mumps serologies evaluated at the preparation stage of kidney Tx were recorded. The patients who were found to have positive hepatitis B surface antigen (HBs Ag) and who had positive HAV, varicella, measles, rubella, mumps and varicella IgM serologies, were excluded from the study.

The majority of the patients who were included in the study arrived at our center from different geographic regions and cities. Vaccine records have been stored on electronic media since 2010 in our country; however, definite vaccine records of patients aged over 10 years could not be reached. The patients who had no vaccination card or whose data could not be reached on electronic media were excluded from evaluation because verbal vaccination reports obtained from families were not considered reliable. Ethics committee approval for the study was obtained from Akdeniz University Faculty of Medicine Ethics Committee (decision number: 23.10.2019/1005). The study was conducted in accordance with the Helsinki Declaration.

Definitions: The diagnoses of primary renal disease leading to end-stage renal disease were collected under 6 main titles: congenital anomalies of the kidney and urinary tract, glomerular diseases, cystic renal diseases, tubulointerstitial diseases, other and unknown etiologies. Kidney Tx performed before the initiation of renal replacement treatment were defined as pre-emptive kidney Tx.

Hepatitis A antibodies (Anti-HAV IgG) (Elecsys Anti-HAV IgG and IgM, Roche Diagnostics, Almanya); hepatitis B surface antigen (Hbs Ag) and hepatitis B surface antibodies (HbsAb) (Elecsys anti-HBs Reagent Kit, Roche Diagnostics, Almanya) were tested using the electroche-
moluminescent immunoassay (ECLIA) method. Serologic tests were evaluated considering the manufacturer's instructions. An anti-HAV IgG value of >10.0 IU/mL and an anti-HBs value of >10.0 IU/mL were considered positive.

Mumps, measles, rubella, and varicella IgG levels were studied in serum samples using enzyme-linked immunosorbent assay (ELISA) in accordance with the manufacturer's recommendations (Chorus Measles IgG, Chorus Mumps IgG, Chorus Varicella-zoster IgG; Diesse Diagnostic, Italy and Immulite 2000 Rubella Quantitative IgG, Siemens, USA). Measles, mumps, and varicella IgG levels above 1.2 IU/mL and rubella IgG levels above 10.0 IU/mL were considered seropositivity.

For cytomegalovirus, nucleic acid extraction and real-time polymerase chain reaction (PCR) were performed using a Cobas Amplicprep/COBAS Taqman (Roche, USA) system. Artus EBV RG PCR (Qiagen, Germany) kits were used for the Epstein-Barr virus.

Statistical Analyses
The data obtained in this study were statistically analysed using the Statistical Package for the Social Sciences (SPSS) for Windows Ver. 21.0. Descriptive statistics including frequency distribution, mean, standard deviation, and median were used to describe the sample. The normal distribution hypothesis was examined. The difference between the mean values of two independent groups was examined using Student’s t-test when parametric test assumptions could be provided. When parametric test assumptions could not be provided, non-parametric alternatives for these tests were used: The Mann-Whitney U test was used for two groups and the Kruskall-Wallis test was used for more than two groups. Categorical data were examined using the Chi-square significance test or Fisher’s exact test. The factors influencing EBV and varicella seropositivity were evaluated using multiple logistic regression analysis. The results were evaluated at a significance level of 95% or using a margin of error of 0.05.

Results
Among 412 pediatric kidney transplant recipients evaluated in the study, 14 children were excluded because they had IgM positivity at the time of presentation (7 hepatitis A, 4 rubella and 3 mumps), and 145 children were excluded because their medical records could not be reached completely. Among 253 pediatric kidney transplant recipients included in the study, 54.9% (n=139) were male, the mean age was 16.7±6.23 years, and the mean age at the time of kidney Tx was 11.4±5.01 years. The median follow-up time was 3.2 (range, 0.4 – 11.1) years. The most common cause of end-stage renal disease was congenital anomalies of the kidney and urinary tract (35.6%) (Table 1). The frequency of glomerular disease for which prednisolone and/or immunosuppressive agents (e.g. cyclosporin, cyclophosphamide) were used was 28.5% (n=72). The age at the time of Tx was lower among subjects who had glomerular disease compared with the others (10.1±5.4 vs. 12.0±4.7, p=0.006).

Hemodialysis was performed in 86 (3.9%) patients and peritoneal dialysis was performed in 73 (28.9%) patients before kidney Tx; 94 (37.2%) patients underwent pre-emptive kidney Tx. The median time waiting for organ Tx under dialysis treatment was 12.0 (range, 1.0–96) months. There was no difference in terms of the age at the time of Tx between the patients who underwent pre-emptive Tx and patients who underwent Tx after receiving dialysis treatment (10.6±0.55 vs. 11.8±0.37, p=0.65). The rate of Tx from living donors was 72.3% and the rate of Tx from cadaver donors was 27.7% (Table 1). The rate of renal Tx from cadaver donors was higher in pediatric kidney transplant recipients who underwent Tx after dialysis treatment (32.0% vs. 23.6%, p=0.028).

Anti-HBs antibodies were found as positive in 277 (89.7%) patients. The mean ages at the time of Tx were found to be similar in patients who had positive and negative anti-HBs antibodies (11.5±4.94 vs. 10.3±5.40, p=0.235). The anti-HBs antibody positivity was found as 91.8% in patients who underwent hemodialysis treatment and 94.5% in patients who underwent peritoneal dialysis. In the pre-emptive patients, the anti-HBs antibody positivity (84.9%) was lower compared with patients who had received hemodialysis and peritoneal dialysis (p=0.037).

Hepatitis A seropositivity was found in 153 (60.5%) patients. The mean age was higher in the renal transplant recipients who had hepatitis A IgG positivity compared with those who had negative hepatitis A IgG (11.97±4.98 vs. 10.5±4.91, p=0.025). Twenty-eight pediatric patients who were born after hepatitis A vaccine was included in the routine vaccination schedule (after April 2012) and turned 2 years of age at the time when kidney Tx was planned. Hepatitis A IgG was found as positive in 20 (71.4%) of these children.

In the study group, CMV seropositivity was found as 92.1% (n=233). The mean age at the time of Tx was found to be higher in patients who were CMV seropositive compared with those who were CMV seronegative (11.7±0.31 vs. 7.72±1.8; p<0.001). The frequency of glomerular disease and the rate of pre-emptive Tx were similar between CMV IgG-positive and negative patients (29.6 vs. 23.5%, p=0.223 and 35.9 vs. 50.0%, p=0.142; respectively).
Epstein-Barr virus IgG was found as positive in 82.2% of the patients (n=208). The rate of EBV seropositivity was found as 59.6% in the kidney transplant recipients aged below 5 years, whereas it was found as 69.8% in the recipients aged below 10 years. The mean age at the time of kidney Tx was higher in patients who had positive EBV IgG (12.0±4.54 vs. 8.14±5.82, p<0.001). The rate of EBV seropositivity was lower in the kidney transplant recipients with a diagnosis of glomerular disease compared with recipients who had other etiologies (75.0% vs. 86.7%, p=0.022). In multiple regression analysis, increased age at the time of Tx was found to be a factor influencing EBV IgG seropositivity (OR=0.737, 95% CI: [0.817–0.938]; p<0.001). The presence of glomerular disease was not found to be a factor that influenced seropositivity (OR=0.875, 95% CI: [0.841–3.589]; p=0.14) (Table 2).
Measles seropositivity was present in 78.7% of the patients in the study group. The seropositivity rate was found as 88.1% for rubella and 61.2% for mumps. All three antibodies were positive in 118 (46.6%) patients. When the patients who had positive measles antibodies were compared with patients who had negative measles antibodies, age at the time of Tx, the pre-emptive Tx rate, and glomerular disease rates were found to be similar between the two groups (p>0.05).

The ages at the time of Tx were similar between the patients who had positive and negative measles, rubella and mumps antibodies (11.5±4.82 vs. 11.2±5.17 years, p=0.61). In addition, there was also no difference in terms of the frequency of glomerular disease and pre-emptive Tx rates (44.4 vs. 55.5%, p=0.68, and 50.0% vs. 50.0%, p=0.44, respectively).

Varicella antibody was found as positive in 182 (71.9%) patients, and the ages at the time of Tx were higher in the seropositive patients compared with the seronegative patients (12.5±4.20 vs. 8.40±5.71, p<0.001). In addition, the varicella seropositivity rate was found as 56.9% in recipients who had glomerular disease, whereas it was 80.0% in patients who had non-glomerular pathology (p=0.004). Both univariate regression analysis and multivariate regression analysis showed that the age at the time of Tx and the presence of glomerular disease were factors that influenced varicella seropositivity (OR=0.860, 95% CI: [0.808–0.915]; p<0.001 and OR=2.502, 1.321–4.739; 0.005) and pre-emptive kidney Tx did not influence varicella seropositivity (OR=1.162, 95% CI: [0.615–2.195]; p=0.643) (Table 3).

| Risk factors                      | Univariate logistic regression analysis | Multivariate logistic regression analysis |
|-----------------------------------|-----------------------------------------|------------------------------------------|
|                                  | OR       | 95% CI       | p       | OR       | 95% CI       | p       |
| Age at the time of Tx            | 0.847    | 0.798–0.898  | <0.001  | 0.860    | 0.808–0.915  | <0.001  |
| Presence of glomerular disease   | 3.024    | 1.667–5.487  | <0.001  | 2.502    | 1.321–4.739  | 0.005   |
| Pre-emptive kidney Tx            | 1.349    | 0.770–2.363  | 0.295   | 1.162    | 0.615–2.195  | 0.64    |

Discussion

In this study, antibody responses against common viral infectious agents were evaluated in children for whom kidney Tx was planned. Hepatitis B virus was the viral agent that had the highest seropositivity rate (89.7%) among vaccine-preventable viral diseases in the present study. In a study conducted in Turkey in 2012, the seropositivity rate for hepatitis B was found as 84.3% (3). In a Brazilian cohort, the vaccination rate for hepatitis B was found as 63.0% in pediatric kidney transplant recipients, whereas it was 88.6% in a European group (2, 5). In patients receiving dialysis treatment, regular supervision by the Ministry of Health in terms of hepatitis B infection influences improvement in seropositivity rates. In our study, anti-HBs antibody positivity was found to be lower in the patients who underwent pre-emptive kidney Tx compared with patients who were under dialysis treatment. In a study in which adult patients receiving hemodialysis treatment were evaluated, it was shown that advanced age (>65 years) influenced unresponsiveness to hepatitis B vaccine (6). However, a relationship between age and hepatitis B antibody positivity was not found in this study in which the pediatric age group was examined.

In the present study, the seropositivity rate for hepatitis A was found as 60.5%. Although hepatitis A virus vaccine has been included in the national vaccination schedule quite newly in Turkey, a hepatitis A antibody test has been performed and seronegative patients have been vaccinated before kidney Tx since 2008 in our center. In an article published by the European Pediatric Dialysis Study Group in 2018, it was reported that hepatitis A serology was studied during dialysis or before Tx only in 13 of 18 centers included in the study group (7). In a study conducted by Jeon et al. (8), hepatitis A antibodies were tested in the follow-up period after Tx and seropositivity was found in 81.2% of the patients who underwent kidney Tx. When patients aged below 40 years were reevaluated in this study, which also included adult recipients, this rate was reduced to 31.8% (8). Similar to the results of the current study, the mean age of patients who were hepatitis A seropositive in our study was higher compared with seronegative patients. In light of this study, it was thought that past infection had a greater effect on hepatitis A seropositivity compared with immunization.

Cytomegalovirus and EBV are viral pathogens that may lead to dysfunction in the transplanted kidney after Tx. It is known that seronegative patients who have not had cytomegalovirus and EBV infections carry a higher risk
compared with seropositive patients (9). In this study, in which pediatric kidney transplant recipients were examined, the seropositivity rate was found as 92.1% for CMV and 82.2% for EBV. As the mean age at the time of Tx increased, CMV and EBV seropositivity rates also increased. In a study conducted in Spain in which 239 pediatric kidney transplant recipients from five centers were evaluated, 54% of patients were found to be CMV seronegative (10). In the United States of America, CMV seroprevalence is about 50%, and some studies showed that age, geographic characteristics, and socioeconomic conditions influenced seroprevalence (11, 12).

In developing countries, EBV seroprevalence is about 90% at the age of 5 years and this rate is lower in developed countries (13). In our study, we found the EBV seropositivity rate as 59.6% in children aged under 5 years. In addition, EBV seroprevalence was found to be lower in patients who had a diagnosis of glomerular disease and therefore used immunosuppressive drugs. However, it should also be noted that the mean age was lower in the patient group who developed ESRD because of glomerular diseases compared with the patient group who developed ESRD because of other causes.

The prevalences for MMR and varicella, for which vaccination after kidney Tx is not possible, were found as 70.4%, 61.2%, 88.1%, and 71.9%, respectively. According to the Cooperative European Paediatric Renal Transplant Initiative (CERTAIN) recording system, the seropositivity rates for MMR and varicella before Tx were found as 77%, 90%, 73%, and 90%, respectively (1). In a study conducted by Dilli et al. (14) in Turkey, the seropositivity rates for MMR and varicella in children with a mean age of 11.8 years were found as 81.6%, 80.0%, 85.5%, and 71.0%, respectively. In the same study, the vaccination rates for MMR and varicella were found as 96.9%, 0.4%, 2.0%, and 1.2%.

In Turkey, the Ministry of Health Public Health Institution vaccination recommendations for individuals for whom solid organ Tx is planned are as follows: In patients who have not been vaccinated for measles, mumps and rubella or who are seronegative, a total of two doses are recommended such that the final dose is administered at least 4 weeks before Tx. Varicella vaccine should preferably be administered 4 months before Tx to individuals who have not had varicella or have not been vaccinated before. A single dose of varicella vaccine is sufficient for children aged below 13 years, and two doses of varicella vaccines with an interval of 1 month should be administered to patients aged 13 years and over. Hepatitis B vaccine and hepatitis A vaccine (two doses) are recommended for all solid organ recipients. In addition, pneumococcal conjugate vaccine (CPA) and pneumococcal polysaccharide (PPA23) vaccine should be administered to patients for whom solid organ Tx is planned, considering the vaccination schedule appropriate for the age group.

Our study has some limitations. Vaccination rates were not included in the study because the vaccination schedule could not be reached in all patients. Therefore, the group that could not develop a vaccine response despite being vaccinated could not be differentiated. Similarly, it is not known which patients obtained seropositivity by immunization and which patients obtained seropositivity by past infection.

Despite these limitations, we think that this study will contribute to the literature because it reflects the frequency of antibody response against viral infectious agents in children with ESRD for whom kidney Tx is planned.

In conclusion, it is important to screen patients with renal failure in terms of vaccine-preventable diseases, to determine patient groups that carry risk, and to provide vaccination for these patients in the early stage. All vaccines should be administered at the appropriate time without waiting for Tx preparation. Also, intermittent serology monitoring in the post-Tx period is important in terms of protecting the transplanted kidney’s functions and providing survival.

**Ethics Committee Approval:** Ethics committee approval was received from Akdeniz University Medical Faculty Ethics Committee for this study (23.10.2019/1005).

**Informed Consent:** Since the data used in the study were obtained from retrospective medical data, written informed consent was not obtained.

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