Assessment of cardiometabolic and other complications in children with chronic kidney disease and renal transplantation

Ph.D. thesis

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Budapest
2018

INTRODUCTION

Cardiometabolic diseases are one of the causes of leading deaths worldwide. Because of that a more detailed mapping of these diseases and personalized therapy is particularly important in terms of health preservation. This finding is especially true for chronic patients. Therefore many research aims to investigate and influence cardiometabolic diseases in detail. Chronic kidney disease (CKD) affects more and more people all over the world, increasing the burden on health. Although relatively uncommon in children, it can be devasting illness with many long-term consequences. Some typical features of pediatric CKD, such as disease aetiology or long-term complications, will not only influence the child's health, but also have long-term impact on the life of the adult that they will become. CKD that start during childhood require appropriate treatments in order to optimize health outcomes.

Our research team has been investigating for a long time the cardiometabolic status in children with chronic kidney disease and renal transplantation to get the more detailed knowledge of the disease and its complications thus to improve long-term survival. Beside other factors, taking into account also the research group results, the caring protocol of children with CKD and renal transplantation is constantly changing. Investigation and therapeutic protocol may change according to the research results. Widely used (but not routinely used in this population, for example: oral glucose tolerance test), relatively new, routinely used in other populations (for examle: continuous glucose monitoring system or polysomnography) or new, unused methods (pulse wave velocity measurement) can be added to the caring protocol.

OBJECTIVES

Our aim was to study cardiometabolic status and other complications in children with chronic kidney disease and renal transplantation. Main questions and aims were the followings:

1. To determine the detailed cardiac morphology and function in children with CKD and after renal transplantation measured by cardiac magnetic resonance (CMR). Are cardiac lesions in childhood CKD reversible after succesful transplantation (Tx)?
2. To monitor glucose levels in children after renal transplantation using the continuous glucose monitoring system (CGMS). Does it provide additional information on the glycemic control of this patient population?
3. To study the safety and efficacy of H1N1 vaccination among the pediatric renal transplant population. What is the rate of protection in this population? Is there any correlation with the development of protection?
4. Would it be useful to introduce additional methods to optimize care of children with CKD and after renal transplantation?

METHODS

CMR study

CMR imaging offers new opportunities for assessing cardiac status. CMR has been validated in healthypopulation, as well as in hypertensive patients. CMR imaging is emerging as the optional method for cardiac assessment in CKD and volume overload patients, because it provides a more accurate characterization of left ventricular borders than echocardiography and enables accurate and more importantly volume-independent evaluation of both myocardial structure and function. Moreover CMR has been considered the „gold standard" for assessing ventricular dimensions in patients with CKD. CMR scans were performed either on a General Electric (GE) Signa 1.5T Infinity (General Electric Medical Systems, Milwaukee, WI, USA) or later on a Philips Achieva 1.5T (Philips Medical System, Best, The Netherlands) magnet. We measured left ventricular ejection fraction (LVEF), left ventricular end diastolic and end systolic volume index (LVEDVi, LVESVi), left ventricular mass index (LVMi) and right ventricular ejection fraction (RVEF).

In our observational study, 15 patients with CKD stages 2-5. and 18 patients with Tx were enrolled. Eight patients were prospectively followed in CKD and also after kidney Tx. Therefore we evaluated cardiac morphology and function in 49 CMR assay of 41 pediatric patients either with CKD or after Tx. CMR parameters were analysed in a cross sectional study (15 CKD and 18 renal Tx patiens) and intraindividual comparison (8 prospectively followed patients). CMR parameters were indexed for m^2 body surface area. Reference values for girls and boys have been obtained from data of Sarikuoch et al. (age groups: 8-15 year and 16-20 year). CMR parameters are presented as percentage, mean ± SD or median (interquartile range) as appropriate. Categorical variables were analyzed by chi-square test. Independent continuous variables were compared by Student's t-test

or Mann-Whitney U-test. In a group of eight patients paired t-test and Wilcoxon signed-rank test were used for intraindividual comparison. A p value < 0.05 was considered as statistically significant.

CGMS study

CGMS is a well-known and useful tool to demonstrate changes in glucose levels is diabetic patients. The CGMS is nowadays widely used in patients with diabetes to detect glucose variability, and such continuous glucose measurements offer the potentional to help patients optimize glycemic control and reduce the risk of hypoglycemia. As CGMS provides a real-time continuous monitoring of glucose levels, it can provide new insights into the glucose homeostasis of kidney transplant patients. Although the use of CGMS in these patients is unknown and furthermore data on CGMS in children with kidney transplantation are missing. Therefore, the aim of this cross-sectional study was to monitor glucose levels of children after renal transplantation using the CGMS to display which information could be provided on the glycemic control of this patient population.

Twenty renal transplant patients were recruited at a routine post-transplant clinic visit. All of them had stable graft function at the time of the study. The CGMS monitoring was performed with a Medtronic minimed CGMS device. The sensor was inserted into the subcutaneous tissue of the anterior abdominal wall. At the time of the CGMS, on the first day of the visit, an additional oral glucose tolerance test (OGTT) was performed. According to OGTT patients were classified into normal glucose metabolism, impaired glucose tolerance (IGT) and new onset diabetes after transplantation (NODAT) group. Insulin levels were analyzed during the OGTT measurement and during the whole CGMS study period. HOMA index was calculated (according to the pediatric standard values published by Allard et al.). Insulin was assessed using the Elecsys insulin assay

from Roche (Roche Diagnostic GmBH, Mannheim, Germany) according to the manufacturer's protocol. The CGMS monitoring consisted of a 72-h monitoring period. Glucose monitoring data for at least a continuous 48-h period were used for analysis. During monitoring, patients received a defined carbohydrate intake (180 g daily) and physical activity was also in the same range.
The main CGMS outcome parameters included mean glucose concentrations, 24-h SD of glucose levels, mean amplitude of glucose excursion (MAGE) and quantification of hypoglycemic and hyperglycemic episodes. T-test was used to compare groups. Pearson's correlation coefficient was calculated between the time after transplantation and glucose SD. Statistical analysis was performed through GraphPad (San Diego, CA, USA) Prism version 5. A p value < 0.05 was considered as statistically significant.

H1N1 vaccination efficacy study

In organ transplant patients, infections may cause severe problems and may lead to graft rejection. In contrast, it is known that vaccination do not increase the risk of rejection among organ transplant recipients. Annual vaccination is recommended for immunocompromised patients for infections diseases such as influenza. Unfortunately, owing to the immunosuppressive therapy, the efficacy of vaccination differs among individuals. Therefore, antibody measurements are needed to check protection rates in these patients.
37 renal transplant patients were recruited at the study. On the first day blood was obtained for antibody titer measurement against 2009-H1N1 influenza A virus. Immediately thereafter, we vaccinated the patients with a single shot of Fluval-P-monovalent vaccine (A/California/7/2009 [H1N1] NYMC X-179/A, 6 μg hemagglutin/0.5 ml doses with aluminium phosphate gel adjuvant; Omnivest, Gödöllő, Hungary). Exactly 21 days after vaccination, patients underwent a physical examination and blood was obtained to evaluate graft

function and antibody titer. Hemagglutination inhibition was used to evaluate antibody titer. We calculated seroprotection rate, seroconversion rate and seroconversion factor. Using international criteria, we determined patients with an antibody titer ≥1:40 or with ≥4-fold elevation to be protected. Patients with an antibody titer ≥1:10 or ≥2-fold elevation were defined as moderately protected. Data are presented as mean ± SD or median (interquartile range). The data analysis was performed using Statistica.6 software (StatSoft, USA). Chi-square and Fisher's exact test were used to assess associations between risk factors and seroprotection and seroconversion rates after vaccination. A p value <0.05 was considered as statistically significant.

RESULTS

CMR study

In our cross-sectional cardiac assessment (based on the mean ± SD), LVEF was higher and in the normal range in Tx patients compared to children with CKD (-0.3 ± 1 vs. -2.1 ± 1.6, Tx vs. CKD, $p < 0.05$). LVEDVi and LVESVi were higher in patients with CKD compared to Tx patients (0 ± 1.7 vs. 2.1 ± 3.1, Tx vs. CKD; 0.2 ± 1.2 vs. 3.1 ± 3.7, Tx vs. CKD, both $p < 0.05$); however, LVEDVi was still not in the normal range after Tx. LVMi was close to the normal range in Tx patients, but was far from the normal range in children with CKD (1.4 ± 1.5 vs. 3.4 ± 2.9, Tx vs. CKD, $p < 0.05$). LVH has been demonstrated in 12 of 15 (80%) patients with CKD, while the prevalence of LVH was 7 of 18 (39%) in the Tx patient group ($p < 0.05$). With regard to RVEF, the difference between the patient groups did not reach the level of significance, noteworthy RVEF was in the normal range also before Tx (-0.9 ± 1.4 vs. -0.9 ± 1.8, Tx vs. CKD, $p=0.35$). In the cross-sectional study, we did not find any patients with low LVEF after Tx and only one with high LVESVi. The low LVEF observed in CKD contrasts with prior echocardiographic studies, which showed elevated contractility and diastolic dysfunction in children with CKD or on dialysis. The further increase in contractility seen post-transplant is consistent with a former echocardiographic study. In intraindividual comparison (8 patients who underwent CMR both before and after Tx) in an average of six months (2.8-16.5 months) after Tx, LVEF, LVEDVi, LVESVi and LVMi improved significantly compared with the values detected before Tx ($p < 0.05$); however, RVEF did not change (p = n.s.) LVH was found in seven of eight children (88%) in CKD and four of eight (50%) after Tx. ($p < 0.05$).

CGMS study

OGTT showed IGT in six (30%) patients, whereas in one patient (5%), NODAT was diagnosed. Seven patients (35%) had a HOMA index showing insulin resistance.

The average lowest glucose level was significantly higher in the IGT group than in the normal group ($p = 0.042$ vs. normal). The average highest glucose level did not differ between the groups, but in the NODAT patient, it tended to be higher than that in the two other groups. The IGT patients presented 0.1 hypoglycemic episode per patient, whereas in the normal group, there was an average of 1 hypoglycemic episode per patient ($p = 0.043$ vs. IGT group) The incidence of hyperglycemic episodes was nearly the same in the normal and IGT groups. In the NODAT patient, 1.5 hypoglycemic and 1 hyperglycemic episode were observed. In the IGT group, both 24-h glucose level SD and MAGE were lower than in the normal group, but the differences were not significant. The patient with NODAT had a 24-h SD of glucose value similar to the average SD in the normal group. MAGE was lower compared with the normal group and was approximately the same as the average MAGE in the IGT group. Correlation between the major CGMS parameters and the time after Tx was analyzed. There was no correlation between time after Tx and mean glucose level, mean highest glucose level, mean lowest glucose level, hyperglycemic episodes, hypoglycemic episodes, or 24-h MAGE. There was a significant correlation between the 24-h glucose SD and the time elapsed after Tx ($r = -0.42$, $p = 0.029$).

H1N1 vaccination efficacy study

None of the patients experienced vaccination-related side effect. All participants developed antibody responses against the pandemic influenza A H1N1 virus. However, only 29.72% of patients showed an appropriately increased antibody titer (≥1:40 = seroprotection). The remaining subjects showed elevations without reaching the recommended 1:40 level. Calculating the seroconversion rate, the situation was even worse, because only 18.75% of patients had ≥4-fold elevation of antibody titer. The seroconversion factor was 1.45 among our patient population, showing a lower level compared with the World Health Organization recommendation (>2.5). Calculating seroprotection and seroconversion, there was no difference between genders. Seroprotected patients were significantly older than those with moderate protection (19 ± 3,9 vs. 14,9 ± 4,9 y, seroprotected vs. moderate protected, p = 0.02). Evaluating the time after Tx, we did not observe significant difference between seroprotected and moderately protected patients (7 ± 4,3 vs. 5,7 ± 3,9 y, seroprotected vs. moderately protected, p = n.s.). Patients receiving 3-drug therapy showed a tendency toward a lower rate of seroprotection compared with patients under 2-drug therapy, however the difference was not significant (21% vs. 39%, 3-drug therapy vs. 2-drug therapy, p = n.s.). Underlying diseases or therapy before Tx did not correlate with seroprotection or seroconversion.

CONCLUSIONS

Cardiovascular diseases (CVD) are getting more common in children with CKD and may lead to death even is childhood. Population-based studies show that minor renal impairment is strongly associated with cardiovascular changes. With the progression of kidney disease, the frequency of cardiovascular complications increases. Although preemptive kidney Tx is the best way to slow the progression of CVD, after succesful Tx, cardiovascular mortality remains significantly higher compared to healthy children.

New onset diabetes mellitus after transplantation (NODAT) is well-known complication of immunosuppressive therapy after Tx being a risk factor for cardiovascular and infectious complications, affecting patient and graft survival. The emergence of new immunosuppressive drugs may lead to replacement of protocols containing nephrotoxic, diabetogenic, hypertonogenic calcineurin inhibitor immunosuppressive medications. But it is not enough to know the new (with better side effects) medications to optimize treatment. The patient or the state of the disease should be well-known. Therefore it is indispensable to use new diagnostic methods and to determine their clinical value. With the help of all these, it will soon be possible in clinical practice personalized immunosuppressive therapy. This therapy takes advantage of the possibilities offered by agents with different mechanism to use combinations that are most effective and have the least number of side effects, taking into account underlying disease, age, immunization state and accompanying diseases.

Renal transplant patients are susceptible to infections and infections may be more severe due to the side effects of immunosuppressive therapy. The deaths of patients with functional kidneys mainly caused by infections. Therefore prevention of infections is particularly important in this population.

Our research aimed at a detailed investigation of three related complications. Our research was carried out in the hope of optimizing the care of children with CKD and renal transplant. Based on our results the following conclusions can be derived.

1. In our study we analyzed the cardiac structure and function in children with CKD and renal Tx by CMR in detail for the first time. The method was well tolerated in children involved in the study. Due to the age of the patients, we did not face the early childhood problems described in the literature. Our data demonstrate that kidney Tx in children is associated with a significant improvement in cardiac structure and function. **CMR might be an appropriate complemetary method for measuring detailed cardiac status in children with CKD.**
2. CGMS analysis showed that under standardized conditions IGT in renal-transplanted children is not characterized by hyperglycaemia, but higher „lowest glucose" level and lower glucose variability. We analyzed the correlation between the main CGMS parameters and the time after Tx. We could clearly demonstrate that 24-h glucose SD significantly decreased with time after Tx. These results indicate that glucose variability might be an important variable in glucose homeostasis of renal-transplanted children. These changes may also have a diagnostic value, but further studies are needed to obtain follow-up data showing whether these parameters have a predictive value. **Based on our conclusions CGMS might be a useful ambulatory tool in detection of glucose metabolism disorder in the post transplant period.**
3. We studied the safety and efficacy of a single shot monovalent whole-virus vaccine against the pandemic 2009-H1N1

influenza A infection. Based on our results, vaccination was safety, none of the patients experienced vaccination-related side effect. But by measuring antibody responses, only 29,72% of the patients achieved a safe seroprotection level. Patients classified as protected by antibody titer elevation were significantly older than those classified as moderate protected. **Taken together our results indicated that even a single vaccination may have protective effects in children with kidney grafts.**

4. Further studies are needed to use the results and to learn where CMR and CGMS studies might be used in clinical practice. Based on our research results, these additional methods can be useful tools in personalized patient care.

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