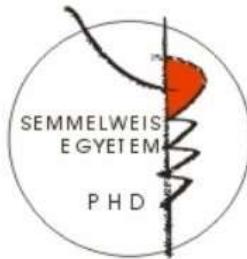


# Cardiovascular consequences of uraemia and renal transplantation in children

Thesis abstract

**Dr. Kis Éva**

Semmelweis University, PhD School - Clinical Medicine



Supervisor: Dr. Reusz György PhD, ScD, professor

Opponents:

Dr. Járai Zoltán, PhD

Dr. Studinger Péter, PhD

PhD theoretical exam:

Chairman: Dr. Rosivall László, PhD, ScD, professor

Members: Dr. med. habil. Kiss István PhD, professor,

Dr. med. habil Szabó László PhD, professor

Budapest, 2011

## **Introduction**

Cardiovascular (CV) risk of patients with end stage renal disease is about three magnitudes higher compared to the normal population. Renal transplantation decreases CV risk compared to the traditional renal replacement therapies however it still remains above that of the age-matched normal population.

The aim of the study was to analyze whether the signs of early arterial damage of children on renal replacement therapy could be evaluated by means of non invasive methods and to seek out the determinants of the arterial damage. We measured the aortic pulse wave velocity (aPWV) to characterize the mechanical characteristics (stiffness) of the aorta in children on renal replacement therapy. The method of measuring the aPWV is the gold standard, as it has the largest amount of epidemiological evidence for its predictive value for CV events in adults. PhD theses were based on results of four.

## **Aims**

Our aims were:

- a) To evaluate arterial stiffness (aPWV) in children on dialysis
- b) To assess the determinants of aPWV in uremic children
- c) To determine aPWV in children who underwent renal transplantation
- d) To evaluate the determinants of aPWV in renal transplant children
- e) To compare aPWV of the renal transplant patients with similar data of patients with end stage renal disease studied previously.
- f) To characterize the relation between the stiffening of the arterial wall (aPWV) and calcium and phosphate metabolism, bone turnover, and the level of the inhibitory protein fetuin-A in children on renal replacement therapy
- g) To determine predictors of increased aPWV in renal transplant children during post-transplant period

## **Methods**

### **Pulse wave velocity measurement**

Aortic PWV was measured by applanation tonometry (PulsePen device, DiaTecne, Milan, Italy) using sequential recordings of the arterial pressure wave at the carotid and femoral arteries.

Gender, age and height matched controls were used to assess aPWV in children.

To compare patients with different levels of growth retardation, PWV was normalized to height (PWV/h), and PWV standard deviation score (PWV SDS) was calculated. A database of 188 healthy subjects was used for the calculations

There was a highly significant correlation between values of PWV SDS and PWV/h ( $r=0.81$ ,  $p<0.05$ ).

### **Laboratory and clinical data**

Serum Ca, P, creatinine were measured by routine laboratory methods (local laboratory reference ranges were used).

Serum parathyroid hormone (PTH), bone alkaline phosphatase (BALP),  $\beta$ -crosslaps, osteocalcin (OC) levels were measured.

Fetuin-A - an important circulating systemic calcification inhibitor glycoprotein - was measured by DRG human fetuin enzyme-linked immunoabsorbent assay (ELISA) kit. (Age and gender matched controls were used.)

Data of serum Ca, P, iPTH and creatinine were reviewed retrospectively before and twelve months after transplantation.

The cumulative dose of calcitriol administered during dialysis and time on dialysis were assessed.

To estimate the potential of calcium-phosphate precipitation CaxP/Fetuin-A was calculated.

Bone turnover activity was estimated as the ratio of bone marker of children on renal replacement therapy and bone marker of the age and gender matched controls.

### **Evaluation of the effect of end stage renal disease**

To characterize the impact of the persistent uremic environment - the “uremic burden” (UB) - we introduced scoring system. High-normal serum calcium ( $\text{Ca} > 2.2 \text{ mmol/l}$ ), elevated serum phosphate ( $\text{P} > 1.8 \text{ mmol/l}$ ), PTH ( $> 180 \text{ pg/ml}$ ), and the time on dialysis beyond 12 months were scored as 1.

### **Patients**

Patients were on renal replacement therapy (dialysis and renal transplantation) at Semmelweis University, 1st Dept. of Pediatrics, Nephrological Care Unit.

### Dialysed patients

Eleven patients receiving maintenance dialysis participated in the study (age: 14.3 (4.1) years, weight: 36.9 (15.5) kg, height: 1.42 (0.21) m). Four children were treated with peritoneal dialysis (PD) and seven with haemodialysis (HD). Two patients had transplants previously, but returned to dialysis due to chronic rejection.

### Renal transplant patients

Twenty-five transplanted patients (age: 15.1 (3.8) years, weight: 46.7 (14.0) kg, height: 1.49 (0.16) m) were examined.

The time spent on dialysis prior to transplantation was 9 (0–60) months. Three of them were transplanted pre-emptively. To assess the effect of sustained end stage renal disease (ESRD) and the actual kidney function on PWV, we divided the transplant patients into groups, according to the time spent on dialysis (cutting point: >1 year on dialysis) and according to the GFR (cutting point: clearance of creatinine (CCl) <90 ml/min/1.73 m<sup>2</sup>).

We compared PWV of the transplant patients to similar data of 11 patients with ESRD studied previously.

### Bone and Ca-P metabolism in children on renal replacement therapy

*Dialysed patients:* Eleven patients with end stage renal disease were examined (age: 13.8 (4.3) years); seven on

continuous ambulatory peritoneal dialysis (CAPD); four on haemodialysis. The time spent on dialysis was 11 (3–78) months at the time of the PWV measurement.

*Renal transplant patients:* Seventeen transplant patients (age: 15.0 (4.2) years) were examined. The time spent on dialysis prior to transplantation was 12 (0–36) months. Five were treated by HD, nine by CAPD prior to transplantation, and three were transplanted pre-emptively. The time since transplantation was 36 (1–166) months.

#### Determinants of arterial stiffness in renal transplant children

Forty-seven transplant patients (age: 15.2 (4.1) years, height: 153 (128) cm, weight: 52.3 (16.8) kg) were examined. The time spent on dialysis prior to transplantation was 11 (0–61) months.

## **Statistics**

Database analysis was performed using the STATISTICA 8.0 (Stat Soft., Inc. USA). Data are presented as mean  $\pm$  standard deviation (SD). In case of data with non-normal distribution, data are expressed as median (range). Laboratory data were compared by Student's t test or analysis of the variance (ANOVA), where appropriate; Mann-Whitney U test was used to compare data. Univariate and multivariate regression

analysis was applied to assess associations between the data. A p value of  $<0.05$  was considered statistically significant.

## **Ethics**

The study conformed to the Helsinki Declaration and was approved by the local ethical committee. Parental informed consent was obtained from all subjects participating in the study.

## **Results**

### Pulse wave velocity in dialysed patients

Patients with ESRD were significantly shorter and lighter than the age matched controls. (height: 1.42 (0.21) vs. 1.56 (0.20) m, weight: 36.9 (15.5) vs. 46.6 (15.6) kg). Further, they were significantly older than their height-and weight-matched healthy pairs. (age: 14.3 (4.1) vs. 10.7 (2.6) years).

There was no difference in hemodynamic parameters of the patient and the control groups.

The PWV of the patients with ESRD did not differ significantly from the age-matched group (5.72 (0.94) vs. 5.01 (1.07) m/s). On the contrary, patients with ESRD had significantly increased PWV compared with the height/weight-matched control group (5.72 (0.94) vs. 4.56 (0.50) m/s).

Based on the novel parameter the PWV/height, the ESRD group differed significantly from both age-matched control and height/weight matched control groups (4.10 (0.75) vs. 3.17 (0.60) vs. 3.23 (0.49) 1/s). Significant correlation could be shown between PWV/height and phosphate level ( $r<0.64$ ,  $p<0.05$ ) and between PWV/height and the UB score (expressed as the sum of the individual risk factors) ( $r<0.61$ ,  $p<0.05$ ).

### Pulse wave velocity in renal transplant patients

Renal transplant patients ESRD were significantly shorter and lighter than the age matched controls (height: 1.49 (0.16) vs. 1.63 (0.15) m, weight: 46.7 (14.0) vs. 56.6 (15.5) kg).

They were significantly older than their height- and weight-matched healthy pairs (age: 15.1 (3.8) vs. 12.2 (3.3) years).

PWV standard deviation score and PWV/height were calculated.

There was a positive correlation between both the PWV SDS and PWV/height values and the calcium x phosphate product (CaxP) before transplantation( $r=0.58$ ,  $p=0.03$  and  $r=0.56$ ,  $p= 0.03$ ) and the cumulative dose of calcitriol administered during ESRD before transplantation ( $r=0.81$ ,  $p=0.002$ , and  $r=0.83$ ,  $p=0.0009$ ). By multivariate regression analysis, the cumulative dose of calcitriol was the main factor that influenced PWV SDS and a PWV/h following transplantation ( $\beta=0.7$ ,  $p<0.05$  ill.  $\beta=0.6$ ,  $p<0.05$  respectively).

Transplant patients with a CCI $>90$  ml/min/1.73 m $^2$  had lower PWV SDS and PWV/h than those with CCI $<90$  ml/ min/1.73 m $^2$  or the ESRD population (PWV SDS: 0.67 (1.08) vs. 1.46 (0.94) vs 1.71 (1.48); PWV/h: 3.47 (0.47) vs. 3.98 (0.51) vs. 4.1 (0.75) 1/s). There was no difference between the CCI  $<90$  ml/min/1.73 m $^2$  group and the ESRD population.

Patients with a dialysis time <1 year had lower PWV SDS and PWV/h than the ESRD patients (PWV SDS: 0.78 (1.04) vs. 1.29 (1.08) vs 1.71 (1.48)).

### Bone and Ca-P metabolism in children on renal replacement therapy

Both dialysed and renal transplant patients proved to be growth retarded compared with the healthy population. Neither systolic nor diastolic blood pressure SD score exceeded the 90th percentile of normal. Clinical data of uremic and transplant children are shown in table.

	<b>D</b>	<b>Tx</b>	<b>Control group</b>	
			<b>C – D</b>	<b>C – Tx</b>
<b>Fetuin-A (g/l)</b>	0.63 (0.37)	0.70 (0.28)	0.967 (0.237)**	0.93 (0.13)**
<b>CaxP/ Fetuin-A (mmol<sup>2</sup>/l*g)</b>	10.9 (7.87)	6.1 (3.54)*	3.38 (1.04)**	4 (0.76)**
<b>iPTH (pg/ml)<sup>&amp;</sup></b>	773 (94- 1588)	46 (10- 137)*	10-65#	
<b>BALP (U/l)<sup>&amp;</sup></b>	713 (130- 2060)	333 (94- 800 )*	304 (82- 442)**	248 (81- 420)
<b>B crosslaps</b>	6701	1701	829	865

(pg/ml) <sup>&amp;</sup>	(222-17620)	(259-4103)*	(350-2890)**	(502-1680)
--------------------------	-------------	-------------	--------------	------------

D-dialysed patients, Tx-renal transplant patients, C-control group

# Laboratory reference range

\*p<0.05 Tx vs. D \*\* p<0.05 Tx or D vs. C

In dialysed patients there was a significant correlation between bone markers (iPTH, BALP,  $\beta$  crosslaps). No correlation was found between CaxP or fetuin-A and PWV/h. However, considering the ratio of CaxP and fetuin-A (CaxP/fetuin-A), it correlated significantly with PWV SDS ( $r=0.65$ ,  $p<0.05$ ).

There was a significant correlation between PWV SDS and BALP ( $r=0.56$ ,  $p<0.05$ ). In transplant patients, only the cumulative dose of calcitriol relates significantly to PWV SDS ( $r=0.47$ ,  $p<0.05$ ).

By multiple regression analysis in dialysed patients CaxP/fetuin-A proved to be the only independent predictor of increased PWV ( $\beta=0.63$ ,  $p=0.04$ ).

#### Determinants of arterial stiffness in renal transplant children

In our renal transplant study population Ca, P, PTH, creatinine levels decreased after transplantation.

Using age and gender matched controls, bone markers were increased even after transplantation: (BALP 267 (61-1981) vs. 213 (68-488) U/l; OC 114 (26-1843) vs. 49 (12-1470) ng/ml, beta-crosslaps 1442 (79-5178) vs. 816 (48-2345) pg/ml).

On the contrary BALP levels decreased two years after transplantation.

The ratio of bone markers of tx to bone marker of the age and gender matched controls ratio decreased significantly after one year in post-transplant period.

(BALP/control: 2.95 (1.15) vs. 1.58 (0.74); OC/control: 3.03 (1.22) vs. 1.80 (2.05); beta-crosslaps/control: 2.94 (1.11) vs. 1.39 (0.62);  $p<0.05$ ).

aPWV was significantly increased in renal transplant children (PWV SDS: 1.13 (1.63),  $p<0.05$ ).

Dividing the transplantation group according to time after transplantation:

In children less than two years after transplantation, there was significant correlation between PWV SDS and BALP ( $r=0.53$ ,  $p<0.05$ ) and BALP and creatinine (first year post-transplant control) ( $r=0.80$ ,  $p<0.05$ ).

In children more than two years after transplantation, there was significant correlation between PWV SDS and cholesterol levels ( $r=0.38$ ,  $p<0.05$ ).

## **Discussion**

In our studies in uremic children, we have shown the necessity of appropriate controls in special paediatric populations. Thus controls matched for both age and height should be used to assess aPWV in children with growth failure. The aPWV normalized for height age (PWV SDS) provides a more universal parameter to avoid the bias caused by growth retardation. As the PWV SDS and PWV/h are closely related, PWV/h is an alternative, simple, age-independent measure of PWV.

Uremic children with more risk factors involving calcium and phosphate homeostasis have higher aPWV, suggesting the role of disturbed calcium metabolism.

Our study demonstrates elevated aPWV as a sign of increased arterial stiffness in children with renal transplantation. Determinants of increased aPWV after transplantation were the calcium x phosphate product (CaxP) and cumulative dose of active vitamin D before transplantation.

By multivariate regression analysis, the cumulative dose of calcitriol was the main factor that influenced arterial stiffness following transplantation. Our data strongly suggest that pharmacological doses of calcitriol may lead to an elevated cardiovascular risk in children with ESRD, and that the present form of management of secondary hyperparathyroidism is far from optimal.

We could also confirm that a shorter period of ESRD goes hand in hand with both lower PWV SDS and PWV/h, whereas transplant children dialysed for >1 year had values similar to those actually on dialysis. These results are in accordance with data from adults demonstrating a positive effect of early transplantation on the cardiovascular system.

Similarly, patients with a better renal function had lower PWV SDS and PWV/h than children with poor creatinine clearance who did not differ from the ESRD population on dialysis.

Combining the two parameters, CaxP/fetuin-A ratio proved to be a much more sensitive marker with a highly significant correlation to PWV SDS. By multiple regression analysis pre-eminent role of this parameter could be suggested in dialysed patients. The ratio of the CaxP product and fetuin-A represents the proportion of the physicochemical propelling force and the inhibition of calcium precipitation.

According to our studies, immediately after transplantation disturbed bone metabolism is responsible for arterial stiffness, later the lipid parameters may influence the process of arterial calcification and pulse wave velocity.

## **Theses**

1. Our study demonstrates increased age and height matched aPWV as a sign of increased arterial stiffness in uremic children. Determinants of increased aPWV in ESRD children are the disturbed Ca-P metabolism and the cumulative dose of calcitriol supplementation.
2. Study on renal transplant children confirmed elevated normalized aPWV. Uremic burden (disturbed calcium-phosphate metabolism) prior to transplantation determines the arterial stiffness in this patient group. After successful transplantation aPWV is found to be lower than that in dialysed patients, which could imply that vascular changes in children are reversible.
3. Determinants of aPWV are fetuin-A and CaxP/fetuin-A levels in ESRD children. CaxP/ fetuin-A ratio may be useful to establish a database that would enable us to identify patients at increased risk for development and progression of vascular calcifications
4. After two year of successful renal transplantation dyslipidaemia may influence arterial stiffness in renal transplant children

## Publications

- Kis E, Cseperekal O, Kerti A, Salvi P, Benetos A, Tisler A, Szabo A, Tulassay T, Reusz GS. Measurement of pulse wave velocity in children and young adults: a comparative study using three different devices. *Hypertension Res.* 2011 Accepted
- Kis E, Cseperekal O, Biro E, Kelen K, Ferenczi D, Kerti A, Szabo AJ, Szabo A, Reusz GS. Effects of bone and mineral metabolism on arterial elasticity in chronic renal failure. *Ped Nephrol.* 2009; 24:2413-2420.
- Kis E, Cseperekal O, Horvath Z, Katona G, Fekete BC, Hrapka E, Szabo A, Szabo AJ, Fekete A, Reusz GS. Pulse wave velocity in end stage renal disease: influence of age and body dimensions. *Pediatr Res.* 2008; 63:95-98.
- Cseperekal O, Kis E, Schaffer P, Othmane TE, Fekete BC, Vannay A, Szabo AJ, Rempert A, Szabo A, Tulassay T, Reusz GS. Pulse wave velocity in

- children following renal transplantation. Nephrology Dialysis Transplantation. 2009; 24:309-315.
- Reusz GS, Cseperekal O, Temmar M, Kis E, Cherif AB, Thaleb A, Fekete A, Szabo AJ, Benetos A, Salvi P. Reference values of pulse wave velocity in healthy children and teenagers. Hypertension. 2010; 56:217-224.

### **Hungarian publications**

- Kis E, Cseperekal O, Cseh A, Tory K, Toth-Heyn P, Sallay P, Szabo A, Reusz Gy. Pulzushullám terjedési sebesség egészséges és vesetranszplantált gyermekknél. Gyermekgyógyászat. 2009; 1:30-35.
- Degi AA, Kerti A, Kis E, Cseperekal O, Reusz Gy. Az ambuláns artériás stiffness index (AASI) vizsgálata vesetranszplantált gyermek esetében. Hypertonia és Nephrologia. 2011; 15:111-115.
- Cseperekál O, Kis E, Szabo AJ, Reusz Gy. Gyakorlati útmutató az érfalrugalmasság mérésére gyermekknél – Mit mivel mérjünk? Hypertonia és Nephrologia. 2010; 14: 183-191.

- Cseprekal O, Kis E, Paszka D, Tory K, Kenesei E, Sallay P, Reusz Gy, Szabo A. A kalcium- és foszfát anyagcsere vizsgálata urémiás és vesetranszplantált gyermekknél. Gyermekgyógyászat. 2009; 1:2-5.
- Cseperekál O, Kis E, Schaffer P, Fekete BC, Othmane T elH, Szabo A, Reusz Gy. Pulzushullám terjedési sebesség egészséges gyermekknél. Hypertonia és Nephrologia. 2008; 12:15–20.