



**UNIVERSITY OF MEDICINE AND PHARMACY OF
CRAIOVA DOCTORAL SCHOOL**

PHD THESIS SUMMARY

**DIAGNOSIS PARTICULARITIES AND OUTCOMES
IN PEDIATRIC POLYCYSTIC KIDNEY DISEASE**

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**CRAIOVA
2020**

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Keywords: polycystic kidneys, hepatorenal cystic syndrome, chronic kidney disease, nephromegaly, nephrectomy, aquaporin, hepatic fibrosis

1. Introduction

The hepatorenal cystic syndrome is a heterogeneous group of severe monogenic conditions that may be detected before birth. Commonly, hepatorenal cystic syndrome present in the neonatal and pediatric age, consists of developmental abnormalities mostly involving the liver and kidney. The changes include the proliferation and dilatation of epithelial ducts in these tissues with abnormal deposition of extracellular matrix. In the liver, increased hepatic fibrosis often associates with cysts lined with biliary epithelium and a variable degree of intrahepatic biliary tract dilatation. Cystic lesions also affect the kidneys and their severity determines the clinical presentation and long term prognosis for the hepatorenal cystic syndrome. Liver disease is an invariable feature of autosomal recessive polycystic kidney disease (ARPKD) and in some cases it can be a predominant clinical feature.

Of all ARPKD neonatal survivors, it is estimated that 40% have severe dual organ disease, with the remainder equally divided into those with severe kidney/mild hepatobiliary disease; mild kidney/severe hepatobiliary disease, and mild dual organ involvement.

2. Current state of knowledge

The most common genetically inherited kidney disease was proved to be autosomal dominant polycystic kidney disease (ADPKD), with an incidence of 1:400 - 1 :1000 in USA. Renal manifestations in ADPKD are the consequences of the fluid-filled cysts located within the kidney, along with a renal function deterioration. Extrarenal manifestations are represented by high blood pressure, intracranial aneurysms and the presence of cysts located in the liver.

In 85% of cases, a mutation of the PKD1 gene causes the onset of ADPKD. Hepatorenal cystic syndrome includes ARPKD with an incidence of 1: 10 000- 1: 40 000 live births. The clinical features of this disease are represented by nephromegaly and pulmonary hypoplasia which will determine the onset of the respiratory distress syndrome and perinatal death in 30 % of the neonates.

3. Personal contribution

Our first study involved 50 patients, who were between 3 months and 16 years of age, with multiple admissions in the Nephrology Department of „Maria Sklodowska Curie” Children's Emergency Hospital from Bucharest, during 6 years (April 14th 2010 -October 24th 2016), to evaluate the hepatorenal cystic (HRC) syndrome. In order to delay the end-stage renal disease and minimize the adverse outcomes we established an early diagnosis of HRC syndrome in children using biochemical, genetic and imagistic techniques along with adequate therapeutic strategies. The women assigned to the

ultrasound test underwent ultrasonographic examination at 16-22 weeks of gestation. The positive ultrasonography results were correlated with genetic testing (PKD1 and PKD2 mutations are known to be associated with autosomal dominant polycystic kidney disease-ADPKD) and also with positive family history of polycystic kidneys.

In case of inconclusive ultrasound results, a postnatal MRI was performed to confirm the diagnosis of polycystic kidney disease. Urinary tract infections (UTIs) diagnosis for symptomatic patients was based on positive urine culture ($\geq 10^5$ col/ml, single germ) and for asymptomatic patients, at least two positive samples in two different days ($\geq 10^5$ col/ml, same germ).

Statistical analysis was performed using Microsoft Office Excel 2010. For numerical variables, statistical mean differences were assessed using Student's t-test and ANOVA, while for categorical variables the chi-square test was used. Statistical difference was considered at p-levels under 0.05 (95% confidence). The graphs showing the results were created using Microsoft Office Excel 2010. Postnatal confirmation of prenatally diagnosed polycystic kidneys at 16-20 weeks gestational age was obtained for 20 patients after they undergone renal ultrasound. Neonatal diagnosis of polycystic kidneys was confirmed in 4 cases, and the postnatal diagnosis was positive in 26 cases for patients admitted to the hospital with non-specific renal symptomatology.

The admission symptomatology was mainly represented by the nephrology evaluation which was essential in the management of children's polycystic kidney disease. For example, a premature infant (gestational age=32 weeks) with positive heredo-collateral history (mother and grandmother were diagnosed with polycystic kidney disease), was tested positive for cystic renal disease after the fetal morphology was performed. It was also done a genetic determination for the presence of PKD1 and PKD2 mutations which are specific to autosomal dominant polycystic kidney disease-ADPKD. However,

the genetic test was negative and a postnatal nephrological evaluation was performed using renal ultrasound.

The image revealed autosomal recessive polycystic kidney disease-ARPKD. Postnatal renal ultrasound revealed: enlarged normally located kidneys (Right kidney-18.2/10.6cm; Left kidney-17/8cm), the loss of bilateral corticomedullary differentiation, renal parenchyma replaced by multiple cysts with a maximum diameter of 2,2cm in the inferior right pole and 2cm in the inferior left pole and right mild mediorenal caliectasis of 9mm. There was no evidence of nephrolithiasis or dilation of pyelocaliceal system, urinary bladder in semirepletion, transonic content, normal vesical wall.

No fluid collection in the peritoneal recesses. Limited visualization of the ureters. There were 50 patients confirmed with polycystic kidney disease, 42 of them were diagnosed with UTIs, 6 were negative for UTIs and in 2 of the cases there was no evidence of it. Autosomal dominant polycystic kidney disease- ADPKD was diagnosed in 10 female patients and 9 male patients, ARPKD (AR pattern) was confirmed at 3 female patients and 4 male patients, both Multicystic Left/Right Dysplastic Kidney and Renal cystic dysplasia with undetermined genetic transmission pattern were positive for 10 female patients and 14 male patients. Our study revealed that 18 out of 19 patients with autosomal dominant polycystic kidney disease (AD) were postnatally diagnosed, unlike those patients with ARPKD who were mainly prenatally diagnosed. These results are in concordance with medical literature which states that only 2% of the patients with ADPKD, experience symptoms before the age of 15 years old. Prenatal diagnosis of ARPKD can be suspected starting with the 15th week of gestational age based on hyperechogenic enlarged kidneys. Oligohydramnios can be diagnosed around the 20th week of gestation accompanied by enlarged kidneys, absent bladder or its reduced dimensions.

Our second research was conducted over a period of three years (July 26, 2015–October 30, 2018) on 22 patients aged between two days and 36

months, diagnosed with polycystic kidneys that presented multiple hospital admissions in the Department of Nephrology, “Maria Skłodowska Curie” Emergency Children’s Hospital, Bucharest, Romania. The nephrectomy sections were obtained from the material of the Department of Pathology of the same Hospital. We have also studied a case of a premature infant born in October 2018 with an estimated gestation date of 32 weeks with a presumptive prenatal diagnosis of right polycystic kidney. The polycystic kidney disease diagnosis was established based on the histopathology performed in the Pathology Department of the Emergency County Hospital of Craiova, and in the Center for Microscopic Morphology and Immunology of University of Medicine and Pharmacy of Craiova along with the biochemical and imagistical investigations. Early diagnosis of polycystic kidney disease (PKD) was based on the positive fetal ultrasonography performed at 16-22 weeks of gestation. Prenatal ultrasonography results were correlated with positive family history of polycystic kidney disease, fetal enlarged kidneys and oligohydramnios. Neonatal diagnosis of polycystic kidney disease was based on Potter’s sequence and respiratory distress syndrome. Potter sequence is due to oligohydramnios includes hypertelorism, micrognathia and epicanthal folds. Autosomal dominant polycystic kidney disease was characterized by late onset with lumbar pain, nephromegaly and recurrent urinary tract infections. Moreover, in order to evaluate the excretion disturbances in PKD patients, as well as the global and individual renal functions, we used dynamic kidney scintigraphy with ^{99m}Tc -diethylenetriaminepentaacetic acid (DTPA).

The data were analyzed after collecting the GFR results (glomerular filtration rate), excretion values, time activity curves and the scintigraphic features. After surgical resection, the overall aspect of the kidneys showed that the normal parenchyma had been mostly replaced by cysts with thin, translucent, walls that contained a clear fluid. Tissue fragments were fixed in 10% neutral buffered formalin and processed for paraffin embedding and

microtome sectioning in the departments of Pathology from the „Maria Sklodowska Curie” Children's Emergency Hospital from Bucharest and Clinical Emergency County Hospital of Craiova. Selected tissue fragments were first re-evaluated in the Research Center for Microscopic Morphology and Immunology from UMF Craiova based on routine hematoxylin-eosin staining. Next, the slides were incubated overnight at 4°C with the primary antibodies[mouse anti-CD34, Dako (Glostrup, Denmark), 1:100; mouse anti-CD45, Dako, 1:100; mouse anti-ki-67, Dako, 1:100; mouse anti-collagen IV, Dako, 1:100; rabbit anti-albumin, Dako, 1:10.000, and mouse anti-Aquaporin 1, Thermo Fischer Scientific (Waltham, Massachusetts, USA), 1:200]. Microscopy confirmed that the parenchyma was mostly replaced by dilated cysts lined by simple cuboidal or simple flattened epithelium, with areas of remnant fetal kidney parenchyma separated by an enriched stroma.

Immunohistochemistry for blood vessels (CD34) revealed normal fine walled blood vessel arcades in the control kidneys, while in most areas from polycystic disease, the blood vessels exhibited enlarged, thickened endothelium, and less collapsed lumens. IOD measurement for the CD34 signal thus showed significant more intense signal in the disease kidneys compared to controls ($p<0.05$). Another factor that contributed to the significant increase in collagen IV immunostaining density ($p<0.05$) was also the presence of fibrosis between the immature tubules in the cases. In the polycystic disease there were dense-diffuse lymph cells accumulations in the interstitium, and the staining for T lymphocytes (CD45) showed this distribution, as well as the significant increase over the scattered cells present in control kidneys ($p<0.05$). Regarding the proliferative capacity of the tissues, our ki-67 staining revealed that the less formed, younger tubules in the pathological state had a higher proliferative index compared to control tissue ($p<0.05$). This proliferative capacity seemed to be retained mostly in the tubular epithelium, and less in the glomeruli. We assessed next the immunoexpression of albumin, which apparently showed a

generalized staining in almost all parenchymal elements of the developing normal and pathological kidneys. There seemed to be less albumin staining in the epithelia of the distal contort tubules, but that distinction was present also in our pathology.

The overall expression level was reduced in polycystic cases ($p < 0.05$), and it could be that this expression decrease might be related to the reduced function of these kidneys. Lastly, we wanted to evaluate the expression of AQP1 as a further putative marker of the water transport function in these kidneys. In the polycystic cases, the signal was more diffuse, in almost all the tubules present regardless of their maturation degree, even in the epithelia of the cysts. A more diffuse staining was present in the glomeruli, with no clear-cut vascular profiles.

The cystogenesis in autosomal dominant polycystic kidney disease (ADPKD) is proved to be the consequence of fluid transport into the lumen of the cyst by the increased level of transepithelial osmotic water permeability of some specific parts of the nephron due to the existence of the protein aquaporin-1. These alterations are represented by a remodelled laminated basement membrane, positive expression of collagen type IV and disruption of the renal parenchyma. Previous studies of interstitial infiltrates in polycystic kidney disease tissues determined the progression to tubulointerstitial fibrosis, leading to end stage renal disease. It has been observed a marked interstitial inflammation highlighted by positive CD 45 lymphocytes. Progressive evolution to renal insufficiency was connected to the increased number of macrophages and monocytes of the interstitial infiltration along with the enlarged renal cysts and the renal interstitial fibrosis.

4. Conclusions

1. This study emphasizes the importance of an early diagnosis (prenatal, neonatal, postnatal).
2. Prenatal diagnosis was based on the genetical investigations (mutations of the PKD1 and PKD2 genes for ADPKD and PKHD1 gene for ARPKD).
3. It has been identified the maternal inheritance pattern of ADPKD, and therefore the anamnesis has a strong role, positive history of ADPKD in the family could help in establishing the diagnosis.
4. Our study has revealed that ADPKD is more common in female patients, and ARPKD in male patients.
5. Prenatal ultrasonography results were correlated with positive family history of polycystic kidney disease, fetal enlarged kidneys and oligohydramnios.
6. Neonatal diagnosis of PKD was considered, when some of the neonates presented palpable flank masses that caused fetal dystocia.
7. The clinical aspects were correlated to the paraclinical investigations regarding the diagnosis of recurrent urinary tract infections of both symptomatic and asymptomatic patients, as well as with the imagistical results.
8. Microscopy confirmed that the parenchyma was mostly replaced by dilated cysts lined by simple cuboidal or simple flattened epithelium, with areas of remnant fetal kidney parenchyma separated by an enriched stroma.
9. Immunohistochemistry for blood vessels (CD34) revealed normal fine walled blood vessel arcades in the control kidneys, while in most areas from polycystic disease, the blood vessels exhibited enlarged, thickened endothelium, and less collapsed lumens.

10. In the polycystic disease there were dense-diffuse lymph cells accumulations in the interstitium, and the staining for T lymphocytes (CD45) showed this distribution, as well as the significant increase over the scattered cells present in control kidneys.

11. When we evaluated the basement membranes (based on anti-collagen IV immunohistochemistry), we found discontinuous basement membranes surrounding the proximal and distal tubules along with the increased expression of collagen IV.

12. AQP1 deficiency will determine the onset of cystogenesis.

13. Our research revealed that most of the AQP1 signal in the control kidney comes from the proximal tubules and the glomerular epithelium, while in the polycystic kidneys the signal was more diffuse in almost all the tubules present.

14. Progressive evolution to renal insufficiency was connected to the increased number of macrophages and monocytes of the interstitial infiltration along with the enlarged renal cysts and the renal interstitial fibrosis.