A STUDY OF PATHOGENETIC FACTORS RELATING TO LESIONS OF THE RENAL PARENCHYMA IN CHILDREN WITH URINARY TRACT INFECTION

Ph.D. Thesis
Éva Károly, M.D.

Semmelweis University
1st Department of Paediatrics
Tutor: Professor Tivadar Tulassay, M.D.

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Supervisors: Professor György Reusz, M.D.,DsC.,
and Andrea Fekete, M.D., Ph.D.

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Tutor: Professor Tivadar Tulassay, M.D., DsC.
1. Introduction

The most common disease entities in paediatric nephrologic practice are urinary tract infections (UTIs). Their treatment always calls for careful and in-depth consideration because of the possible consequences.

It is well known that the risk of renal parenchymal lesions is increased in UTIs. Progressive renal parenchymal lesions can lead to hypertension, pre-eclampsia, renal insufficiency and terminal nephropathy.

In a particular patient it is often difficult to determine the etiology and pathogenesis of a renal parenchymal lesion. We often see malformations, and various risk factors are well known: vesicoureteral reflux (VUR), UTI at early age, delay in the treatment of urinary tract infection, and recurrent UTIs.

However, a consideration of the factors and their careful study do not adequately explain the cause of the renal scars in some patients.

Besides to the bacterial virulence factors the ability of the host organism to respond is also of great
importance. A very special role in the recognition of the bacteria causing UTI-s and in the local immune response is played by the uroepithelium. The early immune response to the invasive pathogens is provided through the natural immunity, in which the toll-like receptors (TLR-s) and in particular the TLR4, play central roles. The TLR4 recognizes the lipopolysaccharide wall of Gram-negative bacteria, including the commonest urinary bacterium, Escherichia coli, and it is therefore very important in the development of UTIs. The TLR4 introduces a signaling cascade, stimulates the natural immune response, enhances the destruction of the bacteria and promotes the regenerative processes.

In UTIs, numerous other factors and mechanisms, including the heat shock proteins (HSPs) take part in the immune response. The HSPs are currently at the centre of medical interest. The members of the HSP70 family are partly constitutive formed in the cytosol (HSC70) and are partly present in stress-induced form (HSP72). They not only decrease tubular damage, but are also involved in the restoration of the renal function and in the activation of the immune system.
It is known from the literature that, genetic polymorphisms apart from the environmental factors, can give rise to a higher risk of infections and sepsis.

An arginine-guanine substitution, \( TLR4 \ A(896)G \), is known in the \( TLR4 \) 896 nucleotide, which results in an aspartic acid – glycine substitution, Asp,99Gly, associated with the higher risk of Gram-negative infections. It has also been reported that the level of HSP72 mRNA decreased in the presence of the \( HSPA1B \ A(1267)G \) polymorphism, and this is associated with the development of different inflammatory diseases.

The role of genetic polymorphisms has likewise emerged in connection with renal diseases.

The role of the renin-angiotensin system has been demonstrated in renal and urinary abnormalities and in different renal diseases, including IgA nephropathy, diabetic nephropathy and autosomal dominant polycystic renal disease. The DD polymorphism of the angiotensin-converting enzyme (\( ACE \)) is associated with a higher ACE activity both in the plasma and in the kidney finally
promoting the development of glomerular necrosis, interstitial fibrosis and renal scarring.

The outcomes of paediatric UTIs are probably greatly influenced by the genetic background. An understanding of the predictive factors involved in the pathomechanism of UTIs and in the development of renal scars can facilitate emergence of an individual care strategy for children with urinary tract infection.

2. Aims

2.1. Clinical data collection

2.1. Data were collected on 103 patients with UTIs at the Paediatric Unit of Jász- Nagykun Szolnok County Hospital with particular regard to the following aspects:

2.1.a. The prevalence of renal parenchymal lesions in children with UTIs.

2.1.b. The pathological changes in the background of UTIs?

2.1.c. The prevalence of renal scarring in children with low-grade VUR or with high-grade VUR.
2.1.d. The prevalence of renal parenchymal lesions in children with serious relapsing UTIs? (3 or more confirmed episodes of pyelonephritis).

2.1.e. Urinary tract malformations other than VUR associated with UTIs.

2.1.2. Data were collected on 77 paediatric patients with VUR to establish the prevalence of renal parenchymal lesions among children with various grades of VUR.

2.2. Genetic examinations

2.2.1. The children with UTIs were examined to establish the prevalence of the \textit{HSPA1B} A (1267)G and \textit{TLR4} A(896)G genetic polymorphisms in comparison with those in healthy controls, and to analyze the possibility of associations between the occurrence of UTI, VUR or renal parenchymal lesion and these two genetic polymorphism.

2.2.2. The children with VUR were examined to establish whether there was any difference in frequency of mutations of the genes \textit{ATG}, \textit{ACE} and \textit{AT1} relative to healthy controls, and to analyze the possibility of
associations between the \textit{ATG} gene M(235) T, \textit{ACE} I/D and \textit{AT1} A (1166)C polymorphisms and VUR or renal scarring.

3. Patients and Methods

3.1.1. UTI patients

The data on the 103 children with UTIs treated at the Pediatric Nephrologic Outpatient Clinic of the Pediatric Unit at the Jász- Nagykun Szolnok County Hetényi Géza Hospital were processed in 2005. Their mean age was 7.3 ± 4.5 years (81 girls, 22 boys). All of them participated in the necessary laboratory examinations (blood count, ESR, CRP, electrolytes, CN, creatinine, urine general sedimentation and urine bacteriological examinations) and various imaging examinations (abdominal and small pelvis US examinations, mictional cystourethrography (MCU) and static renal isotope examination (DMSA).

DNA samples extracted from peripheral blood samples were subjected to the \textit{HSPA1B} A(1267)G and \textit{TLR4} A(896)G polymorphism determinations. As a control, a
randomly selected group of 235 unrelated healthy controls from the Hungarian ethnic population was used. The examinations were carried out with the ethical approval of Semmelweis University.

3.1.2. VUR patients

For the examinations of the polymorphisms $ATI A(1166 \text{ G, } ACE I/D, ATG M (235)T}$ of the renin-angiotensin system genes peripheral blood samples from 77 children (mean age $6.9 \pm 3.2$ years, 33 boys, 44 girls) treated at the Department of Pediatrics University of Szeged, the Pediatric Unit of Pándy P. County Hospital, Gyula, the Pediatric Unit of the Semmelweis City Hospital, Kiskunhalas and at the Pediatric Unit of the Hetényi Géza County Hospital, Szolnok. The necessary laboratory examinations (blood count, ESR, CRP, electrolytes, CN, creatinine, urine general sedimentation and bacteriological examinations) and the imaging examinations (abdominal and small pelvis US, MCU, and DMSA) were performed at the treatment centers.
Blood samples from 80 healthy blood donors supplied by the Blood Bank of the University of Szeged (mean age $33.1 \pm 7$ years, 41 males, 39 females) served as controls.

The examinations were carried out with the ethical approval of the University of Szeged.

3.2. Methods

3.2.1. Restriction fragment length polymorphism (PCR-RFLP) examinations

DNA isolation and genotype determination
The genomic DNA extracted from the peripheral blood samples with phenol / chloroform.

For the detection of $HSPA1B\ A(1267)G$ genetic polymorphisms the DNA samples were amplified with specific primers and the products formed were digested with $Pst\ I$ or $Bsr\ BI$ restriction endonuclease enzyme. For the $TLR4$ examinations $NcoI$ was used for the digestion. The products formed were electrophoretesed on 3% agar gel stained with ethidium bromide and visualised with UV light.
For the examinations of the *ACE* gene I/D polymorphism in addition to the forward and reverse primers an insertion-specific primer was used for the definite heterozygote identification.

### 3.2.2. Real-time PCR method – melting point analysis (light cycler)

For the *ATG M(235)T* and *AT1 A(1166)C* polymorphism examinations the DNA sample was amplified in a light cycler, and the gene polymorphism was determined by evaluation of the melting curve on the basis of melting point analysis in the interval 40-90°C.

### 3.2.3. Statistics

In the statistical analysis on the children with UTIs use was made of the median, the mean, the sample size and the homogeneity determination (Prekopa András: Valószínűségmélet műszaki alkalmazásokkal. Műszaki Könykiadó, Budapest, 1962).

The data relating to the *HSP72*, and the *TLR4* polymorphisms were evaluated by means of the STATISTICA 6. Software (Stat. Soft Inc., USA). The
linkage and the distribution calculations were performed with Arlequin software (http://anthropologie.unige.ch/arlequin). The data on the gene polymorphisms of the renin-angiotensin group were processed with the use of SRSS 9.0 software.

Differences were considered to be statistically significant at p<0.05.

4. Results
4.1. Processing of the data on the children with UTIs or VUR
a. Renal parenchymal lesions were detected in 40/103 (38.8%) of the UTIs patients.
b. VUR was demonstrated in 40/103 (48.5%) of the children with UTIs.
c. Renal parenchymal lesions were observed in 27% of the children with mild-medium-grade VUR and in 76% of those with high-grade VUR.
d. Renal parenchymal lesions were confirmed in 62.9% of the children with recurrent UTI (3 or more episodes of pyelonephritis).
e. Other urinary tract anomalies associated with the UTIs were found in 9.4% of the group without VUR (pyelon duplex, agenesia renis, vesical diverticulum or hypospadiasis) and in 24% of the group with VUR/pyelon duplex, hypoplasia renis (unilateral), multicystic dysplasia or vesical diverticulum).

f. Renal parenchymal lesions were observed 70% of the children with high-grade VUR, but in only 30% of those with mild-medium-grade VUR.

4.2. The possible associations between the UTIs, VUR, the renal parenchymal lesions and the polymorphisms of HSP72 and TLR4?

a. The HSPA1B(1267)GG genotype was detected more frequently in the UTIs patients than in the healthy controls (p=0.0001), and also more frequently in the UTIs children with renal parenchymal lesions (p=0.012). It was likewise more frequent in the children with VUR, but not significantly so.

b. The HSPA1B (1267) G allele was carried significantly more frequently in the UTIs patients (=0.0001), and also in the UTIs patients with renal parenchymal lesions.
(p=0.049) than in the healthy controls. It was similarly more frequent among the patients with VUR, but not significantly.

c. The prevalence of the \( TLR4 \) (896) AG genotype was significantly higher in the children with UTIs than among the healthy controls (p=0.031), same was true for the UTIs without VUR (p=0.03). This genotype was also more frequent among the children with UTIs and renal parenchymal lesions, but not significantly so.

d. The \( TLR4 \) (896) G allele was significantly more prevalent in the UTIs children (p=0.041) and also in the UTIs children without VUR (p=0.03) than in the healthy controls, whereas it was non-significantly more frequent in the UTIs children with a damaged renal parenchyma.

e. The children with UTIs exhibited the \( HSPA1B \) (1267) AG and \( TLR4 \) (896) AG genotypes significantly more frequently than did the healthy controls.

4.3. The possibility of renin-angiotensin system gene mutation frequency differences between children with VUR and healthy controls.
a. As concerns the ATG M(235)T polymorphism, there was no difference either in the genotype or the allele frequency between the VUR children and the controls, or between the VUR children with or without renal scarring.

b. As concerns the I/D polymorphism of the ACE gene, the ID and DD genotypes were significantly more frequent in the group with serious VUR as compared with healthy controls (p<0.05), while the DD polymorphism of the ACE gene was more frequent the VUR children with renal scarring than among the VUR children without renal parenchymal lesions (p=0.01).

c. For the AT1 A(1166)C polymorphism there was no difference in either the genotype or the allele frequency between the VUR children and the healthy controls, or between the VUR children with or without renal parenchymal lesions.

5. Discussion and summary of the results

5.1. Pediatric UTIs, often the cause of complications (hypertension, eclampsia of pregnancy, renal
insufficiency) have been at the centre of the interest in the pediatrics and paediatric nephrology for decades. The goal in the treatment of UTIs is to prevent renal damage and late complications. Recurrent UTIs and VUR is a known risk factors for renal scarring.

- The data on the 103 children with UTIs revealed an association with VUR in 48.5% of the cases, but other urinary tract anomalies also occurred significantly frequently. Other urogenital abnormalities were seen in 24% of the VUR children.

- In the 103 children with UTIs, the frequency of renal parenchyma involvement was 38%. The frequency was very high (76%) in those with high-grade VUR and in the patients with 3 or more episodes of pyelonephritis in the history (62.9%).

The processing of the data on the 103 children with UTIs treated at the Jász-Nagykun Szolnok County Hospital led to findings in accord with previous national and international experience. Among children with UTIs, it is necessary to reckon with the presence of urogenital
anomalies, and primarily VUR during the setting-up of the examination and treatment strategy.

Renal parenchymal lesions can be verified in a high proportion of children with UTIs, but in even higher proportions among those with severe VUR and recurrent pyelonephritides. These observations verify the risk-enhancing character of VUR (particularly severe VUR) and recurrent UTIs.

5.2. The genetic examinations on the 103 children with UTIs demonstrated that the HSP A1B (1267) GG genotype and the carriage of the HSPA1B (1267) G allele led to a susceptibility to uropathogenetic infections and predispose to renal parenchymal lesions. In the event of this genetic constellation, the production of HSP72 (the function of which is to repair the protein and the tissue) will be impaired, and thus the renal regeneration may be damaged, which may finally result in fibrotic lesions of the kidney. Accordingly, more aggressive examinations and treatment principles can be recommended in order to prevent irreversible renal damage in UTI patients with
the \textit{HSPA1B} (1267) GG genotype and the \textit{HSPA1B} (1267) G allele.

Carriage of the \textit{TLR4} (896) AG mutation and the \textit{TLR4} (896) G allele is associated with an enhanced risk of UTIs, and is primarily characteristic of UTI patients without VUR. Our data show that the carriers of this genetic variation are at high risk of urinary tract inflammations, confirming the crucial role of \textit{TLR4} in Gram-negative bacterial infections.

5.3. The \textit{ATG} M(235)T and \textit{ATI} A(1166)C polymorphisms of the 3 examined renin-angiotensin genes were not associated with either VUR or renal parenchymal lesions. At the same time, the ID and DD genotypes in the I/D polymorphism of the \textit{ACE} gene were characteristic of the high-grade VUR patients and those with renal parenchymal lesions. Hence, carriage of the D allele in VUR can be considered a genetic risk factor for renal scar development.
6. Conclusions

Our clinical observations have provided Hungarian data confirming that the high-grade VUR and recurrent UTIs significantly increase the risk of renal scar development in children. The predisposing genetic factors play a very important part in the development of pediatric UTIs and renal parenchymal lesions. TLR4 has a significant role in the recognition of the pathogens and the early immune response, HSP72 with its repairing functions and the ACE gene polymorphisms with their part in the renal fibrotic processes are probably also such predisposing factors of UTI and the complex processes of later renal scarring due to the UTIs. The knowledge of these factors may be of great importance in the future as it can facilitate a personally developed examination, treatment and care strategy in the therapy of children with UTIs.
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THE AUTHOR’S PUBLICATIONS IN THIS SUBJECT


THE AUTHOR’S OTHER PUBLICATIONS