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Influence of Plasminogen Activator Inhibitor -1 Gene Polymorphism on Renal Scarring After First Febrile Urinary Tract Infection in Infants

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ABSTRACT

Background: The pathogenesis of renal scarring (RS) after first febrile urinary tract infection (UTI) in children is multifactorial. In addition to well-known risk factors, a role for genetic predisposition has been suggested. **Aims**: To determine whether deoxyribonucleic acid (DNA) polymorphisms at the plasminogen activator inhibitor -1 (PAI-1) gene were associated with evolution to RS following a febrile UTI in infants. **Materials and Methods**: Our research included 100 infants, 84 girls and 16 boys, ages up to 1 year with a first febrile UTI, increased inflammatory parameters and positive urine culture treated at the Pediatric Clinic II of the University Clinical Center Sarajevo (UCCS). The diagnostic was based on the imaging studies: ultrasonography, voiding cystourethrography (VCUG) and initial and control static renal scintigraphy (DMSA renal scan), to assess the renal parenchymal damage (RPD). The polymorphisms of the PAI-1 were determined based on polymerase chain reaction technique. The distribution of PAI-1 genotypes and the allele frequencies were compared between different groups of patients with febrile UTI. **Results**: Results presented that 66 infants had acute pyelonephritis (APN) and 22 had vesicoureteral reflux (VUR). On initial DMSA renal scan examination, we detected no RPD in any patient. After 6 months, the repeat DMSA renal scan revealed the presence of RPD in 18 (27%) out of 66 infants with APN. Distribution of PAI-1 genotypes was not different between various groups of patients with febrile UTI. **Conclusions**: The results of our study have not shown that individual genetic variation in PAI-1 is an independent variable that predispose same of children for RS after first febrile UTI. Maybe that yet unknown gene polymorphisms together with geographical and /or socio-economic differences can influence on the development of RS.

Keywords: urinary tract infection, plasminogen activator inhibitor -1, renal scarring.

1. INTRODUCTION

Acute pyelonephritis in children has been shown to be associated with RS and has been associated with development of hypertension and renal functional impairment that is responsible for 10%-24% of all children entering end-stage failure in Europe (1). The most important risk factors for renal scars are male gender, younger age of the child, P-fimbriatus Escherichia coli, VUR, recurrent UTIs, genetic predisposition and delayed antibiotic treatment (2, 3). However, the pathogenesis of RS is still controversial. Numerous clinical trial data show that the cellular, genetic and inflammatory mechanisms play a major role in the progression of renal disease (4, 5, 6). At the same time, they are involved in bacterial cleaning and tissue damage, which leads to the development of fibrotic kidney changes.

Medical scientific thought failed to resolve puzzle why acute febrile inflammation of the urinary system causes RPD only in some children, while others do not experience those consequences. It is certain that the individual characteristics and properties of the gene depends to some extent whether children after febrile UTI, or not is the subject to the creation of renal parenchymal scars (7).

We have conducted a set of examination to prove the associations between genetic polymorphisms of PAI-1 gene and RS in infants with first febrile UTI. Hemostasis is a complex defense mechanism. It creates number of factors, which, on the one hand, stimulate coagulation and, on the other hand, fibrinolysis. The most important role of fibrinolysis is

the plasmin formation from the circulating plasminogen at the site of the blood clot. Two plasminogen activators catalyze plasmin formation: urokinase plasminogen activator (uPA) and tissue plasminogen activator (tPA). In blood plasma, also exist factors that inhibit the effect of plasmin activators, such as PAI. There are two subtypes of PAI: PAI-1 and PAI-2. For PAI-1 is typical to create a covalent complex with tPA and uPA. Sources of plasma PAI-1 are still unknown. For now, it is known that hepatocytes, endothelial cells, adipocytes and megakaryocytes synthesize and secrete PAI-1 in the blood circulation (8, 9). One of the several factors that affect the plasma levels of PAI-1 in human is PAI-1 genotype. High levels of the PAI-1 in plasma correlate with polymorphic variations in the guanine base number (4 G opposite 5G) in the promoter at position -675. Polymorphism in the promoter region of PAI-1 gene is associated with elevated levels of PAI-1. Individuals with the 4G allele have higher plasma levels of PAI-1 than those with 5G allele. PAI-1, as a multifunctional glycoprotein with impressive properties in the mobilization orchestrate different cell types in the alleviation of inflammation. PAI-1 affects the adhesion and migration of cells, especially macrophages, acts on the cell proliferation, stimulates angiogenesis and mitogenesis, and inhibits apoptosis. Thus, PAI-1 plays a significant role in inflammatory and malignant processes. It is fact that PAI-1 promotes renal fibrosis, but how it does is not understood completely.

2. MATERIALS AND METHODS

A total of 100 infants with first febrile UTI (84 girls and 16 boys), ages up to 1 year were included in the study. The study was performed at the UCCS, Pediatric Clinic II. Children with neurogenic bladder, lower urinary tract obstruction, renal hypoplasia, ectopic kidney and recurrent UTI were excluded from the study group. We have defined febrile UTI by the clinical and laboratory findings including fever, followed by non-specific clinical symptoms typical of this age in terms of the UTI, increased erythrocyte sedimentation rate, positive C-reactive protein, pathological findings of urinalysis and positive urine culture. Laboratory tests were performed using standard laboratory techniques at the UCCS. Ultrasonography and initial 99m-Tc-labeled dimercapto-succinic acid (DMSA) renal scanning were performed no later than 10 days after admission to hospital. Scintigraphical images were obtained 3 hours after the injection of the weight-scaled dose of 99m Tc-DMSA. Focal or diffuse areas of decreased uptake in the first scan, without evidence of cortical loss, were considered indicative of APN. VCUG was performed within the first 2 weeks of acute UTI (10, 11). The International System of Radiological Grading of Vesicoureteral Reflux (12) graded detected VUR severity on VCUG from I to V. All children with positive DMSA renal scanning results for APN were scheduled for repetition of the study between 6-12 months to detect any RS at the site of the original APN. Renal scarring was defined as decreased uptake with distortion on the contours or cortical thinning with loss of parenchymal volume. The applied protocol for the interpretation of the findings on the control DMSA renal scan is taken of Slovenian researchers (13).

PAI-1 genotype analyses

The gene polymorphism of PAI-1 gene was determined based on amplification by the PCR as it is done at the Institute of Biotechnology and the Genetic Engineering on the Faculty for Natural Sciences and Mathematics in Sarajevo. Genomic DNA was extracted from the ethylene diamine tetra acetic acid (EDTA) (14). The PAI-1 gene and alleles were identified based on PCR amplification. PCR was performed on extracted DNA by standard method, using primers. The whole process is automatized. PAI-1 genotypes were determined as 4G/4G, 4G/5G and 5G/5G. The distribution of PAI-1 genotypes and the allele frequencies were compared between different groups of patients with first febrile UTI.

The Ethics Committee of UCCS approved the study.

Statistical analysis

Statistical analyses were performed using *IBM Statistics SPSS v19.0* with package of tools for medical research and *MedCalc v10*. Fisher's exact test was used to compare the patient's characteristics related to gender. Hardy-Weinberg equilibrium was used to analyze genetic influence of the observed gene. Allele genotype frequencies among different groups of patients were compared using two proportion and Chi-squared tests. Student's *t* -test was used for comparison of quantitative data. A *P* -value of less than 0.05 was set as significant.

3. RESULTS

Our study treated 100 infants (16 boys and 84 girls) aged 1 month to 12 months with a proven first febrile UTI. Inflammation of the renal parenchyma with first febrile UTI was demonstrated in 66 (66%) of 100 children. Out of 66 patients with APN 22 (33%) had VUR: six (27%) were boys and 16 (73%) were girls, which made a statistically significant difference in the prevalence of VUR by gender of children ($\chi 2 = 5.677$; $p = 0.0034$). Out of 16 boys with UTI, four (25%) had renal scar, compared to 14 (16.6%) girls out of 84 female infants. There was a statistically significant difference in the prevalence of RS in relation to the gender ($\chi 2 = 4.254$; $p = 0.042$). In infants with renal scars was discovered VUR in only nine (50%) children with equal prevalence in both genders. VUR level III was predominant, in four (44.45%) patients. Patients' characteristics are presented in Table 1.

The expected distribution of genotype in our study population was not in Hardy-Weinberg equilibrium (HWE) considering that functional mutations (polymorphisms) are exposed to selective pressure (χ^2=18-627, p=0.000016). The nineteen infants had typical genotype PAI-1 5G5G. Risk homozygous genotype of PAI-1 4G4G had 10 patients. The largest moderate heterozygous genotype of PAI-1 4G5G had 71 infants. The differences between three genotypes were not found to be significant (p>0.05). The distribution of PAI-1 genotypes did not differ between the patients with APN and patients with lower urinary tract infections (Table 2).

The distribution of the 4G4G, 4G5G and 5G5G genotypes of the PAI-1 gene polymorphism were similar

	Total UTI patients (n=100)	Patients with AP (n=66)	Patients with lower UTI (n=34)	Patients with VUR (n=22)	Patients with renal scar (n=18)	Patients without renal scar (n=82
Male/female ratio	16/84	12/54	4/30	6/16	4/14	12/70

Table 1. Patients' characteristics

	PAI-1 genotypes, n (%)			Allele frequency, n (%)	
Groups	4G4G	4G5G	5G5G	4G	5G
Patients with APN (n=66)	9(13.64)	44(66.66)	13(19.70)	62 (47)	70 (53)
Patients with lower UTI (n=34)	1(2.94)	27(79.41)	6(17.65)	29 (42.6)	39 (57.4)
P	0.181	0.272	0.983	0.666	

Table 2. Comparison of genotype and allelic frequencies of PAI-1 gene between patients with APN and lower urinary tract infection. Chi-squared=7.55; P=0.469; P>0.05

between the patients with VUR and renal scar-positive findings and groups of patients with VUR and without renal scar (P >0.05). The 4G and 5G allele frequencies of patients between these two groups were also similar (P>0.05) (Table 3).

	PAI-1 genotypes, n (%)			Allele frequency, n (%)	
Groups	4G4G	4G5G	5G5G	4G	5G
Scaring +VUR (n=9)	1(11.11)	5(55.56)	3(33.33)	7 (38.9)	11 (61.1)
Non-scaring + VUR (n=13)	1(7.69)	10(76.93)	2(15.38)	12 (46.15)	14 (53.85)
P	0.631	0.554	0.638	0.866	

Table 3. Comparison of genotype and allelic frequencies of PAI-1gene between patients with VUR and renal scarring and VUR without renal scarring on dimercaptocuccinic acid study. Chi-squared=0.257; P=0.389; P>0.05

Comparison of patients with renal scarring and those without scarring showed evidence of higher 4G5G and lower 4G4G and 5G5G genotype frequency in both groups of patients without statistical significance (P>0.05). The higher 5G allele frequency and lower 4G allele frequency of the scar group also did not reach to a significant level compared with the non-scarred group (P > 0.05) (Table 4).

	PAI-1 genotypes, n (%)			Allele frequency, n (%)	
Groups	4G4G	4G5G	5G5G	4G	5G
Scaring (n=18)	2(11.11)	12(66.67)	4(22.22)	16 (44.4)	20 (55.6)
Non-scaring (n=82)	8(9.76)	59(71.95)	15(18.29)	75 (45.73)	89 (54.27)
P	0.795	0.872	0.886	0.965	

Table 4. Comparison of genotype and allelic frequencies of PAI-1 gene between infants with and without RS on dimercaptocuccinic acid study. Chi-squared=2.205; P=0.444; P>0.05

4. DISCUSSION

Urinary tract infections are among the most prevalent bacterial infections in children. Among febrile infants, the prevalence of UTI is approximately 7% (15). Some prospective studies using DMSA scintigraphy have shown that 15%-40% of children will have renal scarring after a febrile UTI (16, 17). In our study 18 (18%) infants had renal scarring after a febrile ITU (Table 1).

Studies of human genetics and genetic technologies have recently went through dynamic and dramatic development and were imposed as a powerful tool in the genesis of many diseases, even those that previously have been linked to the genetic basis. So is the case with the UTI. The development of RPD is determined by genetic and non-genetic factors, of which many are not scientifically investigated. In the human genome are hidden secrets of successful diagnosis and treatment of disease. What seems impossible today tomorrow will become a reality. Consulting the literature about UTI and RS in children were found false positive results in genetic association studies due to great dependence on the thoroughness of the studies.

Number of open questions and dilemmas related to the relationship between APN, VUR and RS exist. Although the kidney normally does not produce PAI-1, PAI-1 synthesis by both resident and intrarenal inflammatory cells occurs in several acute and chronic kidney diseases. One of the factors that affect the PAI-1plasma levels in humans is PAI-1 genotype. Studies have shown that people with homozygous 4G/4G have a 25% higher concentration of PAI-1 in plasma than those of genotype 5G/5G. According to previous research, 4G allele is a risk factor for deep vein thrombosis and myocardial infarction in younger person and risk factor of vascular complications in patients with diabetes mellitus (18, 19). Animal experiments show that the PAI-1 is powerful fibrotic molecule. It is not clear how PAI-1 promotes renal fibrosis. It seems that PAI-1 directly modulates cell functions leading to a vicious cycle of inflammation, fibroblasts activation and scar tissue accumulation. PAI-1 is present in the most aggressive kidney disease as thrombotic microangiopathy, crescentic glomerulonephritis, proliferative glomerulonephritis, diabetic nephropathy, lupus nephritis, chronic allograft nephropathy (20) and chronic progressive renal disease (21). RPD occurs only in part of the children's population because of serious inflammation. It is certain that PAI-1 genotype may be a predictor of progression of chronic renal disease (21, 22, 23). Study of Roelofs and colleagues in experimental animals (mice) had shown that PAI-1 is critically involved in host defense against Escherichia coli, modeled neutrophils influx and thus stimulates APN (24). Eddy and Fogo believe that PAI-1 has a specific physiological role in the body's defense of APN (21). However, Danish researchers Kristine Jessen et colleagues did not find association between polymorphisms of different cytokines including PAI-1 and outcome in patients with gram-negative sepsis, caused by Escherichia coli and UTI in elderly patients (25).

PAI-1 plays the role in other numerous pathological and physiological processes. One of the aims of the present study was to investigate the effect of polymorphisms of PAI-1 gene on clinical expressions on the first febrile

UTI in 100 infants, the occurrence of RPD, VUR and their common interaction. During inflammation, PAI-1 is released by various cytokines. Some of the cytokines have a pro-inflammatory while others have anti-inflammatory effect and the relationship between these cytokines are a significant factor in the development of kidney damage in acute UTI. Individual differences in cytokine levels are the result of their genetic polymorphism, which affects the change in the inflammatory response.

The study of polymorphisms in genes PAI-1 detected three polymorphisms. In our study the largest was moderate heterozygous genotype of PAI-1 4G5G that was present in 71 infant. It can be expected that in the particular circumstances, these healthy patients with moderate heterozygous genotype mutate into risk homozygous genotype. As could be seen genetic constitution of 71 infant's is vulnerable. Applying different statistical tests did not reveal any significant difference in the distribution of specific single nucleotide polymorphisms of PAI-1 gene between patients with inflammation of the lower urinary tract and patients with APN, between infants with proven existence of VUR and those with renal scars and between infants with renal scars and other without renal scar ($p > 0.05$) (Table 2, 3, 4). Remains a mystery why acute inflammation results in development of renal scars in some children, while in others full resolution without scarring occurs. In children younger than a year with the first febrile UTI evaluated in our study, the independent predictors for RS are proven to be age and gender of the infant, high non-specific inflammatory parameters, gram-negative bacteria, proven APN on static renal scintigraphy and VUR. The obtained results suggest that the UTI is multifactorial disease.

5. CONCLUSIONS

The topic of RS in the first febrile UTI in infants has not been the subject of research in Bosnia and Herzegovina. We conclude that genetic polymorphisms of PAI-1 components are dependent prognostic indicators of RS after first febrile UTI in infants. However, it remains a mystery what factors of the immune defense mechanisms impair the efficiency of the response to the invasion of pathogenic microorganisms in the urinary system. The results lead us to believe that probably other genetic polymorphisms exist that may manifest effect through interaction with still unexamined factors associated with geographic and demographic diversity among the human populations. It is obvious, however, that they still needed more clearly designed and wider and deeper clinical studies to determine their predictive value in children with urinary infections.

- Declaration of interest: All authors declare no conflicts of interest.

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