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Research Article

Polymorphisms of the TNF-α, ACE, PAI-1 and MTHFR Genes, and Renal Scarring in Infants with First Febrile Urinary Tract Infection

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Abstract

Background: There is no doubt that genetic predisposition to Febrile Urinary Tract Infection (FUTI), recurrent UTI and Renal Scarring (RS) exists in addition to well-known risk factors.

Aims: To determine whether Deoxyribonucleic Acid (DNA) polymorphisms in the Tumor Necrosis Factor alpha (TNF-α), Angiotensin-Converting Enzyme (ACE), Plasminogen Activator Inhibitor – 1 (PAI-1), and Methylenetetrahydrofolate Reductase (MTHFR) gene were associated with evolution to renal scars following a first FUTI in infants.

Methods: The study included 100 children (84 girls, 16 boys) with first FUTI at age up to 1 year. The study was performed at the Clinical Center University of Sarajevo (CCUS), Pediatric Clinic. The diagnosis was based on the clinical, laboratory findings and imaging procedures: ultrasonography, voiding cystourethrography (VCUG) and, initial and control static renal scintigraphy (DMSA renal scan). The gene polymorphisms of TNF-α, ACE, PAI-1, and MTHFR were determined based on amplification by the Polymerase Chain Reaction (PCR). The distribution of these four genotypes and the allele frequencies were compared between different groups of patients with first FUTI.

Results: Results showed that 66 infants had Acute Pyelonephritis (APN), 22 had Vesicoureteral Reflux (VUR) and 18 had RS. We did not find significant difference in the individual representation of specific polymorphism of the examined genes between patients with APN, VUR and RS. But, when we used the regression analysis we were found that all investigated genes together have influence in 67% of cases in the prediction of APN, VUR and RS in children with first FUTI.

Conclusions: Although our study supports the belief that genetic variations in TNF-α, ACE, PAI-1 and MTHFR genes together may predispose renal scar, more extensive and comprehensive research and study of the role of polymorphism of different genes in RS after first FUTI is necessary.

Keywords: Gene polymorphism; Renal scarring; Urinary tract infection

Introduction

Urinary Tract Infection (UTI) is a common health problem in children. It is usually divided into upper urinary tract infection or Acute Pyelonephritis (APN) and lower UTI or cystitis. Acute pyelonephritis is characterized by bacterial infection of the renal parenchyma. APN can lead to permanent Renal Scar (RS) formation, which has been associated with long-term kidney problems in some children [1,2]. After the assessment of Febrile Urinary Tract Inceftion (FUTI), approximately 30 to 50% of children who underwent voiding cystourethrograms have Vesicoureteral Reflux (VUR) [2]. The association between acute pyelonephritis, VUR and renal scar is well-known [3]. Reflux nephropathy which is mainly associated with high-grade reflux causes renal scars and can result in end-stage renal disease in 3% to 25% of cases [4]. Approximately 10% of children with a RS will develop hypertension in adolescence or early adulthood [5]. Also, renal scarring after APN in children may lead to preeclampsia during pregnancy and proteinuria [6]. The most important risk factors for renal scars are male gender, younger child's age, P-fimbriatus Escherichia coli, VUR, recurrent UTIs, genetic predisposition and delayed antibiotic treatment [7,8]. 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 [9-12]. At the same time, they are involved in bacterial cleaning and tissue damage, which leads to the development of fibrotic kidney changes. We should not ignore the fact that the nephropathogenic bacteria with its genetic potential aimed at its own survival, lead a tough fight against the host immune system. Host inflammatory response leads to the affected site an army of immune agents to destroy pathogen which causes the disease. The innate immune system response provides front-line defense against microbial insult and leads to activation of the adaptive immune system [13,14]. At low concentrations, endotoxin stimulates the immune response of a body that has a protective role. On the contrary, a high concentration of endotoxin leads to excessive activation of the immune system and responses that have harmful effects to the body, the so-called „cytokine storm" or the cytokine release syndrome [15]. The relationship between proinflammatory and anti-inflammatory cytokines is a significant factor in the development of kidney damage in acute urinary tract infection.

The progress of science and technology has contributed to the development of molecular medicine, which causes the disease to be investigated at the level of genome. Individual differences in cytokine levels, cytokine receptors, chemokines, cell adhesion molecules, and phagocytes are the result of their genetic polymorphism, which affects the inflammatory response, the progression of renal disease and different consequences of UTI in children. Genetic studies have shown that polymorphisms of nucleotides (Single Nucleotide Polymorphismus -SNPs) exist on all genes. Polymorphism is the replacement of nucleotides in the Dex+oxyribonucleic Acid (DNA) molecule. This is a common occurrence in the human population [16]. Of major importance are the SNPs that affect gene expression as a factor in changing the structure of the protein. To date, 84.7 million SNP polymorphisms have been discovered in the human genome [17]. It is important to point out that any such change will not lead to functional disorders in the body [18]. Cytokine gene polymorphisms have influence on the levels of cytokine gene expression and cytokine levels in body fluids and tissues [19]. There is no doubt that a genetic predisposition to UTI, recurrent UTI and renal scarring exists. Also, the genetic complex can explain large variations in the individual response to urinary tract infection. Many genes have been studied as a potential candidate gene for prediction of renal parenchymal damage, but not one of them has strong scientific evidence for direct link for occurrence of RS. Genes that have been shown to predispose patients to recurrent UTI and renal scarring include Angiotensin-Converting Enzyme Insertion/Deletion (ACE I/D) gene, Interleukin (IL)-8, receptor CXCR1 and CXCR2 genes, IL-10-1082 A/G gene, heat shock protein 72 (HSPA1B) gene, Transforming Growth Factor (TGF)-β1 gene, Toll-Like Receptor (TLR) pathway genes, and Vascular Endothelial Growth Factor (VEGF) gene [20-22].

We have conducted a set of examination to prove the associations between genetic polymorphisms of the Tumor Necrosis Factor Alpha (TNF-α), Angiotensin-Converting Enzyme (ACE), Plasminogen Activator Inhibitor -1 (PAI-1), and Methylenetetrahydrofolate Reductase (MTHFR) gene and RS in infants with first FUTI. One of the key cytokines in the inflammatory process is the tumor necrosis factor alpha, which is mainly produced by macrophages, T lymphocytes, and natural killer cells [23]. TNF-α is a multifunctional cytokine which has different roles in the human body: it regulates immune response, inflammation and apoptosis, plays a significant role in the pathogenesis of infectious and autoimmune diseases, neoplasms and dysplasia, stimulates the release of other proinflammatory cytokines, chemokines, and growth factors (granulocyte-macrophage colony-stimulating factor (GM-CSF), macrophage colony-stimulating factor (M-CSF) and monocyte chemoattractant protein-1 (MCP-1) [24,25]. Gene for TNF-α is located on the 6th chromosome (6p21). It has more polymorphisms (715). The most frequently investigated polymorphism is at position -308, where guanine was replaced by adenine [26].

The renin-angiotensin system, in addition to hemodynamic effects, has a significant contribution to inflammation and tissue fibrosis. Angiotensin II has a positive effect on the: blood pressure, volume of liquid, concentration of sodium, regulation of intraglomerular pressure, achieving interaction between the Renin-

Angiotensin System (RAS) and the transforming growth factor beta (TGF-β), secretion of renin and aldosterone, kallikrein-kinin stimulation system, renal prostaglandin synthesis, release of macrophages and T-cells, stimulation of thirst, and secretion of sympathomimetic substances (adrenaline, noradrenaline) in the adrenal gland [27-29], stimulation or permitting the proliferation of myofibroblasts and mesangial cells, synthesis of extracellular matrix ,stimulation of TGF-β expression and stimulation of the plasminogen activator inhibitor. On that path it encourages prosclerotic activity and contributes to the pathogenesis of renal disease. Interaction between the RAS system and TGF-β attempts to explain the special sensitivity of the kidney to fibrosis [30,31]. Genes that have been shown to predispose patients to recurrent UTI and renal scarring include *Angiotensin-Converting Enzyme Insertion/Deletion* (*ACE I/D*) gene which is located within the chromosome 17 (q23 region) [22].

Hemostasis is a complex defense mechanism that protects the integrity of the vascular system of injured and damaged tissue. It involves a number of factors, which, on the one hand, stimulate coagulation and, on the other hand, fibrinolysis [32]. The most important role of fibrinolysis is the formation of plasmin from the circulating plasminogen at the blood clot site. Two plasminogen activators catalyze plasmin formation: urokinase Plasminogen Activator (uPA) and tissue Plasminogen Activator (tPA). In blood plasma there are also factors that inhibit the effect of plasmin activators. Such as a plasminogen activator inhibitor (PAI) [33]. There are two subtypes of PAI: PAI-1 and PAI-2. For PAI-1 is typical to form a covalent complex with tPA and uPA. Plasma PAI-1 sources are still unknown. Under physiological conditions, PAI-1 is released into the circulation and the extracellular space by only a few cells: liver cells, smooth muscle cells, adipocytes, and platelets are the major sources of PAI-1. Under pathological conditions, however, several other tissues secrete quite large amounts of PAI-1: tumor cells, endothelial cells in response to inflammatory cytokines, and other inflammation-activated cells [34]. The most significant factor that affect the plasma levels of PAI-1 in human is PAI-1 genotype. The human PAI-1 gene is located on the chromosome 7 (q21.2-22). High levels of the PAI-1 in plasma correlate with polymorphic variations in the guanine base number (4G opposite 5G) the promoter at position -675. Individuals with the 4G allele have higher plasma levels of PAI-1 than those with 5G allele. It is assumed that this polymorphism is associated with a higher incidence of arterial thrombosis [35].

PAI-1, as a multifunctional glycoprotein with impressive properties in the mobilization of 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 [36-39].

In humans, the folate and methionine cycles both generate products essential to cellular survival. These two cycles intersect at the enzyme 5, 10-Methylenetetrahydrofolate Reductase (MTHFR) which belongs to the group of major regulatory enzymes in the metabolism of homocysteine (Hcy) and is one of the most important enzymes in the physiology of a human organism [40]. For the normal course of homocysteine degradation, folic acid and B12 are necessary as cofactors. Homocysteine is formed by demethylation of methionine, which comes into the body through food [41]. Gene for MTHFR is located on the chromosome 1 (p36.3). Severe MTHFR deficiency (MIM #607093) is inherited in an autosomal recessive manner and is the most common inborn error of folate deficiency with ~200 patients known [42]. The methylenetetrahydrofolate reductase gene polymorphism is associated with lighter and severe hyperhomocysteinemia. Methylenetetrahydrofolate reductase (MTHFR) is an enzyme that breaks down the amino acid homocysteine. If the MTHFR gene that codes for this enzyme mutates, it may contribute to a variety of health conditions ranging from depression to some cancers.

To date, 40 mutations of the MTHFR gene have been identified in people with homocystinuria. The greatest clinical relevance is the mutations C677T and A1298C [43]. Mutation of the gene for MTHFR C677T, including lack of folic acid and vitamin B12 leads to moderate hyperhomocysteinemia. This polymorphism involves the substitution of cytosine with thymine at position 677, resulting in an amino acid change from alanine to valine in the enzyme [44]. This leads to thermolability and reduced and prevented enzymatic activity which the conversion of Hcy to methionine results in a disorder of growth, differentiation and cell function because of the impossibility for the synthesis of important biological substances, since the body uses methionine to make proteins and other important compounds. Normal type (CC) MTHFR gene provides instructions for the production of methylentetrahydrofolate reductase enzyme [45]. Researchers investigating the ethnic and geographical distribution of the C677T *MTHFR* gene polymorphism in newborns across 16 regions of Europe, Asia, Australia, the Middle East, and the Americas reported that the TT genotype was more frequently observed in China, Mexico, and Southern Italy, with a particularly low frequency observed in newborns of African origin [46]. It is certain that Hcy can cause pathological changes in the blood vessels, so, also, in the kidney, but the mechanisms of that action have not been fully elucidated. The possible pathogenic mechanism by which Hcy causes damage to blood vessels are: damage to the endothelium of the blood vessels, encouragement and participation in oxidative metabolism of Low Density Lipoprotein (LDL), increased platelets aggregation mediated by thromboxane, inhibition of thrombomodulin expression on cell surface and activation of protein C, improved binding of lipoprotein to fibrin, amplify smooth muscle cell proliferation and collagen accumulation [47-50]. Studies has suggested a con-

nection between *MTHFR* gene polymorphisms, especially C677T and A1298C (rs 1801131), and several pathological conditions, including kidney disease, cardiovascular disease, neural tube defects, cancer, inflammatory bowel disease, type 2 diabetes, Down syndrome, headache, epilepsy, Alzheimer's disease, schizophrenia, liver diseases, and numerous chronic diseases and disorders of regulation of embryonic development [51-58].

Materials and Methods

Patients and study design

This prospective and descriptive study was based on data from patient medical records and included a total of 100 children (84 girls, 16 boys) with first FUTI, at age up to 1 year. The study was performed at the Clinical Center University of Sarajevo (CCUS), Pediatric Clinic. Children with congenital and acquired urological abnormalities, recurrent UTIs, renal failure, and afebrile UTIs were excluded from the study. We have defined FUTI by the clinical and laboratory findings including fever, followed by non-specific clinical symptoms typical for this age in terms of the UTI, increased erythrocyte sedimentation rate, positive C-reactive protein, pathological findings of urinalysis and positive urine culture.

Methods

Laboratory tests were performed using standard laboratory techniques at the CCUS. Ultrasonography (USG) of the urinary system was performed within the first 3 days of admission by expert pediatric radiologists using an ultrasound machine General Electric Volson 730 PRO with a convex probe of 5.0 MHZ and a linear multifrequency probe with a central frequency of 7.5 MHZ at the CCUS. USG findings of APN included altered parenchymal echogenicity, pelvicalycial dilatation or fullness, undifferentiated corticomedullary junction, and renal enlargement. APN was defined as a UTI associated with a positive static renal scintigraphy with 99m-Tc-labeled dimercapto-succinic acid (DMSA renal scan). The DMSA renal scintigraphy was performed during the first week from admission by injection of 2 MBq/kg Tc- 99m DMSA. To allow the cortical uptake, we waited 3 hours before imaging [59]. An abnormal acute DMSA scan suggesting APN was defined as the presence of focal or diffuse areas of decreased cortical uptake, with preservation of the renal contour [60]. VUR was identified by conventional voiding cystourethrography (VCUG) within the first 2 weeks of acute FUTI [61]. The VUR was graded from I to V according to the International Reflux Study in children [62]. All children with positive initial DMSA renal scanning results for APN were scheduled for repeated DMSA after 6-12 months in order 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 [63]. We evaluated the degree of renal scarring as: mild, moderate and severe.

TNF- α, ACE, PAI-1 and MTHFR Genotype Analyses

The gene polymorphisms of TNF- α, ACE, PAI-1, and MTHFR and alleles were determined based on amplification by the polymerase chain reaction (PCR) and performed at the University of Sarajevo - Institute for Genetic Engineering and Biotechnology, Sarajevo. Genomic DNA was isolated from the leukocytes of peripheral blood samples. The PCR was performed on extracted DNA by standard method, using primers. The whole process is automatized. ACE genotypes were determined as II, ID, DD, TNF-α genotypes as GG, AG, AA, PAI-1 genotypes as 4G4G, 4G5G, 5G5G and MTHFR as CC, CT, and TT. The distribution of TNF- α, ACE, PAI-1, and MTHFR genotypes and the allele frequencies were compared between different groups of patients with first FUTI.

The Ethics Committee of CCUS approved the study. Written informed consent was obtained from all the parents before inclusion to the study.

Statistical Analysis

Statistical analysis was performed using statistical software for medical studies 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. Statistical methods of regression analysis were used to examine the relationship between four variables of interest.

Results

Figure 1. Control static scintigraphy of the kidney with Tc-99m DMSA in a girl aged 10.5 months with the first FITU: the right kidney is smaller than the left with inhomogeneous distribution of radiopharmaceuticals. Along the lateral edge of the upper third of the right kidney, there is a noticeable weakening of the activity fixation, with an irregular cortical line. This corresponds to the presence of a mild scarring change on the renal parenchyma

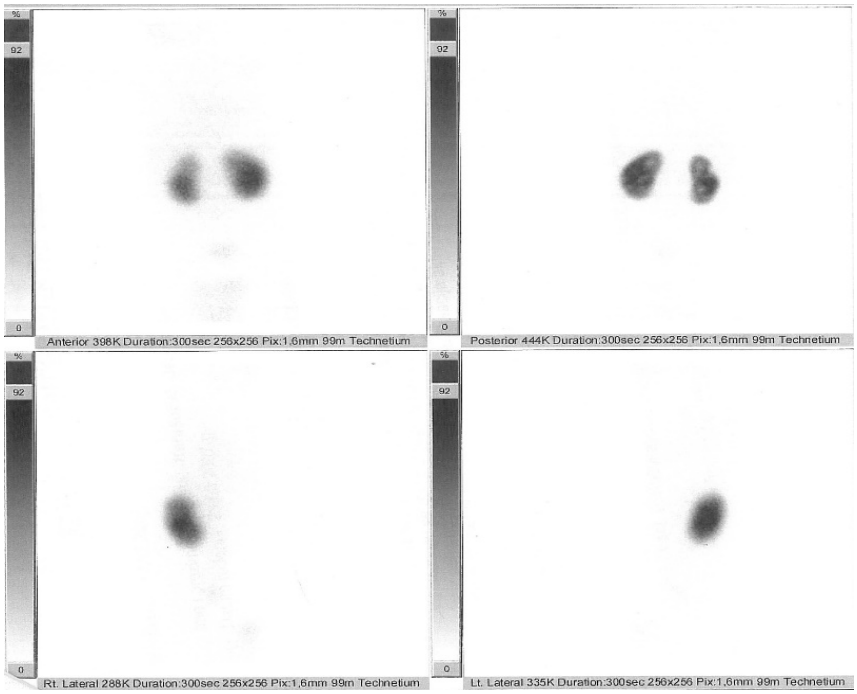

Table 1: Patients' characteristics.

	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	Dec-54	Apr-30	Jun-16	Apr-14	Dec-70

Table 2: Numerical representation of genetic polymorphisms of the tested TNF-α, ACE, PAI-1 and MTHFR genes

	Typical genotype	Risk homozygous genotype	Moderate risk heterozygous genotype	Total
TNF-α	7	5	88	100
ACE	21	26	53	100
PAI-1	19	10	71	100
MTHFR	19	4	77	100
Total	66/16.5%	45/11.25%	289/72.25%	400

Table 3: Common influence of all four TNF-α, ACE, PAI-1 and MTHFR genes tested as predictors of acute pyelonephritis in infants

with the first febrile urinary tract infection. (regression analysis).

Logiste regression			
Dependent Y	Therapy group I level (ITU -0, AP-1) N-100		
Method	Enter		
Sample size	100		
Cases with Y=0	34 (34.00%)		
Cases with Y=1	66 (66.00%)		
The overall model			
OR for a null model	28,207		
OR for the actual model	1,24,831		
Chi-square	13,376		
SS	4		
P	P = 0.0497		
Coefficients and standard errors			
Variable	Coefficient	Standard error	P
ACE	-0.4131	0.5132	0.4209
MTHFR	0.9058	0.6558	0.1672
PAI-1	0.01012	0.5580	0.9855
TNF-α	-0.8188	0.8712	0.3473
Constanta	0.6613		
OR and 95% confidence interval			
Variable	OR	95% CI	
ACE	0.6616	0.2420 – 1.8089	
MTHFR	24.739	0.6842 – 8.9455	
PAI-1	10.102	0.3384 – 3.0156	
TNF-α	0.4410	0.0800 – 2.4319	
Classification table (limit value p = 0.5)			
Actual result	Prediction		The percentage of true
	0	1	
Y = 0	2	32	5.88 %
Y = 1	1	65	98.48 %
The percentage of accurately classified cases			67.00 %
ROC curve analysis			
The area under the ROC curve (AUG)	0.611		
Standard error	0.058		
95% confidence interval	0.508 – 0.706		

Table 4: Common influence of all four TNF-α, ACE, PAI-1 and MTHFR genes tested as predictors of renal scarring in infants with the

first febrile urinary tract infection. (regression analysis).

Logiste regression			
Dependent Y	Therapy group I i II levels: Scar: 0-no, 1-yes N=100		
Method	Enter		
Sample size	100		
Cases with Y=0	82 (82.00%)		
Cases with Y=1	18 (18.00%)		
The overall model			
OR for a null model	62,279		
OR for the actual model	92,526		
Chi-square	11,753		
SS	4		
P	P = 0.0374		
Coefficients and standard errors			
Variable	Coefficient	Standard error	P
ACE	-0.8588	0.7989	0.2824
MTHFR	0.2165	0.6672	0.7456
PAI-1	0.1781	0.6415	0.7813
TNF-α	-0.4397	11.634	0.7055
Constanta	-14.260		
OR and 95% confidence interval			
Variable	OR	95% CI	
ACE	0.4237	0.0885 – 2.0281	
MTHFR	12.417	0.3358 – 4.5917	
PAI-1	11.949	0.3398 – 4.2019	
TNF-α	0.6442	0.0659 – 6.2999	
Classification table (limit value p = 0.5)			
Actual result	Prediction		The percentage of true
	0	1	
Y = 0	82	0	100.00 %
Y = 1	18	0	0.00 %
The percentage of accurately classified cases			82.00 %
ROC curve analysis			
The area under the ROC curve (AUG)	0.543		
Standard error	0.0765		
95% confidence interval	0.440 – 0.643		

Table 5: Common influence of all four TNF-α, ACE, PAI-1 and MTHFR genes tested as predictors of renal scarring in infants with acute

pyelonephritis (regression analysis).

Logiste regression			
Dependent Y	Therapy group I level: AP-1 Therapy group II level: Scar: no -0, yes-1 N=66		
Select			
Method	Enter		
Sample size	66		
Cases with Y=0	48 (72.73%)		
Cases with Y=1	18 (27.27%)		
The overall model			
OR for a null model	24.307		
OR for the actual model	74.441		
Chi-square	19.866		
SS	4		
P	P = 0.003		
Coefficients and standard errors			
Variable	Coefficient	Standard error	P
ACE	-0.7426	0.8333	0.3729
MTHFR	-0.0891	0.7149	0.9008
PAI-1	0.2016	0.6838	0.7681
TNF-α	-0.03885	12.826	0.9758
Constanta	-0.8841		
OR and 95% confidence interval			
Variable	OR	95% CI	
ACE	0.4759	0.0929 – 2.4371	
MTHFR	0.9148	0.2253 – 3.7140	
PAI-1	12.234	0.3202 – 4.6736	
TNF-α	0.9619	0.0779 – 11.8818	
Classification table (limit value p = 0.5)			
Actual result	Prediction		The percentage of true
	0	1	
Y = 0	48	0	100.00 %
Y = 1	18	0	0.00 %
The percentage of accurately classified cases			74.73 %
ROC curve analysis			
The area under the ROC curve (AUG)	0.571		
Standard error	0.0811		
95% confidence interval	0.443 – 0.692		

Table 6: Common influence of all four TNF-α, ACE, PAI-1 and MTHFR genes tested as predictors of renal scarring in infants with VUR

(regression analysis).

Logiste regression			
Dependent Y	Therapy group II level: Scar: 0-no, 1-yes		
Select	VCUG: VUR no-0, VUR yes-1 N=22		
Method	Enter		
Sample size	22		
Cases with Y=0	13 (59.09%)		
Cases with Y=1	9 (40.91%)		
The overall model			
OR for a null model	29.767		
OR for the actual model	66.577		
Chi-square	13.190		
SS	4		
P	P = 0.004		
Coefficients and standard errors			
Variable	Coefficient	Standard error	P
ACE	-12.782	13.989	0.3609
MTHFR	0.2941	15.380	0.8483
PAI-1	0.6283	11.579	0.5874
TNF-α	1,56,634	1,99,44,416	0.9937
Constanta	-0.3896		
OR and 95% confidence interval			
Variable	OR	95% CI	
ACE	0.2785	0.0180 – 4.3215	
MTHFR	13.420	0.0658 – 27.3480	
PAI-1	18.744	0.1938 – 18.1332	
TNF-α	6.35E+06	0.0000 – 0.0000	
Classification table (limit value p = 0.5)			
Actual result	Prediction		The percentage of true
	0	1	
Y = 0	11	2	84.62 %
Y = 1	7	2	22.22 %
The percentage of accurately classified cases			59.09 %
ROC curve analysis			
The area under the ROC curve (AUG)	0.607		
Standard error	0.126		
95% confidence interval	0.379 – 0.805		

Discussion

Urinary tract infections are among the most prevalent bacterial infections in children. Some prospective studies using DMSA scintigraphy have shown that 15%-40% of children will have renal scarring after a FUTI [64,65].

Our study involved 100 infants (84 girls and 16 boys) aged from 1 to 12 months with a proven first FUTI. Inflammation of the renal parenchyma (APN) with first FUTI was demonstrated in 66 (66%) of 100 children, with the technique of renal static scintigraphy. 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 ($\chi^2 = 5.677$; $p = 0.0034$). The frequency of VUR in children with first FUTI varies from country to country from 26% to 59.3% [66,67]. On the control DMSA, performed at the earliest, 6 months after the first DMSA, the presence of renal scarring was found in 18 (27%) infants. 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 ($\chi^2 = 4.254$; $p = 0.042$). The data provided in the literature show extremely low percentage (9.5%) in USA [68], or high percentage of children (40%) in Switzerland [69], while RS after FUTI in certain countries cannot be accepted as reliable. The reason for this is the non-uniformity of the studies and the impossibility of their comparison. The results of our study in infants with renal scarring revealed VUR in 9 (50%) children with equal prevalence in both genders. The risk of renal scarring is highest in children under 1 year of age with FUTI and high-grade vesicoureteral reflux [65].

Reflux is not the only precondition for renal damage. It has been shown that the renal scar is formed exclusively at the sites of acute pyelonephritis (Figure 1). This is confirmed by the primary role of acute kidney inflammation in etiology of renal damage [6,39,70]. Our results support these findings. Patients' characteristics are presented in Table 1. Studies of human genetics are passing dynamic and dramatic development and have been imposed as a powerful tool in the genesis of many diseases and even those that have not previously been linked to the genetic basis. This is also the case with UTIs. The development of RS is determined by genetic and non-genetic factors, of which many are not scientifically investigated. In the human genome are hidden secrets of successful disease diagnosis and treatment. What seems impossible today, tomorrow will become a reality [39].

By studying literature that examines the relationship between urinary tract infections and renal scars in children, it is apparent that the results are mutually contradictory and very unsuitable for comparison. These wide variations may be due to different sample sizes and the inclusion of heterogenous patient populations with variations in age, gender distribution, diagnostic criteria for UTI, afebrile, febrile and recurrent urinary infections, reflux grade, comorbid urological anomalies, genetic background and renal scarring. In the worldwide literature, we did not find similar articles which investigated the polymorphisms of the four investigated genes as in our study, and their relationship with renal parenchymal scars in children with the first FUTI. Also, even in Bosnia and Herzegovina, this issue has not been investigated so far, except one article that examines influence of PAI-1 gene polymorphism on renal scarring after first FUTI in infants [39]. The issue of urinary tract infections requires to be studied as affected by the natural, social, economic, cultural and civilization circumstances and particularities of each individual social and state community. The influence of these factors on the natural and social being of human species is subject to continuous changes. This dimension of study is lacking in the existing medical literature. Medical science has made great progress in illuminating all the complexities of urinary tract disease. However, a number of fundamental issues in the field of UTI in children have remained unanswered, which is subject to contradictory interpretations in professional literature. So, we decided to examine children younger than one year old because they are of particular interest in pediatric nephrology due to the increased risk of developing renal scars after first FUTIs.

To science is offered the only interpretation that the polymorphism of cytokine genes can be considered as an integral part of the potential risk factors for urinary system diseases. Also, antimicrobial peptides and epithelial cell functions have not been well studied. We examined genetic polymorphisms of the TNF-α, ACE, PAI-1 and MTHFR genes. 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 seven infants had typical genotype TNF-α GG. Risk homozygous genotype of TNF-α AA had 5 patients. The moderate risk heterozygous genotype of TNF-α AG had 88 infants. Twenty one infants had typical ACE II genotype. Risk homozygous genotype of ACE DD had 26 patients. The moderate risk heterozygous genotype of ACE ID had 53 infants. The nineteen infants had typical genotype PAI-1 5G5G. Risk homozygous genotype of PAI-1 4G4G had 10 patients. The moderate risk heterozygous genotype of PAI-1 4G5G had 71 infants. The nineteen infants had typical genotype MTHFR CC. Risk homozygous genotype of MTHFR TT had 4 patients. The moderate risk heterozygous genotype of MTHFR CT had 77 infants. The differences between three genotypes in the all 4 tested genes were not found to be significant ($p>0.05$).

We came to interesting results. We detected 12 polymorphisms and a total of 400 genotypes of TNF-α, ACE, PAI-1 and MTHFR genes. Of this: typical genotypes represented 66 (16.5%), risky homozygous 45 (11.25%) and moderately risky heterozygous genotypes 289 (72.25%) (Table 2). TNF-α plays an important role in stimulating extracellular matrix deposition in kidney tissue and the formation of renal scarring [24,25]. The plasma concentrations of TNF-α released by inflammatory processes depend on the course of the disease and its consequences. The study from Ireland

demonstrated for the first time in 2004 the association between vesicoureteral reflux and TNF-α AA genotype which have increased risk to reflux nephropathy [71]. In literature, we are confronted with contradictory data on the involvement of genomic polymorphisms for TNF-alpha in pathogenesis of renal damage in children with first FUTI. Also, TNF-α has been identified as a significant factor in the pathogenesis of many autoimmune diseases and cancer [72]. The role of the gene in the Renin-Angiotensin System (RAS), which contributes to the progression of kidney disease was investigated on the genes for the synthesis of renin, angiotensinogen, ACE and angiotensin receptor gene. The greatest interest of the researchers evoked the polymorphism of the ACE I/D gene polymorphism. In the medical literature, spears are broken, about the role of polymorphisms of the ACE gene in the formation of renal damage after UTIs. There are three genotypes of ACE gene II, ID and DD. Each of them can determine the activity of tissue and plasma converting angiotensin I. People with DD genotype have the highest concentrations of ACE, while those with genotype II have the lowest concentration. Experts do not share the same opinion in the interpretation of the ACE I/D gene polymorphism on renal scar formation. Savvidou and colleagues suggest that D allele polymorphism of the ACE gene is associated with UTI which are related with severe renal scarring in young children [73]. Also some other researchers believe that the DD genotype of the ACE gene represents a unfavorable prognostic factor for renal disease. Studies from Spain, USA, Japan, Korea, Turkey and India have argued that ACE gene polymorphism belongs to the risk factor for renal parenchymal damage in patients with congenital abnormalities of urinary system, especially VUR [31,74-79].

Interestingly, the meta-analysis of the selected 10 studies by Zhou and associates from different parts of the world came to the conclusion that D allele and DD genotype are risk factor for VUR and renal scarring only in Turkish children and for that population could be a valid predications for these diseases [80]. Obviously further exploration of this issue is needed, but it is not disputed that genes controlling the renin-angiotensin system play a role in the process of kidney fibrosis.

PAI-1 is upregulated by inflammatory cytokines and may therefore be regarded as a marker for an ongoing inflammatory process. Animal experiments confirm that PAI-1 is a potent fibrotic molecule. It is fact that PAI-1 promotes renal fibrosis in humans, but how it does is not understood completely. Study by Roelofs and Eddy and their associates on experimental animals have shown that PAI-1, stimulates acute pyelonephritis [81,82]. Increased plasma levels of PAI-1 and PAI-1 4G / 4G genotype are present in most aggressive renal diseases, including: thrombotic microangiopathy, crescentic glomerulonephritis, proliferative glomerulonephritis, diabetic nephropathy, lupus nephritis, and chronic allograft nephropathy [83]. However, Danish study by Kristine Jessen and colleagues didn't found association between TNF-α, IL-1β, PAI-1, uPA, CD14 and TLR4 polymorphisms and outcomes in gram-negative sepsis in older patients, most induced by Escherichia coli due to UTI [84]. The role of PAI-1 in protecting the organism from Escherichia coli infection remains unclear. PAI-1 4G / 4G genotype represents an increased risk of vascular complications in patients with diabetes mellitus, especially when associated with ACE D / D genotype. All shows that PAI-1 significantly affects glomerular proliferative lesions and sclerosis [85].

UUnfortunately, in the recent literature we did not find any articles dealing with the connection between genetic polymorphism of the MTHFR gene and renal scars after FUTI and VUR. Noiri and associates identified an increased frequency of CT and TT genotypes in male hemodialysis patients with type 2 diabetes mellitus [86]. The same scientists consider that the predisposition of male patients with MTHFR polymorphism for the development of diabetic nephropathy is affected by their increased smoking preference, homocysteine level correlation with the amount of free fat and testosterone, and the direct metabolic link between creatinine and homocysteine. The fact that homocysteine levels reflect the amount of muscle mass and the concentration of creatinine explains why men have higher concentrations of homocysteine and creatinine than women [87]. However, conflicting evidence also exists. Zhong et al. reported no association with diabetes development in Asian, Caucasian, and African cohorts [88]. There are many unknowns about MTHFR gene mutations and their clinical relevance, especially between FUTIs and RS.

In our study none of the treated infants had all the typical genotypes. It can be expected that in the particularly unfavorable social and health circumstances of these patients the moderately heterozygous alleles will be mutated in risky homozygous genotypes. So, the genetic constitution of every 100 infants is vulnerable. We didn't find significant difference in the individual representation of specific polymorphism of the examined genes between patients with acute pyelonephritis, vesicoureteral reflux and renal parenchymal damage. But, when we used the regression analysis we found that all investigated genes TNF-α, ACE, PAI-1 and MTHFR together have influence in 67% of cases in the prediction of acute pyelonephritis, vesicoureteral reflux and renal scarring in children with first FUTI (Tables 3- 6). The obtained results suggest that there are likely to be other genomic polymorphisms that could have an effect on interaction with the still unknown factors associated with geographic and sociodemographic differences among the human population (multifactorial disease).

Conclusions

Pathogenesis of the kidney scarring after acute pyelonephritis in children is multifactorial and controversial. Medical scientific thought failed to reveal the secret why acute febrile inflammation of the urinary system causes renal parenchymal scars only in some children, while others is going to full resolution without

scarring. Identifying patients with risk factors for acute renal damage remains a challenge for medical professionals because clinical indicators explain only part of the individual risk. The other factors likely to include the risk of illness are internal genetic variability. Science just begins a research and study of the genetic conditions for the occurrence of urinary tract infections, pyelonephritis and renal scarring. Each patient is a separate world with genetically induced characteristics of its psychological, physical and immunological constitution. In the human genome there are secrets of successful diagnosis and treatment of the disease. We highlight the need for a greater understanding of UTI pathogenesis as we strive to develop better management and therapeutic strategies. Although our study supports the belief that genetic variations in TNF-α, ACE, PAI-1 and MTHFR genes may predispose renal scar, more extensive and comprehensive research and study of different genes polymorphism role in renal parenchymal damage after first FUTI is necessary. It will not be surprising if, in the future, genetic access becomes a clinically important diagnostic predictor of an exit from tunnel of the FUTIs. If the genetic basis of different types of UTIs were discovered, individual therapy could be directed according to the genetic type.

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