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ASSOCIATION OF *TGF-β1* AND *TNF-α* GENES POLYMORPHISMS WITH THE KIDNEY SCARS FORMING RISK IN CHILDREN WITH VESICOURETERAL REFLUX

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Matrix accumulation in the tissue is the main pathological feature of fibrosis. *TGF-β1* stimulates the production of extracellular matrix protein and induces fibrosis in different tissues. TNF-α is a proinflammatory cytokine produced in the kidneys by proximal tubular cells, mesenchymal cells, interstitial fibroblasts and macrophages. The aim of this study was to investigate the association of *TGF-β1* and *TNF-α* genes polymorphisms with the risk of developing kidney scars in children with vesicoureteral reflux (VUR). DNA samples analyzed in this study were extracted by a phenol-chloroform method from the peripheral blood of 50 children with VUR and 70 healthy controls. The genotyping was performed by the PCR/RFLP method. Results of this study have shown that homozygous genotype T/T is more frequent in the patient group ($\chi 2 = 13.92$, $p = 0.0009$). It can be concluded that the presence of the genotype T/T on the position -509 in the promoter region *TGF-β1* gene is

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a risk factor for the development of kidney scars in children with VUR (TT vs. CT + CC: OR = 19.4615; CI 2.420-156.477; p = 0.0003). However, there was the absence of association of the promoter region polymorphism of the *TNF-α* gene region with the risk of developing kidney scars of children with VUR.

Keywords: gene polymorphism, PCR, TNF-α, TGF-β1, VUR, kidney scars

INTRODUCTION

Urinary tract infection is one of the most common bacterial infections in children (RUSHTON, 1997). Large population surveys showed that 8% of girls and 2% of boys had at least one bacterial urinary tract infection by the age of seven (HELLSTRÖM *et al.*, 1998; MARILD and JODAL, 1998).

Children who have had an infection of kidney parenchyma have a risk of persistent kidney damage which may result in kidney scarring, hypertension, recurrent urinary tract infections, reflux nephropathy, complications in later pregnancies or, in the worst case, renal insufficiency (RUSHTON, 1997; AKMAN *et al*., 2009). The presence of vesicoureteral reflux (VUR) is considered as a risk factor for a parenchymal kidney infection and subsequent scarring (NAKAI *et al.*, 2003; MOHANAN *et al.*, 2008). Due to dysfunction of the ventricular mechanism of the vesicoureteral compound, manifested by the return of urine from the urinary bladder to the ureter, VUR is the most common anomaly of the urinary tract in children. The ureterovesical junction normally functions as a one-way folding valve which slowly collapses during the filling phase, preventing the retractable return of urine into the ureter and kidney (AKMAN *et al*., 2009; PURI *et al*., 2017).

VUR can be primary and secondary. Primary VUR is caused by an abnormality of the vesicoureteral compound, where the submucosal portion of the ureter is shorter, and the compression of the bladder muscle during micturition is not sufficient to prevent the urine being returned to the ureter. Secondary VUR is a result of anatomic and/or functional abnormality of the lower urinary tract (COOPER, 2009). VUR is diagnosed in 1% of otherwise healthy children (CELIK *et al.*, 2013), 30-40% of children after symptomatic urinary tract infections (PURI *et al*., 2017; COOPER, 2009), and 7-35% of infants with prenatally diagnosed hydronephrosis (SKOOG *et al.*, 2010).

Genetic studies conducted on people with primary VUR indicate a possible familial character of the disorder (BARTIK *et al*., 2015; GIANNOTTI *et al*., 2011; PAREKH *et al*., 2002; PIRKER *et al*., 2006). The incidence of VUR in monozygotic twins is 80-100% and in dizygotic 35-45% (KAEFER *et al*., 2000), which is strong evidence that the disease might be hereditary. Although the majority of recorded cases show an autosomal-dominant way of inheritance with incomplete penetrance (VAN EERDE *et al*., 2007), cases with recessive (WENG *et al*., 2009) and X-linked inheritance are also recorded (NASERI *et al*., 2010). Also, the association of the polymorphism of the gene for cytokines with VUR in children have been demonstrated (KORDI-TAMANDANI *et al*., 2013).

Transforming growth factor beta (TGF-β) is a multifunctional cytokine. It is important for the proliferation, differentiation and growth of the cells. *TGF-β* activation is also a trigger of several events that promote fibrosis, such as the transcription of the matrix-disintegrating enzymes inhibitors and matrix-binding receptors, the transformation of fibroblasts into myofibroblasts, trans-differentiation of tubular epithelial cells into myofibroblasts, and chemotaxis of fibroblasts and monocytes (FIDAN *et al*., 2013). *TGF-β* induces the epithelial-mesenchymal transition process and inhibits endothelial cell apoptosis leading to atrophy of glomerular capillaries that accompany

fibrosis. Increased expression of TGF-β is a feature of all human and experimental kidney fibrosis models (LEE *et al*., 2014).

TGF-β1 was the most studied in the pathogenesis of fibrotic renal impairment, stemming from the fact that it is the main factor in the modification of the extracellular matrix. Contemporary studies have indicated a link between the polymorphism of the *TGF-β1* gene both with urinary tract infection and with VUR (VAN EERDE *et al*., 2007; KOWALEWSKA-PIETRZAK *et al*., 2008; WILDBRETT *et al*., 2013).

The two most commonly studied *TGF-β1* gene polymorphisms are polymorphisms in the gene promoter at the -800 position (G-800A), where guanine is replaced with adenine, and at position -509 (C-T) where replacements of cytosine with thymine occur. The meta-analysis of polymorphism -509 (C/T) has shown that carriers of T alleles have a significantly increased risk of almost 36% for developing asthma (YONGGANG *et al*., 2010). Also, this study concluded that this risk is significantly higher in the Asian population than in the white population, suggesting that, besides genetic factors, the incidence of disease can also be influenced by factors such as the environment. At position 915 (G-915C) in codon 25, guanine is replaced in cytosine, resulting in the change of the amino acid of arginine into proline. A link between arginine homozygous genotype (+915 G/G) and liver fibrosis was found in patients with chronic hepatitis C infection (POWELL *et al*., 2000). Also, the relationship between deletion polymorphism 713-8delC at intron 4 and lower bone density in osteoporosis was established (BERTOLDO *et al*., 2000). In-depth understanding of these polymorphisms could lead to better disease prognosis in patients who have an increased risk of progression of the disease.

Tumour necrosis factor alpha (TNF-α) is a proinflammatory cytokine produced in the kidneys by proximal tubular cells, mesenchymal cells, podocytes, interstitial fibroblasts and macrophages (KITA *et al*., 1993; RAMESH *et al*., 2006; ROSA *et al*., 2012; ZAGER *et al*., 2006). Important stimulants for the production of TNF-α are lipopolysaccharides (LPS) of Gram-negative bacteria. TNF-α stimulates the production of proinflammatory cytokines (IL-1 and IL-6), increases the cytotoxicity of macrophages, has a mitogenic and chemotactic effect on fibroblasts. Also, TNF-α modulates extracellular matrix (ECM) turnover in the HK-2 human proximal tubule cell line by increasing matrix metalloproteinase 9 (MMP-9) in addition to inhibitor of metalloproteinase-1 (TIMP-1) suppression (NEE *et al*., 2004). Moreover, TNF-α contributes to the development of renal scar tissue by releasing of IL-1β and TGF-β from inflammatory cells.

The gene encoding TNF-α is located on the p arm of chromosome 6 (6p21.3). This segment of chromosome 6 also contains the genes of the major histocompatibility complex (MHC) (MULLER *et al*., 1987; HORIUCHI *et al*., 2010).

Some of the important polymorphisms found in the promoter region of the *TNF-α* gene, intensively examined in autoimmune and infectious diseases, are in the position: -1031 (T/C),-863 (C/A), -857 (C/A), -851 (C/T), -419 (G/C), -376 (G/A), -49 (G/A), -163 (G/A). The polymorphisms -419, -163, -49 are rare in the white population (MIRA *et al*., 1999; ELAHI *et al.*, 2009); -308 A allele is associated with a more serious inflammatory reaction in many viral diseases such as hantavirus-induced nephropathy (MAKELA *et al*., 2001) and dengue-induced haemorrhagic fever (FERNANDEZ-MESTRE *et al*., 2004). A study that examined patients with *Helicobacter pylori* infection revealed that women with the -308 (G/G) genotype have an increased risk of developing an ulcer in the duodenum (KUNSTMANN *et al*., 1999). Studies based on patients with solid tumours and haematological malignancies have shown the association between elevated levels of TNF-α in plasma and poor clinical outcome. For example, in patients with non-Hodgkin's lymphoma,

where polymorphism *TNF-α* -308 (G/A) and lymphotoxin alfa (*LT-α*) +252 A /G polymorphism gene were studied, a higher presence of highly productive haplotype (A allele) and a poor prognosis in terms of outcome of the disease were seen in patients compared to controls (SEIDEMANN *et al*., 2005).

The aim of this study was to investigate the association of polymorphisms *TGF-β1* and *TNF-α* genes with the risk of developing kidney scars in children with VUR.

MATERIAL AND METHODS

Samples

DNA samples analyzed in this study were extracted from white blood cells of 50 patients and 70 controls.

The patient group consisted of children of both sexes aged from 4 months to 14 years with vesicoureteral reflux and renal scars who was treated at the Clinic for Children's Internal Diseases, Clinical Center of Niš, Serbia.

Control group included 70 healthy persons of both sexes aged from 18 to 25 years. Criteria for inclusion in the research was the absence of the previous history of VUR, renal scars as well as other acute and chronic diseases.

Ethical approval has been obtained from the Ethics Committee of the Faculty of Medicine, University of Niš, Serbia 01-2857-9.

The molecular genetic research was carried out at the Laboratory for Functional Genomics and Proteomics of the Institute for Biomedical Research, Faculty of Medicine, University of Niš, Serbia.

Blood samples were collected from all participants via venipuncture and then kept in EDTA-coated tubes to be used for DNA extraction at the time of clinical examination. Extraction of DNA from white blood cells was done using a standard phenol-chloroform method. The DNA quality was determined with 260/280 optical density (OD) ratios in all samples, which were stored at − 20 °C until use.

Genotyping

Polymerase chain reaction (PCR)

Molecular-genetic testing involved the analysis of the polymorphism located in the promoter regions of *TNF-α*-308 G/A and *TGF-β*-509 C/T gene using polymerase chain reaction/restriction fragment length polymorphism (PCR/RFLP) technique. The priming sequences and the length of the amplified PCR fragments are given in Table 1.

Table 1. Sequences of primers and length of amplified PCR fragments

	Sequences of primer		Amplified fragment
TNF-α	5'- AGG CAA TAG GTT TTG AGG GCC AT-3'	F	117bp
	5'- ACA CTC CCC ATC CTC CCT GCT – 3'	R	
TGF-β1	5'-GGA GAG CAA TTC TTA CAG GTG -3'	F	120bp
	5'- TAG GAG AAG GAG GGT CTG TC - 3'	R	

PCR reaction was carried out according to the manufacturer's protocol (KAPA2G Fast HotStartReadyMix (2x) PCR Kit; 2x ReadyMix contains Taq DNA polymerase, PCR buffer,

dNTPs, and MgCl2). A target DNA (200 ng) and 0.50 μM final of each primer were added to the reaction mixture (25 μL). The amplification was performed on Eppendorf Mastercycler EP gradient S (Applied Biosystems, Foster City CA, USA) under the following conditions: initial denaturation at 95 °C for 2 min, followed by 35 cycles of denaturation at 95 °C for 15 s, annealing at 60 °C for 15 s, and an extension at 72 °C for 15 s, and final extension at 72 °C for 30 s. The PCR products were separated using 2 % agarose (Invitrogen, USA) gel electrophoresis and visualized with an ultraviolet transilluminator after staining with DNA safe stain HoeferMacroVue UVis-20 (Amersham Biosciences Corp. Piscataway, USA).

Restriction fragment length polymorphism (RFLP)

Restrictive endonuclease PCR amplifications were cut into smaller fragments, depending on the presence of restriction site polymorphisms. DdeI restriction enzyme was used for *TGF-β1* while NcoI restriction enzyme was used for the digestion of *TNF-α*. The digestion was carried out in a water bath at 37^0C overnight. Upon completion of the digestion, the DNA fragments obtained were identified by vertical electrophoresis at 8% polyacrylamide gel (PAGE).

Polymorphism for *TGF-β1*- 509 C/T: The homozygous genotype C/C is detected on the gel as two fragments of length 74bp and 46bp, the C/T heterozygous genotype is confirmed by the presence of three fragments of 120bp, 74bp and 46bp, and the homozygous genotype T/T is shown as one non-aligned fragment length 120bp.

Polymorphism for *TNF-α*-308 G/A: Fragments 97bp and 20bp represent the homozygous genotype G/G while the fragments 117bp, 97bp, and 20bp represent the heterozygous genotype G/A. Homozygous genotype A/A is represented by a 117bp fragment.

Statistical analysis

Statistical analyses were performed using the SPSS 16.0 software package (IBM Corp., Armonk, NY, USA) with $p < 0.05$ considered significant. Chi-square test of independence was used to determine the difference in the distribution of different alleles and genotypes between the group of children with VUR and healthy control group and the correlation between continuous variables was assessed using Pearson's correlation coefficient.

RESULTS AND DISCUSSION

This study included 50 children with vesicoureteral reflux and 70 healthy controls. The *TGF-β1* polymorphism in the-509 C/T and *TNF-α* polymorphism in the-308 G/A sites were genotyped in all the subjects.

Polymorphism for TGF-β1-509 C/T

RFLP electrophoresis on 8% polyacrylamide gel for *TGF-β1* is shown in Figure 2. The genotype distribution and allele frequencies of the -509C/T SNP in the *TGFβ1* gene promoter among patients and healthy controls are presented in Table 3. The frequency of T allele-509 was statistically higher in the group of patients than in the control group (59% vs. 41%, $\chi^2 = 4.61$, $p<0.05$). Heterozygous genotype C/T was present in the control group with 77.1%, and in the patient group with 64%. Homozygous genotype C/C was found within 14% of the patients and 21.4% among the control, while the percent of homozygous genotype T/T in the control was 15% compared to the 22% in the patient group. Statistical analysis indicates that homozygous genotype T/T was obviously more frequent in the patient group ($\chi^2 = 13.92$, $p = 0.0009$) and that the

correlation of the homozygous genotype T/T with the kidney scars forming risk was evident (TT vs.CT + CC: OR = 19.4615; CI 2.420-156.477; p = 0.0003).

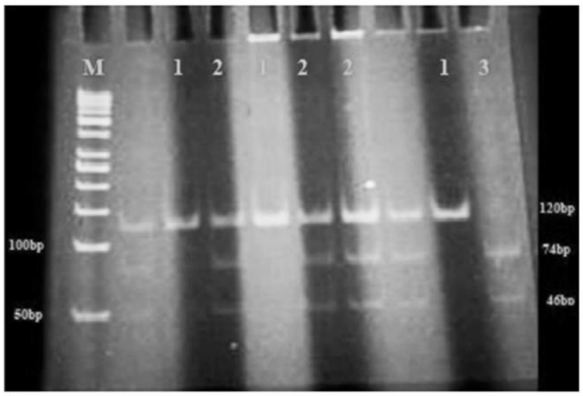

Figure 1. Representative photograph of RFLP electrophoresis on 8% polyacrylamide gel for *TGF-β.* PCR product (120 bp) was digested with DdeI restriction enzyme. 1) T/T genotype 120bp, 2) C/T genotype 120bp, 74bp and 46bp, 3) C/C genotype74bp and 46bp, M) DNA size marker 100bp and 50bp

Table 2. The genotype and allele frequencies of the -509 C/T SNP in the TGF-β1 gene promoter among the patients (VUR) and healthy controls (K); OR – odds ratio, CI – confidence interval

TGF-β1	VUR (n=50)	K(n=70)
allele		
C	0.46 (46)	0.6 (84)
T	0.54 (54)	0.4 (56)
χ^2=4.61(p=0.032)		
OR=1.761(CI1.05-296; p<0.05)		
genotype		
CC	0.14(7)	0.21 (15)
CT	0.64 (32)	0.77 (54)
TT	0.22 (11)	0.02 (1)
χ^2= 13.92 (p= 0.0009)		
(Yates) = 11.422 p= 0.003		
TT vs. (CT+CC): OR=19.4615	(CI 2.420-156.477; p=0.0003)	
*T- vs.*CC: OR=1.675	(CI 1.63-4.47; p>0.20)	

However, analyzes did not show statistical significance when comparing genotypes containing T allele with genotype C/C (T- vs. CC: OR = 1.675; CI 0.63-4.47; p>0.20).

Polymorphism for TNF-α-308 G/A

Genotyping at position -308G>A is represented in Figure1. At this position, homozygous genotype G/G was found in 40 patients (80%) and 48 controls (68.6%), heterozygous genotype G/A was observed in 10 patients (20%) and 22 controls (31.4%) while homozygous genotype A/A

was not found. The distribution of *TNF-α* genotype and allele frequency among the patients and controls are summarized in Table 2. The frequencies of G and A alleles were 84% and 16% in the control group and 90% and 10% in the group of patients, respectively.

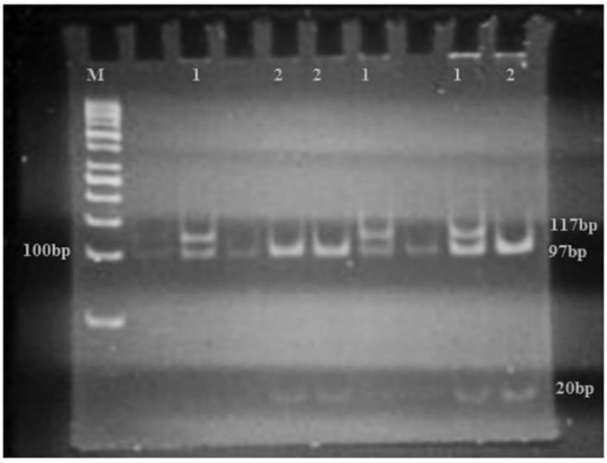

Figure 2. Representative photograph of RFLP Electrophoresis on 8% polyacrylamide gel for *TNF-α.* PCR product (117 bp) was digested with Ncol restriction enzyme. 1) GA genotype 117bp, 97bp and 20bp, 2) GG genotype 97bp and 20bp, M) DNA size marker 100bp; AA genotype not found

The results revealed no significant difference in genotype distribution and allele frequencies of *TNF-α-308 G/A* polymorphism between the group of patients with vesicoureteral reflux and the control group (all $p>0.05$).

TGF-β1 regulates the production of the extracellular matrix and induces the process of fibrosis in various tissues. T allele at -509 is associated with a higher circulating TGFβ-1 concentration, and those homologous to T allele have a higher level of TGFβ-1 and a decreased proliferation of T cells (SHAH *et al*., 2006), therefore it is a logical assumption that an elevated level of TGFβ-1 in plasma reflects an increased transcription and translational activity when *TGFβ-1* expression is activated locally in response to inflammation.

Table 3. Allelic and genotypic frequencies of TNF-α gene among patients with VUR and healthy controls (K)

TNF-α	VUR (n=50)	K(n=70)
allele		
G	0.9 (90)	0.84 (118)
A	0.1 (10)	0.16 (22)
$\chi^2 = 1.65$ (p=0.20)		
genotype		
GG	0.8 (40)	0.69 (48)
GA	0.2 (10)	0.31 (22)
AA	0	0
$\chi^2 = 1.95$ (p=0.16)		

An increased concentration of TGF-β1 in the urine of children with urinary tract infection (FARMAKI *et al*., 2005) was found, and it is observed that TGF-β1 is secreted shortly after infection onset. Also, by inducing VUR *in vitro*, it has been found that hydrodynamic pressure increases the secretion of TGF-β1 from tubular epithelial kidney cells, which interfere with the development of metanephric mesenchymal (MARUYAMA *et al*., 2005). Many studies have dealt with the possible role of DNA polymorphisms in the renin-angiotensin system in progressive kidney damage. The data indicate that *TGF-β1* and angiotensin II regulate the expression of each other (LI *et al*., 2013). All this supports the hypothesis that *TGF-β1* might be an important factor in the pathogenesis of urinary tract infections, VUR and kidney scars. Polymorphisms in the *TGF-β1* gene are associated with an increased risk of the occurrence and higher severity of the number of diseases such as colorectal cancer (STANILOVA *et al*., 2018), asthma (YONGGANG *et al*., 2010), osteoporosis (TAHA *et al*., 2017), rheumatoid arthritis (ZHOU *et al*., 2014), psoriasis vulgaris (EL-HADIDI *et al*., 2018), and others. However, many studies have found no association with the polymorphisms of this gene with diseases such as pancreatic cancer (FARAHBAKHSH *et al*., 2017), ischemic stroke (KUMAR *et al*., 2016), glioma (VIEIRA *et al*., 2015).-509 T allele is associated with numerous conditions of the kidney disease, such as IgA nephropathy (VOUNG *et al*., 2009), chronic allograft nephropathy (SHO *et al*., 2008), chronic progressive renal failure (KHALIL *et al*., 2005), and other diseases such as rheumatic heart disease (SHOU *et al*., 2004) and endometriosis (HSIEH *et al*., 2005). Various studies have shown that the incidence of allele C in the healthy population is 55-70%, and allele T 30-45% 8 (YONGGANG *at al*., 2010). The results of allele analysis in the healthy population obtained in our study showed the presence of 60% C allele, and 40% T alleles, which is in compliance with the literature data.

According to a study which included children in the UK (COTON *et al*., 2002), variants in the gene for *TGF-β1* may have an important impact on the pathogenesis of renal parenchymal scars. Research has shown that there is a link between *TGF-β1* -800 G/A, -509 T/T and $Leu^{10}\rightarrow$ Pro genotype and kidney scarring after urinary tract infections. The genotypes G/A -800 and Leu^{10} →Pro C/T are much more frequent, while the incidence of T/T-509 genotype is lower in a group of children who have not developed renal scarring compared to a group of children who developed renal scars after a urinary infection.

In this study, in 50 patients with renal parenchymal scarring in the presence of VUR, the homozygous genotype C/C is represented by 14%, which is less compared to a healthy control group (21.4%). The results obtained are in accordance with the study data of a group of Irish children with reflux nephropathy (SOLARI *et al*., 2005).

The results of this research show that the frequency of the T/T genotype is significantly higher in the patient group compared to the control group (22% vs. 1.5%), which is in concordance with the study conducted on a population of Polish children (KOWALEWSKA *et al*., 2008), as well as the study which included British children (COTON *et al*., 2002).

On the other hand, Jim and associates found a reduced T/T genotype frequency in Korean patients with renal impairment compared to patients without damage (YIM *et al*., 2007).

The response to the kidney infections includes both the epithelium and the local immune system. TNF is one of the first immunological mediators that occurs after infection (PARAMESWARAN and PATIAL, 2010; DUQUE and DESCOTEAUX, 2014). By stimulating the synthesis of other proinflammatory growth factors and cytokines (interleukin-1b, TGF-β, etc.) it leads to interstitial fibrosis and parenchymal damage.

Since it has been established that *TNF-α* is located within the central MHC complex, many speculate on the genetic association between certain *TNF-α* allele and susceptibility to diseases.

A significant link was observed in the case of *TNF-α* -308 G/A, -238 C/T and -1031 G/A polymorphisms with an increased risk of cervical cancer (LI *et al*., 2018).

The risk of the kidney cell carcinoma is 6.5 times higher in patients with G/A genotype at the site -238 and 2.9 times higher in those with G/A genotype at the +488 locus when comparing the tissue of patients with renal cell carcinoma and the healthy control tissue (NAKAJIMA *et al*., 2001).

The single-nucleotide polymorphism of the *TNF* gene at the -308promoter region is associated with changes in the production and transcription of this cytokine (UMAPATHY *et al*., 2018). Allele A (*TNF2*) is associated with higher levels of TNF, therefore inflammatory nephropathy may be more common in patients with allele A due to the increased production of this cytokine. Allele -308A is associated with a greater predisposition for developing several inflammatory diseases, such as areata alopecia (CRISTINA *et al*., 2012) and systemic erythema lupus (YANG *et al*., 2017).

However, there are studies that do not associate this polymorphism with certain diseases, for example, a study conducted in the Brazilian population did not found a significant difference in the frequency of alleles and genotypes between controls and patients with sepsis and the occurrence of septic shock (PASKULIN *et al*., 2011). Also, a study done in the Iranian population has no association with this pulmonary fibrosis polymorphism (FOROZAN *et al*., 2006).

Association studies of kidney diseases have shown variable results: Umapathy and associates (UMAPATHY *et al*., 2018) observed the association of *TNF-α* polymorphism with diabetic nephropathy, while Dabhi and associates (DABHI *et al*., 2015) failed to prove this.

Kim and associates (KIM *et al*., 2004) did not find any difference in the distribution of genotype between patients with nephrotic syndrome and the control group. Solari and associates (SOLARI *et al*., 2004) found a greater proportion of *TNF-α* A/A genotype in patients with reflux nephropathy compared to a healthy control group, suggesting that this genotype could be associated with progression of renal impairment in patients with VUR, however, Pardo and his associates did not manage to prove this (PARDO *et al*., 2007).

In our study, in 50 patients with kidney parenchymal scars in the presence of VUR, the most common genotype was homozygous G/G with 80%, heterozygous was represented with 20% while homozygous genotype A/A was absent. Statistical analysis of data revealed no significant difference in the frequency of genotypes between the control group and the patient group. We had not been able to prove the connection between this polymorphism and renal impairment in patients with VUR, as opposed to the study by Solari and associates (SOLARI *et al*., 2004), which might be explained by the fact that we included patients with all the stages of renal damage, not only moderate or severe forms. Our data confirmed the previous hypothesis that *TNF-α* polymorphism is not associated with the progression of the disease (PARDO *et al*., 2007).

The results of the analysis of the frequency of genotypes in the healthy population obtained in our study showed the presence of homozygous genotype G/G with 68.6%, heterozygous genotype G/A with 31.4% and the absence of homozygous genotype A/A, which is completely consistent with the literature data (PARDO *et al*., 2007; YADAV *et al*., 2016).

A healthy control group in this study had a -308A allele frequency of 15.7%. This is similar to the distribution published by other authors, which ranged from 11-20% (SOLARI *et al*., 2004). Unlike to the previously published results, our data showed that allele-308A (TNF2) is not more

frequent in VUR patients compared to the control group, which did not support the idea that polymorphism might be associated with progression of renal damage after infection. Recent studies had shown an increased level of TNF-α in fetal renal dysplasia (CALE *et al.*, 2000). Therefore, this allele could be associated with the development of the irregular position of the urethra leading to the VUR.

It is well known that the prognosis of renal impairment in patients with VUR can be affected not only by genetic factors but also by the frequency and severity of urinary tract infections as well as the age of the patient when the first infection occurs. Further research is needed to determine the influence of each factor, including genetic ones.

CONCLUSION

The results of the present study showed the significant difference in the incidence of T/T genotype between patients with VUR and the control group, which support the hypothesis that the genetic variation of the *TGF-β1* gene affects the pre-position for the formation of renal scars after urinary tract infections. However, there was no significant difference in the distribution of the C and T allele frequency at -509 in the gene promoter for *TGF-β1* between the control group and the patient group. Moreover, -308G/A polymorphism in the *TNF-α* gene might not be associated with a high risk of renal scarring in children with VUR.

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ASOCIJACIJA POLIMORFIZAMA *TGF-β1* i *TNF-α* GENA SA FORMIRANJEM BUBREŽNIH OŽILJAKA KOD DECE SA VEZIKOURETERALNIM REFLUKSOM

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Izvod

Akumulacija matriksa u tkivu je glavna patološka karakteristika fibroze. *TGF-β* stimuliše proizvodnju proteina vanćelijskog matriksa i indukuje fibrozu u različitim tkivima. TNF-α je proinflamatorni citokin koji u bubrezima proizvode proksimalne tubularne ćelije, mezenhimalne ćelije, intersticijalni fibroblasti i makrofazi. Cilj ovog rada bio je ispitivanje povezanosti polimorfizama u genima *TGF-β1* i *TNF-α* kao mogući faktor rizika za razvoj bubrežnih ožiljaka kod dece sa vezikouretalnim refluksom (VUR-om). Genomska DNK analizirana u ovoj studiji izolovana je fenol-hloroformskom metodom iz limfocita periferne krvi 50 dece sa VUR-om i 70 zdravih kontrola. Genotipizacija je rađena PCR/RFLP tehnikom. Rezultati ovog istraživanja su pokazali da je homozigotni genotip T/T frekventniji u grupi pacijenata ($\chi 2 = 13.92$, $p = 0.0009$). Može se zaključiti da je prisustvo homozigotnog genotipa T/T na poziciji -509 promotorskog regiona *TGF- β1* gena faktor rizika za razvoj bubrežnih ožiljaka kod dece sa VUR-om (TT vs.CT + CC: OR = 19.4615; CI 2.420-156.477; p = 0.0003). Osim toga, primećeno je odsustvo povezanosti polimorfizma u promotorskom regionu *TNF-α* gena sa rizikom za razvoj bubrežnih ožiljaka kod dece sa VUR-om.

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