ORIGINAL ARTICLE

URINARY EXCRETION OF TGF-B1 AND VEGF IN CHILDREN WITH VESICOURETERAL REFLUX

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ABSTRACT

The aim of this study was to investigate the relation between urinary TGF-β1, urinary VEGF and renal scarring resulted from VUR.

Materials and methods: This study included 141 patients with VUR and 34 healthy sex and age matched children. The statistical analysis consisted of descriptive statistical parameters, KruskalWallis, Mann-Whitney tests and ROC analysis.

Results: The urine levels of TGF-β1 and VEGF were significantly increased in children with VUR, compared to the controls. The levels of TGF-β1 urine excretion in children with renal scarring were higher compared children no renal scarring. The indicators of VEGF urine excretion in children with renal scarring compared to indicators in children no renal scarring, were lower, however exceeded the indicators in children of control group. The area under the ROC curve for TGF-β1 was 109.9, for VEGF was 207.6.

Conclusions: The study allowed to substantiate and propose non-invasive methods for early diagnosis of renal scarring in children with VUR.

KEY WORDS: vesicoureteral reflux; renal scarring; TGF-β1; VEGF, children

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INTRODUCTION

One of the most frequent form of congenital urinary tract abnormalities in children is the vesicoureteral reflux (VUR) [1]. According to International Reflux Study Committee the frequency of the nephrosclerosis development with VUR among children of the European population is 25-40% and leads to the development of a terminal stage of *chronic kidney disease* (CKD) in 40-50% of patients [2, 3]. According to modern literature, the frequency of terminal CKD in children with renal scarring (RS) constantly grows around the world [4, 5]. About 25% of children with renal scarring owing to VUR need carrying out a hemodialysis and kidney transplantation [6].

Over the past years, scientists from different countries conducted a lot of research on the analysis the renal parenchyma in children with VUR [7, 8]. Despite this, the search for new markers of renal tissue damage in patients with VUR is not stopped. Today it is known that the development of RS is a cascade *kidney remodeling processes*, which is characterized by functional and morphological changes [9].

Experimental and not numerous clinical trials demonstrated that immuneinflammatory mechanisms are involved in formation of RS with proinflammatory and profybrotic cytokines, that results in inflammation of the interstition involving tubules and blood vessels with the subsequent remodelation of the tubulointerstitial kidney tissue that causes fibrosis of a parenchyma and an atrophy of tubules [10]. Authors note that transforming growth factor β1 (TGF-β1) is a powerful profibrotic cytokine. TGF-β1 strengthens a fibroblastic proliferation and an epitelial-mezenximal transformation of endothelial cells that strengthens the fibrosis of kidney parenchyma [11, 12]. At the same time, the researchers established that podocytes synthesized vascular endothelial growth factor (VEGF) induces cellular adhesion and suppresses proliferation of fibroblasts and also supports a peritubular bloodflow, stimulates angiogenesis [13].

Despite intensive researches, the problem of noninvasive diagnostics of nephrosclerosis, especially its preclinical stages, is still being up to date.

THE AIM

In this study we defined the level of biological markers of the fibrogenesis (TGF-β1) and angiogenesis (VEGF) in urine, depending on existence of signs of RS and also established diagnostic predictive of the level of these indicators on formation of RS in children with VUR.

MATERIAL AND METHODS

GENERAL INFORMATION

A prospective study was carried out from 2014 to 2019 on 141 children (38 boys and 103 girls) from 1 to 17 years of age with VUR and full clinical-laboratory remission of pyelonephritis.

The patients divided into the groups according to grade of VUR and whether or not combined with RS. The I group

Table I. General information on patients with VUR and pyelonephritis

Characteristic	I group (n = 24)	II group (n = 87)		III group (n = 30)		Overall (n = 141)
		II A (n = 69)	II B (n = 18)	III A (n = 9)	III B (n = 21)	
Age, (%)						
6 months – 2 years	8 (33.3)	27 (39.1)	2 (11.1)	3 (33.3)	1 (4.8)	41 (29.1)
3 – 6 years	6 (25.0)	27 (39.1)	2 (11.1)	2 (22.2)	6 (28.6)	43 (30.5)
7 - 11 years	6 (25.0)	10 (14.5)	10 (55.6)	3 (33.3)	7 (33.3)	36 (25.5)
12 - 17 years	4 (16.7)	5 (7.3)	4 (22.2)	1 (11.1)	7 (33.3)	21 (14.9)
Male/Female	4/20	13/56	7/11	4/5	11/10	39/102
Index urinary tract infection, no. (%)	20 (83.3)	23 (33.3)	-	2 (22.2)	-	45 (31.9)
The incidental course of pyelonephritis Recurrent pyelonephritis	4 (16.7)	46 (66.7)	18 (100.0)	7 (77.8)	21 (100.0)	96 (68.1)
Bilateral VUR, no./total no. (%)	7/24 (29.2)	15/69 (21.7)	3/18 (16.7)	2/9 (22.2)	7/21 (33.3)	34/141 (24.1)

VUR - vesicoureteral reflux; no - absolute number;

Table II. Laboratory data in children with VUR

Characteristic	I group (n = 24)	II group		III group	
		II A (n = 69)	II B (n = 18)	III A (n = 9)	III B (n = 21)
Hemoglobin, g/l*	126 (116; 134)	117 (111; 129)	124 (120; 131)	120 (107; 122)	116 (108; 124)
	MW test: $p_{I-II} = 0.2429$; $p_{II-III} = 0.6782$; $p_{I-III} = 0.1917$;				
ESR, mm/h*	5.5 (4.0; 15,5)	5.0 (3.0; 11.0)	7.0 (4.0; 10.0)	5.0 (3.0; 7.0)	7.0 (4.0; 11.0)
	MW test: $p_{I-II} = 0.6441$; $p_{II-III} = 0.9155$; $p_{I-III} = 0.7873$;				
Serum creatinine, μmol/l*	67.6 (58.7; 86.0)	68.0 (57.4; 90.0)	72.7 (60.4; 83.9)	63.6 (53.1; 68.3)	100.0 (61.9; 140.0)
	MW test: $p_{I-II} = 0.6804$; $p_{II-III} = 0.1094$; $p_{I-III} = 0.1717$;				
GFR, mL/min/1.73 m^{2}*	80.0 (56.0; 96.0)	80.0 (61.0; 95.0)	85.0 82.0; 108.0)	89.0 (68.0; 92.0)	73,0 (51.0; 94.0)
	MW test: $p_{I-II} = 0.5007$; $p_{II-III} = 0.2006$; $p_{I-III} = 0.6198$;				

VUR - vesicoureteral reflux; ESR - *erythrocyte sedimentation rate*; GFR - glomerular filtration rate; MW - Mann Whitney for paired comparison; Data are presented in the form Me (Lq; Uq)

comprised 24 children had I grade VUR, no RS; the II group comprised 87 patients had II and III grade VUR: among them 69 children no RS (IIA group), 18 – with RS (IIB group), the III group comprised 30 patients had V grade VUR: among them 9 children no RS (IIIA group), 21 patients had RS (IIIB group). The control group included 31 healthy children (19 girls and 12 boys) of similar age that had no complaints, clinical signs, data anamnesis which would confirm presence of any chronic disease or congenital defects of urinary system organs or an acute disease.

Complex assessment of the patient condition presupposed studying of the anamnesis, full objective inspections and laboratory tool researches for verification of the diagnosis by KDIGO 2012 recommendations [14]. VUR was diagnosed according to the voiding cystourethrography and graded according to International Study Classification [15, 16].

Renal scarring was diagnosed with dimercaptosuccinic acid (DMSA). It was noted that DMSA scan was performed 3-4 months after exacerbation period of pyelonephritis.

TGF-β1 and VEGF urinary excretion levels were analyzed with the ELISA (enzyme-linked immunosorbent assay). TGF-β1 levels were determined with the help of standard sets (Catalog No: BMS249/4 and BMS249/4TEN human TGF-beta1, Bender MedSystems GmbH, Austria), VEGF levels – (Catalog No: BMS277/2 and BMS277/2TEN human VEGF-A, Bender MedSystems GmbH, Austria) according to manufacturers' instructions.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study was approved by the ethics committee of the Kharkiv National Medical University, Ukraine. Information consents were signed by all participants and/or their parents gave written informed consent (Protocol No.8 dated October 3, 2018). Research was conducted in accordance with the World Medical Association's Helsinki Declaration.

Table III. Urine excretion of TGF-β1 and VEGF in children with VUR

Group	Parameter	
	TGF-β1, pg/ml	VEGF, pg/ml
I group (n = 24)	77.5* (26.7; 140.1)	206.6* (126.5; 265.7)
II A group (n = 69)	70.2* (28.7; 105.5)	222.0* (150.6; 358.6)
II B group (n = 18)	139.5* (117.2; 215.9)	178.4* (137.3; 203.2)
III A group (n = 9)	30.7* (29.1; 40.5)	244.2* (189.3; 295.8)
III B group (n = 21)	108.6* (56.2; 133.5)	124.9* (104.9; 152.6)
Controls group (n = 31)	14.0 (3.1; 29.2)	40.9 (27.2; 59.3)

*p<0.05 in comparison with control group; TGF-β1 - transforming growth factor-beta1; VEGF - vascular endothelial growth factor; Data are presented in the form Me (Lq; Uq)

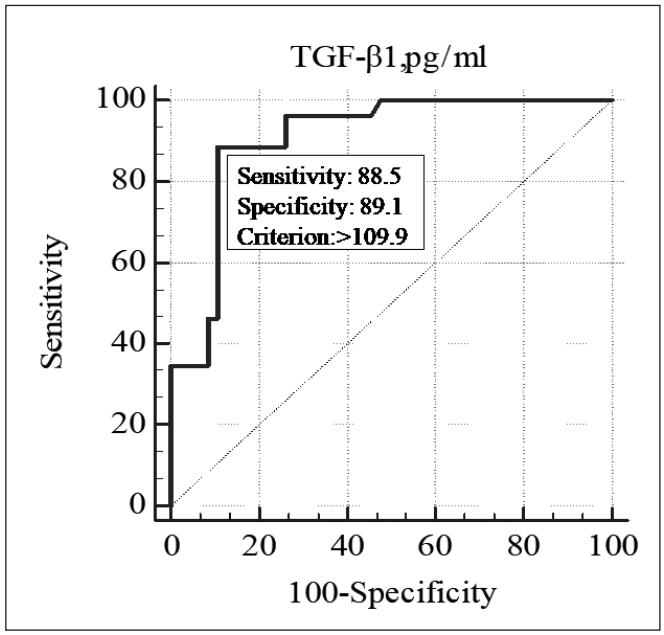

Fig.1. Receiver operating characteristic (ROC) curve of *TGF-β1* for the prediction of renal scarring in children with VUR.

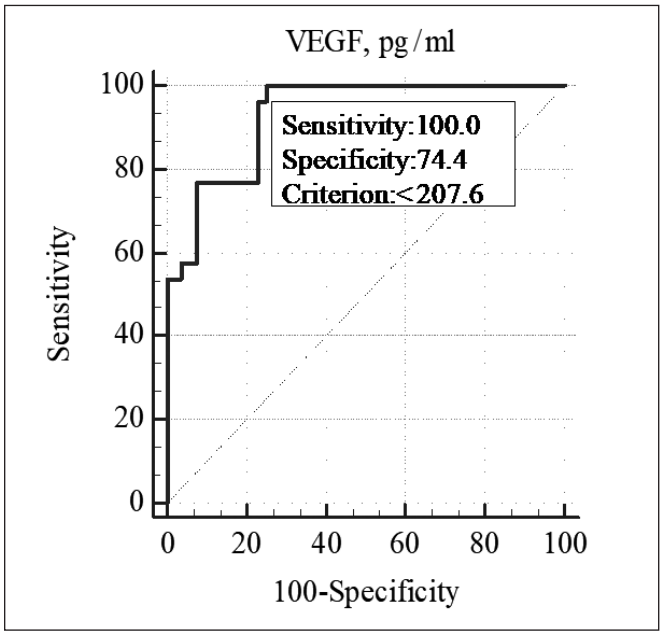

Fig.2. Receiver operating characteristic (ROC) curve of VEGF for the prediction of renal scarring in children with VUR.

STATISTICAL ANALYSES

Statistical analysis was performed using «STATISTICA 7». Median (interquartile range IQR) values and frequencies are provided for the description of continuous and categorical variables, respectively. The Kruskal-Wallis test was used for intergroup comparisons. The MannWhitney U test was performed to compare differences between two independent groups.

Receiver operating characteristic (ROC) curves were drawn for variables to determine the optimal «cut-off» values to predict an endpoint. Statistical «cut-off» values were calculated by minimizing the distance between the point with specificity=1 and sensitivity=1 and various points on the ROC curve. The areas under the ROC curve for urinary TGF-beta1 (pg/ml) and VEGF (pg/ml) were calculated. The sensitivity, specificity and predictive values were calculated in comparison to the control group. The results were evaluated in the confidence interval of 95%, and significance was evaluated at the level of p<0.05.

RESULTS

There were 141 children aged 1 to 17 years with VUR and full clinical-laboratory remission of pyelonephritis. Analysis of the age characteristics of the children of the main group showed that 41 children (29.1%) were aged 6 months to 2 years, 43 children (30.5%) from 3 to 6 years, 36 children (25.5%) from 7 to 11 years and 21 of patients (14.9%) at the age from 12 to 17 years. 102 (72.3%, p<0.05) of the children were girls (Table I).

The results of the voiding cystourethrography demonstrated more frequent high grade VUR (III, IV and V) in surveyed patients than lowgrade VUR (I, II) (82.9% and 17.1%, p<0.05, respectively). At all grades of VUR, unilateral affect is probably observed more often than bilateral (all p<0.05). The average age of the disclosure of the disease was 4.1 ± 2.1 years.

In 39 (27.7%) of the examined children were found signs of RS. In patients of I group no signs of RS were found. Depending on a reflux grade, the specific weight of patients

with RS, particularly in 18 (20.7%) children of II group and 21(70.0%) children of III group increases ($p<0.05$). At the high grade of VUR the risk of RS is higher, than in grades I-II (RR = 8.00, 95% CI [1.16; 55.44]).

There were no statistically significant differences between the groups in hemoglobin, erythrocyte sedimentation rate, serum creatinine. Meanwhile, with increasing grading of reflux, the amount of glomerular filtration rate decreased, but the changes were not statistically significant ($p>0.05$) (Table II).

Patients in all groups had significantly higher urinary TGF-β1 level when compared to control group (KW H=30.72, p=0.0001, MW p_{cI}=0.0078; p_{cIIA}=0.0000; p_{cI-IB}=0.0002; p_{cIIIA}=0.0047; p_{cIIIB}=0.0001). However, TGF-β1 in patients showed increasing trend that became statistically significant in children with VUR and RS when compared to children with VUR no RS (MW p_{IIAIIB}=0.0067, $p_{IIIAIIIB}$=0.0057) (Table III).

Patients in all groups had significantly higher urinary VEGF level when compared to control group (KW H=43.134, p=0.0003, p_{cI}=0.0003; p_{cIIA}=0.0000; p_{cIIB}=0.0000; p_{cIIIA}=0.0022; p_{cIIIB}=0.0003). However, VEGF in patients showed decreasing trend that became statistically significant in children with VUR and RS when compared to children with VUR no RS, but exceeded data of indicators in children of control group (MW p_{IIAIIB}=0.0354, $p_{IIIAIIIB}$=0.0101, p_{cIIB}=0.0000, p_{cIIIB}=0.0003).

The obtained data became the reason for the determination of the urine VEGF by logistic regression using the ROC-analysis method, with the determination of the specificity and sensitivity of the method with the subsequent prediction of RS in children with VUR.

The obtained data became the reason for the determination of the urine TGFβ1 and VEGF by logistic regression using the ROC-analysis method, with the determination of the specificity and sensitivity of the method and subsequent prediction of RS in children with VUR. The area under the ROC curve (AUC) for TGF-β1 was 0.906. The optimal «cut-off» for TGF-β1 was >109.9 pg/ml (sensitivity, 88.5 %; specificity, 89.1 %) (Fig. 1). The area under the ROC curve (AUC) for VEGF was 0.932. The optimal «cut-off» for VEGF was <207.6 pg/ml (sensitivity, 100.0 %; specificity, 74.4 %) (Fig. 2).

DISCUSSION

In our study, the age group of under 6 children was prevailed (59.6%, $p<0.05$) that corresponds to statistics on age distribution of patients with VUR [17, 18]. At distribution for sex, irrespective of age in all groups of the examined children the girls prevailed over the boys ($p<0.05$). Similar to results of other published studies in the literature [19].

According to M. Życzkowski et al., there is an increased risk of permanent kidney damage, especially at high grade of VUR [20]. Scientists of the different countries reported, that children with grade III or IV, V reflux are more likely to have larger RS [21, 22]. We established that risks of emergence of RS in children with high grade of VUR (III, IV, V) are eight times higher, than children with VUR of I-II grades. In accordance with the literature, this has confirmed the hypothesis that as VUR stage increases, the risk for RS also increases.

In our study, urinary TGF-β1 level was found to be increased in patients with VUR compared to the control group. Urinary TGF-β1 levels in children with RS ware significantly higher compared to patients no RS. Merrikhi A. showed a relationship between urin TGF-β1 and RS and emphasized the importance of this indicator [23]. Silva A. et al. reported that, urinary levels of TGF-β1 were significantly higher in patients with reduced DMSA uptake on technetium-99m DMSA scintigraphy [24]. In the context of its known profibrotic effects, these findings suggest that TGF-β1 contributes to chronic tubulointerstitial fibrosis.

The results of present study show that urinary VEGF levels were higher in all patient with VUR compared to health children. Indicators of urine excretion of VEGF in children with RS, compared to indicators of patients no RS, were significantly higher, however exceeded indicators in children of control group that can point to remodeling of a kidney blood-flow even in patients no RS. Avgustin N. et al. noted a role of VEGF as early predictor of progressing of CKD in patients with glomerular diseases and defined that increase in VEGF levels provides progressing of renal scarring [27].

The conducted ROC-analysis allowed to determine the "cut-off" value of TGF-β1 and VEGF in which the most likely development of renal scaring in children with VUR is still in the onset of the disease.

CONCLUSIONS

Our study suggests that the established urine excretion level of fibrogenesis and angiogenesis, can be used as non-invasive test for monitoring RS associated with VUR. That will help to prevent the development RS, to define tactics of treatment and observation of patients with VUR.

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The Authors declare no conflict of interest.

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