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Features of systemic immune-inflammatory response in children with pyelonephritis depending on the manifestations of undifferentiated connective tissue dysplasia

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Abstract
Urinary tract infections are the most common in children under the age of 3 years ranking second among all infectious diseases. Pyelonephritis is a nonspecific bacterial inflammation of the kidney with predominantly focal tubulointerstitial damage and severe involvement of the renal calyces and pelvis. Pyelonephritis which occurs in children with modified somatic status is of special interest. Particular attention should be paid to undifferentiated connective tissue dysplasia (UCTD). The aim of the research was to study the effect of underlying UCTD on the features of systemic immune-inflammatory response in children with pyelonephritis. 160 children at the age of 3-15 years (the average age - 8.6±1.4 years) with pyelonephritis underwent clinical and laboratory examinations. There were 80 children with manifestations of UCTD and 80 children without manifestations of connective tissue dysplasia. The analysis of paraclinical indicators of systemic immune-inflammatory response in children with pyelonephritis revealed their significant activation in patients with underlying UCTD as well as in patients without it. The maximum deviations of the given parameters from normal values were detected in younger children ($p<0.05$). With age, the signs of cytokine burst reduced, however, they prevailed among children with UCTD of all age groups. The levels of tumor necrosis factor (TNF-α) and interleukin 8 (IL-8) increased in all age groups compared to healthy children ($p<0.001$). In children of both groups, alongside with the activation of pro-inflammatory response, an anti-inflammatory interleukin 10 (IL-10) increased as well ($p<0.001$). Thus, the severity of systemic immune-inflammatory response was higher in children with pyelonephritis and UCTD manifestations.

Keywords: Pyelonephritis, connective tissue dysplasia, cytokines

1. Introduction
Among diseases of the kidneys and urinary tract in children, their bacteria-induced inflammatory lesions are the most common accounting for 77-89% of all pediatric hospitalizations to the nephrological department thereby ranking second and third in the reported morbidity after respiratory diseases [1, 15]. In Ukraine, according to statistical data, the incidence rate of chronic pyelonephritis (PN) in 2013 was 5.51 cases per 1,000 children, while in Ivano-Frankivsk region it was 6.27 cases per 1,000 children.

PN which occurs in children with modified somatic status is of special interest. Particular attention should be paid to undifferentiated connective tissue dysplasia (UCTD). According to E.V. Zemtsovskyi (2000), UCTD is a separate nosologic syndrome which combines the external phenotypic features of connective tissue dysplasia and pathological changes in one or several internal organs developing due to a large number of different multifactorial and multilevel effects on the body. The prevalence of UCTD among children and adolescents is 9-80% depending on age, sex, ethnic and clinical study groups; however, in practice, it is diagnosed in 1.8-2.4% of cases only [10].

The presence of underlying UCTD determinates the features of developing various somatic diseases as well as changes their pathomorphism, manifestations and clinical course thereby requiring a modified strategy of clinical observation, prognosis of the clinical course and selection of treatment tactics [12, 13, 14].

In literature, there are currently only a few reports dealing with the features of the mechanisms of the development and clinical manifestations of local inflammatory process in the kidneys and its systemic manifestations in children on the background of connective tissue dysplasia [2, 3, 11]. Our research is devoted to the study of this problem.

2. The Aim of the Research

The aim of the research was to study the effect of underlying UCTD on the features of systemic immune-inflammatory response in children.

2.1. Materials and Methods

160 children at the age of 3-15 years (the average age - 8.6±1.4 years) with PN underwent clinical and laboratory examinations. All the children were divided into two groups: Group I included 80 children with manifestations of UCTD; Group II included 80 children without manifestations of connective tissue dysplasia. Among patients of Group I, there were 20 children under the age of 6 years, 31 children at the age of 6-12 years and 29 children older than 12 years of age. Among patients of Group II, there were 21 children under the age of 6 years, 29 children at the age of 6-12 years and 30 children older than 12 years of age. The control group included 20 apparently healthy children of the same age. Among patients of both groups, girls predominated (93, IP=58.1)

The diagnosis of PN was verified in accordance with the Order of Ministry of Health of Ukraine of November 03, 2008 No 627 "Protocol for treatment of children with urinary tract infections and tubulo-interstitial nephritis" [9]. The presence of UCTD was determined according to screening diagnostics proposed by T. Miolkovska-Dmitrova and A. Karkasheva (1985) being completed by T.I. Kadurina and V.N. Gorbunova [8].

The serum level of C-reactive protein (CRP) was determined applying the latex agglutination method using a reagent kit "Granum" (Ukraine) according to the method of the manufacturer.

Quantitative evaluation of inflammation was obtained according to the parameters of the leukocyte intoxication index (LII) proposed by Ya. Kalf-Kalif. The latter one is the ratio of cells the number of which increases in inflammation [neutrophilic leukocytes – myelocytes, juvenile cells (metamyelocytes), band cells, segmented cells] to cells which may decrease in the number in case of inflammation [lymphocytes, monocytes, eosinophils]: the LII=(4M+3JC+2BC+SC)x(PC+1):(L+MO)x(E+1), where M - myelocytes, JC - juvenile cells, BC - band cells, PC - plasma cells, SC - segmented cells, L - lymphocytes, MO - monocytes, E - eosinophils. In healthy people, the LII is within the range of [0.3 -1.0].

The increase in the index to 2.0 - 3.0 conditional units is known to be an indicator of body intoxication as well as already formed infectious process. The value within 4.0-0.0 conditional units indicates the predominant bacterial component of intoxication. The decrease in the LII during treatment under conditions of inflammation indicates an appropriate therapeutic tactics and rapid clinical improvement. The increase in the LII in positive clinical dynamics indicates insufficient effectiveness of therapeutic measures and possible clinical deterioration in the patient's condition.

Cytokine levels (IL-8, IL-10, TNF-α) were determined using a reagent kit "Vector-Best" (Russia); the results were evaluated according to the method of the manufacturer.

To assess the confidence level of the results objectively, variational statistical analysis of the obtained results using the Pentium II processor, the Statistica 8.0 software package and the Microsoft Excel package was applied. The parametric data were presented as M±m. The dynamics within the groups was assessed using the paired t-test (the Student's t-test). During the statistical processing of the data, the arithmetic mean (M), the standard deviation (δ), the standard error of the arithmetic mean (m), statistical significance of research results (p) were calculated. To determine the correlations between individual variables, the Pearson correlation coefficient (r) was calculated. The obtained indicators were converted to SI units.

3. Results and Discussion

The analysis of anamnestic data revealed that within a year the exacerbation rate of PN in children with manifestations of connective tissue dysplasia was 3.28±0.09 events per year, while in children without manifestations of UCTD - 2.05±0.09 events per year. Clinical manifestations of exacerbated PN in patients of both groups included mainly toxic and pain syndromes. Toxic syndrome included hyperthermia, headache, hyperhidrosis, low appetite, nausea, vomiting. However, their severity depended on the child's age as well as the presence of underlying UCTD (Table 1). In particular, in patients with modified somatic status, PN was significantly more common accompanied by hyperthermia (52.5% versus 33.7%, $p<0.05$), low appetite (83.8% versus 38.8%, $p<0.05$), headache (86.3% versus 43.8, $p<0.05$), hyperhidrosis (63.8% versus 47.5%, $p<0.05$). With age, the reduction in the number of children with symptomatic toxic syndrome was observed in both Group I and Group II. Pain syndrome manifested itself as intermittent abdominal pain and sudden lumbar pain. The symptoms of pain and abdominal syndromes prevailed significantly among patients with underlying UCTD ($p<0.05$) with a clear tendency towards the predominance in younger children ($p<0.05$). In addition, the severest lumbar pain was observed in children of both groups being older than 12 years of age ($p<0.05$).

The analysis of paraclinical indicators of systemic immune-inflammatory response in children with PN revealed their significant activation in patients with underlying UCTD as well as in patients without it (Table 2). Thus, the analysis of the LII revealed its increase in children of all age groups ($p<0.001$) with no significant difference between them except Group I (in children under the age of 6 years, the LII was higher as compared to children older than 12 years of age, $p<0.001$). In children with manifestations of connective tissue dysplasia, the LII was significantly higher as compared to children without them: in children under the age of 6 years, it was twice higher ($p<0.001$); in children at the age of 6-12 years, the index was 1.6 times higher ($p<0.05$); in children older than 12 years of age, the LII was 1.2 times higher ($p<0.05$).

One of the acute-phase markers of inflammation possessing the most informative value is CRP. The serum level of CRP increased significantly in children of all age groups with no significant difference between them in the presence of UCTD manifestations as well as in their absence ($p<0.001$). Nonetheless, CRP was predominantly detected in younger children with underlying UCTD.

The analysis of cytokine status in children with PN revealed the increase in all studied parameters in patients with PN as compared to those in healthy individuals ($p<0.05$). However, qualitatively unidirectional changes were quantitatively unequal. Thus, there was observed a clear tendency to the predominance of IL-8, IL-10 and TNF-α in patients with underlying UCTD of all age groups. The maximum deviations of the given parameters from normal values were detected in younger children ($p<0.05$). With age, the signs of cytokine

burst reduced, however, they prevailed among children of all age groups belonging to Group I.

Elevated serum level of IL-8 is an objective criterion for inflammation. The increase in the serum levels of IL-8 by 3.3 times was observed in children under the age of 6 years belonging to Group I [to (89.86±1.78) pg/ml (p<0.001)] and by 2.3 times in Group II [to (75.47±2.05) pg/ml (p<0.001)] as compared to the control group; the parameters were significantly higher than those in children with manifestations of connective tissue dysplasia (p<0.001).

With age, the serum level of IL-8 in children with UCTD manifestations tended to reduce: from (89.86±1.78) pg/ml in children under the age of 6 years to (81.08±2.95) pg/ml (p<0.05) in children at the age of 6-12 years and to (74.75±1.89) pg/ml (p<0.001) in children older than 12 years of age. Elevated serum level of IL-8 was observed in children of the control group as well. It increased by 3.3 times in children under the age of 6 years (p<0.001), by 2.9 times in children at the age of 6-12 years (p<0.001) and by 2.7 times in children older than 12 years of age (p<0.001).

Similar, but less pronounced intensity of pro-inflammatory response of the body was observed in children of Group II: the levels of TNF-α and IL-8 increased in all age groups compared to healthy children (p<0.001). In children under the age of 6 years, the serum level of TNF-α was the highest [(47.41±1.69) pg/ml] with a further reduction: in children at the age of 6-12 years, it reduced to (42.17±1.54) pg/ml (p<0.001) and in children older than 12 years of age, it reduced to [(40.09±2.43) pg/ml (p<0.001)]. The serum levels of IL-8 reduced with age as well: from (75.47±2.05) pg/ml in children under the age of 6 years to (57.91± 3.02) pg/ml (p<0.001) in children older than 12 years of age.

In children of both groups, alongside with the activation of pro-inflammatory response, the anti-inflammatory defense increased as well: the level of IL-10 increased significantly in all age groups (p<0.001). However, the reduction in anti-inflammatory response with age was observed in children of both group (regardless of the presence of UCTD signs). The levels of IL-10 reduced significantly: [from (59.51±1.76) pg/ml to (43.42±1.84) pg/ml (p<0.001)] in Group I and [from (49.53±2.98) pg/ml to (36.64±1.25) pg/ml (p<0.001)] in Group II – in children under the age of 6 years and those older than 12 years of age, respectively.

Such features of cytokine response in children of different age groups can be explained by the fact that in chronic inflammatory processes, TNF-α is produced continuously thereby inhibiting the activity of IL-10 that results in immunological imbalance as well as the prolongation of the inflammatory process. Therefore, with increasing duration of disease the activity of IL-10 reduces. Low CRP levels serve as an evidence of the depletion of body reserves in patients with chronic inflammatory diseases.

There was a strong positive correlation between IL-8 and CRP in the presence of UCTD signs (r=+0.93, p<0.001) as well as in their absence (r=+0.98, p<0.001). A strong inverse correlation between pro-inflammatory (IL-8) and anti-inflammatory (IL-10) cytokines (r=-0.93, p<0.001) was observed; a moderate negative correlation (r= -0.64, p<0.05) was observed in children of Group II (Fig. 1, 2).

Table 1: Clinical characteristics of children with pyelonephritis depending on their age and the presence of UCTD signs

	UCTD								without UCTD							
	Total number, n=80 (1)		3-6 years of age, n=20 (2)		6-12 years of age, n=31 (3)		older than 12 years of age, n=29 (4)		Total number, n=80 (5)		3-6 years of age, n=21 (6)		6-12 years of age, n=29 (7)		older than 12 years of age, n=30 (8)	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Low-grade fever	38	47.5*	7	18.4	15	39.5'	16	42.1^	53	66.3	12	22.6	18	33.9	23	43.3ǂ
Fever	42	52.5 *	17	40.5	14	33.3	11	26.2	27	33.7	13	48.1	8	29.6	6	22.2ǂ
Low appetite	67	83.8*	28	41.8	21	31.3	18	26.9	31	38.8	14	45.2	9	29.0	8	25.8
Headache	69	86.3*	27	39.1	23	33.3	19	27.5	35	43.8	15	42.9	11	31.4	9	25.7
Hyperhidrosis	51	63.8*	24	47.1	14	27.5'	13	25.5^	38	47.5	18	47.4	10	26.3	10	26.3
Nausea	74	92.5	10	13.5	34	45.9	30	40.5	63	78.6	9	14.3	25	39.7•	29	46.0
Vomiting	52	65.0*	28	53.8	13	25.0'	11	21.2^	35	43.8	20	57.1	9	25.7•	6	17.1
Intermittent abdominal pain	50	62.5*	32	64.0	10	20.0	8	16.0	27	33.8	16	59.3	6	22.2•	5	18.5ǂ
Sudden lumbar pain	74	92.5	18	24.3	26	35.1	30	40.5^	68	85.0	14	20.6	25	36.8•	29	42.6

Notes: * statistical significance between groups p1-5 <0.05;
' statistical significance between groups p2-3 <0.05;
^ statistical significance between groups p2-4 <0,05;
• statistical significance between groups p 6-7, 6-8 <0.05;
ǂ statistical significance between groups p 6-7, 6-8 <0.05;

Table 2: Parameters of systemic immune-inflammatory response in children with pyelonephritis depending on the signs of undifferentiated connective tissue dysplasia

Parameter	Healthy children	UCTD			without UCTD		
	n=20 (1)	3-6 years of age, n=15(2)	6-12 years of age, n=12(3)	older than 12 years of age, n=11 (4)	3-6 years of age, n=14 (5)	6-12 years of age, n=12 (6)	older than 12 years of age, n=12 (7)
TNF-α, pg/ml	14.55±0.35	62.93±3.95*	56.83±3.65*	54.75±3.56*	47.41±1.69ǂ*	42.17±1.54*	40.09±2.43ǂ*
IL-8, pg/ml	27.31±0.39	89.86±1.78*	81.08±2.95*	74.75±1.89◊*	75.47±2.05ǂ*	65.33±4.18*	57.91±3.02◊*
IL-10, pg/ml	23.42±0.62	59.51±1.76°*	50.92±2.01ǂ*	43.42±1.84◊*	49.53±2.98ǂ*	40.33±2.27*	36.64±1.25◊*
CRP, g/l	2.25±0.16	14.05±1.02ǂ*	12.38±0.32*	11.27±0.61*	10.12±0.72*	9.72±0.61*	8.24±0.68*
LII	0.73±0.06	6.45±0.38*	5.61±0.34*	5.53±0.49*	4.55±0.51*°	4.38±0.49*ǂ	3.95±0.49ǂ*

Notes:
1.* - probability of difference between the given indicator and that in healthy children p<0.001;
2.◊ p 2-4, 5-7 <0.001;
3. ° p 2-3<0.01;
4. ǂ p 2-4, 5-6, 5-7 <0.05.

Parameter	TNF-α	IL-8	IL-10	CRP	LII
TNF-α		+0.94	-0.75	+0.86	+0.97
IL-8			-0.93	+0.93	+0.86
IL-10				-0.59	-0.43
CRP					+0.81
LII					

Fig 1: Correlation matrix between the parameters of systemic immune-inflammatory activity and the parameters of systemic inflammation in children with pyelonephritis with the signs of undifferentiated connective tissue dysplasia

Parameter	TNF-α	IL-8	IL-10	CRP	LII
TNF-α		+0.89	-0.38	+0.97	+0.84
IL-8			-0.64	+0.98	+0.77
IL-10				-0.43	-0.21
CRP					+0.79
LII					

Fig 2: Correlation matrix between the parameters of systemic immune-inflammatory activity and the parameters of systemic inflammation in children with pyelonephritis without the signs of undifferentiated connective tissue dysplasia

4. Conclusions

1. In patients with underlying UCTD, the clinical course of PN was accompanied by more symptomatic nonspecific inflammatory toxic syndrome the manifestations of which were the most pronounced in younger children.
2. In children with PN of all age groups, there was observed a significant increase in proinflammatory cytokines (TNF-α, IL-8, $p<0.001$) as well as anti-inflammatory cytokine IL-10 indicating the activation of the immune system. With age, in the course of the prolongation of the inflammatory process, the activity of anti-inflammatory response reduced (the level of IL-10 reduced in older children).
3. The severity of systemic immune-inflammatory response was higher in children with PN and UCTD manifestations which can be attributed to more pronounced structural and morphological changes in the focus of inflammation.

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