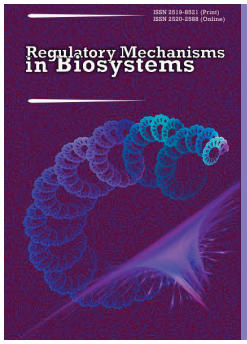

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Peculiarities of leukocyte apoptosis modulation in children with pyelonephritis

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One of the leading places among inflammatory diseases of the urinary tract of children belongs to pyelonephritis, the course of which presents in most cases as a severe infectious disease threatening the patient's life, which is the main reason for development of chronic kidney failure. This study was conducted to compare apoptosis stages in peripheral blood of children of different age categories with pyelonephritis depending on etiological factor and complications. The problem of mechanisms underlying immune system misregulation, especially functional activity of leukocytes in children with pyelonephritis, have not been explored in recent years. Assessment of leukocytes (neutrophils) apoptosis stages in peripheral blood of children of different age categories with pyelonephritis depending on complications and etiological factor was the aim of present study. The children's peripheral blood samples were analysed and assessed using a flow cytofluorimeter. The present study demonstrates an increase of the level of apoptotic cells at an early stage of apoptosis in children of all age categories with chronic pyelonephritis, which can be explained by associations of a wide range of pathogens and the presence of sequelae. An increase in the number of apoptotic cells in the late stage of apoptosis is observed in children aged 1 month – 8 years, in children 8–18 years, the amount of apoptotic cells is reduced by 1.5 times. The study of apoptosis stages allows complete characterization of the dynamics of the apoptotic process and supplementation of the pathogenesis of pyelonephritis in children. Such studies will make it possible to affect apoptosis modulation to regulate or correct it and encourage the finding of innovative solutions in the treatment related to influence on the immune response. We conclude that enhancement of peripheral blood leukocyte apoptosis in chronic form of pyelonephritis especially in young children is due to the polyetiology of this form of pyelonephritis and the development of complications.

Keywords: Child age categories; sequelae; immune blood cells; flow cytometry; urinary tract infections.

Introduction

Among inflammatory diseases in children, urinary tract pathology takes the first place by the frequency of disease detection. One of the prominent places belongs to pyelonephritis (Chen et al., 2006; Spencer et al., 2014; Whiteside et al., 2015; Olson, 2016). Acute pyelonephritis (APN) in most cases is a severe infectious disease posing a threat to the patient's life and is one of the prominent reasons for chronic pyelonephritis (CPN) development and chronic kidney failure. In the complicated course when urinal sepsis progresses against the background of purulent pyelonephritis, mortality reaches 80% (Greineder et al., 2007; Wang et al., 2008; Oktem et al., 2009). The wide distribution of acute pyelonephritis, its frequent transformation to chronic form, low efficiency of treatment of chronic forms, high incidence among women of childbearing age, which limits their reproductive abilities and is the reason for the birth of children predisposed to kidney disease – all this determines the great medical and social importance of the problem – diagnostics and treatment of acute pyelonephritis (Zhanga et al., 2001; Delogue et al., 2008).

In recent years, the idea of the mechanisms of programmed cell death has changed dramatically, which allowed us to isolate phagocytosis, necrosis and apoptosis into independent forms, mediated by the presentation of cluster molecules or death signals on the cell membrane. Thus, of particular importance is the discovery of the phenomenon of cell apoptosis, the results of which established that a genetic program in the cells of the body that provides their life cycle, under certain physiological or pathological conditions triggers the process of apoptosis, programmed cell death. There are a number of signaling molecules, most of which regulate other important functions of the body (Ostapchenko et al., 2010).

The apoptosis mechanisms are very complex, including a variable cascade of molecular events. Research demonstrates that there are two basic pathways of realization of apoptosis: the extrinsic way or way of receptor of death and the intrinsic or mitochondrial way. There is proof that these two pathways are united and that the molecules of one way can influence those of the other. There is an additional way that includes the T-cell mediated cytotoxicity and perforin-granzyme dependent cellular elimination (Elmore et al., 2007).

The ability of the host to feel encroachment of pathogenic organisms and to react adequately in the fight against an infection has an important value for survival. The innate immune system developed as a primary protection of the organism from invading microorganisms. Toll-like receptors (TLRs) are part of innate immune defence. There are ten member families of TLR, that are identified in humans, and some of them recognize specific microbial products, such as bacterial DNA, peptidoglycan, lipopolysaccharides, and bacterial lipoproteins.

A transmission of signals of TLR is the basis of innate immune response to a microbiosis. These receptors are expressed in tissues participating in an immune function (leucocytes of spleen and peripheral blood, lungs, gastrointestinal tract). It is known that after activation of TLR a wide spectrum of proinflammatory cytokines are secreted (interleukin-1, tumour necrosis factor), profibrogenic mediators, the different components of cellular microorganism, in particular, monocytic chemotactic protein-1, and growth factors. This effect increases the freeing of numerous oxygen radicals and lipid mediators. On the whole, these factors result in development of local damage and participate in progress of sclerotic changes in kidney tissue. TLR4 is mainly activated by a lipopolysaccharide. TLR5 senses bacterial flagellin (Hennessy et al., 2010).

CD45 is an evolutionarily extremely well preserved tyrosine phosphatase receptor protein, which is exclusively expressed on all the nucleated cells of the hematopoietic system. CD45 is one of the key players in the administration of the T lymphocyte receptor, which signals the activation of protein tyrosine kinases Src Lck and the Fyn families. T and B lymphocytes dysfunction can be caused by the decline in CD45 levels. Such a deficiency is important in infectious diseases (Rheinländer et al., 2018). Chronic kidney disease is determined according to the presence or absence of kidney damage and the level of kidney function regardless of the type of kidney disease (diagnosis). Among individuals with chronic renal disease, the stages are determined based on the level of renal function, in particular the glomerular filtration rate (GFR). So there are 5 stages of chronic kidney disease according to the American Kidney Foundation classification (G1–G5, K/DOQI clinical practice guidelines for chronic kidney disease: evaluation, classification, and stratification).

Teixeira et al. (2013) reported an investigation on whether there was a confirmed decline of kidney function which correlated with the changes of levels of $CD4^+$ T, $CD8^+$ T and B cells in older patients. It is possible to observe that G3a (GFR = 45–59 mL/min/1.73 m^2) under light to moderate chronic kidney insufficiency in accordance with the classification of The American Kidney Foundation was related to considerably lower median percentage of $CD8^+$ T-cells, while substantial distinctions were not discovered in regard to the percentage of $CD4^+$ T-cells, percentage of B-cells ($CD19^+$) or correlations of CD4/CD8 between individuals with similar kidney functions. And also a tendency was observed towards a higher median percentage of cells of $CD4^+$ and $CD19^+$ in persons with G2 (GFR = 60–89 mL/min/1.73 m^2). This stage was slightly lower compared with the percentage in individuals with the G3a stage. Patients had median correlation of CD4/CD8 higher with the G2 stage.

Today the problem of mechanisms underlying regulation of the immune system, especially functional activity of leukocytes in children with pyelonephritis remains relevant (Roger et al., 2010; Tittel et al., 2011).

Adjustment of tyrosine phosphorylation is provided by the mutual action of protein tyrosine kinases and phosphatases. CD45 first and prototype of prototype receptor-like tyrosine phosphatase protein, is expressed on all nucleated hematopoietic cells and plays a central role in this process. The latest achievements showed that modulation of CD45 function can have therapeutic advantages with many diseases (Hermiston et al., 2003).

In the literature we come across strong evidence of interconnection between violations of apoptosis processes and pyelonephritis development, which leads to decrease in immune response efficiency and manifested depression of immune mechanisms connected with induction of apoptosis in peripheral blood lymphocytes (Maheswari et al., 2013; Anis, 2016). Apparently excessive accumulation of apoptosis cells due to their utilization by phagocytes with the complement participation leads to antigens of cells being identified as alien, change in the spectrum of dominant humoral factors with increase in anti-inflammatory cytokines fractions capable of long-term maintenance of immune response (Marius, 2005; Joshi et al., 2015).

Saad et al. (2014) reported that the average amount of circulatory CD3 T-lymphocytes was significantly reduced in patients as compared to healthy controls. In addition, the number of CD4 cells and the ratio of CD4 cells to CD8 were considerably mionectic in the group with ESRD (End Stage Renal Disease). But the CD8 T-cells in the group with ESRD did not differ significantly from those observed in the control group. Among the subsets of cells B; there was a significant decrease in the total number of cells ($CD19^+$) B, innate B1 cells ($CD19^+$, $CD5^+$), ordinary B2 cells ($CD19^+$, $CD5^-$) and memory B cells ($CD19^+$ $CD27^+$) in children with ESRD were comparable to healthy people from the control group. Apoptosis of B-lymphocytes and T-lymphocytes in ESRD patients shows that there were significant increases in T and B-lymphocytes as compared to control.

Though problems of leukocytes (neutrophils) programmed cellular death in children with pyelonephritis depending on age and complications remain out of focus, in this connection there is no doubt that study of neutrophils apoptosis stages and programmed cellular death induction mechanisms will help to complete the picture of pyelonephritis pathogenesis peculiarities in children. Thus study of leukocytes apoptosis modulation in children of various age categories is a promising area which ensures elimination of potentially dangerous cells, damaged by the organism of children.

The purpose of the given study is the assessment of periphery blood leukocytes (neutrophils) apoptosis in pyelonephritis in children of various age categories depending on etiological factor and complications.

Materials and methods

The study was approved by the ethics committee of the Kharkiv National Medical University, Ukraine. Information consents were signed by all participants of the study before it began. All participants or their parents gave written informed consent. The study involved the microbiological analyses of *E. coli*, *E. faecalis*, *K. pneumoniae*, *P. mirabilis*, *P. aeruginosa* and other strains isolated from the urine of 83 children with acute and chronic pyelonephritis in the period of exacerbation, aged from 1 month to 18 years, who were examined and treated in the Nephrological Department of Kharkiv Clinical Children's Hospital No. 16. The children were hospitalized between 1st October 2018 and 25th February 2019. Patients with pyelonephritis and co-existing diseases (dysmetabolic nephropathy, renal cyst, pyelectasis, hydronephrosis) were included in this study. Examination of children with pyelonephritis was carried out on the day of the child's admission to the hospital before treatment. Patients with glomerulonephritis, cystitis and other acute or chronic inflammatory diseases and all participants or their parents who did not give written informed consent were excluded from the study.

All patients were divided into three age groups. In each group, patients were also divided according to the diagnosis of acute or chronic pyelonephritis. The I group included children of the age category from 1 month to 3 years and 11 months (n = 37: APN n = 26 and CPN n = 11); the II group included children from 4 to 7 years and 11 months (n = 16: APN n = 10 and CPN n = 6); the III group included children from 8 to 18 years (n = 30: APN n = 16 and CPN n = 14). The diagnosis, criteria of severity of PN, and basic therapy were determined according to the protocol of diagnosis and treatment of children with urinary tract infections using standardized and unified methods.

152 strains of microorganisms were isolated from the patients. Pure cultures of microorganisms were isolated by the bacteriological method. The identifications of pure cultures of *E. coli* (n = 53); *K. pneumoniae* (n = 30), *P. mirabili*s (n = 12) were carried out using Micro-La-Test ENTEROtest, *P. aeruginosa* (n = 5) was identified using NEFERMtest and *E. faecalis* (n = 47) was confirmed by EN-COCCUStest diagnostic kits (ErbaMennheim®) and others (n = 5). All strains of microorganisms were sampled at admission to hospital before the start of antibacterial therapy and isolated from the urine of patients.

Whole blood preparations for determination of leukocytes (neutrophils) apoptosis: 3 mL of blood samples were drawn from the peripheral vein into sterile tubes with K3 EDTA (Vacumed®) after an overnight fast in children with pyelonephritis (n = 83): with acute pyelonephritis (APN) n = 52 and chronic pyelonephritis (CPN) n = 31.

Apoptosis was measured according to the manufacturer's instructions (BD Biosciences). The assessment of cells' apoptosis stages was carried out with simultaneous introduction of Annexin V FITC, CD45 PE and 7-AAD (7-aminoactinomycin D) markers into the sample. All reagents were made by BD, USA.

For this 5 μL of Annexin V and 10 μL of CD45 PE and 7-AAD were added μL resuspended in 1^x solution of Annexin V binding buffer of whole blood and incubated for 15 min at indoor temperature (20–25 ºC) in the dark. 400 μL of 1^x Annexin V binding buffer solution was added to each sample. The samples were analyzed on flow cytofluorimeter "FACS Calibur" ("BD", USA).

Samples were analyzed on a FACS Calibur flow cytometer (BD, USA). For error minimization in the samples 20000 $CD45^+$ cases were analyzed (CD45 positive cells). Measurement results were assessed with the help of "CELLQuestPro" ("BD", USA) software development.

The analysis on Dot plots of the obtained data allows one to identify four different states (type, degree of damage) of cells: 1 – living cells (AnnexinV-7AAD-cells) (left lower quadrant); 2 – cells in the initial (early) stage of apoptosis when the cell membrane is still not broken (Annex-

inV + 7AAD – cells) (left upper quadrant); 3 – dead cells undergoing late apoptosis/necrosis with cell membrane defect (AnnexinV+7AAD+) (right upper quadrant); 4 – dead necrotic cells (AnnexinV-7AAD+) (right lower quadrant). In the assay of leukocytes' apoptosis the following cell populations were found: ANX V-/7AAD-, ANX V+/7AAD-, ANX V+/7AAD+, ANX V-/7AAD+ ,which were regarded as alive, early apoptotic and late apoptotic and necrotic, respectively. The axis on the dot plots is the logarithm of the fluorescence intensity with the minimum at the point "10^0" and the maximum at the point "10^4".

Statistical analysis was performed using Statistica 9 (StatSoft Inc., USA). All groups were subjected to the Gaussian distribution model. We established that the distribution in each apoptosis stage is normal. We assessed data as mean ± SE. The multiple-comparison approach, namely the one-way ANOVA, were used to identify statistically significant differences among the groups. The differences between the groups were considered significant at $P < 0.05$ using the Tukey test.

Results

As the result of the research conducted, the number of living cells in children of various age categories with chronic form of pyelonephritis was found to be reliably lowered, especially in children of the age category 0–3 years and comprised 69.6 ± 0.87%. The amount of leukocytes at the early stage of apoptosis (Fig. 1a, b) in children of the age category 0–3 years (I group of children) with acute form of pyelonephritis was 4.40 ± 0.08% on average; from 4 to 7 years (II group of children) – 3.30 ± 0.06% and from 8 to 18 years (III group of children) – 3.45 ± 0.07% (Fig. 2a, b).

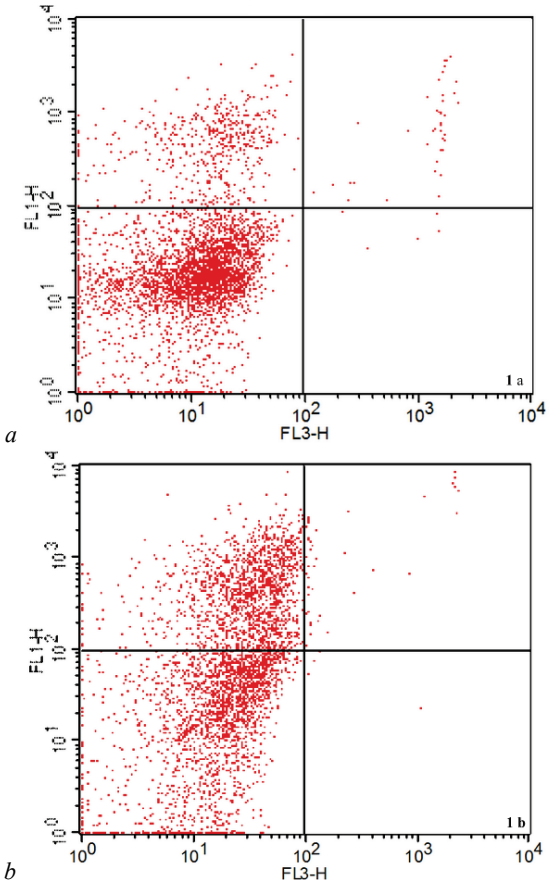

Fig. 1. Assay of leukocytes apoptosis based on ANX V-FITC and 7-AAD in children of the I group with pyelonephritis: *a* – acute form, *b* – chronic form

As grouping factor we used monoculture or mixed culture causing pyelonephritis in children. The multivariate test showed that $P < 0.001$ for acute pyelonephritis and $P < 0.001$ for chronic pyelonephritis ,which rejected a hypothesis that there is no difference between groups.

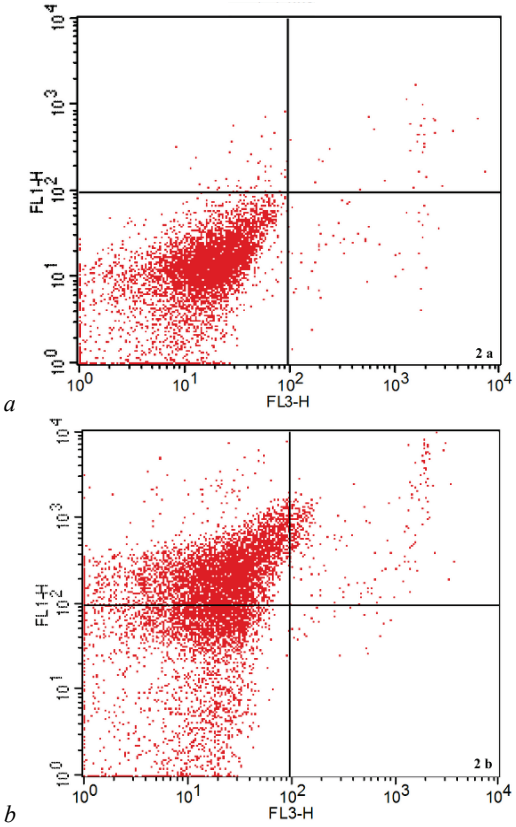

Fig. 2. Assay of leukocytes apoptosis based on ANX V-FITC and 7-AAD in children of the III group with pyelonephritis: *a* – acute form, *b* – chronic form

In children with chronic form of pyelonephritis (Table 1) leukocytes rates at apoptosis early stage are significantly high: in the I group of children – 29.3 ± 0.38%, in the II group of children – 23.2 ± 0.43% and in the III group of children – 17.2 ± 0.51%, which can be explained, on the one hand, by polyetiology of pyelonephritis chronic form – such microorganisms associations as *E. faecalis* and *E. coli*; *K. pneumoniae* and *E. coli*; *E. faecalis* and *K. pneumoniae*; *P. mirabilis* and *K. pneumoniae*; *E. coli* and *P. aeruginosa* were most often isolated.

Table 1

The leukocytes percentage involved in different stages of apoptosis in children with pyelonephritis by different age categories

Apoptosis stage	Age categories, n	Acute pyelonephritis			Chronic pyelonephritis		
		I* (n = 26)	II (n = 10)	III (n = 16)	I (n = 11)	II (n = 6)	III (n = 14)
Alive cells	x ± SE	92.9 ± 0.38	96.1 ± 0.45	94.4 ± 0.35	69.7 ± 0.74	76.4 ± 0.55	78.1 ± 0.75
	Min–Max	89.4–97.6	93.2–98.3	92.4–97.8	66.3–74.2	74.7–78.1	74.8–85.8
	P value		0.0078**			0.0032**	
Cells in the initial stage	x ± SE	4.5 ± 0.09	3.3 ± 0.06	3.5 ± 0.07	29.3±0.38	23.3±0.44	17.3±0.51
	Min–Max	3.7–5.6	3.1–3.6	3.1–3.9	26.9–31.2	22.1–24.4	13.4–19.9
	P value		0.0001**			0.0011**	
Cells in late apoptosis	x ± SE	0.9 ± 0.04	1.9 ± 0.04	1.6 ± 0.05	1.5 ± 0.05	1.8 ± 0.04	1.2 ± 0.09
	Min–Max	0.6–1.3	1.1–1.5	1.1–1.9	1.2–1.8	1.7–1.9	0.5–1.9
	P value		0.0001**			0.5386***	
Dead necrotic cells	x ± SE	0.2±0.02	0.3 ± 0.06	0.4 ± 0.02	0.5 ± 0.03	0.4 ± 0.09	0.4 ± 0.06
	Min–Max	0.1–0.4	0.1–0.6	0.3–0.6	0.4–0.7	0.1–0.8	0.1–0.9
	P value		0.0004**			0.8327***	

Note:* – I – age 0–3 years, II – 4–7 years, III – 8–18 years; ** – $P < 0.005$;*** – no significant differences.

On the other hand, the increased leukocytes apoptosis modulation in chronic form of pyelonephritis can be explained by the appearance of complications: thus in children of the I group infection of urinary tract (54.5%), dysmetabolic nephropathy (18.2%), kidney dysplasia (9.1%) were most often diagnosed. In children of the II group – infection of the urinary tract (66.6%), neuro-muscular dysfunction of the bladder

(16.7%), polycystic kidney disease (16.7%), in children of the III group besides infection of the urinary tract (37.7%) dysmetabolic nephropathy (43%), kidney dysplasia (7.1%) and neuro-muscular bladder dysfunction were diagnosed (Table 2).

In young children, complications of acute pyelonephritis were 15.4%, which is 6.5 times less than in chronic pyelonephritis. In children aged 4–7 years, complications occurred in 20% of cases, half of which was pyelectasia and the other half were dysmetabolic nephropathy. In older children with acute pyelonephritis, nearly half the cases of complications involved dysmetabolic nephropathy.

Table 2
Percentage of diseases which complicate the course of pyelonephritis in children in different age

Complication	Age category					
	I*		II		III	
	APN** (n = 26)	CPN** (n = 11)	APN (n = 10)	CPN (n = 6)	APN (n = 16)	CPN (n = 14)
Dysmetabolic nephropathy	11.5	18.2	10.0	0.0	50.0	43.0
Neuromuscular bladder dysfunction	3.9	0.0	0.0	16.7	0.0	7.1
Pyeloectasis	0.0	0.0	10.0	0.0	0.0	7.1
Kidney dysplasia	0.0	9.1	0.0	0.0	0.0	7.1
Polycystic kidney disease	0.0	0.0	0.0	16.7	0.0	0.0
Urinary tract infection	0.0	54.5	0.0	66.6	0.0	35.7
Purine metabolism disorder	0.0	9.1	0.0	0.0	0.0	0.0
Uretrohydronephrosis	0.0	9.1	0.0	0.0	0.0	0.0
Total	15.4	100.0	20.0	100.0	50.0	100.0

Note: * I – age 0–3 years, II – 4–7 years, III – 8–18 years; ** APN – acute pyelonephritis; ** CPN – chronic pyelonephritis.

Discussion

Research of apoptosis stages allows more complete characterisation of apoptosis process dynamics and complete pyelonephritis pathogenesis in children. Study of different apoptosis stages with the help of flow cytofluorimetry allows one to influence its modulation to a certain extent with the purpose of regulation or correction. Definition of the role of apoptosis in leukocytes' disorder in children of various age categories with pyelonephritis promotes the search of new ways of treatment connected with the influence on immune response, efficiency of such medications can be assessed with the help of definition of apoptosis modulation. This study found that the percentage of leukocyte apoptosis (including neutrophil apoptosis) was significantly higher in patients with pyelonephritis than in healthy controls. Moreover, the highest activity of early-stage leukocyte apoptosis is observed in children aged 0–3 years with acute pyelonephritis. At the same time, it was found that in children with chronic pyelonephritis, apoptic leukocyte count, including neutrophils, increased, which can be explained by the polyetiology of the disease and the occurrence of complications.

Some studies have found an increase in apoptosis of monocytes and lymphocytes (Joza et al., 2001) .However, other studies have shown an increase in leukocyte apoptosis (Kumagai et al., 2000) and reduction of apoptosis of B lymphocytes (Mattsby-Baltzer et al., 2012) or no difference in apoptosis (Holmstrom et al., 2000). In general, in this study, the percentages of leukocyte apoptosis at different stages are significantly higher in young children than in older children and contros.

Peculiarities of induction of leukocytes apoptosis of peripheral blood of patients with various forms of acute pyelonephritis were studied by Khodyreva et al. (2010). They found that the purulent complications of acute pyelonephritis were accompanied by a significant increase in the number of apoptotic cells among primary isolated peripheral blood lymphocytes, and in the cultivation of leukocytes revealed significant differences in the increase of the number of apoptotic cells. They also determined their level was higher in patients with purulent complications than in healthy people . In patients with acute pyelonephritis with uncomplicated course of infectious-inflammatory process, the level of leukocyte apoptosis in cultures was not significantly different from the ordinary level. In the available literature, there are many reports regarding the determination of immune cell apoptosis in various purulent-inflammatory processes. Thus, Mayer et al revealed activation of apoptosis in the culture of peripheral blood lymphocytes of patients with chronic renal failure (Meier et al., 2012). Roger and colleagues (2010) reported the change in sensitivity to apoptosis of lymphocytes found during cultivation in septic shock due to acute pyelonephritis. As a rule, only the early apoptosis stage is taken into account in the studies devoted to the study of apoptosis, since, using flow cytometry, only at this stage can apoptotic cells be distinguished from necrotic ones. Therefore, it is known that pyelonephritis is a common cause of sclerostenosis of the renal cortex and development of hypoplastic kidneys in children. Specialists from Stockholm have reported that observed changes in the kidney developed due to pyelonephritis in experimental rats, and it was found that four days after infection there was a temporary increase in apoptotic cells among cortex cells outside the inflammatory areas, but no increase in apoptotic cells was observed 10 days after infection. Only a few apoptotic cells were detected in the kidneys of the control animals. This data suggests that inhibition of cell proliferation and increased apoptosis may contribute to the loss of renal parenchyma after childhood pyelonephritis (Serlachius et al., 2017). The processes of modulation of immune cell apoptosis in pyelonephritis in children depend on many causes, including the action of etiological factors. Scientists in Korea report that the immunopathogenesis of each of the following diseases is different: asymptomatic bacteriuria, cystitis and acute pyelonephritis, which are classified as urinary tract infections. The age predisposition and gender are at the forefront of acute pyelonephritis: most children (over 70–80%) with acute pyelonephritis have an age of 1–2 years, with a predominance of boys. After 1–2 years of age, girls predominante in the structure of morbidity. This finding suggests that the immature immune response of infants may be related to the pathogenesis of acute pyelonephritis.

Researchers have shown that the main etiological factor of acute pyelonephritis is *E. coli*, which together with other uropathogens originate from the normal microflora of the host, which is constantly changing due to environmental factors. Therefore, uropathogens can have characteristics different from those of extraneous bacterial pathogens. Although uropathogens resistant to antibiotics , including strains which produce beta-lactamase of broad spectrum of action, are increaing in Korea and worldwide, insufficiency of treatment is rarely found in immunocompetent children. The immunopathogenesis of acute pyelonephritis remains unknown. Intact bacteria may not be pathogens in renal cell damage; rather, smaller substances produced during bacterial replication can cause renal cell injury and scarring. In addition, substances from host cells, such as proinflammatory cytokines, can be involved in renal cell damage (Kyung-Yil, 2016).

In general, it is well known that an infant's kidney is more vulnerable to infection than an adult kidney and that acute pyelonephritis in infancy and early childhood shows a delay in kidney growth leading to chronic renal failure. To understand the mechanism underlying the damage to the renal cortex, Korean scientists experimentally caused a urinary tract infection, and after histopathological examination, including inflammation, fibrosis, and tubular atrophy, they determined the apoptosis index in the cortical tubules and cortical cells, they found that the number of cells was significantly increased in non-inflammatory areas of the cortex during the first week. The index decreased in the third week, the expression of growth factor protein was localized in the inflammatory region, and expression of the growth factor protein was not observed in the tubules of the non-inflammatory region, which indicates a delay in the growth of kidneys in experimental infant rats. Such a reaction was associated not only with the inflammatory response itself, but also with increased apoptosis of tubular cells in the non-inflammatory region. They revealed that the antimicrobial therapy used does not eliminate inflammation and does not prevent growth retardation (Sung et al., 2000). Thus, evidence of changes in the sensitivity to apoptosis of leukocytes or kidney cells in various forms of pyelonephritis is confirmed by many researchers. It is known that the process of disintegration of apoptotic cells takes a long time and involves a phase of impaired permeability of the cell membrane, called the late stage of apoptosis. In the culture of such cells, the apoatosis index will be greater, the longer their complete disintegration. In addition, it is doubtful that some of the blood leukocytes in the culture die by necrosis. In this

case, swelling and impaired permeability of the cell membrane occurs at an early stage of cell apoptosis, which makes it possible to quantify the presence of necrotic cells. But in various studies these cells are considered a late stage of apoptosis, assuming that cell decay after a violation of the integrity of the cell membrane during apoptosis and necrosis in vitro is not much different and it is almost impossible to assess the contribution of these processes. Therefore, our studies on the stages of leukocyte apoptosis in children of different age categories with acute and chronic pyelonephritis allows us to more fully characterize the dynamics of the apoptotic process and complement the pathogenesis of pyelonephritis in children. The study of the various stages of apoptosis by flow cytofluorimetry can in some way influence its modulation for regulation or correction. Determining the role of apoptosis in leukocyte injury, including neutrophils, in pyelonephritis in children of all ages helps to find new approaches to treatment associated with the immune response, and to evaluate the effectiveness of such drugs by determining the modulation of apoptosis. According to the results of the conducted research the increase of apoptosis cells level on the early stage in all children of various age categories with chronic pyelonephritis is due to associations of agents and presence of complications, in most cases accompanied by urinary tract infection. The increase of apoptosis cells at the late stage of apoptosis can be found in children of the I and II age categories, in children of the III group content of apoptosis cells decreases 1.5 times.

Conclusions

The present study demonstrates the reenforcement of periphery blood leukocytes apoptosis in chronic form of pyelonephritis especially in children of early age, which is related to the polyetiology of this form of pyelonephritis and the presence of sequelae. This study reveals changes that occur at the level of leukocytes (neutrophils) apoptosis by applying the method of fluorescence labeling of CD45 with subsequent separate analysis of them on a flow cytofluorimeter. This technique allows one to evaluate the patterns of the different stages of leukocytes apoptosis in all ages of children with pyelonephritis. As a result expressed readiness for early apoptosis of leukocytes was established in children with chronic pyelonephritis and an increased percentage of apoptotic cells at the late stage of apoptosis in children with pyelonephritis aged from one month to seven years were established. The obtained data indicates that the stimulation of different stages of leukocyte apoptosis depends on the child's age, the form of pyelonephritis and the pathogenicity factors of the causative agent and the type of infectious process.

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