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The role of nitric oxide synthase and cystatin C in the mechanisms of antimicrobial protection in children with urinary tract infections considering the etiological factor

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A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of the article

The aim of the study was to investigate the main etiological factors of urinary tract infections in children, the role of nitric oxide synthase and cystatin C in the mechanisms of antimicrobial protection in children with acute and chronic urinary tract infections.

Materials and methods. The study groups consisted of 84 children (mean age – 10.0 ± 1.3 years). The main group was divided into subgroups: the first subgroup – 17 children with acute pyelonephritis, the second subgroup – 21 patients with chronic pyelonephritis, the third subgroup – 16 patients with acute cystitis, the fourth subgroup – 10 patients with unspecified urinary tract infections.

The control group consisted of 20 relatively healthy children. The levels of inducible NO-synthase (NOS2) and cystatin C were measured by enzyme-linked immunosorbent assay. The etiological pathogen was identified in the urine of 200 patients with urinary tract infections.

Results. *Escherichia* coli was identified as the dominant pathogen in 46.7 % of cystitis patients and in 66.6 % of chronic pyelonephritis patients. The next most frequently detected etiological agent in children with acute (27.3 % of cases) and chronic (25.6 %) pyelonephritis and unspecified urinary tract infection (32.2 %) was *Enterococcus faecium*. *Proteus mirabilis* was found in 26.6 % of patients with cystitis.

The level of NOS2 in all the studied subgroups was significantly higher than that in the control group ($P < 0.01$). A statistically significant increase in the level of cystatin C in the main group ($P < 0.05$) was determined. The cystatin C-to-NOS2 ratios in the studied subgroups were 1.5–2.0 times lower than those in the control group ($P < 0.05$).

Conclusions. The change in the spectrum of pathogens has been determined, which was a premise of the need for constant bacteriological monitoring. The development of the primary inflammatory process in the urinary tract occurred amidst a certain dysfunction of the immune system, which was manifested in an insufficient quantitative response of cystatin C, as well as high serum levels of inducible NO-synthase in the patients.

Key words: children, urinary tract infections, cystatin C, inducible nitric oxide synthase.

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Роль синтази оксиду азоту та цистатину С у механізмах антимікробного захисту в дітей з інфекціями сечовивідної системи, враховуючи етіологічний фактор

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Мета роботи – дослідити основні етіологічні чинники розвитку інфекцій сечовивідної системи у дітей, роль синтази оксиду азоту та цистатину С у механізмах антимікробного захисту в дітей, хворих на гострі та хронічні інфекції сечовивідної системи.

Матеріали та методи. Група дослідження – 84 дитини (середній вік – 10,0 ± 1,3 року). Основну групу поділили на підгрупи: перша – 17 хворих на гострий пієлонефрит; друга – 21 пацієнт із хронічним пієлонефритом; третя – 16 дітей, хворих на гострий цистит; четверта – 10 осіб із неуточненими інфекціями сечовидільної системи.

У контрольну групу включили 20 умовно здорових дітей. Дослідження вмісту індуцибельної NO-синтази (NOS2) та цистатину С здійснили методом імуноферментного аналізу. Етіологічний патоген визначили в сечі 200 хворих на інфекції сечовидільної системи.

Результати. Патоген, що домінує, у 46,7 % хворих на цистит та 66,6 % пацієнтів із хронічним пієлонефритом – *Escherichia coli*. Наступний за частотою виявлення етіологічний збудник у дітей, хворих на гострий (27,3 % випадків), хронічний (25,6 %) пієлонефрит і неуточнену інфекцію сечової системи (32,2 %), – *Enterococcus faecium*. *Proteus mirabilis* виявили у 26,6 % хворих на цистит.

Рівень NOS2 у всіх підгрупах дослідження достовірно вищий за показник контрольної групи ($p < 0,01$). Визначили статистично значуще підвищення вмісту цистатину С в основній групі ($p < 0,05$). Коефіцієнти співвідношення рівня цистатину С щодо NOS2 у групах спостереження нижчі в 1,5–2,0 раза порівняно з групою контролю ($p < 0,05$).

Висновки. Визначили зміну спектра патогенів, що обґрунтовує необхідність здійснення постійного бактеріологічного моніторингу. Розвиток первинного запального процесу в сечовивідних шляхах відбувається на тлі певної дисфункції імунної системи, що виявляється як недостатня кількісна реакція з боку цистатину С, а також на тлі високого вмісту індуцибельної NO синтази в сироватці крові хворих.

Ключові слова: діти, інфекції сечовивідної системи, цистатин С, індуцибельна NO синтаза.

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Urinary tract infection (UTI) is the leading disease in childhood [14], second only to acute respiratory diseases. Research on the bacterial factor spectrum of urinary tract infection and also on the peculiarities of certain parts of the immune system functioning could help to understand the links in the disease pathogenesis. That would also serve to identify the factors that contribute to the chronicity of the process, especially when it occurs without premorbid abnormalities (developmental defects of the system) and, of course, help improve the quality of care for this cohort of children [6].

Despite the fact that bacteria regularly enter the bladder, UTI does not always develop due to the local mechanisms of the bladder wall protection, such as mucus production and secretion of antimicrobial peptides by the uroepithelium, which limit or prevent the attachment of bacteria to uroepithelial cells [10]. Uroepithelium has many antibacterial factors that increase the synthesis and effectiveness of antibacterial factors. Nitric oxide (NO) is one such factor that is an element of innate immune protection [16].

NO is a small hydrophobic molecule with antibacterial properties, which easily penetrates by diffusion through bilayer lipid membranes. NO is formed by the enzyme nitric oxide synthase (NOS) in the reaction when the amino acid L-arginine and oxygen are converted to NO and L-citrulline. There are three main isoforms of NOS derived from individual genes: two constitutive isoforms and one inducible (iNOS) isoform. However, different host cells enzymatically produce NO by inducing NO-synthase (NOS2/iNOS) during infection, and NO plays a key role in the innate immune response [9,17]. Numerous studies also indicate that the toxic radical of NO, produced via the activation of iNOS, plays an important role in protecting the host from bacterial infections, including UTI [10], and iNOS can be induced in a wide variety of immune cells, including macrophages, neutrophils, and epithelial cells. According to data [12], an increase in both NO and iNOS levels was found during bacterial infection in patients with UTI, which is a logical confirmation. But the main source of antibacterial NO is the activity of the host's iNOS [16]. Given the fact that the NO molecule is unstable and has a very short half-life, in order to assess the activity of the "punishing sword of the immune system", it is desirable to study NO by the activity of iNOS [16].

Cystatin C is another important protein for the immune system. Although clinically it is mainly used as a biomarker of renal function [8], more and more scientific evidence suggests that cystatin C is involved in numerous immune processes [13], performs an immunoregulatory role at both the cellular and molecular levels, including antigen presentation, cytokine secretion, nitric oxide synthesis, etc. [19]. Under the influence of various mediators of inflammation, cystatin C, in turn, affects inflammation and the immune response induced by it, which protects the macroorganism from the penetration of microorganisms and parasites that use cysteine proteases to enter the body. Studies in mice have confirmed that cystatin C can significantly induce the formation of NO from macrophages, but its role in NO production depends on the activation of the iNOS pathway [21]. All of the above was the factor that motivated us to conduct this study of the urinary system.

Aim

The aim of the study was to investigate the main etiological factors of urinary tract infections in children, the role of nitric oxide synthase and cystatin C in the mechanisms of antimicrobial protection in children with acute and chronic urinary tract infections.

Materials and methods

We examined 84 children aged 6 to 14 years (the mean age was 10.0 ± 1.3) who were hospitalized to the Zaporizhzhia Regional Children's Clinical Hospital during 2018–2020. The main study group included 64 children with primary urinary tract infections. Patients with urinary tract abnormalities, as well as patients who received antibacterial therapy prior to the experiment, were excluded from the study. The children were divided into groups according to the classification and taking into account the criteria for the diagnosis of UTI, according to the EUA guidelines, 2021 (levels of evidence I, II) [5] and the order of the Ministry of Health of Ukraine No. 627 dated 03.11.2008 [20].

The main group children were divided into four subgroups: the first included 17 children with acute pyelonephritis, the second – 21 patients with chronic pyelonephritis, the third – 16 patients with acute cystitis, the fourth – 10 patients with urinary tract infections unspecified. The control group included 20 relatively healthy children, representative by sex and age, without any inflammatory signs of the urinary system.

The serum NOS2 concentrations in patients included in the study were detected by enzyme-linked immunosorbent assay (ELISA) using a commercial kit for NOS2 (Cloud-Clone Corp., USA).

Measurement of serum cystatin C in patients included in the study was performed using a commercial ELISA kit BioVendor Human Cystatin C (Czech Republic).

Additionally, we determined the presence of the pathogen in the urine of 200 children aged 3 to 18 years (the mean age was 12.0 ± 1.4 years) with UTI who were hospitalized to the Zaporizhzhia Regional Children's Clinical Hospital during 2018-2020. Conventional methods of inoculating urine into ready-made nutrient media (Columbia Blood Agar, BioMerieux, France, and Selective Chocolate Agar, BioMerieux, France) were used to identify bacterial strains. Verification of the pathogen was performed on a microbial detection analyzer "BioMerieux", France. Pathogenic flora was detected in 185 (95.5 %) patients through bacteriological examination, an etiological factor was not identified in 15 (7.5 %), therefore, they were excluded from further participation in the study.

The results obtained were processed by the method of variation statistics using statistical packages Excel and Statistica 13.0 (StatSoftInc., No. JPZ8041382130ARCN10-J). The method of correlation analysis with the calculation of Spearman's rank correlation coefficient was applied. The non-parametric Mann–Whitney test (U) was used to assess differences between indicators. Differences were considered significant at P values of <0.05.

All human studies complied with the ethical standards of the Institutional and National Research Committee and the 1964 Declaration of Helsinki and its subsequent amendments or comparable ethical standards. Informed consent

Table 1. The main pathogens of UTI taking into account the clinical form of the disease

Pathogens/nosological entity	Unspecified UTI, n = 87		Acute pyelonephritis, n = 44		Chronic pyelonephritis, n = 39		Cystitis, n = 15	
	abs.	%	abs.	%	abs.	%	abs.	%
Escherichia coli	44	50.6	27	61.3	26	66.6	7	46.7
Enterobacter cloacae	2	2.3	–	–	–	–	–	–
Enterococcus faecium	28	32.2	12	27.3	10	25.6	1	6.7
Enterococcus faecalis	3	3.4	2	4.5	–	–	–	–
Klebsiella pneumoniae	7	8.1	2	4.5	2	5.1	3	20
Proteus mirabilis	3	3.4	1	2.2	1	2.5	4	26.6

Table 2. The serum levels of NOS2 and Cystatin C in the examined children

Indicator, units of measurement	Control group, n = 20	Study group, n = 64	Subgroup 1, n = 17	Subgroup 2, n = 21	Subgroup 3, n = 16	Subgroup 4, n = 10
NOS2, ng/ml	0.160 (0.100; 0.195)	0.32 (0.24; 0.42)**	0.39 (0.27; 0.45)**	0.36 (0.23; 0.43)**	0.26 (0.20; 0.31)*	0.31 (0.25; 0.41)**
Cystatin C, ng/ml	572 (543.7; 663.2)	738.8 (586.7; 846.3)*	708.3 (580.4; 832.6)*	772.3 (622.3; 851.5)*	718.9 (635.5; 784.2)*	584.6 (536.3; 704.2)
Cystatin C-to-NOS2 ratio, c. u.	3575 (5430; 3400)	2308.75 (2444.60; 2015.00)*	1816.1 (2149.6; 1850.0) **	2145.3 (2705.6; 1980.2)*	2765.0 (3177.5; 2529.6)*	1885.8 (2145.2; 1717.5)**

*: P < 0.05 compared with the control group; **: P < 0.01 compared with the control group.

was obtained from all individual participants included in the study. A complete set of data on children, their parents and physicians confirming the results of this study was not publicly available due to limited initial ethics approvals.

Results

The results of the study on the spectrum of pathogens that dominated as the etiological factor in the development of UTI in children are presented in *Table 1*.

As can be seen from *Table 1*, *Escherichia coli* was the dominant pathogen and occurred with a frequency of 46.7 % in cystitis and 66.6 % in chronic pyelonephritis. Therefore, *Escherichia coli* continued to occupy a leading place in the structure of pathogens, although this prevalence was significantly lower as compared to reports of other authors [7]. According to the results of further ranking of the etiological significance of pathogens, *Enterococcus faecium* took second place, which with varying frequency, but still was the second in the groups of children with acute and chronic pyelonephritis and UTI. At the same time, the analysis showed that children with acute cystitis comprised a distinct group with only 6.7 % of Gram-positive isolates, while all other nosological entities represented Gram-positive flora in 25.6–37.9 % of cases. In our opinion, the obtained fact should be taken into account when prescribing empirical therapy.

The next stage of our work was to study the level of serum iNOS in patients with inflammatory diseases of the urinary tract. The examination results are given in *Table 2*.

As can be seen from the data in *Table 2*, the development of UTI was accompanied by a 2-fold increase in serum NOS2. The data obtained seem logical, especially if we take into account the protective nature of the NOS2 increase aimed at inactivating the bacterial agent. Afterwards, we analyzed the serum NOS2 level, considering the selected groups. As expected, the level of NOS2 in patients of all subgroups was significantly higher than that in the control group (P < 0.01), but without statistical intersubgroup differences.

In the next stage of our study, the cystatin C level was measured as a marker of immune system activation. It should be noted that the results obtained were slightly different from those as to the analysis of the iNOS level. For example, we noted a significantly increased cystatin C level in the main study group (P < 0.05) due to its statistically significant increase in subgroups of children with acute cystitis and chronic pyelonephritis. In children with unspecified UTI, its level did not differ statistically from the control group (P > 0.05).

Based on the data indicating that cystatin C induces NO production by macrophages regardless its inhibitor activity [21], and the realization of this role is due to activation of the iNOS pathway [21], we additionally calculated the cystatin C-to-NOS2 ratio. The data obtained are shown in *Table 2*. As shown in the table, the figures derived by calculating this ratio in the study subgroups were 1.5–2.0 times lower than those in the control. It is worth noting that the highest ratio was revealed in the subgroup of patients with acute cystitis.

Discussion

In general, *Escherichia coli*, of course, remains the leader among the pathogens that cause UTI in children, but the percentage of its detection is much lower (56.2 %) than described in the world literature (80–90 %) [7]. This has been confirmed by the data on 54–67 % of *Escherichia coli* reported by Balighian E., Burke M. in children with UTI [1].

Instead, the role of enterococci in the development of inflammatory diseases of the genitourinary system is underestimated. For instance, according to data published by the American Academy of Pediatrics (Pediatrics in Review, 2018), only 3–9 % of patients had bacteria of the genus *Enterococcus*. Budnik T. V. et al. [3] have found that enterococci accounted for approximately 7 % of cases. Bezruk V. V. [2] has revealed that cocci caused UTI from 1 % to 12 % of cases. At the same time, we have found that bacteria of the genus Enterococcus were the cause of inflammatory diseases of the genitourinary system in 25.6–35.6 % of cases. And only in 6.7 % of patients with cystitis, it was isolated as a leading pathogen. The data obtained indicate that in the case of ineffective traditional initial empirical antibiotic

therapy, the inflammatory process should be suspected to be caused by bacteria of the genus *Enterococcus*. This, in turn, requires appropriate replacement of antibiotic therapy.

The results obtained regarding the role of bacteria of the genus *Klebsiella* have been confirmed in other studies. So, Eric Balighian et al. [1] have shown in a work that it accounts for 6–7 % of UTI cases. But meanwhile, S. Sadeghi-bojd et al. [15] have reported that *Klebsiella* was the leading pathogen in approximately 12 % of patients with UTI. However, T. V. Budnik et al. [3] and J. R. Watson et al. [18] have reported that this pathogen occurred in 2.0 % and 3.3 % of UTI, respectively.

The percentage of *Proteus mirabilis* as an etiological factor of UTI was quite high in patients with cystitis (26.6 %), while it accounted for only 4.5 % in previous studies of G. O. Lezhenko, O. E. Pashkova [11]. In a study of R. Eremenko et al. [4], the percentage of the genus *Proteus isolates* was 11.2 % of all UTI. This suggests that *Proteus mirabilis* remains an atypical microflora in upper UTI but becomes one of the leading pathogens in lower UTI.

Therefore, at the present stage, the microbial spectrum of UTI pathogens is very diverse, and the data of researchers are heterogeneous, and sometimes opposite, indicating the need for constant local bacterial monitoring to improve the effectiveness of antibacterial therapy.

According to the literature, low levels of iNOS are present in the blood under normal physiological conditions, but it is expressed in response, for example, to microbes and/or inflammatory cytokines. After induction, iNOS produces constantly high amounts of NO, which can limit bacterial growth or help to defend against invading pathogens [16]. These data were logically confirmed by the results of our work, as we indicated the increased level of iNOS within 200 % in all studied groups of children in response to bacterial agent contamination.

The aim of the study on the cystatin C level in children of these groups was, first of all, to identify its possible effect on NO generation by activating the iNOS pathway. The comparisons definitely showed the increased level of cystatin C in the main study group. However, we later found that this increase was due to children with chronic pyelonephritis and acute cystitis, while in children of other groups, its values did not differ statistically from those obtained in the control group. Moreover, the calculated ratio showed a relative decrease in the level of cystatin C in relation to the amount of iNOS allowing to predict the activation of other signaling pathways.

It is possible that the lack of quantitative response of cystatin C in acute inflammatory processes of the urinary tract is a prerequisite for the development of bacterial inflammation, especially given its integral role in the immune response, both innate and adaptive immunity. In this context, disorders of expression and localization of cystatin C may be both accidental and effector factors of pathological processes [21].

Conclusions

1. At present, there is a change in the range of pathogens – agents of urinary tract infections in children, and this is a premise of the need for constant bacteriological monitoring for effective antimicrobial therapy.

2. The development of urinary tract infection occurs amidst high levels of serum inducible NO synthase in patients. This reaction is aimed at activating the synthesis of NO in order to limit the bacterial growth and defend against invading pathogens.

3. The development of the primary inflammatory process in the urinary tract occurs amidst a certain dysfunction of the immune system, which is manifested in an insufficient quantitative response of cystatin C, which may be an effector factor in the pathology.

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References

[1] Balighian, E., & Burke, M. (2018). Urinary Tract Infections in Children. *Pediatrics in Review, 39*(1), 3-12. https://doi.org/10.1542/pir.2017-0007
[2] Bezruk, V. V. (2015). Infektsii sechovoi systemy u ditei: etiolohichna struktura, vikovi ta henderni osoblyvosti [Urinary tract infection in children: etiological structure, age and gender characteristics]. *Zdorov'e rebenka – Child`s Health*, (7), 113-117. [in Ukrainian].
[3] Budnik, T. V., & Mordovets, Ye. M. (2016). Rezultaty vyvchennia mikrobiolohichnoho portreta infektsii sechovoi systemy v ditei m. Kyieva ta Kyivskoi oblasti [The results of the study of microbiologic pattern of the urinary tract infection in children from Kyiv and Kyiv region]. *Pochky – Kidneys,* (2), 21-25. [in Ukrainian].
[4] Eremenko, R., Barmatz, S., Lumelsky, N., Colodner, R., Strauss, M., & Alkan, Y. (2020). Urinary Tract Infection in Outpatient Children and Adolescents: Risk Analysis of Antimicrobial Resistance. *The Israel Medical Association journal : IMAJ*, *22*(4), 236-240.
[5] 't Hoen, L. A., Bogaert, G., Radmayr, C., Dogan, H. S., Nijman, R., Quaedackers, J., Rawashdeh, Y. F., Silay, M. S., Tekgul, S., Bhatt, N. R., & Stein, R. (2021). Update of the EAU/ESPU guidelines on urinary tract infections in children. *Journal of pediatric urology*, *17*(2), 200-207. https://doi.org/10.1016/j.jpurol.2021.01.037
[6] Khan, A., Jhaveri, R., Seed, P. C., & Arshad, M. (2019). Update on Associated Risk Factors, Diagnosis, and Management of Recurrent Urinary Tract Infections in Children. *Journal of the Pediatric Infectious Diseases Society*, *8*(2), 152-159. https://doi.org/10.1093/jpids/piy065
[7] Korbel, L., Howell, M., & Spencer, J. D. (2017). The clinical diagnosis and management of urinary tract infections in children and adolescents. *Paediatrics and international child health*, *37*(4), 273-279. https://doi.org/10.1080/20469047.2017.1382046

[8] Lameire, N., Van Biesen, W., & Vanholder, R. (2017). Epidemiology of acute kidney injury in children worldwide, including developing countries. *Pediatric nephrology (Berlin, Germany)*, *32*(8), 1301-1314. https://doi.org/10.1007/s00467-016-3433-2
[9] Lee, M., Rey, K., Besler, K., Wang, C., & Choy, J. (2017). Immunobiology of Nitric Oxide and Regulation of Inducible Nitric Oxide Synthase. *Results and problems in cell differentiation*, *62*, 181-207. https://doi.org/10.1007/978-3-319-54090-0_8
[10] Leung, A. K. C., Wong, A. H. C., Leung A. A. M., & Hon, K. L. (2019). Urinary Tract Infection in Children. *Recent Patents on Inflammation & Allergy Drug Discovery, 13*(1), 2-18. https://doi.org/10.2174/1872213X13666181228154940
[11] Lezhenko, G. O., Pashkova, O. Ye., Rudova, O. I., & Rubanova, Ye. I. (2014). Dosvid zastosuvannia tsefiksymu v likuvanni infektsii sechovoi systemy u ditei. [Experience of Cefixime application for the treatment of urinary tract infection in children]. *Zdorov'e rebenka – Child`s,* (3), 12-16. [in Ukrainian].
[12] Logadottir, Y., Hallsberg, L., Fall, M., Peeker, R., & Delbro, D. (2013). Bladder pain syndrome/interstitial cystitis ESSIC type 3C: high expression of inducible nitric oxide synthase in inflammatory cells. *Scandinavian journal of urology*, *47*(1), 52-56. https://doi.org/10.3109/00365599.2012.699100
[13] Magister, S., & Kos, J. (2013). Cystatins in immune system. *Journal of Cancer*, *4*(1), 45-56. https://doi.org/10.7150/jca.5044
[14] Millner, R., & Becknell, B. (2019). Urinary tract infections. *Pediatric Clinics of North America*. *66*(1), 1-13. https://doi.org/10.1016/j.pcl.2018.08.002
[15] Sadeghi-Bojd, S., Naghshizadian, R., Mazaheri, M., Ghane Sharbaf, F., & Assadi, F. (2020). Efficacy of Probiotic Prophylaxis After The First Febrile Urinary Tract Infection in Children With Normal Urinary Tracts. *Journal of the Pediatric Infectious Diseases Society*, *9*(3), 305-310. https://doi.org/10.1093/jpids/piz025
[16] Svensson, L., Poljakovic, M., Demirel, I., Sahlberg, C., & Persson, K. (2018). Host-Derived Nitric Oxide and Its Antibacterial Effects in the Urinary Tract. *Advances in microbial physiology*, *73*, 1-62. https://doi.org/10.1016/bs.ampbs.2018.05.001
[17] Vumma, R., Bang, C. S., Kruse, R., Johansson, K., & Persson, K. (2016). Antibacterial effects of nitric oxide on uropathogenic Escherichia coli during bladder epithelial cell colonization--a comparison with nitrofurantoin. *The Journal of antibiotics*, *69*(3), 183-186. https://doi.org/10.1038/ja.2015.112
[18] Watson, J. R., Sánchez, P. J., Spencer, J. D., Cohen, D. M., & Hains, D. S. (2018). Urinary Tract Infection and Antimicrobial Stewardship in the Emergency Department. *Pediatric emergency care*, *34*(2), 93-95. https://doi.org/10.1097/PEC.0000000000000688
[19] Xu, Y., Ding, Y., Li, X., & Wu, X. (2015). Cystatin C is a disease-associated protein subject to multiple regulation. *Immunology and cell biology*, *93*(5), 442-451. https://doi.org/10.1038/icb.2014.121
[20] Ministry of Health of Ukraine. (2008, November 3). *Pro zatverdzhennia protokolu likuvannia ditei z infektsiiamy sechovoi systemy i tubolointerstytsialnym nefrytom* [On the approval of the treatment protocol for children with urinary system infections and tubulointerstitial nephritis (No. 627)]. https://zakon.rada.gov.ua/rada/show/v0627282-08#Text
[21] Zi, M., & Xu, Y. (2018). Involvement of cystatin C in immunity and apoptosis. *Immunology letters*, *196*, 80-90. https://doi.org/10.1016/j.imlet.2018.01.006