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The level of antimicrobial peptides in different clinical forms of urinary tract infections in children

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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. To study the content of antimicrobial peptides in the serum of children with urinary tract infections depending on the clinical form of the disease and to establish their pathogenetic role in the development of various clinical forms of pathology.

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 study of the content of cathelicidin, hepcidin and lactoferrin was performed by enzyme-linked immunosorbent assay.

Results. The development of urinary tract infection was accompanied by a statistically significant increase in the content of cathelicidin ($P < 0.05$). The highest level of serum cathelicidin was registered in children of the first ($P < 0.05$) and third subgroups ($P < 0.05$). In the other two subgroups, the level of LL-37 had only a trend towards increasing ($P > 0.05$). The level of hepcidin in the main study group was statistically lower than in the control group ($P < 0.05$).

The development of chronic pyelonephritis and acute cystitis occurred amid a statistically significant decrease in hepcidin levels by 2.5 and 1.7 times ($P < 0.01$ and $P < 0.05$, respectively). The level of lactoferrin in the general group was within the control group figures ($P > 0.05$), however, there was a statistically significant decrease in serum lactoferrin in a subgroup of children with unspecified urinary tract infections ($P < 0.05$).

We determined a relationship between hepcidin and lactoferrin levels in the investigated groups and found a clear direct relationship in a subgroup of children diagnosed with chronic pyelonephritis ($r = 0.58$, $P < 0.01$).

Conclusions. Each nosological form of urinary tract infection has its own configuration of antimicrobial peptides. The analysis of the relationship between hepcidin and lactoferrin, the antimicrobial peptides that limit the access of the pathogens to serum iron, indicates the synchronization of the body's defense mechanisms aimed at eliminating the pathogen.

Key words: children, urinary tract infection, antimicrobial peptides, cathelicidin, hepcidin, lactoferrin.

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Рівень антимікробних пептидів при різних клінічних формах інфекцій сечовивідної системи у дітей

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Мета роботи – дослідити вміст антимікробних пептидів у сироватці крові дітей, хворих на інфекції сечовидільної системи, залежно від клінічної форми захворювання, а також встановити їхню патогенетичну роль у розвитку різних клінічних форм патології.

Матеріали та методи. У дослідження залучили 84 дитини (середній вік – 10,0 ± 1,3 року). Основну групу поділили на підгрупи: 1 – 17 хворих на гострий пієлонефрит; 2 – 21 пацієнт із хронічним пієлонефритом; 3 – 16 дітей, хворих на гострий цистит; 4 – 10 осіб із неуточненими інфекціями сечовидільної системи. Контрольну групу склали 20 умовно здорових дітей. Вміст кателіцидину, гепсидину та лактоферину в сироватці крові пацієнтів, залучених у дослідження, вивчали методом імуноферментного аналізу за допомогою комерційних наборів.

Результати. Виникнення інфекцій сечовидільної системи супроводжувалося статистично значущим збільшенням вмісту кателіцидину ($p < 0,05$). Найвищий рівень сироваткового кателіцидину зареєстрували в дітей першої ($p < 0,05$) і третьої підгруп ($p < 0,05$). У двох інших підгрупах рівень LL-37 мав лише тенденцію до зростання ($p > 0,05$). Рівень гепсидину в основній групі дослідження статистично нижчий за показник контрольної групи ($p < 0,05$).

Розвиток хронічного пієлонефриту та гострого циститу визначали на тлі статистично значущого зниження рівня гепсидину в 2,5 та 1,7 раза ($p < 0,01$ і $p < 0,05$ відповідно). Рівень лактоферину в сироватці крові дітей з основної групи відповідав показникам групи контролю ($p > 0,05$), але виявили статистично значуще зниження рівня сироваткового лактоферину в підгрупі дітей, хворих на неуточнені інфекції сечовидільної системи ($p < 0,05$).

Встановили взаємозв'язок між рівнями гепсидину та лактоферину в підгрупах обстежених дітей. Виявили чітку пряму залежність у підгрупі дітей із діагностованим хронічним пієлонефритом ($r = 0,58$, $p < 0,01$).

Висновки. Кожній нозологічній формі інфекції сечовивідних шляхів притаманна своя конфігурація вмісту антимікробних пептидів. Аналіз взаємозв'язку між гепсидином і лактоферином, тобто антимікробними пептидами, що обмежують доступ патогенів до сироваткового заліза, вказує на синхронізацію захисних механізмів організму, спрямовану на елімінацію збудника.

Ключові слова: діти, інфекція сечовидільної системи, антимікробні пептиди, кателіцидин, гепсидин, лактоферин.

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The problem of urinary tract infections (UTIs) in children, despite the progressive development of science, remains relevant. Among the bacterial diseases of pediatric patients, UTIs occupy a leading position [5] and, according to data [4], accounts for 5 % to 14 % of all visits to the pediatrician and has a clear gender orientation. Thus, in girls, inflammation of the urinary system is more common and is up to 7 years – 7.8 %, and up to 16 years – 11.3 %, while in boys – 1.7 % and 3.6 %, respectively. The high frequency of recurrences, which is 30–50 %, causes some embarrassment [13].

Usually, the recurrent episode of the disease is caused by the same strain of the bacterium as the primary, i. e. pathogenic microorganism persists in the host, despite etiotropic therapy [14]. Combined with rapidly increasing antibiotic resistance, a significant problem in the treatment of infection in the future is growing, which encourages the search for alternative treatments and/or protection of the body.

The innate immune system plays an important role in protecting the urinary tract from infections, both directly and by activating the adaptive immune system. Compared to the adaptive, the innate immune system has a faster response to the penetration of microbial agents [5]. Local defense mechanisms such as the bladder wall and uroepithelium are also important in limiting the attachment and penetration of pathogens [13].

Antimicrobial peptides (AMPs) are a significant component of the innate immune system, produced by immune cells and expressed by epithelial cells throughout the body. These are short, positively charged oligopeptides that have a variety of structures and functions [2]. They provide instant protection due to their antimicrobial activity against a wide range of pathogens, as well as promote immunomodulation or alternative effects on the pathogens [14]. Although AMPs are quite common in the human body, they are quite limited in the urinary tract and the kidneys. Spencer J. D. et al. (2013) in their work indicates that the antimicrobial peptides described in the urinary tract include defensins, cathelicidin (LL-37), hepcidin and ribonuclease 7 as well as proteins that have antimicrobial properties – Tamma–Horsfall protein, lactoferrin and a leukocyte secretory proteinase inhibitor. Bacteria, in turn, have already developed mechanisms to counteract the antimicrobial effect of endogenous antibiotics, but resistance to these AMPs is still ineffective [14].

All of the above was the motive that prompted us to conduct this study.

Aim

To study the content of antimicrobial peptides in the serum of children with urinary tract infections depending on the clinical form of the disease and to establish their pathogenetic role in the development of various clinical forms of pathology.

Materials and methods

Our study included 84 children aged 6 to 14 years (mean age 10.0 ± 1.3), who were hospitalized in the Zaporizhzhia Regional Children's Clinical Hospital during 2018–2020. The main study group consisted of 64 children with primary urinary tract infections. Patients with urinary tract abnormalities, as well as patients who started antibacterial therapy before the study, were excluded from the study. The division of children into groups took place in accordance with the classification and taking into account the criteria for the diagnosis of UTI in accordance with EUA guidelines, 2021 (level of evidence 1, 2) [8] and in accordance with the order of the Ministry of Health of Ukraine No. 627 from 03.11.2008 [18].

The main group (children) was 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 signs of inflammation of the urinary system.

The study of the content of cathelicidin, hepcidin and lactoferrin in the serum of patients included in the study was performed by enzyme-linked immunosorbent assay (ELISA) using commercial kits Hycult Biotech, LL-37, Human, ELISA (Netherlands), Hepcidin-25 (human) (H – 8r, pI) Enzyme Immunoassay Kit: Extraction Free, (USA) and Human LTF/LF (Lactoferrin) ELISA Kit, Elabscience, (USA), respectively. The studies were conducted on the basis of the Training Medical and Laboratory Center of the Zaporizhzhia State Medical University (the Head is professor A. V. Abramov).

The obtained results 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 in performance. Differences were considered significant at $P < 0.05$.

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 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, which consisted in determining the levels of cathelicidin, hepcidin and lactoferrin in the serum of children with urinary tract infections depending on the form of the disease, are shown in *Table 1*.

As can be seen from the data shown in *Table 1*, the development of urinary tract infection was accompanied by a statistically significant increase in the content of cathelicidin ($P < 0.05$) in the serum of the patients under our supervision. At the same time, our attention was drawn to the rather high coefficient of variability of the indicator studied in the main group, which indicated

Table 1. The content of LL-37, hepcidin and lactoferrin in the serum of the children with urinary tract infections who were under observation

Indicator, units of measurement	Control group, n = 20	Main group, n = 64	Subgroup 1, n = 17	Subgroup 2, n = 21	Subgroup 3, n = 16	Subgroup 4, n = 10
LL-37, ng/ml	1.34 (1.18; 1.66)	1.65 (1.33; 2.03)*	1.84 (1.52; 2.35)*	1.58 (1.31; 1.90)	1.9 (1.35; 2.19)*	1.64 (1.3; 1.73)
cV, %	8.3	43.7				
Hepcidin, ng/ml	17.3 (7.4; 23.4)	11.3 (5.5; 21.0)*	16.2 (7.6; 22.4)	6.8 (3.0; 12.7)**	9.9 (5.3; 16.4)*	20.95 (5.5; 23.4)
cV, %	62.7	80.9				
Lactoferrin, ng/ml	10.93 (8.90; 11.4)	9.1 (6.5; 11.6)	9.7 (6.8; 11.9)	10.3 (7.7; 11.2)	8.6 (6.3; 11.7)	6.4 (4.5; 9.9)*
cV, %	20.4	40.8				

*: P < 0.05, the significance of the difference compared to the control group; **: P < 0.01, the significance of the difference compared to the control group.

the heterogeneity of the group. In our opinion, this was primarily due to the different location of the lesion and the duration of the process. Therefore, further analysis of the content of cathelicidin in the serum was performed taking into account the clinical form of the disease. As can be seen from the data in *Table 1*, the highest level of serum cathelicidin was registered in children of the first and third subgroups, i. e. in children with acute pyelonephritis (P < 0.05) and acute cystitis (P < 0.05). In the other two subgroups, the level of LL-37 had only a trend towards increasing (P > 0.05).

Another picture was found in the study of the level of hepcidin in the serum of children with inflammatory diseases of the urinary system. As can be seen from *Table 1*, the level of hepcidin in the main group of the study was statistically lower than in the control group (P < 0.05), which according to O. E. Abaturov et al. (2018) is associated with a high risk of infection. Against this background, high indicators of the coefficient of variability attracted attention – a feature that was studied in the main group and, in contrast to the previous indicator, in the control group. The discovered fact emphasized the role of hepcidin in many processes in the body and its significant biological role. Accordingly, we analyzed its content in the serum of children taking into account the clinical form of UTI, and found a fundamentally different picture. We found a downward trend in the indicator studied. Thus, the development of chronic pyelonephritis and acute cystitis occurred against the background of a statistically significant decrease in hepcidin levels by 2.5 and 1.7 times (P < 0.01 and P < 0.05, respectively). In the other two subgroups, the level of the specified antimicrobial peptide in the serum of children under our supervision did not differ significantly from the control group.

The next step in our work was to analyze the data obtained on the content of lactoferrin in the serum of children who were included in the study. As can be seen from *Table 1*, the level of lactoferrin in the serum of children who were included in the general group was within the control group figures (P > 0.05). However, the coefficient of variability indicated certain heterogeneity of the group. The analysis of lactoferrin content taking into account the clinical form of the pathology revealed that this was due to a statistically significant decrease in the level of serum lactoferrin in a subgroup of children with unspecified UTIs (P < 0.05).

Then we determined the relationship between hepcidin and lactoferrin levels in the subgroups of children studied. The analysis of the relationship between these AMPs showed its absence in the first subgroup of children (r = 0.13, P > 0.05), the presence of inverse interdependence, although weak, in the third and fourth subgroups (r = -0.38, P < 0.05 and r = -0.37, P < 0.05, respectively) and the presence of a clear direct relationship in a subgroup of children diagnosed with chronic pyelonephritis (r = 0.58, P < 0.01).

Discussion

Antimicrobial peptides occupy a leading position in the chain of non-specific mechanisms of the innate immune system. It has been proven that some AMPs have a level of antibacterial activity that exceeds antibacterial drugs due to instantaneous synthesis in response to pathogen expansion and a wide range of action [9]. Therefore, research aimed at studying AMP is relevant and promising in the areas of disclosure of pathogenetic mechanisms of infectious pathology and the development of new therapeutic measures.

The study emphasized that the development of bacterial inflammatory processes in the urinary tract occurs against the background of different changes in the content of AMP in the serum of sick children.

The world literature in most sources describes a significant increase in the level of cathelicidin in the serum of the patients with urinary tract infections [3,10,12]. Thus, according to I. H. Babikir et al. (2018), the highest level of cathelicidin was observed in the patients with upper UTI (i. e. pyelonephritis). The data of our work also confirm this. We found that the development of acute inflammatory processes in the urinary tract, primarily topically localized, leads to a significant increase in the level of LL-37. The growth pattern of LL-37 is aimed at inactivating the bacterial agent. Today, this peptide is considered the main biologically active antimicrobial peptide, especially if we take into account its direct microbicidal, immunomodulatory and antibiofilm activity [1]. The antibiotic activity of LL-37 is indicated by J. Krahulec et al. (2010), Y. Kai-Larsen et al. (2010). The authors showed that LL-37 has an inhibitory effect on E. coli biofilms due to the interaction of AMP with CsgA – the main subunit of fimbriae. Due to the binding of LL-37 to monomeric CsgA AMP inhibits polymerization and, consequently, reduces the formation of biofilms [10].

Patients with unspecified urinary tract infection and chronic pyelonephritis did not increase its content, which is possible, and was a factor in promoting the transformation and chronicity of the process. In favor of this assumption, it is evidenced by the knowledge that an important effect of LL-37 is antibiofilm activity [1], which is a factor that prevents chronicity. However, this conclusion needs further study.

Hepcidin is a small peptide belonging to the defensin family, also known as hepatic antimicrobial peptide-1 (LEAP-1), produced in the liver and excreted into the urine. [16]. Despite the fact that the liver is the main site of synthesis of hepcidin [7], its expression should not be underestimated in peripheral organs, and especially in the kidneys. Daher R. et al. (2019) in their in vitro study confirmed the bacteriostatic activity of renal hepcidin against uropathogenic *Escherichia coli*. Also, the authors found that uropathogenic *E. coli* has a reversible effect on renal hepcidin and weakens its effect. Based on the above data and in accordance with our results, we can assume that the lack of increase in the level of hepcidin or its quantitative insufficiency is a leading factor that allows to start a bacterial inflammatory process in the urinary tract. This assumption is supported by the knowledge that hepcidin has a broad-spectrum antimicrobial action and plays an important role in iron homeostasis. Thus, R. Daher, Z. Karim (2017) in their work showed that hepcidin not only has a direct bacteriostatic effect on uropathogenic *E. coli*, but also effectively stimulates several host kidney protection systems due to the fact that it promotes the mobilization of iron and its accumulation including in the epithelial cells of the kidneys [6]. Against this background, insufficient activity of the specified AMP or its quantitative insufficiency leads to free access to serum iron of pathogens that use iron for life and reproduction, and ultimately contributed to the development and chronicity of UTIs. At the same time, J. Yan et al. (2019) in their work describes the increased level of hepcidin in the serum of children with UTIs, but it should be noted that the average age of patients in this study did not exceed 3 years, whereas in our study it was 10.0 ± 1.3 years [17].

Lactoferrin, like hepcidin, is a protein that is involved in limiting the availability of iron by chelation. It is a protective AMP (glycoprotein), which is an important part of the innate protection of the host, which demonstrates antimicrobial, anti-inflammatory, antioxidant and immunomodulatory properties [15]. Lactoferrin has a direct antimicrobial effect due to damage to the membrane of pathogenic cells, and also affects the growth of bacteria by eliminating iron from free access for bacterial pathogens [16]. Against the background of the above, we observed no statistically significant changes in the serum of children with UTIs, except for one subgroup, i. e. in children diagnosed with unspecified UTIs, where we observed a statistically significant decrease in its content. Thus, the development of both acute and chronic inflammatory bacterial process in the urinary tract in the most groups of children under our supervision, occurred against the background of the intact of the specified antimicrobial peptide. Therefore, we observed a lack of activation of the body's protective response aimed at reducing access to ferric iron, which is necessary for pathogens to live and reproduce. In addition, D. Kell et al. (2020) in his work describes that lactoferrin under certain conditions can be used by bacteria in order to obtain ferric iron from it [11].

Given that hepcidin and lactoferrin are the proteins involved in iron restriction, we decided to determine the relationship between hepcidin and lactoferrin levels in the subgroups of children studied. Thus, according to the results of the comparisons, it is possible to say that each nosological form of UTIs has its own configuration of antimicrobial peptides, but the general feature is the lack of reaction to increase the synthesis of certain AMP, or its inhibition, which obviously serves as a basis for pathology.

Conclusions

1. Each nosological form of urinary tract infection has its own configuration of antimicrobial peptides, but the general feature is the lack of response to increase the synthesis of certain AMP, or its inhibition, which, apparently, is a certain basis for the development of pathology.

2. The analysis of the relationship between hepcidin and lactoferrin, i. e. antimicrobial peptides that limit the access of pathogens to serum iron, showed its absence in children with acute pyelonephritis ($r = 0.13$, $P > 0.05$), the presence of a weak inverse relationship in children with acute cystitis and unspecified urinary tract infection ($r = -0.38$, $P < 0.05$ and $r = -0.37$, $P < 0.05$, respectively), and clear direct relationship in children diagnosed with chronic pyelonephritis ($r = 0.58$, $P < 0.01$), which indicates the synchronization of the body's defense mechanisms, aimed at eliminating the pathogen.

Prospects for further research. It should be noted that the study needs further follow-up to obtain additional data on the functioning of the mechanisms of the innate immune system in children with urinary tract infections and the identification of the factors that contribute to the chronicity of the pathology.

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