The role of endogenous antibacterial peptides in pneumonia occurrence among children of young age


Abstract. The comprehensive examination included 204 children with community-acquired pneumonia aged 2 months to 3 years. It was found that in young children with community-acquired pneumonia, the main etiologic factor is bacteria Streptococcus pneumoniae (36.8 %). The content of endogenous antimicrobial peptides was identified in the serum of 20 young children with pneumonia and in 17 children in the control group. It is proved that the development of pneumonia in young children occurs on the background of the reduction in the blood serum levels of β1-defensin and cathelicidin LL-37. The lowest values of LL-37 were identified in children with pneumonia caused by Streptococcus pneumoniae. The analysis of the content of vitamin D metabolites in the serum showed that in children with pneumonia, concentration of 25-hydroxyvitamin D was 1.4 times lower compared with healthy children (p < 0.05). Established deficiency of vitamin D metabolites in young children with community-acquired pneumonia serves as an important pathogenetic factor for cathelicidin LL-37 deficiency in the blood serum, which was confirmed by 3.7-times decrease in the percentage of LL-37 compared with vitamin D metabolites in this cohort of patients.

Keywords: community-acquired pneumonia; β1-defensin; cathelicidin LL-37; 25-hydroxyvitamin D; young children

Introduction

Acute respiratory infections of lower respiratory tracts — is a leading reason of child-under 5 years disease incidence in the world and makes up 34–40 occurrences among 1000 children a year [1]. Pneumonia is also a leading reason for child of young age deaths in the world. Every year it takes approximately 1.4 million children under 5 years old [2]. An important role in supporting a barrier function of respiratory epithelium and, as a cause, in avoidance of inflammatory diseases of respiratory tracts, play endogenous opiate peptides, in particular: defensin and cathelicidin LL-37, which are secreted by epithelial cell, neutrophils, monocytes and lymphocytes [3, 4]. These peptides are non-specific factors of humoral immunity and display a line of actions, including endotoxin-neutralizing and immunomodulatory action, and also providing protection against a wide spectrum of microorganisms: gram-negative and gram-positive bacteria, fungus, viruses and the simplest [5]. The relevance of antimicrobial peptides to protect the host’s organism from infection was illustrated on animal models and acknowledged by clinical observations, which demonstrate the change of their expression in cases of different diseases of respiratory tract [6]. The activation of NEUTS during infectious and inflammatory processes leads to a quick release of defensins, which later are found in plasma and other organism liquids. Cytokine and defensin activation disorder leads to penetration of originator even in small amounts of it [7].

Cathelicidin has an important role in an innate immunity in protection from bacterial infections, developing antimicrobial activity against gram-negative and gram-positive bacteria, fungus, some viruses and the simplest, and also doing a synergetic antimicrobial effect along with the defensins. The change of concentration...
LL-37 in blood serum is seen in a range of diseases, including inflammatory diseases of respiratory tracts [8, 9]. Defensin and cathelicidin output is strengthened by vitamin D [10]. Nowadays there is a proof, that 1,25(OH)2D regulates the efficiency of immunity response and has an anti-inflammatory action [11]. This way vitamin D strengthens organism’s protection against bacterial infections [12]. A connection between vitamin D deficiency and the frequency of respiratory disease development was found [13, 14]. In V. Wayse and his co-authors’ study (2004) a raise of severe infection of lower respiratory tracts development risk among children with subclinical vitamin D deficiency was shown [15].

The purpose: to determine intension of endogenic antimicrobial peptides among children of young age, children sick with pneumonia and factors, which affect it.

Materials and methods
We did a complex examination of 204 children of age from two months to 3 years (an average age of patients was 1.6 ± 0.3 years), sick with pneumonia.

A mandatory complex of examination included chest organs roentgenography, a general blood test examination, general urinalysis, microbial examination of oral swab. Examination of microbial spectrum of tunica mucosa biomaterial was conducted before the antimicrobial therapy was prescribed when the child was directed to the in-patient hospital on a bacteriological analyzer VITEK 2 Compact (BioMérieux, France) with the use of AES software: Global CLSI-based + Phenotypic. β1-defensins content in blood serum was examined with immune enzymometric analysis with the use of commercial kit Defensin Beta 1 (Elisa, Germany). The exa-mination of cathelicidin level LL-37 was conducted by the immune enzymometric analysis with the use of commercial kit LL-37 (Hycultbiotech, Netherlands). The examination of 25-hydroxvitamin D was conducted by IFA with the help of commercial kit IDS OSTEIA 25-Hydroxy Vitamin D test. The control group included 17 healthy children, semblant by age.

Received results were processed by the method of variation statistics with the usage of analysis package program Statistica for Windows 6.0 with calculating of arithmetical mean (M), standard deviation (σ) and average mistakes (m). To evaluate difference in measurements in comparable groups we used Student’s t-test. The differences were considered meaningful, if $p < 0.05$.

Results
Considering that the main ways of lower respiratory tracts infection of children in young age are oral aspiration and breathing in microbial aerosol, and also, given information from the literature that the respiratory microflora has the same structure, and biomass of which is decreasing from upper to lower tract [16, 17], we did an analysis of oral microbiological “scenery” features of children in young age, who are sick with out of hospital pneumonia. According to the results of microbiological examination we established diagnostically meaningful colonization of upper respiratory tracts by pathogenic microflora with 124 (60.8 %) out of 204 kids (fig. 1). Microflora that was dominating among children who were sick with out-of hospital pneumonia, was *Streptococcus pneumoniae* — 75 children (36.8 %). Almost six times less we saw grammegative bacteria *Klebsiella pneumoniae* — 14 patients (6.9 %) and *Haemophilus influenzae* — 13 kids (6.4 %). Other origins were seen in rare cases.

According to this, children of young age, who were sick with out-of hospital pneumonia, had colonization of upper respiratory tracts by *Streptococcus pneumoniae* bacteria.

It is well-known, that *Streptococcus pneumoniae* is facultative origin, which inhabits nasopharynx of a person, where it is exposed to a set of antimicrobial peptides, which are a part of innate immunity response. Antimicrobial peptides connect with negatively charged teichoic acids on the membranes of Gram-positive bacteria through electrostatic interaction, which leads to the lysis of microbial cells, creating a first line of defense [18, 19]. That’s why it is possible that the susceptibility of strains of *Streptococcus pneumoniae* to antimicrobial peptides has a certain role in determining their ability to colonize [20].

So the next step of our work was to determine endogenous antimicrobial peptides in the blood serum of 20 infants, sick with out-of hospital pneumonia (tab. 1).

![Figure 1. Microflora structure, selected from oral swab from children of young age, who were sick with out-of hospital pneumonia](image)

Table 1. Contents of endogenous antimicrobial peptides in the blood serum of 20 infants, sick with pneumonia (M ± m)

<table>
<thead>
<tr>
<th>Indicator</th>
<th>Sick with pneumonia, n = 20</th>
<th>The Control group, n = 17</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>β1-defensin, pg/ml</strong></td>
<td>100.7 ± 18.2</td>
<td>123.6 ± 15.9</td>
</tr>
<tr>
<td><strong>LL-37, ng/ml</strong></td>
<td>0.10 ± 0.01*</td>
<td>0.30 ± 0.08</td>
</tr>
<tr>
<td><strong>25(OH)D, mME/ml</strong></td>
<td>75.0 ± 10.0*</td>
<td>104.8 ± 6.7</td>
</tr>
</tbody>
</table>

*Note: * — $p < 0.05$ — in comparison to the control group.*

The choice of β1-defensins as a subject of our research is because it is the main factor of innate immunity and so, the antimicrobial barrier system MALT. Defensins take part in all the phases of lungs response, including the initial pathogen destruction. The peptide β1-defensins predominantly shows activity regarding *Moraxella catarrhalis* and *Streptococcus pneumoniae*, as well as has a special meaning in avoiding transition of commensal bacteria to opportunistic pathogens [21].
The study of β-defensins content in blood serum of infants, sick with out-of-hospital pneumonia, showed the presence of tendency to reduction of its level compared to control group (tab. 1). It is possible, that the received result is conditioned by wide constitutively expression of LL-37 with the help of epithelial cells and takes part in innate antimicrobial protection. At the same time, the induction of expression of specified antimicrobial peptide because of inflammatory stimulus is barely happening [22]. Moreover, if other defensins can directly recognize specific lipids in the pathogen membranes [23, 24], then β-defensin becomes active only when conformation changes after the reduction of its disulfide bonds [25, 26]. In addition, the production of β-defensin in the epithelial cells of respiratory tracts is reducing in the condition of acidosis [27], which naturally is developed among children, sick with pneumonia [28].

In the course of further work, we investigated the content in the blood serum of children who were under observation, catelcidin LL-37. The multifunctional role of LL-37 is realized due to its ability to recognize and interact with various molecular targets and immune cells [22]. In addition, LL-37 modulates innate immunity by stimulating macrophages to phagocytic bacteria [29]. In addition to antimicrobial activity, LL-37 also activates mechanical features, such as permeability and bacterial uptake by epithelial cells [4].

As a result of the study, it was found that the LL-37 content in the group of infants with out-of-hospital pneumonia was 3 times lower than the control group. The lowest values of LL-37 were found in children with pneumococcal pneumonia. It is possible to assume that a decrease in the content of LL-37 in the blood serum of infants is one of the main causes of increased susceptibility to Streptococcus pneumoniae. One possible factor contributing to the decrease in LL-37 activity is that the cations of specific antimicrobial peptide interact with mucin anions, which is a component of airway mucus, resulting in a decrease in the antimicrobial activity of LL-37 [30]. It was found that LL-37 induces the virulence of Streptococcus of group A due to increased production of virulence factors, which is mediated by the component of the regulatory system CsrRS [31]. In year 2014 J.J. Velarde and coauthors identified the smallest fragment of LL-37 (RI-10) required for binding to CsrRS. This fragment can directly bind to the sensory kinase CsrS, which leads to the activation of expression of virulence factors of the microorganism [32]. It was previously found that such a peptide does not possess antibacterial activity [33, 34]. In the opinion of I. Grylllos and coauthors (2008), LL-37 has a paradoxical effect, stimulating the regulated CsrRS expression of the virulence gene, thereby increasing the pathogenicity of group A Streptococcus during an infectious disease. The ability of Streptococcus of group A to perceive and respond to LL-37 may partially explain the susceptibility of humans as a biological species not only to Streptococcus of group A, but also to streptococcal infection in general [31]. Based on the data obtained, it was suggested that in the conditions of LL-37 deficiency an inversion of its action is observed, that is, instead of the expected bactericidal effect, the virulence of the microorganism increases [35].

An additional factor that may influence the expression and activity of LL-37 may be metabolic or respiratory-metabolic acidosis. Abou Alaiwa and coauthors (2014) showed that the antibacterial activity of LL-37 depends on the pH of the airways. At the same time, a decrease in pH from 8 to 6.8 in the airways reduces the activity of LL-37 [36]. It is known that pH modulates the state of human oligomerization of LL-37. At acid pH, LL-37 is monomeric, at physiological pH cathecidin aggregates [37]. In the work of Singh, D. and coauthors (2014), it was found that the enhancement of LL-37 signal transduction by the Toll-like receptor 3 (TLR3) is regulated by pH [38]. Upon acidification by endosomes, the oligomerized LL-37 disassociates into LL-29 (a natural LL-37 fragment lacking the C-terminal part) [39], which is unable to transmit TLR3 signals [38]. In this case, inhibition of cathepsins, which includes proteases, whose activity is activated by endosome acidification, resulted in an increase in the half-life of LL-37 from cells [38].

**Discussion**

It is known that vitamin D plays an important role in regulating LL-37 expression. Respiratory epithelial cells convert vitamin D into its active metabolite calcitriol, which has a 100 times greater affinity for the vitamin D receptor than calcidiol [40]. The process of formation of calcitriol is catalyzed by the enzyme α₁-hydroxylase, that is present in the mitochondria of renal tubular cells [41]. The interaction of calcitriol with epithelial cells of the respiratory tract leads to active synthesis of catelicidin protein, which prevents penetration of pathogens into the lower respiratory tract [40].

In the works of P.T. Liu and coauthors (2006) describes the vitamin-D-dependent pathway of the TLR2/1-associated pathway for the synthesis of antimicrobial peptides. It has been shown that activation of human macrophage TLR with increased expression of the vitamin D receptor, promotes the induction of LL-37 expression. According to the authors, the findings confirm the relationship between TLR and vitamin D-mediated congenital immunity and suggest that differences in a person’s ability to produce vitamin D affect susceptibility to microbial infection [42]. It was shown by the researchers from New Zealand (2011), that in patients with pneumonia, severe 25-hydroxyvitamin D-deficiency (<30 nmol/L) correlated closely with a higher 30-day mortality compared to patients with a sufficient level (>50 nmol/L) [43].

Considering that the low level of vitamin D supply is associated with a high risk of developing respiratory tract infections [44, 45], the next step of our work was to determine the content of vitamin D metabolites in the serum of children in the observation groups (tab. 1).

The analysis of the content of vitamin D metabolites in blood serum showed that among children, sick with out-of-hospital pneumonia, the concentration of 25-hydroxyvitamin D was 1.4 times lower than in healthy children and averaged 75.0 ± 10.0 mIU/ml vs 104.8 ± 6.7 mIU/ml, accordingly (p < 0.05). Taking into account that vitamin D induces LL-37 expression; we determined the ratio of LL-37 to 25-hydroxyvitamin D. It was found that in the group of children with pneumonia, a 3.7-fold decrease...
in the percentage of LL-37 in relation to vitamin D (0.12 ± 0.04 % against 0.44 ± 0.12 %, according, p < 0.05). So, on the background of low levels of vitamin D among children with pneumonia, there was not enough LL-37 synthesis.

One of the pathogenetic mechanisms for reducing calcitriol levels is a decrease in the activity of 1α-hydroxylase [46]. In numerous experimental studies, it was shown that the development of metabolic acidosis suppresses the synthesis of 25-hydroxyvitamin D 1α-hydroxylase in the proximal tubules of the kidneys by inhibiting parathyroid hormone-dependent adenylate cyclase, which leads to a decrease in serum vitamin D levels [47–49] and, as a consequence, a decrease in the synthesis of LL-37 [50].

Conclusions

1. The development of out-of-hospital pneumonia among infants occurs at the background of a low blood serum level of a number of endogenous antimicrobial peptides (β-defensin and LL-37).

2. A significant pathogenetic factor of the deficiency of cathelicidin LL-37 in the blood serum of infants with out-of-hospital pneumonia is the deficiency of vitamin D metabolites.

Conflicts of interests. Authors declare the absence of any conflicts of interests that might be construed to influence the results or interpretation of their manuscript.

References


5. Aleshina GM, Kokryakov VN, Shamova OV. Covremennaya kontseptsiya ob antimikrobnyih peptidah kak molekulyarnyih faktorah immunite-


14. Bredgen KA. Antimicrobial peptides: pore formers or metabolic inhibi-


Роль ендогених антимікробних пептидів у виникненні пневмонії в дітей раннього віку

Резюме. Проведено комплексне обстеження 204 дітей віком від 2 міс до 3 років із позалікарняною пневмонією. Установлено, що в дітей раннього віку, хворих на позалікарняну пневмонію, значний дефіцит метаболітів вітаміну D у дітей раннього віку, хворих на позалікарняну пневмонію, є значним патогенетичним фактором дефіциту кателіцидина LL-37 в сироватці крові, що підтверджувалося сниженням концентрації 25-гідроксивитамина D до 1,4 раза нижче порівняно зі здоровими дітьми (р < 0,05). Установлено, що в дітей, батьків хворих на позалікарняну пневмонію, концентрація 25-гідроксивитамина D була в 1,4 раза нижча порівняно зі здоровими дітьми (р < 0,05). Установленний дефіцит метаболітів вітаміну D у дітей раннього віку, хворих на позалікарняну пневмонію, є значним патогенетичним фактором дефіциту кателіцидина LL-37 у сироватці крові, що підтверджувалося виявленням в даний когорті хворих у 3,7 раза процентрного вмісту LL-37 щодо метаболітів вітаміну D.

Ключові слова: позалікарняна пневмонія; β-дефензин; кателіцидин LL-37; 25-гідроксивитамін D; діти раннього віку