

The Influence of Insufficiency of Vitamin D on Activity of Endogenous Antimicrobial Peptides at Children of Early Age with Acute Bacterial Diseases of the Respiratory System

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Abstract

Determination of content of vitamin D and antimicrobial peptides (cathelicidin, β 1- defensins, lactoferrin and hBPI) in blood serum of 67 children with acute bacterial diseases of a respiratory system aged from 1 month up to 3 years taking into account the level of providing with vitamin D is carried out. The research showed that decrease in maintenance of a number of antimicrobial peptides in blood serum against the background of deficiency of vitamin D acts as risk factor of development of acute bacterial diseases of a respiratory system of children of early age.

Keywords: acute bacterial diseases of a respiratory system, antimicrobial peptides, vitamin D, children of early age

Introduction

The epithelium of respiratory tracts represents primary link of protection of a macroorganism at contact with a great number of pathogenic agents which protective

arsenal is presented not only in the form of a physical barrier, but also the specific receptors and antimicrobial substances which are a part of the congenital immune system [1, 5, 16]. Secretion of endogenous antimicrobial peptides acts as one of such protective factors: defensins, cathelicidin and other larger antimicrobial proteins, such as lactoferrin and bactericidal/permeability-increasing protein [1, 7] which possess not only a wide range of antimicrobial activity against viruses, bacteria and fungi but also have for many antibiotics bactericidal effect [4, 13].

In the last years in literature the increasing attention is paid to a nonconventional role of vitamin D in our organism. In addition to traditional concepts about its participation in phosphorus-calcium exchange and a skeletal homeostasis, great interest is attracted by studying of its influence on activity and regulation several other aspects human health [20]. So, great interest is directed to a potential role of vitamin D in development resistance to infections of a respiratory path, due to its direct influence on the immune system [9, 18]. It is proved that the immune system of our organism is capable to produce CYP27B1 enzyme which participates in transformation of the circulating form 25(OH)D₃ into an active form – 1,25(OH)₂D which in turn leads to induction of antimicrobial peptides, for providing fuller response to penetration of pathogens [11].

The most of the literary data concerning interrelation of security with vitamin D and synthesis of antimicrobial peptides in the conditions of respiratory infection is contradictory therefore today there is relevant a further studying of a pathogenetic role of vitamin D during acute inflammatory bacterial diseases of a respiratory system at children of early age.

Objective: to estimate influence of deficiency of vitamin D on the level of endogenous antimicrobial peptides in blood serum at children with acute bacterial diseases of respiratory system of early age.

Methods

observation there were 67 children aged from 1 month up to 3 years (average age $1,6 \pm 0,1$ years old), with acute inflammatory bacterial diseases of a respiratory system.

The assessment of influence with vitamin D children of groups of observation was carried out according to recommendations of the International society of endocrinologists and according to recommendations about prevention and treatment by deficiencies of vitamin D for the population of the countries of Central Europe (2013) (tab.1) [10, 21, 22].

Table 1
Contents assessment 25 hydroxyvitamins D in blood serum (reference data)

Concentration 25(OH)D ₃ ng/ml/MI	Concentration 25(OH)D ₃ nmol/l	
≥10	≥25	The severe deficiency of vitamin D.
10-20	25-50	The deficiency of vitamin D.
21-29	51-75	The insufficient vitamin D (suboptimal levels).
30-100	76-250	The satisfactory supply of vitamin D.
≤100	≤250	The excess vitamin D.

The conducted of level of contained levels vitamin of D children who were under observation and also on the basis of the provided reference values allowed to distribute patients on the following groups: the I group - 12 patients with deficiency of vitamin D (level serum 25(OH)D₃ was ranging from 21,5 up to 33,5 nmol/ml), the II group - 26 patients about suboptimal levels vitamin D (from 58.0 to 69.0 nmol/ml) and 29 patients with satisfactory values of vitamin D and entered into the III groups (from 100,0 nmol/ml to 120,0 nmol/ml). It should be noted that the preventive grant of vitamin D within the first 3 years was received by 85% of children of the III group. Children of I and II group only in 10% and 35% respectively received irregular doses of vitamin D both on the frequency of reception and on the level of a preventive dose.

The control group was made by 17 children representative on age, without manifestations of acute respiratory diseases with the except for congenital malformations of a respiratory system, organic pathology of a cardiovascular system, anomalies of development of the central nervous system and other rough malformations and also without clinical laboratory signs of deficiency of vitamin D.

The research of content of vitamin D in blood serum was conducted by means of a method of the immunoassay analysis with determination of content by 25 hydroxyvitamins D with use of the commercial Vitamin D OSTEIA 25-Hydroxy sets which is the main circulating form of vitamin D and is considered the main indicator of contained levels the specified vitamin D a human body [21]. Determination of level of antimicrobial peptides in blood serum of children of groups of observation, namely a cathelicidin (LL37), β1-defensins, bactericidal/permeability-increasing protein (hBPI) and lactoferrin also carried out with use of commercial LL37 sets (Human, ELISA), Defensin Beta 1 (Elisa, Germa-

ny), Human BPI (Hyculbiotech, the Netherlands) and Human Lactoferrin of production Hyculbiotech (Netherlands) respectively.

Statistical Analyses

The mathematical analysis and statistical data processing were carried out with use of the license Statistic for Windows 13,0 software package, the serial number JPZ8041382130ARCN10-J. For all types of the analysis statistically significant considered distinctions $p < 0,05$. Quantitative signs were presented in the form of Me (Q25;Q75) taking into account distribution which differed from normal. Reliability of distinctions estimated by means of nonparametric criterion Mann-Whitney's U-criterion for independent selections. For assessment of interrelations between indicators used a method of the correlation analysis with calculation of coefficients of correlation of Spirmen.

Results and discussion

Being based on the data of literature indicating that vitamin D plays an important role in regulation of synthesis of antimicrobial peptides in particular activation of TLR of human macrophages with the raised expression of a receptor of vitamin D promotes induction of synthesis of the last [14]. We content was determined of antimicrobial peptides taking into account providing with vitamin D at children with acute bacterial diseases of a respiratory system. Results of the conducted researches it is shown in table 2.

Apparently from tab.2 in all groups of the children who were under observation of providing with antimicrobial peptides was different. In particular, authentically low levels of a LL-37 in blood serum were noted in group of children with deficiency and suboptimal vitamin D levels ($p < 0,01$). In group of children with satisfactory levels of vitamin D only the tendency to decrease in the specified peptide ($p > 0,05$) in comparison with indicators of control group was noted. That is, accurate inverse relation of LL-37 level in blood serum from a condition of contained levels vitamin D of children with acute bacterial diseases of a respiratory system is established. It is known that the main circulating form of vitamin D - 25(OH)D₃ is a necessary condition for production activation to a cathelicidin [6], as well as the fact that levels of serum 25(OH)D₃ of the 100th nmol/l are necessary for ensuring sufficient synthesis of LL-37 with cells of the immune system [1]. That is at children with insufficient levels of vitamin D in blood serum does not happen to sufficient synthesis to cathelicidin and consequently its anti-infectious properties at the local level are not implemented, promotes colonization of a respiratory path pathogens.

Table 2
The level of antimicrobial peptides in blood serum taking into account contained levels of vitamin D of children with acute bacterial diseases of a respiratory system
Me (Q25; Q75)

	I group n=12	II group n=26	III group n=29	Control group n=17
LL-37, нг/мл	0,08(0,05; 0,10)**	0,09(0,05; 0,11)**	0,17(0,11; 0,21)	0,20(0,2;0,3)
β 1-defensins, пг/мл	73,5(54,0; 85,0)**	81,0(59,0; 102,0)*	88,0(61,0; 121,0)	90(78;170)
Lactoferrin нг/мл	7,6(6,0;8,1) **	6,7(4,0;8,8) **	6,3(3,7;9,1) **	3,5(3,0;6,8)
hBPI, нг/мл	1385(1020; 1650)**	1590(1100; 1950)**	1800(1200; 2350)*	2500(1650; 4700)

Addition:

*- a significant ($p < 0,05$) difference compared to the corresponding indicator of the control group;

** - a significant ($p < 0,01$) difference compared to the corresponding indicator of the control group.

The obtained data confirm the assumption that vitamin D had influence and on synthesis β 1- defensins in epithelial cells of respiratory tracts, providing big protection against penetration of pathogenic microflora [13,17,19]. The similar trend was established by us concerning features of providing children with acute bacterial diseases of a respiratory system β 1- defensins (tab.2). The conducted analysis showed that development of bacterial diseases of a respiratory system happened against the background of statistically significant decrease in contents β 1- defensins in blood serum as among children to deficiency of vitamin D ($p < 0,01$) and among children about suboptimal levels of contained levels with the specified vitamin ($p < 0,05$). Children III groups which had satisfactory levels of vitamin D, the statistical difference between indicators of group of control of a certain peptide in blood serum was not noted ($p > 0,05$). Besides, the specified data were confirmed by work of Hansdottir S. et al. (2008) in which the author showed that against the background of insufficient for synthesis vitamin D, which plays an important role in regulation of synthesis of antimicrobial peptides, in particular β 1- defensins [8], there is a decrease in a constitutive endogenous expression of the specified peptide that in turn leads to colonization of airways pathogenic microflora and promotes heavier course of a disease.

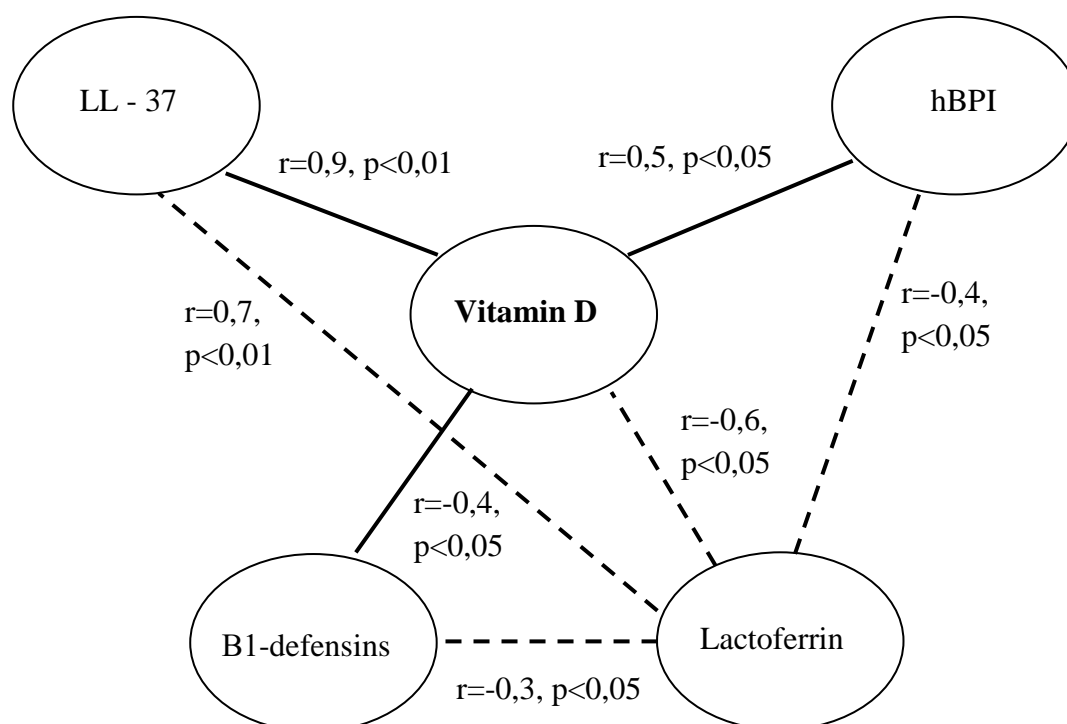
In the analysis of maintenance of a laktoferrin in blood serum of children from groups of observation, we noted that development of inflammatory processes of respiratory organs occurred against the background of reliable growth of the contents of the last ($p < 0,01$) on the relation in values of control group (tab.2). The results received by us have a certain logic, lactoferrin performs functions of the regulator of activity of immunocompetent cells which role considerably increases

in conditions of insufficient activity of endogenous antimicrobial peptides. Besides, Mayeur S. et al. (2016) in the researches confirmed antimicrobial properties of a laktoferrin, due to binding with this protein of free ions of iron that limits amount of iron for bacterial absorption and growth of pathogenic microorganisms is suppressed [15]. Therefore, the specified peptide was classified as a component of the first line of anti-infectious protection of a macroorganism [12,15].

At the present stage the role of bactericidal/permeability-increasing protein of cells (hBPI) is up to the end not defined in pathogenesis of inflammatory processes of respiratory organs at children of early age. But it is proved that this protein participates in protection of a respiratory path against sharp respiratory infections and development of chronic diseases of lungs [2, 3]. Therefore we analysed the content of the specified peptide at the children who were under our observation (tab.2). At establishment of level of security of children from groups of observation of hBPI we noticed the trend similar to that that it was established also for a cathelicidin LL-37 and β - defensins, however the expressiveness of changes of the specified peptide was more essential and is statistically significant. So, it was established that the maintenance of hBPI in I and II group was the lowest and statistically authentically differed from indicators of group of control ($p < 0,01$). At children of the III group low indicators of content of peptide in blood serum in relation to indicators of control group were also noted ($p < 0,05$). According to us, decrease in the specified peptide is perhaps connected with insufficient synthesis of the last against the background of considerable colonizations pathogenic microflora in the conditions of deficiency of other antimicrobial peptides and vitamin D. So, it agrees to literary data, the highest basal hBPI levels are found in proximal departments of respiratory tracts and acts as the most powerful barrier to prevention of invasion of pathogens into distal departments of a respiratory path. At the same time, if infection happened, the maintenance of hBPI can decrease that leads further to activation of other protective functions of a respiratory system for elimination of a bacterial respiratory infection [2].

At the following stage of our work the correlation analysis of interrelation and influence of providing with vitamin D on products of antimicrobial peptides in blood serum of children with acute bacterial diseases of a respiratory system confirmed the leading role of vitamin D in regulation of synthesis of antimicrobial peptides and susceptibility to a microbic infection (fig. 1).

The provided data in fig. 1 show existence of direct correlation dependence not only between vitamin D content in blood serum and the level of antimicrobial peptides, but also between the content of peptides which were investigated at children of group of observation (within $r = 0,4 - 0,6$, $p < 0,05$).



Picture 1. The correlation matrix of dependence content of vitamin D and products of antimicrobial peptides in blood serum of children with acute bacterial diseases of a respiratory system.

The set of these results allows to assume, that communication "vitamin D - antimicrobial peptide" in the conditions of decrease in level of vitamin D in blood serum fully does not carry out a protective role that leads further to decrease in inflammatory immunoreactivity of an organism and acts as one of factors of further colonization of a patoganema and more severe disease.

Thus, vitamin D acted as an integrative factor which maintenance realizes providing children, patients acute bacterial diseases of a respiratory system of the majority of antimicrobial peptides. It in turn allows to control inflammatory reactions to infection with pathogens and to regulate adaptive immune responses for protection of a macroorganism against further microbic colonization.

Conclusions

1. The content in blood serum of such antimicrobial peptides as LL-37, β -defensins and hBPI directly depends on the level of contained levels vitamin D of children with acute bacterial diseases of a respiratory system.

2. In the conditions of insufficient amount of antimicrobial peptides at children from groups of observation the leading role in regulation of adaptive immune responses is obviously undertaken by lactoferrin which level grows

against the background of deficiency of vitamin D and a number of antimicrobial peptides.

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