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EXTRACELLULAR DNA AND PLASMA NUCLEASE ACTIVITY EFFECT ON THE COURSE OF THE MICROBIAL INFLAMMATORY PROCESS IN THE RESPIRATORY TRACT IN CYSTIC FIBROSIS

Research article

Abstract

Background. Cystic fibrosis is a hereditary multi-organ disease. The involvement of the respiratory system due to chronic inflammation and a chronic infectious process is the main cause of death in 90% of cases. Currently, chronic inflammation in the respiratory tract in cystic fibrosis is being actively studied and new approaches to therapy are being developed. Circulating extracellular DNA/cell-free DNA (eDNA/cf DNA) is present in the bloodstream and other biological fluids of both healthy and sick individuals. The eDNA concentration is increased during exacerbation of several illnesses and in cases of exposure to damaging factors. The eDNA concentration is regulated by the endonucleases activity.

Objectives. Study of the eDNA concentration and endonuclease activity in the plasma of children with cystic fibrosis.

Methods. The concentration of eDNA was determined in 115 children, and nuclease activity was determined in 117 children. The control group included 49 healthy children of the same age and sex. The eDNA was extracted by phenolic extraction. The concentration of extracellular DNA was measured by fluorescence with an intercalating dye PicoGreen (Invitrogen). The DNAse 1 activity in plasma samples was measured by means of radial diffusion. The data are presented as a median (Me) and quartiles (Q1 - Q3). Statistical analysis was performed using a nonparametric Mann-Whitney U test to compare the eDNA concentration and nuclease activity in groups of children with cystic fibrosis and control, and also to analyze the relationship between the eDNA concentration and the nuclease activity with the clinical characteristics of patients.

Results. The eDNA concentration was lower than that of healthy children ($p<0.05$). The nuclease activity in CF patients did not differ from that of the control group. There was a trend to decrease of the eDNA concentration with the age. Nuclease activity decreased significantly with the age ($p<0.05$). In patients with exacerbation of the bronchopulmonary process, a decrease in nuclease activity was demostrated ($p <0.05$). The eDNA concentration in patients with $FEV_1> 80\%$ does not differ from the control group. The eDNA concentration decreased along with a decrease of lung function ($FEV_1 <80\%$). Patients with deteriorated lung function and increased requirement for bronchodilators had low nuclease activity.

Conclusion. Children with cystic fibrosis had lower eDNA concentration in comparison with the control. In patients with normal spirometry parameters, eDNA probably plays a metabolic role and its composition differs from that of the eDNA of patients with impaired lung function. The nuclease activity of blood plasma in patients with cystic fibrosis decreased with age, and at the same time the progression of the disease was observed. Changes of the eDNA concentration and composition, as well as the nuclease activity in CF patients, can be important for predicting the course and severity of the disease. The interpretation of the qualitative composition of the eDNA requires further study to determine its role in the development of the chronic inflammatory process in CF. The study of eDNA and nuclease activity in CF can be used for development of new pathogenetic treatment modalities.

Keywords: cystic fibrosis, childhood, cfDNA, eDNA, nuclease activity, lung function.

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ВЛИЯНИЕ ВНЕКЛЕТОЧНОЙ ДНК И НУКЛЕАЗНОЙ АКТИВНОСТИ ПЛАЗМЫ НА ТЕЧЕНИЕ МИКРОБНО-ВОСПАЛИТЕЛЬНОГО ПРОЦЕССА В ДЫХАТЕЛЬНОМ ТРАКТЕ ПРИ МУКОВИСЦИДОЗЕ

Научная статья

Аннотация

Муковисцидоз является наследственным полиорганным заболеванием, в 90% случаев поражение дыхательной системы, в результате хронического воспаления и хронического инфекционного процесса, является основной причиной смертности. В настоящее время хроническое воспаление в респираторном тракте при муковисцидозе активно изучается и создаются новые подходы к терапии. Циркулирующая внеклеточная ДНК (вкДНК) присутствует в кровотоке и других биологических жидкостях у здоровых и больных людей. Повышение концентрации вкДНК наблюдается при ряде патологий в период обострения и при воздействии повреждающих факторов. Концентрация вкДНК регулируется активностью эндонуклеаз.

Цель. Исследование концентрации вкДНК и эндонуклеазной активности в плазме детей с муковисцидозом.

Методы. Концентрация плазменной вкДНК исследована у 115 детей, а уровень нуклеазная активности у 117 больных. Контрольная группа составила 49 здоровых детей соответствующего возраста и пола. ВкДНК выделяли методом фенольной экстракции. Концентрацию внеклеточной ДНК измеряли методом флюоресценции с интеркалирующим красителем PicoGreen (Invitrogen). Уровень активности ДНКазы 1 в образцах плазмы был измерен методом радиальной диффузии. Данные представлены как медиана (Me) и квартели (Q1 - Q3). Статистический анализ проводили при помощи непараметрического теста U-критерия Манна–Уитни для сравнения уровней концентрации вкДНК и нуклеазной активности в группах детей с муковисцидозом и контрольной, а также для анализа взаимосвязи между концентрацией вкДНК и уровнем нуклеазной активности с клиническими характеристиками больных.

Результаты. Показатель вкДНК оказался ниже значений группы здоровых детей ($p<0,05$). Уровень нуклеазной активности у больных МВ не отличался от контрольной группы. С возрастом наблюдалась тенденция к снижению концентрации вкДНК. Нуклеазная активность достоверно снижалась с возрастом ($p< 0,05$). Также отмечалось снижение нуклеазной активности при обострении бронхолегочного процесса ($p<0,05$) При оценке функции легких по данным спирометрии и показателю $ОФВ_1>80\%$, содержание вкДНК не отличалось от показателей контрольной группы. При снижении функции легких ($ОФВ_1<80\%$) концентрация вкДНК понижалась. Больные со сниженной функцией легких и повышенной потребностью в бронходилятаторах имели низкую нуклеазную активность.

Заключение. Дети с муковисцидозом имели низкую концентрацию вкДНК в сравнени с контролем. У пациентов с нормальными показателями спирометрии вкДНК вероятно играет метаболическую роль и отличается по составу вкДНК пациентов со сниженной функцией легких. Снижение уровня нуклеазной активности с возрастом происходит одновременно с прогресссированием заболевания. Изменение концентрации и состава вкДНК, а также уровень нуклеазной активности у больных МВ может иметь значение для прогнозирования тяжести течения заболевания. Расшифровка качественного состава вкДНК при муковисцидозе требует дальнейшего изучения для определения его роли в развитии хронического воспалительного процесса. Изучение вкДНК и нуклеазной активности при МВ может быть использовано в разработке нового патогенетического лечения.

Ключевые слова: муковисцидоз, детство, внеклеточная ДНК, нуклеазная активность, функция легких.

Cystic fibrosis (CF) is a serious hereditary disease involving virtually all body systems. In cystic fibrosis as a result of impaired conduction of the chlorine channels, the rheology of secretions alters, which leads to dysfunction of the bronchopulmonary and digestive systems mainly. The abnormalities in the bronchopulmonary system predispose to secondary infection and the formation of chronic inflammation. The study of inflammation markers in various diseases, including cystic fibrosis, is of great interest.

Extracellular DNA (eDNA) is a DNA fraction that is not bound to cells. The number of studies related to the role of eDNA and DNAse activity in the human organism is increasing steadily. In many studies, eDNA was considered as a diagnostic marker for various pathological conditions. Data on the qualitative and quantitative composition of extracellular DNA in malignancies, during pregnancy (fetal DNA), autoimmune diseases, radiation damage, acute and chronic inflammatory diseases, and critical conditions are accumulated. In most papers, a concentration increase of extracellular DNA was observed in acute diseases and

in exacerbation of the chronic process, while a decrease of eDNA concentration occurred more often in chronic diseases without exacerbation [1].

The objective of this study was to determine the role of eDNA and nuclease activity of blood plasma in respiratory tract involvement in children with cystic fibrosis.

1. Study object

The study included 117 children with cystic fibrosis, 70 females and 47 males. The mean age of patients was 6.9 ± 4.25 years. The children were divided by convention into four age groups: 0 - 3 years (34 patients), 4 - 7 years (43 patients), 8 - 11 years (24 patients), 12 - 15 years (11 patients), 16 - 18 years (5 patients). The cystic fibrosis was diagnosed according to the diagnostic criteria of the European Consensus 2017 and the National Consensus "Cystic Fibrosis: definition, diagnostic criteria, treatment" 2016 . Children underwent a comprehensive clinical examination according to the Clinical Guidelines [2]. Additional specific analysis was performed in a homogeneous group of 37 children with a mutation of the *CFTR delF508* gene in a homozygous state. The control group included 49 healthy children of the same age and sex, with no signs of acute respiratory illness during the previous 2 months. The concentration of eDNA was determined in 115 children, and nuclease activity was determined in 117 children. The study protocol was approved by ethical committee of FSBSI "MGRC". Informed voluntary consent was obtained from all the study participants.

2. Study methods

Blood samples were collected in heparin-containing vials with green lids, incubated at ambient temperature for 30 min, centrifuged at 4000 rpm, then 2 ml or more of plasma were transferred into a test tube and stored at -18-20°C until analysis.

The eDNA was extracted by phenolic extraction. The concentration of extracellular DNA was measured by fluorescence with an intercalating dye PicoGreen (Invitrogen). Cells were removed from the blood by centrifugation at 460 × g, followed by mixing 3 ml of plasma with 0.3 ml of a solution containing 1% sodium lauroyl sarcosinate, 0.02 M EDTA, and 75 μg/ml RNAse A (Sigma-Aldrich; Merck KGaA , Darmstadt, Germany), incubation for 45 min, with exposure to proteinase K (200 μg/ml, Promega Corporation, Madison, WI, USA) for 24 hours at 37°C. After two cycles of purification with a saturated solution of phenol, the DNA fragments were precipitated by adding two volumes of ethanol in the presence of 2M ammonium acetate. The precipitate was then washed with 75% ethanol twice, dried and dissolved in water. The concentration of the eDNA (eDNA index) was determined by measuring the fluorescence intensity on 'LS 55' (PerkinElmer, Inc., Waltham, MA, USA) after staining the DNA with PicoGreen (Invitrogen, Thermo Fisher Scientific, Inc., Waltham, MA, USA). The relative standard error of the eDNA index was 10 ± 4%. The DNAse 1 activity in plasma samples was measured by means of radial diffusion

The sweat test was performed using the conductivity method on the Nanodact EliTechGroup Inc., USA (normal - <50 mmol/l, grayzone - 50-80 mmol/l, positive >80 mmol/l). A microbiological study of the respiratory tract secretion was performed in the FSBI Gamaleya National Research Center for Epidemiology and Microbiology of the Ministry of Health of Russia. In children aged 6 years and older the spirometry was performed using the EasyOne Pro® spirograph. The forced expiratory volume for 1 sec (FEV_1) and forced vital capacity (FVC) was evaluated. The analysis of obtained values was performed using the reference values according to G. Polgar, V. Promadhat for children and the working group ECSC (European Coal and Steel Community) for adults [3], [4]. The results were expressed as a percentage of the reference value: obtained value/reference value × 100%. Physical development was assessed using the WHO Anthro and WHO Anthro plus programs.

The statistical analysis was performed using the IBM® SPSS® Statistics Version 17.0 software. The data were analyzed for the accordance of the studied values distribution to the normal distribution. The data are presented as a median (Me) and quartiles (Q1 - Q3). Statistical analysis was performed using a nonparametric Mann-Whitney U test (to evaluate the differences between two independent samples). Differences were considered statistically significant at $p < 0.05$.

3. Results

According to the past medical history, the diagnosis was suspected on the basis of an increase of immunoreactive trypsinogen (IRT) in newborns during neonatal screening for cystic fibrosis (IRT/RTI scheme). Eighty four (71.8%) children had the positive results of neonatal screening. Five (4.3%) children had negative results of neonatal screening. The screening data of 28 (23.9%) patients were not available. The diagnosis of cystic fibrosis was confirmed by the positive sweat test. The median of sweat electrolytes concentration was 110 mmol/L for the first determination and 104 mmol/L for the second determination (97-119 mmol/L and 81.5-117.5 mmol / L). The mean age at diagnosis was 1.8 ± 2.3 years. In 13 (11.1%) patients the disease manifested with the meconial ileus.

A genetic study was performed in 111 patients. Two mutations in 85 patients, one known mutation in 24 patients were identified, and 2 patients had unidentified genotype. The allele frequency of mutation *F508del* was 51.8%, and 34 homozygotes for this mutation was identified. In addition, the I and II class mutations, forming a "severe" phenotype, were identified in 26 patients. IV and V class mutations, defining the "mild" phenotype were identified in 20 patients. Five patients had an "unspecified phenotype".

At the time of the study, 35 (29.9%) patients had signs of exacerbation of the bronchopulmonary process. Polypous sinusitis was diagnosed in 10 (8.5%) patients. Infections caused by Pseudomonas aeruginosa and other non-fermenting Gram-negative bacteria (NFGN) were diagnosed in 35 (30.4%) patients, staphylococcal infection had 80 (69.6%) patients, in the F 508 del mutation homozygous group 38.2% and 70.6% respectively.

Median of eDNA was 452.00 (208.00-764.00) ng/ml in the cystic fibrosis group and 641.90 (368.00-1250.30) ng/ml in the control group (p = 0.0005). The median of nuclease activity was 15.10 (8.40-21.20) units of activity (UA) in the cystic fibrosis group, and 14.00 (7.70-22.00) UA, (P = 0,4329) in the control group (Table 1)

Table 1 – The parameters of the eDNA concentration and plasma nuclease activity, Me (Q1-Q3)

Parameter	Patients	Control	p
n	115	49	
eDNA	452.00 (208,00-764,00)	641.90 (368,00-1250,30)	0.0005
n	117	49	0.4329
Nuclease activity	15.10 (8.40-21.20)	14.00 (7.70-22.00)	

There were no significant differences in the eDNA concentration in the age groups (p> 0.05). The maximum values of eDNA concentration (4443 ng/ml) were observed in the age group 8 - 11 years. The minimum values of eDNA (0 ng/ml) were in the age group under 3 years (Figure 1).

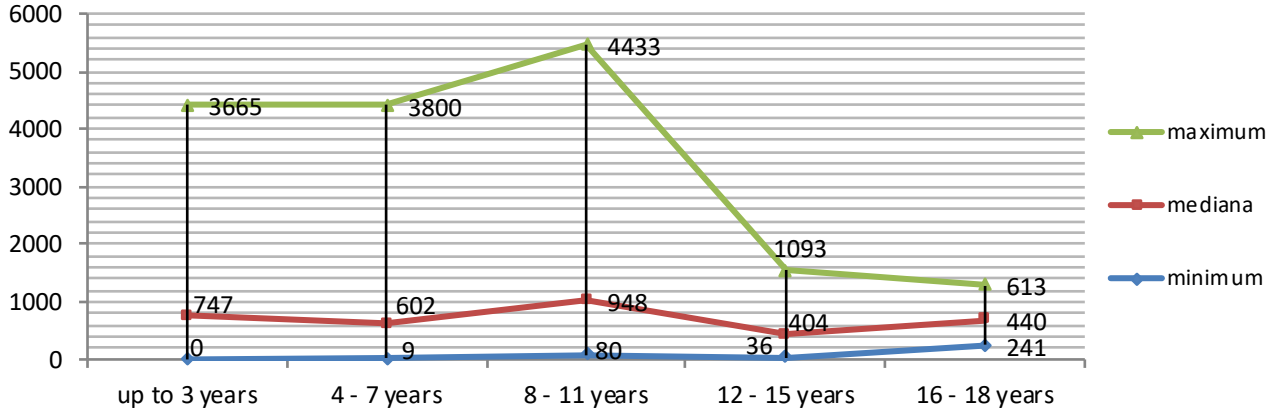

Figure 1 – Distribution of eDNA values (ng/ml) in different age groups

There was a trend to decrease of the eDNA concentration with the age, while nuclease activity decreased significantly with the age (p <0.05) (Figure 2).

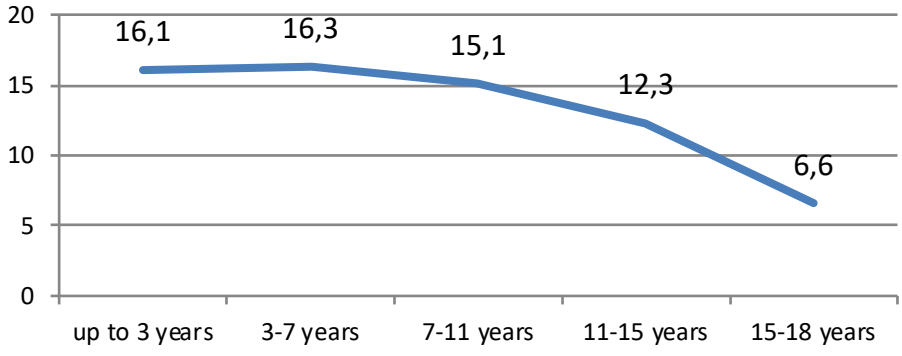

Figure 2 – The parameters of nuclease activity in different age groups (UA)

There were no significant differences in eDNA concentration and nuclease activity in the different sex groups (p> 0.0500).

A trend to lower eDNA concentrations in patients with "severe" mutations compared to "mild" ones was revealed (p = 0.0906). There was no relation between the level of nuclease activity and the genotype (Table 2)

Table 2 – The eDNA concentration and the nuclease activity in different classes of mutations (N = 80), Me (Q1-Q3)

Mutations	"Severe" (1)	Homozygotes F508del (2)	"Mild mutations" (3)	p
n	26	34	20	
eDNA	314.00 (189.00-945.00)	319.00 (187.40-687.00)	562.50 (290.50-1176.50)	$P_{2,3} = 0.0906$
Nuclease activity	16.10 (8.40-19.90)	16.30 (10.40-23.70)	15.90 (8.40-19.60)	$P_{2,3} = 0.2592$

On spirometry, the best values of FEV_1 were observed significantly more often in children with a median eDNA concentration of 230 ng/ml (189-200). There was no significant relation between the FEV_1 values and the nuclease activity. There was a trend to increase of eDNA along with the FVC increase (Table 3).

Table 3 – Relation between FEV_1/FVC and eDNA concentration (ng/ml)/nuclease activity (UA), Me (Q1-Q3)

Parameter	FEV_1				p		
	< 60 (1)	60 - 80 (2)	80 - 100 (3)	> 100 (4)	P1,2 P1,3 P1,4	P2,3 P2,4	P3,4
n	4	5	14	4			
eDNA	670,00 (363.20-1220.00)	230.00 (189.00-260.00)	344.50 (155.00-757.00)	967.50 (430.50-1457.50)	0.1111 0.2771 0.8857	0.5593 0.0317	0.1922
Nucl. act.	10.45 (6.05-15.80)	7.90 (7.50-15.10)	12.25 (7.10-16.90)	15.55 (7.10-25.95)	0.5556 0.6451 0.4857	1 0.7302	0.7209
Parameter	FVC						
	< 60 (1)	60 - 80 (2)	80 - 100 (3)	> 100 (4)	P1,2 P1,3 P1,4	P2,3 P2,4	P3,4
n	4	6	14	4			
eDNA	1083.00 (533.00-1633.00)	474.00 (230.00-807.00)	294.00 (156.00-727.00)	551.00 (310.00-1457.50)	0.2571 0.2771 0.8857	0.9679 0.0667	0.1922
Nucl. act.	6.05 (5.30-6.80)	15.90 (14.10-17.50)	12.25 (7.10-16.90)	15.55 (7.10-25.95)	0.6095 0.6451 0.4857	0.9044 0.7619	0.7209

No differences of the eDNA concentration and nuclease activity in patients infected with Pseudomonas aeruginosa and other NFGN in comparison with those with chronic staphylococcal infection were observed. However, a significantly higher level of nuclease activity was observed in patients infected with Gram-negative bacteria versus those infected with Staphylococcus aureus ($p < 0.05$). Analysis of eDNA concentration and nuclease activity in the group of patients with the F508del/F508del genotype, depending on microbiological status of the patients, demonstrated higher values of the nuclease activity in the Pseudomonas aeruginosa and other NFGN group ($p = 0.0411$) (Table 4).

Table 4 – The eDNA concentration (ng/ml) and nuclease activity (UA) and relation with respiratory tract microflora in the general group of patients with cystic fibrosis and in those with the F508del/F508del genotype, Me (Q1 -Q3)

Parameter	Microbiological status of the respiratory tract in the general group		p
	Staphylococcal infection	Pseudomonas aeruginosa and other NFGN infection	
n	66	35	
eDNA	503.5 (196.00-963.00)	361.00 (226.00-687.00)	0.6087
Nuclease activity	13,55 (8.12-17.77)	16.10 (7.10-25.40)	0.2979
Microbiological status of the respiratory tract in the group of patients with the F508del/F508del genotype			
n	18	13	p
eDNA	268.00 (160.50-533.50)	328.00 (276.00-687.00)	0.563
Nucl. act.	15.10 (9.70-22.50)	25.40 (10.50-28.70)	0.018

No significant change of eDNA concentration in patients with exacerbation of bronchopulmonary process, in comparison with those in remission was observed (Table 5). While in patients with exacerbation of the bronchopulmonary process, a decrease in nuclease activity was demonstrated ($p < 0.05$) (Table 5)

Table 5 – The eDNA concentration and nuclease activity in exacerbation and remission of bronchopulmonary process in the general group and in the group of patients with F508del/F508del genotype, Me (Q1-Q3)

Disease stages	eDNA concentration	p	Nuclease activity	p
General group				
Exacerbation (n=35)	350.00 (193.00-807.00)	0.7979	13.17 (7.10-18.60)	0.0362
Remission (n=77)	454.0 (208.00-759.00)		16.44 (9.70-23.20)	
Patients with F508del/F508del genotype				
Exacerbation (n=13)	328.00 (158.00-493.00)	0.8068	10.40 (7.50-20.20)	0.0217
Remission (n=21)	318.00 (208.00-687.00)		22.85 (11.20-25.40)	

A statistical analysis was also performed to determine the dependence of the eDNA concentration and nuclease activity on other markers of inflammation. The eDNA concentration and the level of nuclease activity did not correlated with the ESR parameter. In cases of leukocytosis of more than 10 x 10^9 there was an increase of the eDNA concentration in the blood plasma, the relationship between leukocytosis and the of nuclease activity was not demonstrated (Table 6).

Table 6 – The eDNA concentration (ng/ml) and nuclease activity (UA) and relationship with ESR and the blood leukocyte count, Me (Q1-Q3)

Parameter	ESR (mm/h)		p	Leukocytes		p
	< 15 mm/h	> 15 mm/h		< $10x10^9$	> $10x10^9$	
n	37	9		52	15	
eDNA	493.00 (255.00-685.00)	328.00 (138.00-738.00)	0.567	289.50 (161.00-577.00)	532.00 (320.00-1384.00)	0.0619
Nucl. act.	16.10 (7.90-23.20)	10.40 (8.40-15.10)	0.4346	15.10 (8.40-21.20)	15.60 (7.50-16.30)	0.3138

Therapy with basic drugs such as hypertonic saline, steroids, azithromycin and other antibiotics is not associated with changes in the eDNA concentration and the nuclease activity. In the group of patients regularly treated with bronchodilators, the median of nuclease activity was 13.00 UA. (7.50-17.50), and in the group without bronchodilators treatment the median nuclease activity was 16.30 (10.40-22.85) UA, which is significantly higher (Table 7).

Table 7 – The eDNA concentration (ng/ml) and the nuclease activity (UA) during the continuous use of bronchodilators, Me (Q1-Q3).

Parameter	Bronchodilators treatment	No bronchodilators treatment	p
n	55	53	
eDNA	371.60 (190.20-740.00)	467.00 (243.00-945.00)	0.4664
Nuclease activity	13.00 (7.50-17.50)	16.30 (10.40-22.85)	0.03557

4. Discussion

Circulating extracellular DNA (eDNA) is present in the bloodstream and other biological fluids of both healthy and sick individuals. The eDNA concentration is increased in exacerbation of several illnesses (autoimmune, cardiovascular, oncological diseases) and in cases of exposure to various damaging factors [5],[6].

The eDNA actively interacts with the organism cells, activating the NF-kB signaling pathway through the TLR9 receptors and stimulates inflammatory response. The eDNA concentration is regulated by the endonucleases activity [6].

It is known that in patients with cystic fibrosis the eDNA accumulates in bronchial secretion, resulting in further increase of sputum viscosity. It is known that disorder of mucociliary clearance is the basic cause of respiratory tract damage in cystic fibrosis. The secretion of the mucous membranes of respiratory tract in patients with CF is viscous due to a derangement of the chlorine channel activity caused by mutations in the CFTR gene (data from the National Consensus "Cystic Fibrosis: Definition, Diagnostic Criteria, Therapy" (2016)). Therefore, regular mucolytic therapy, primarily with Borns alpha, is indicated as a pathogenetic treatment [2].

The initial assumption made by the authors of the study, that the extracellular DNA (eDNA) concentration in plasma of patients with cystic fibrosis was increased in comparison with healthy control was not confirmed. The eDNA concentration was lower than that of healthy children (Table 1). In addition, the nuclease activity of blood plasma in patients with cystic fibrosis decreased with age ($p < 0.0001$), and at the same time the progression of the disease was observed.

We showed, that eDNA concentration in patients with $FEV_1 > 80\%$ does not differ from the control group. eDNA concentration decreases along with a decrease of lung function ($FEV_1 < 80\%$). Patients with deteriorated lung function and increased requirement for bronchodilators had low nuclease activity, which, probably, was compensatory.

There are several hypotheses concerning the origin of extracellular DNA, the main ones being the following: formation of an extracellular nucleic acids pool as a result of cell death (the "cell death hypothesis") and active DNA secretion by living cells (the hypothesis of "metabolic DNA") [7],[8],[9]. Apparently, in CF, the first process and an increase of the second process activity occur. Predominance of the first or second process depends on the stage and duration of the disease. Decrease of the eDNA concentration along with increased damage to blood cells DNA could be due to activation of blood plasma endonucleases [10]. However, in our study no clear relationship between the eDNA concentration and nuclease activity is demonstrated. However, the researchers do not exclude that in a chronic process caused by a disease or external exposure, when the endonuclease activity of blood plasma increases, the eDNA concentration does not reflect the real level of DNA damage and the death of damaged cells [10]. The study of the concentration of GC-rich rDNA as a part of the circulating extracellular DNA in peripheral blood plasma will provide an explanation of the observed patterns. Thus an increase of the GC-rich ribosomal DNA (rDNA) concentration is a marker of chronic processes in the remission stage, which are accompanied by an increase of the cell death rate, but do not result in a significant increase in the total concentration of circulating DNA [5], [11]. These patterns probably occur during a chronic microbial inflammatory process in the respiratory tract of CF patients and require further study.

Determination of the composition of extracellular DNA will help to evaluate the biological activity of this molecule in relation to cells of patients with CF. It was found that oxidized and GC-enriched eDNA fragments result in oxidative stress in human cells, double-strand breaks of cell nuclei DNA, activation of reparation systems and adaptive response. The oxidized fragments penetrate more effectively into human cells and stimulate the production of active oxygen forms, which can provoke the progression of the disease [5].

Previously, it was shown that injection of a high-molecular DNA preparation in combination with proteins activates the humoral and cellular immunity responses, stimulates the phagocytic activity of blood monocytes against both gram-positive (S. aureus) and gram-negative (Y. pseudotuberculosis) microorganisms, increasing the survival rate of mice after the injection of lethal doses of the pathogen [12]. In addition, it is noted that upon the DNA injection to intact animals, their serum cholesterol levels decrease, the production of prostaglandin E increases, and the reproductive functions enhance. The authors explain the result by the fact that the nitrogenous compounds (adenine and guanine) forming the DNA are the structural basis for low-molecular biologically active coenzymes and cofactors which limit biological processes in all organs and tissues of the body. In this regard, they have a significant and versatile effect on the body cells, increasing their metabolic pool without a marked increase in oxygen consumption " [12].

It is possible that in patients with normal spirometry parameters, eDNA plays a metabolic role and its composition differs from that of the eDNA of patients with impaired lung function. Decrease of the eDNA concentration and alteration of its composition can be important for predicting the course of the disease. Probably, not only the quantity, but also the composition of eDNA plays a role in the pathogenesis of lung damage in cystic fibrosis. Interpretation of the qualitative composition of the eDNA requires further study to determine its role in the development of the chronic inflammatory process in CF. In the future, it is possible to develop algorithms for predicting the severity of CF, depending on the eDNA qualitative and

quantitative composition. In addition, the study of eDNA in CF can be used for development of new pathogenetic treatment modalities.

5. Conclusions

1. In the blood plasma of patients with cystic fibrosis, the concentration of extracellular DNA (eDNA) is lower compared to the healthy children.
2. In patients with normal lung function (according to FEV_1 values), the eDNA concentration does not differ from control, which suggests the metabolic role of the eDNA. Impairment of lung function is associated with a decrease of the eDNA concentration in the blood plasma.
3. The nuclease activity of blood plasma in patients with cystic fibrosis decreased with age ($p < 0.0001$), and at the same time the progression of the disease was observed.
4. During an exacerbation of the bronchopulmonary process, and a continuous therapy of bronchodilators a lower level of plasma nuclease activity was observed.

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References

1. Козлов В.А. Свободная внеклеточная ДНК в норме и при патологии / В.А. Козлов // Медицинская иммунология. – 2013. - №15(5).- С. 399-412. https://doi.org/10.15789/1563-0625-2013-5-399-412
2. Клинические рекомендации. Кистозный фиброз (муковисцидоз) у детей. Союз педиатров России, 2016. – 58с.
3. Miller M.R. Standardisation of spirometry /Miller M.R., Hankinson J., Brusasco V. et al. // Eur. Respir. J. – 2005. - 26 (2). – P.319-337. https://doi.org/10.1183/09031936.05.00034805.
4. Quanjer P.H. Lung volumes and forced ventilatory flows. Report Working Party Standardization of Lung Function Tests, European Community for Steel and Coal. Official Statement of the European Respiratory Society / P.H. Quanjer, G.J. Tammeling, J.E. Cotes and all. // Eur Respir J. – 1993.- № 6 Suppl. 16. – P. 5-40. doi: 10.1183/09041950.005s1693.
5. Костюк С.В. Изменение свойств внеклеточной ДНК периферической крови и частоты TCR-мутантных клеток при действии на организм человека ионизирующей радиации / С.В. Костюк, И.А. Замулаева, Р.К. Агапова и др. // Радиационная биология. Радиоэкология. - 2008. - Т. 48. - С. 5- 13.
6. Костюк С.В. Фрагменты внеклеточной ДНК усиливают транскрипционную активность генома мезенхитмальных стволовых клеток человека, активируют TLR-зависимый сигнальный путь и ингибируют апоптоз / С.В. Костюк, Е.М. Малиновская, А.В. Ермаков и др. // Биомедицинская химия, 2012. - том 58. - № 6. - С. 673-683.
7. Pisetsky D.S. The Origin and Properties of Extracellular DNA: From PAMP to DAMP / D.S. Pisetsky // Clinical immunology (Orlando, Fla). 2012 - №144(1). – P. 32-40. doi:10.1016/j.clim.2012.04.006.
8. Dimetrie L. Origin, translocation and destination of extracellular occurring DNA — A new paradigm in genetic behavior / Dimetrie L. Peters, Piet J. Pretorius// Clin. Chim. Acta. - 2011 - №12; 412(11-12). – P.806-11. doi: 10.1016/j.cca.2011.01.026.
9. Gahan P.B. Circulating nucleic acids in plasma and serum. Recent developments // P.B. Gahan, R.Swaminathan // Ann. N. Y. Acad. Sci. – 2008. – Vol. 1137. – P. 1–6.
10. Костюк С.В. Роль внеклеточной ДНК в функциональной активности генома человека: автореферат дис. доктора биологических наук: 03.02.07 : Место защиты: Мед.-генет. науч. центр РАМН 12.05.2014 / Костюк Светлана Викторовна. - Москва, 2014. - 46 с.
11. Вейко Н.Н. Изменение свойств внеклеточной ДНК периферической крови при ревматоидном артрите / Н.Н. Вейко, С.В. Костюк, Н.О. Шубаева и др. // Иммунология. – 2007 – Т. 28 №3. – С. 389–394.
12. Беседнова Н.Н. Иммунотропные свойства дезоксирибонуклеиновой кислоты из молок лососевых рыб / Н.Н. Беседнова, Ю.И. Касьяненко, Л.М. Эпштейн и др. // Антибиотики и химиотерапия. – 1999. – № 10. – С. 13–15.

References in English

1. Kozlov V.A. Svobodnaja vnekletochnaja DNK v norme i pri patologii [Free extracellular DNA in normal state and under pathological conditions] / V.A. Kozlov // Medicinskaja immunologija [Medical immunology]. – 2013. - № 15(5). – P. 399-412. https://doi.org/10.15789/1563-0625-2013-5-399-412 [in Russian]
2. Klinicheskie rekomendacii. Kistoznyj fibroz (mukoviscidoz) u detej. Sojuz pediatrov Rossii [Clinical recommendations. Cystic fibrosis (mukoviscidoz) in children. The Union of pediatricians of Russia], 2016. – 58 p. [in Russian]
3. Miller M.R. Standardisation of spirometry /Miller M.R., Hankinson J., Brusasco V. et al. // Eur. Respir. J. – 2005. - 26 (2). – P.319-337. https://doi.org/10.1183/09031936.05.00034805.
4. Quanjer P.H. Lung volumes and forced ventilatory flows. Report Working Party Standardization of Lung Function Tests, European Community for Steel and Coal. Official Statement of the European Respiratory Society / P.H. Quanjer, G.J. Tammeling, J.E. Cotes and all. // Eur Respir J. – 1993.- № 6 Suppl. 16. – P. 5-40. doi: 10.1183/09041950.005s1693.

5. Kostjuk C.B. Izmenenie svojstv vnekletochnoj DNK perifericheskoj krovi i chastoty TCR-mutantnyh kletok pri dejstvii na organizm cheloveka ionizirujushhej radiacii [Change in the properties of extracellular DNA of peripheral blood and the frequency of TCR-mutant cells under the action of ionizing radiation on the human body] / C.B. Kostjuk, I.A. Zamulaeva, R.K. Agapova and others // Radiacionnaja biologija. Radiojekologija. [Radiation Biology. Radioecology] - 2008. - T. 48. - p. 5- 13. [in Russian]

6. Kostjuk S.V. Fragmenty vnekletochnoj DNK usilivajut transkripcionnuju aktivnost' genoma mezenhitmal'nyh stvolovyh kletok cheloveka, aktivirujut TLR-zavisimyj signal'nyj put' i ingibirujut apoptoz. [Extracellular DNA fragments enhance the transcriptional activity of the genome of human mesenchymal stem cells, activate the TLR-dependent signaling pathway, and inhibit apoptosis] / S.V. Kostjuk, E.M. Malinovskaja, A.V. Ermakov and others // Biomedicinskaja himija [Biomedical Chemistry] 2012. - 58 (6). - P. 673 - 683. [in Russian]

7. Pisetsky D.S. The Origin and Properties of Extracellular DNA: From PAMP to DAMP / D.S. Pisetsky // Clinical immunology (Orlando, Fla). 2012 - №144(1). – P. 32-40. doi:10.1016/j.clim.2012.04.006.

8. Dimetrie L. Origin, translocation and destination of extracellular occurring DNA — A new paradigm in genetic behavior / Dimetrie L. Peters, Piet J. Pretorius // Clin. Chim. Acta. - 2011 - №12; 412(11-12). – P.806-11. doi: 10.1016/j.cca.2011.01.026.

9. Gahan P.B. Circulating nucleic acids in plasma and serum. Recent developments // P.B. Gahan, R.Swaminathan // Ann. N. Y. Acad. Sci. – 2008. – Vol. 1137. – P. 1–6.

10. Kostjuk S.V. Rol' vnekletochnoj DNK v funkcional'noj aktivnosti genoma cheloveka : avtoreferat dis. doktora biologicheskih nauk: 03.02.07 [The role of extracellular DNA in the functional activity of the human genome: the author's abstract of the thesis of the Doctor of Biological Sciences] : defense of thesis : Med.-genet. nauch. centr RAMN 12.05.14 / Kostjuk S.V. - Moskva, 2014. - 46 p. [in Russian]

11. Vejko N.N. Izmenenie svojstv vnekletochnoj DNK perifericheskoj krovi pri revmatoidnom artrite [Change in properties of extracellular DNA of peripheral blood in rheumatoid arthritis] / Vejko N.N., Kostjuk C.V., Shubaeva N.O. and others // Immunologija [Immunology] – 2007 – T. 28 .- №3. – P. 389–394. [in Russian]

12. Besednova N.N. Immunotropnye svojstva dezoksiribonukleinovoj kisloty iz molok lososevyh ryb [Immunotropic properties of deoxyribonucleic acid from milt of salmonids] / Besednova N.N., Kas'janenko Ju.I., Jepshtejn L.M. and others // [Antibiotics and chemotherapy] – 1999. – № 10. – P. 13–15. [in Russian]