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Role Of Genetic Polymorphism Of Cytokines In The Development Of Community-Acquired Pneumonia In Newborns And Children In The First Year Of Life

Kamalov Zainitdin[1*], Suleymanova Lola[2], Rakhmankulova Zukhra[3], Ruzibakieva Malika[4], Alimova Makhbuba[5], Irsalieva Fatima[6]

[1] MD, PhD, Prof., Head of Lab., Institute of Immunology and Human Genomics, Academy of Sciences of the Republic of Uzbekistan, E-mail: zay_kamal@rambler.ru, Tashkent, Uzbekistan
[2]Assistant, Department of Neonatology, Tashkent Pediatric Medical Institute, E-mail: neonatolog88@mail.ru, Tashkent, Uzbekistan.
[3]MD, PhD, Associate Professor, Department of Neonatology, Tashkent Pediatric Medical Institute, E-mail: razuxra55@inbox.ru, Tashkent, Uzbekistan
[4]MD, PhD, Institute of Immunology and Human Genomics, Academy of Sciences of the Republic of Uzbekistan, Tashkent, Uzbekistan
[5] PhD, Institute of Immunology and Human Genomics, Academy of Sciences of the Republic of Uzbekistan, E-mail: makhbuba @rambler.ru, Tashkent, Uzbekistan
[6]MD, PhD, Professor of the Department of Allergology, Clinical Immunology and Nursing of the Tashkent Medical Academy. Member of the Academy of Allergy and Clinical Immunology EAACI, E-mail: irsalieva73@mail.ru, Tashkent, Uzbekistan. https://orcid.org/0009-0006-0114-2870, Tashkent, Uzbekistan

[1]zay_kamal@rambler.ru https://orcid.org/0000-0001-6551-3155
[2]neonatolog88@mail.ru https://orcid.org/0000-0002-4521-8965
[3]razuxra55@inbox.ru, https://orcid.org/0000-0002-4285-9599
[4]malika-ruz@hotmail.com , https://orcid.org/0000-0002-5982-945X
[5]E-mail: makhbuba @rambler.ru,
[6]irsalieva73@mail.ru https://orcid.org/0009-0006-0114-2870

ABSTRACT

One of the most promising approaches to assessing genetic predisposition to respiratory diseases is to identify their association with certain candidate genes. Studying the relationship

[*]**For correspondence**
Zainitdin S. Kamalov – MD, PhD, Prof., Head of Lab., Institute of Immunology and Human Genomics, Academy of Sciences of the Republic of Uzbekistan, 100060, Tashkent
https://orcid.org/0000-0001-6551-3155

between the polymorphism of these genes and the production of cytokines will make it possible to predict the risk of developing pathology, and the nature and severity of its course.

Purpose of the study. To study the features of polymorphism of the cytokine genes IL-4 IL-17, and TNF-α in newborns and children in the first year of life with community-acquired pneumonia.

Materials and methods. We examined 138 children, of which the 1st main group consisted of 72 newborns and children in the first year of life with community-acquired pneumonia, and the 2nd control group included 66 practically healthy children. Genotyping of polymorphic regions of immune response genes was carried out using the polymerase chain reaction (PCR) method with allele-specific primers (NPF "Litekh", Moscow) and electrophoretic detection of reaction products in agarose gel.

Results. It has been established that the -308(G/A) TNF polymorphism contributes to the susceptibility to the development of community-acquired pneumonia in newborns and children of the first year of life and, like IL-4 (-590) C/T rs 2243250 C C, is one of the prognostic factors for the development of the pathology under study.

Conclusions. The data obtained may be one of the prognostic factors for the development of community-acquired pneumonia in newborns and children in the first year of life.

Keywords: newborns, children of the first year of life, community-acquired pneumonia, cytokines, gene polymorphism.

INTRODUCTION

The problem of pneumonia persists throughout the world due to the high prevalence of the disease and the lack of a tendency to improve treatment outcomes, despite the use of the latest antibacterial drugs. Community-acquired pneumonia makes a significant contribution to the morbidity and frequency of hospitalization of children due to its fairly high frequency, polyetiology and severity of the course, as a result of which it seems to be a central problem in paediatrics and infectious pathology [3,4,6].

In addition, mortality in community-acquired pneumonia does not decrease, and the number of patients with a protracted, asymptomatic course, as well as with severe complications of the disease, is steadily growing. The high incidence and often complicated course determine the relevance of studying the clinical and diagnostic aspects of community-acquired pneumonia [1]. An important task in revealing the pathogenetic links in the development and course of various diseases and identifying predisposition to diseases at earlier stages is the study of genetic polymorphisms that control the activity of cytokines [2,13].

Modern scientific literature increasingly publishes information about the significant role of individual genetic predisposition in the development of lung diseases [8,11]. At the same time, one of the most promising approaches to assessing genetic predisposition to respiratory diseases is to identify their association with certain candidate genes. In particular, based on modern data on the pathogenesis of inflammatory damage to the respiratory tract, one of these candidate genes are the genes of pro- and anti-inflammatory cytokines [5,14].

Currently, there is information about a large number of genes that influence the severity and outcome of pneumonia. The presence of a mutant gene is by no means the cause of the disease. However, the individual combination of various variants of polymorphic genes can partly determine the nature of the inflammatory response and specific immunological reactions during

the introduction of infectious agents [7,12]. However, this area of research into acute respiratory pathology in children, in particular community-acquired pneumonia, is practically undeveloped. This determined the relevance of the study.

Thus, the study of molecular genetic mechanisms in community-acquired pneumonia in newborns and children in the first year of life is an important area, the solution of which will allowdetermine the prognosis for the development of pathology before the appearance of its clinical manifestations.

Target. To study the features of polymorphism of the cytokine genes IL-4, IL-17, and TNF-α in newborns and children in the first year of life with community-acquired pneumonia.

MATERIALS AND METHODS

We examined 138 children, of which the 1st main group consisted of 72 children in the first year of life with community-acquired pneumonia, of which 38 were newborns, and the 2nd control group included 66 practically healthy children. By definition, community-acquired pneumonia (home, outpatient) is pneumonia that develops outside the hospital or in the first 72 hours of hospitalization. The International Classification of Diseases, Injuries and Causes of Death, 10th revision (ICD-10) was used in this work. The diagnosis of community-acquired pneumonia was established based on reliable criteria proposed by A.G. Chuchalin et al. [9,10]. In 100% of cases, the diagnosis of community-acquired pneumonia in both groups was verified by chest x-ray data.

vacutainers were used to collect bloodBeckton- Dickinson) with anticoagulant/preservative 15% tripotassiumEDTA (Ethendianin- tetraaceticacid). Blood for further processing could be stored for up to 24 hours at a temperature not exceeding +4 ℃.

To obtain genomic DNA, a two-step method of blood cell lysis was used. Further purification of buffy cell lysates is based on the method of alcohol-salt treatment according to S. Miller et al. (1988) as modified by a laboratory at Stanford University.

Genotyping of polymorphic regions of immune response genes was carried out using the polymerase chain reaction (PCR) method with allele-specific primers (NPF "Litekh", Moscow) and electrophoretic detection of reaction products in agarose gel. 3 SNPs were tested : IL-4 (-590) S/T, IL-17 G-197A, TNF- α -308G/A. Identification of amplification products and their distribution concerning the length marker was carried out in ultraviolet light (310 nm) after electrophoresis for 15 minutes at a voltage of 300 V (in both cases the run was 3-) and staining with ethidium bromide 4 см.

The distribution of genotypes at the studied polymorphic loci was studied using logistic regression analysis and testing for compliance with Hardy–Weinberg equilibrium using Fisher's exact test. The correspondence of patients and controls by gender and age was taken into account. Differences were considered statistically significant at $p < 0.05$.

Statistical processing of the obtained data was carried out using Microsoft Excel, SISA 9.17® software packages and the *SISA* statistical software package,*Arlequin3.5.2.* and several formulas.

RESULTS

In this work, clinical-instrumental and clinical-laboratory studies in children with pneumonia were carried out based on the 5th City Clinical Children's Hospital. Immunological and genetic studies were carried out at the Institute of Human Immunology and Genomics of the Academy

of Sciences of the Republic of Uzbekistan. In total, we examined 138 children. We carried out genotypingIL-4 (-590) C/T rs2243250 in the group of children with community-acquired pneumonia (n = 72) and a comparative analysis of the results obtained in comparison with the control group (n = 66) was carried out. The control group consisted of practically healthy children in the first year of life and newborns born from healthy mothers with a favourable pregnancy, with a normal Apgar score at birth, and with a physiological course of the early adaptation period.

As a result of the analysis, it was found that the T allele was found significantly more often in the control group (32.58%) compared to the group of patients with community-acquired pneumonia (17.36%) with OR = 0.435, 95% CI = 0.247 >0.435 > 0.765 (Table 1).

Analysis of the frequency distribution of the homozygous genotype TT IL-4 (-590) C/T rs 2243250 did not show significant differences. This table shows that in the group of patients with community-acquired pneumonia, the homozygous TT genotype was found in 4.17% of cases, and the control group in 9.09% of cases.

In turn, significant differences were shown for the heterozygous CT genotype, which, according to OR indicators, was registered as a protective genotype OR = 0.405, 95% CI = 0.198 > 0.405 > 0.826, $\chi 2 = 6.313$ (p = 0.011986).

Table 1.IL-4 (-590) C/T rs 2243250 genotypes in the group of sick children with community-acquired pneumonia

Genotype	Patients, n=72	Patients, %	Genotype	Control, n=66	Control, %	$\chi 2$	OR (95% CI)
C	119	82.64	C	89	67.42	8.586 (p=0.003387)	1.308 >2.3> 4.044
T	25	17.36	T	43	32.58		0.247 >0.435> 0.765
CC	50	69.44	CC	29	43.94	9.152 (p=0.002484)	1.442 >2.9> 5.83
CT	19	26.39	CT	31	46.97	6.313 (p=0.011986)	0.198 >0.405> 0.826
TT	3	4.17	TT	6	9.09	1.37 (p=0.241876)	0.104 >0.435> 1.814

Note: χ^2 – Pearson reliability indicator; O R – relative risk.

Thus, the frequency of occurrence of the CT genotype in the control group was 46.97%, and in the group of sick children with community-acquired pneumonia - 26.39% of cases. This table shows that in sick children with community-acquired pneumonia, allele C occurred with a frequency of82.64%, and in the control group - in 67.42% of cases. The CC genotype was observed in sick children in 69.44% of cases, and in the control group – in 43.94% of cases.

Thus, for the genotype CC IL-4(-590) C/T rs2243250,true significance was revealed as well as for the allelic variant C, which carries a prognostic predisposing value.

At the next stage, we analyzed the frequency of distribution of alleles and genotypes of the IL-17 gene in sick children with community-acquired pneumonia (Table 2).

Table 2.Distribution of alleles and genotypes of the IL-17 G-197A rs2275913 gene in sick children with community-acquired pneumonia

Genotype	Patients, n = 72	Patients, %	Genotype	Control, n = 66	Control, %	χ2	OR (95% CI)
G	118	81.94	G	112	84.85	0.418 (p=0.517846)	0.428 >0.81> 1.533
A	26	18.06	A	20	15.15		0.652 >1.234> 2.335
GG	49	68.06	GG	48	72.73	0.36 (p=0.54857 5)	0.383 >0.799> 1.665
GA	20	27.78	GA	16	24.24	0.223 (p=0.636603)	0.56 >1.202> 2.579
A.A.	3	4.17	A.A.	2	3.03	0.127 (p=0.721205)	0.225 >1.391> 8.598

Note. χ^2 – Pearson reliability indicator; O R – relative risk.

Our studies showed that no significantly significant differences were found in the distribution of alleles and genotypes of IL-17 G-197A rs2275913 in patients with community-acquired pneumonia and controls. Thus, the frequency of occurrence of the G allele in the group of sick children was 81.94%, and in the control group – 84.5%. Allele A was found in sick children in 18.06% of cases, and in the control group – in 15.15% of children. The GG genotype in sick children occurred in 68.06%, and in the control group, its frequency was slightly higher (72.73%). The frequency of occurrence of the GA genotype in the group of sick children was 27.78%, and in the control group - 24.24%. The AA genotype in children of the main and control groups was found in the smallest quantities, which amounted to 4.17% and 3.03%, respectively.

Thus, the data obtained indicate that in this sample of sick children with community-acquired pneumonia of moderate severity, the studied polymorphism of the IL-17 gene G-197A rs2275913 does not play the role of a protector or a predisposing marker.

In a comparative study of the distribution of allele frequencies and genotypes of polymorphic markers of the *TNFα -308G/A* rs1800629 gene in groups of patients and controls (Table 3), a statistically significant increase in the frequency of the A allele was established in sick children with community-acquired pneumonia, compared with the control group (15.28% and 8.42%, respectively; OR = 1.961; 95% CI: 0.989 > 1.961 > 3.888; $\chi2 = 3.82$ (p = 0.050656)). At the same time, *the G* allele of the polymorphism under study was found significantly less frequently compared to the control group (84.72% and 91.58%, respectively; OR = 0.51; 95% CI: 0.257 >0.51 > 1.011; $\chi2=3.82$ (p=0.050656)).

Further, a comparative analysis of *TNFα -308G/A* genotypes according to the GG genotype revealed significant differences between patients and the control group (OR = 0.46; 95% CI: 0.221 >0.46> 0.96; $\chi2 = 4.382$ (p = 0.036316)). It was found that the GG genotype in children with community-acquired pneumonia was significantly less common (69.44%) than in children of the control group (83.16%).

Table 3.Distribution of frequencies of alleles and genotypes of the TNF- A -308G/A rs1800629 gene in patients with community-acquired pneumonia

SNP	Group	Allele	Allele frequency,%	χ2	OR (95% CI)	Genotype	Genotype frequency,%	χ2	OR (95% CI)
FNO-a rs1800629	Group of patients	G	84.72	3.82 (p=0.050656)	0.257 >0.51> 1.011	GG	69.44	4.382 (p=0.036316)	0.221 >0.46> 0.96
		A	15.28		0.989 >1.961 > 3.888	GA	30.56		1.042 >2.173> 4.531
						A.A.			
	Control group	G	91.58			GG	83.16		
		A	8.42			GA	16.84		
						A.A.			

Note. χ^2 – Pearson reliability indicator; O R – relative risk.

When analyzing the heterozygous GA genotype, differences were revealed between the frequency of occurrence in patients and the control group (OR = 2.173; 95% CI: 1.042 >2.173 > 4.531; χ2 = 4.382 (p = 0.036316)). Thus, the GA genotype was found in sick children in 30.56% of cases, and in the control group much less frequently - in 16.84% of children.

Thus, the data obtained indicate that the -308(G/A) TNF polymorphism contributes to the susceptibility to the development of community-acquired pneumonia in newborns and children of the first year of life and, like IL-4 (-590) C/T rs 2243250 C C, is one of the prognostic factors for the development of the pathology under study.

DISCUSSION

One of the leading places in the structure of morbidity in children today is occupied by respiratory pathology [15]. Community-acquired pneumonia is the most common form of bronchopulmonary diseases and remains one of the leading causes of death from infectious diseases. Despite the fact that significant progress has been made in its diagnosis and treatment in our country and in the world over the past decades, this disease still remains an acute problem not only in pulmonology, but also in pediatrics as a whole [16]. Thus, according to WHO data, about 155 million cases of pneumonia in children are registered annually in the world, and about 1.4 million of them die before the age of five. Obviously, the risk of developing an infectious disease is genetically determined. Currently, there is evidence of a large number of genes influencing the severity and outcomes of community-acquired pneumonia [17].

One of the most relevant and promising areas in the assessment of genetic predisposition to many recurrent diseases, in particular respiratory diseases, is to identify their association with candidate genes. Active research in recent years has made it possible to identify some candidate genes involved in the development of pulmonary pathology. These markers include genes whose expression products may be involved in the pathogenesis of development, through which the effects of inflammation are realized [4]. Candidate genes for pro-and anti-inflammatory cytokines are among the most significant.

In this regard, it can be assumed that the polymorphism of genes encoding pro-and anti-inflammatory cytokines affects the development and clinical course of respiratory diseases in children.

This article presents data indicating that the -308(G/A) TNF polymorphism contributes to the predisposition to the development of community-acquired pneumonia in newborns and children of the first year of life and, like IL-4 (-590) S/T rs 2243250 SS, is one of the prognostic factors for the development of community-acquired pathology. The conducted studies can be used in the development of prognostic markers of pathology in children and optimization of treatment tactics and preventive measures with an individual approach for each patient.

CONCLUSION

1. It was found that the polymorphism of the TNFa -308G/A rs1800629 gene contributes to the predisposition to the development of community-acquired pneumonia with a severe course in newborns and children of the first year of life, while a statistically significant increase in the frequency of the A allele and the heterozygous GA genotype was found. The protective value is carried by the allele G and the homozygous genotype GG.

2. The study of IL-17 G-197A rs2275913 polymorphism showed that in children with community-acquired pneumonia, regardless of the severity, no alleles and genotypes of prognostic significance were found, which suggests that the studied gene polymorphism does not play a significant role in the development of this pathology.

3. It has been established that IL-4 (-590) S/T rs2243250 SS polymorphism makes a certain contribution to the development of community-acquired pneumonia in newborns and children of the first year of life, regardless of the severity. For the CC genotype, the true significance was revealed regardless of the severity, as well as for the C allele variant, which carries a prognostic predisposing value. For mild pneumonia, the heterozygous CT genotype, and for severe pneumonia, the T allele carry a protective value.

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Rakhmankulova Z. Zh.; research concept and design, interpretation of data-Karimdzhanova G.; approval of the final version of the article-Kamalov Z. S.; technical processing of the article – Suleymanova L. I.

REFERENCES

1. Baigozina E. A. Polymorphism of interleukin-1 family genes as a factor in the pathogenesis of nosocomial pneumonia // Medical sciences. 2015. No. 2. pp. 121-124.
2. Pikuza O.I., Rizvanova F.F., Generalova E.V., Kravtsova O.A. Polymorphism of pro- and anti-inflammatory cytokine genes and acute bronchitis in children // Russian Bulletin of Perinatology and Pediatrics. 2017. No. 5. pp. 136-138.
3. Rizvanova F.F., Pikuza O.I., Generalova E.V., Fayzullina R.A., Kravtsova O.A. Gene polymorphism and community-acquired pneumonia in children // Pediatrics. 2018. T. 16. No. 8. P. 70-73.
4. Samsygina G.A. On the use of antibiotics in outpatient practice for respiratory pathology in children // Pediatrics. G.N. Speransky. 2018. T. 97 No. 2. P. 220-221.
5. Tatochenko V.K. Community-acquired pneumonia in children - problems and solutions // Russian Bulletin of Perinatology and Pediatrics. 2021. No. 1. pp. 9-21.
6. Chuchalin A.G. Pneumonia: an urgent problem of medicine of the 21st century // Pulmonology. 2015. T. 25, No. 2. pp. 133–142.
7. Chuchalin A.G., Sinopalnikov A.I., Kozlov R.S., Tyurin I.E., Rachina S.A. Community-acquired pneumonia in adults: practical recommendations for diagnosis, treatment and prevention // Journal for continuing medical education of doctors. 2013. No. 2. pp. 92-123.
8. Shilova N. A., Fetisova I. N., Mezhinsky S. S., Chasha T. V., Panova I. A., Kulida L. V., Ratnikova S. Yu. Polymorphism of genes of the detoxification system and the major histocompatibility complex hair class in very premature newborns with congenital pneumonia // Russian Bulletin of Perinatology and Pediatrics. 2020. No. 5. pp. 47-53.
9. Esposito S., Patria MF, Tagliabue C. et al. CAP in children // European Respiratory Monograph: Community-Acquired Pneumonia. 2014. P.130-139.
10. Kimberly S. S., Burton L. H., Christa M. G. Communityaguiredpneumoniainchildren//Am. Fam. Physician. 2012. No. 86. P.661-667.
11. Kumar S., Wang L., Fan J. et al. Detection of 11 common viral and bacterial pathogens causing community-acquired pneumonia or sepsis in asymptomatic patients by using a multiplex reverse transcription - PCR assay with manual (enzyme hybridization) or automated (electronic microarray) detection // J. Clin. Microbiol. 2008. No. 46. P. 3063-3072.
12. Michelow IC, Olsen K, Lozano J et al. Epidemiology and clinical characteristics of community-acquired pneumonia in hospitalized children//Pediatrics. 2004. No. 113. P.701-707.
13. PrittB.S., AubryM.C. Histopathologyofviralinfectionsofthelung // SeminDiagnonPathol.2017. Vol. 6.P. 510–517.
14. WattanathumA. Interleukin-10 Haplotype associated with increased mortality in critically 111 patients with sepsis from pneumonia but not in patients with extrapulmonary sepsis. 2005. No. 128(3). P.1690-1698.
15. Grunewaldt A., Hügel K., Bellinghausen K. et al. Do not underestimate the lethality of community-acquired pneumonia! MMW – Advances in Medicine 162, 39–42 (2020).

16. Nair GB, Niederman MS. Updates on community acquired pneumonia management in the ICU. PharmacolTher. 2021 Jan;217:107663. doi: 10.1016/j.pharmthera.2020.107663. Epub 2020 Aug 15. PMID: 32805298; PMCID: PMC7428725.
17. de Benedictis FM, Kerem E, Chang AB, Colin AA, Zar HJ, Bush A. Complicated pneumonia in children.Lancet. 2020 Sep 12;396(10253):786-798. doi: 10.1016/S0140-6736(20)31550-6. PMID: 32919518.