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Cytogenetic Changes In The Hereditary Apparatus of Children of Early Age Suffering From Complicated Pneumonia Combined With Iron Deficiency Anemia

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Cytogenetic changes of the hereditary apparatus in children of early age suffering from complicated pneumonia combined with iron deficiency anemia have been studied. Using comparative analysis of changes in the genetic apparatus of children suffering from CP combined with IDA, we proved their dependence on the severity of anemia. We found an increase in the frequency of associations of aerocentric chromosomes and chromosomal aberrations in children with complicated pneumonia compared with control and dependence of these parameters on the severity of iron deficiency anemia. It has been established that chromosomes 21, 15 and 14 have the greatest ability to form associations, the lowest - 12 and 13 in each group of sick children. Among the types of chromosomal aberrations identified chromatic (single fragments), chromosomal (paired fragments isolated dicentrics and abnormal monocentrics), deletions and chromosome disruption. In the spectrum of chromosomal aberrations we proved the advantage of deletions of long arm of the 4th and short arm of the 5th and 6th chromosomes.

Keyword: Children, pneumonia, anemia, associations of acrocentric chromosomes.

1. Introduction

Pneumonia is one of the urgent problems of child pulmonology due to its high prevalence and mortality. Particularly noteworthy are cases of pneumonia course with aggravated premorbid background or accompanying pathology [1, 2]. Among many factors that may complicate pneumonia course is iron deficiency. In recent years there has been an increase in the number of patients with pneumonia among children of early age, occurring in combination with iron deficiency anemia (IDA) [2]. One of the least covered aspects of the problem still remains cytogenetic homeostasis in above mentioned combined pathology. Currently, connection between changes in chromosomal apparatus of cell and inflammation has been established [5, 6, 9]. In particular, increase of chromosomal rearrangement in such inflammatory bronchopulmonary diseases as chronic bronchitis, bronchial asthma, acute pneumonia has already been described in literature [5, 11]. Among factors that violate genetic stability in inflammation of bronchi and lungs, are the following: influence of infection, including direct cytogenetic effect of viruses and indirect effects of bacterial and viral agents, increased release of inflammatory mediators, quantitative and functional deficiency of cellular link of immunity. However, relationship of cause and effect between inflammatory process and cytogenetic alteration are not completely understood. In this regard, we

have investigated the changes in the chromosome apparatus of children at this comorbidity. Alongside with this, main attention was concentrated on the study of frequency of associations of acrocentric chromosomes (AACh) and chromosomal aberrations (ChA) in peripheral blood lymphocytes as markers of immunogenetic status of a person.

2. Materials and Methods.

The object of study was peripheral blood of 25 children aged from two months to three years. Patients were divided into five equal groups. The 1st group was made from healthy children, the 2nd group consisted of children suffering from complicated pneumonia (CP) without anemia, the 3rd, 4th and 5th groups were for patients with complicated pneumonia with anemia of mild, moderate and severe stages respectively.

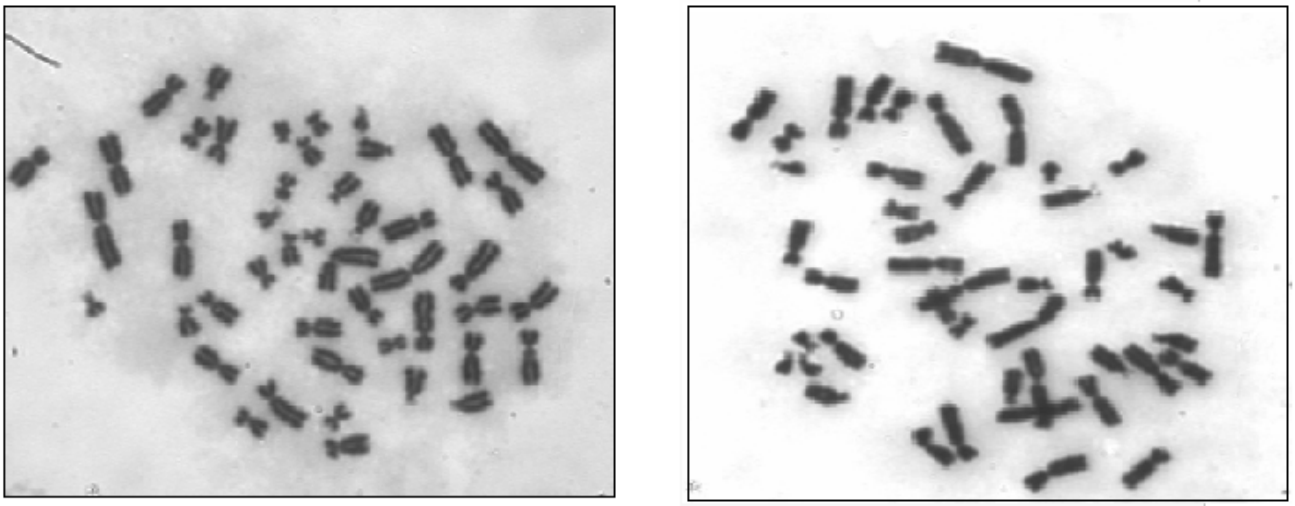
Cultivation of peripheral blood lymphocytes and preparing of chromosomes preparations were carried out by the guidelines approved by the Ministry of Health of Ukraine [3, 4]. Metaphase plates with a good spread of chromosomes were analyzed. The identification of chromosomal abnormalities, the number of associations of acrocentric chromosomes and chromosomal aberrations were carried out at the rate of 100 cells per patient using optoelectronic complex "Metaskan-2". Statistical analysis of the obtained results was carried out on personal computers using the package of statistical programs Microsoft Excel 7.0. Digital results were statistically processed using the method of variation analysis. Chance of variations was assessed using parametric Fisher-Student's criterion [8].

3. Results and Discussion

Comparative analysis of changes in the genetic apparatus of children suffering from CP combined with IDA, their dependence on the severity of anemia has been proved. We found 437 AACh 513 metaphase cells of patients, and in control group this index was lower at 4.05 times (38 AACh in 180 cells). With an increase of iron deficiency we noted tendency to growth of AACh frequency. The most pronounced variations of the given index have been defined in children from the 5th group.

The first stage of work was to study the overall distribution of AACh depending on the number of chromosomes associated with them. In the control group AACh from two and three chromosomes were more common, associations comprised of four acrocentric chromosomes were fewer in number. In children suffering from CP without IDA increase of two-acrocentric AACh in 1, 21 times has been registered compared with control group ($p>0,05$), but fewer were three-chromosome AACh. In the group of children with complicated pneumonia in combination with mild iron deficiency anemia increased incidence of AACh has also been noted, composed of two acrocentric chromosomes in 1, 16 times in comparison with the control group ($p>0,05$). If to speak about number of three-chromosome AACh, the tendency to increase has been discovered in comparison with that of children from the 2nd group. At the same time, in children from the 3rd group four-chromosome AACh were noted as opposed to children in the 2nd group. In children from the 4th group association, comprised of two acrocentric chromosomes occurred with equal frequency in comparison with the control group (Fig.1a). Fewer were three-chromosome AACh, but significantly increased the frequency of four-chromosome AACh in 1,60 times (Fig. 1b). With the growth of severity of IDA (the 5th clinical group) tendency to increasing of two- and four-chromosome associations was recorded. However, compared with the frequency of AACh in children with CP combined with moderate IDA, the above mentioned indexes were lower, that may indicate breakdown of the compensatory capacity of the immune system.

Average number of AACh in one cell of the control group was (0,23±0,03)%. In children with CP. without anemia average frequency of AACh in one cell was (0,63±0,03)%, ($p<0.001$) and further increased with the growth of the severity of IDA: up to (0,70±0,08)% in mild ($p<0.001$) to (0,85±0,10)% in average ($p<0.001$), and to (1,50±0,07)% ($p<0.001$) in severe degree of IDA.



(a) **(b)**

Fig 1: Metaphase plates of children suffering from complicated pneumonia combined with iron deficiency anemia of moderate (a) and grave (b) stages with associations of aerocentric chromosomes. Colouring by Himza. Increase h1000

All AACh according to the number of chromosome associations were divided into three groups (G, D i G+ D), each of them having three subgroups depending on the number of chromosomes in AACh (Table 1).

Table 1: Indicators of acrocentric chromosomes associations in peripheral blood lymphocytes of children with complicated pneumonia in combination with iron deficiency anemia, M±m

Frequency of cells with associations depending on chromosome group, (%)	Groups of examined children				
	I, n=5	II, n=5	III, n=5	IV, n=5	V, n=5
Group D					
2 associations	14,42±2,23	20,79±2,42	18,04±1,91	18,04±2,17	18,15±1,89
3 associations	12,56±1,73	9,36±0,73	9,83±3,56	9,08±0,75	10,40±1,39
4 associations	-	-	3,33±0,00	8,0±0,00	3,02±0,55
Group G					
2 associations	12,56±1,73	7,88±2,16	6,64±1,07*	7,34±0,67*	8,46±0,52
3 associations	12,88±3,79	5,84±0,84	7,32±1,70	7,18±1,80	5,68±0,69
4 associations	-	-	-	8,7±0,00	-
D+G groups					
2 associations	27,73±2,54	37,40±3,54	38,69±4,14	30,44±1,83	30,88±1,26
3 associations	32,88±4,30	18,0±1,11*	17,37±1,53*	24,40±2,58	16,94±0,56*°
4 associations	19,09±0,91	-	7,94±1,95*	13,89±2,14	7,90±0,60*°

Notes:
1. * - data are trustworthy regarding control ($p<0,05$);
2. ° - data are trustworthy between the 4th and 5th groups ($p<0,001$).

In children with complicated pneumonia without anemia, the frequency of chromosome associations of Group D with two acrocentrics prevailed the same associations in control at 30.64%. When analyzing peculiarities of children with CP on the background of mild, moderate and severe IDA, significant differences were not found, but their frequency was higher than the

control at 20.0%. The frequency of three-acrocentric AACh in children from the 2nd, 3rd and 4th groups was registered respectively in 1.34, 1.27 and 1.38 times less than in controls, and in children from the 5th group it insignificantly increased in 1.15 times compared with that of the 4th group. In children from the 1st and 2nd groups four-chromosome AACh was not found and it has been registered in children with CP combined with IDA. Four-chromosome AACh was observed most frequently in children with complicated pneumonia on the background of moderate IDA to 58.38% compared to those in children from the 3rd and 4th groups.

In healthy children, the frequency of two-chromosomes AACh from G group met the most frequently. In children with complicated pneumonia without anemia, the index was 37.26% lower compared with the control. In children with CP combined with mild IDA, the least amount of AACh was registered compared with controls at 47,13% ($p<0,05$). In all patients under study advantage of AACh, joining chromosome of groups D i G was registered. Particularly

demonstrative in this respect was the frequency of four-chromosome AACh (Table 1.). The next stage of work was investigation of association ability of chromosomes depending on their group membership. IN each group of sick children the greatest ability to associations demonstrated chromosomes 21 (16,28%), 15 (17.31%) and 14 (19.24%), the lowest - chromosomes 22 (13,22%) and 13 (11, 43%). It should be noted that the number of associations of chromosomes of D group predominated over the same of G group. When analyzing the peculiarities of D group in children with CP on the background of moderate and severe IDA significant differences were not found. Among the combination of chromosomes from D and G groups chromosomes 14 and 21 were mainly associated. Other chromosomes joined with the same frequency, although chromosome 21 more often was associated with chromosomes of D group than 22 one. Besides in combinations of chromosomes of G group greater tendency to associate chromosome 21 compared with chromosome 22 has also been noted. The latter can be explained by the fact that in the interphase nucleus satellite associations are formed during prolonged conjugation of homologous loci of heterochromatic areas of satellite filaments that are transferred through mitosis and recorded on metaphase plates [10].

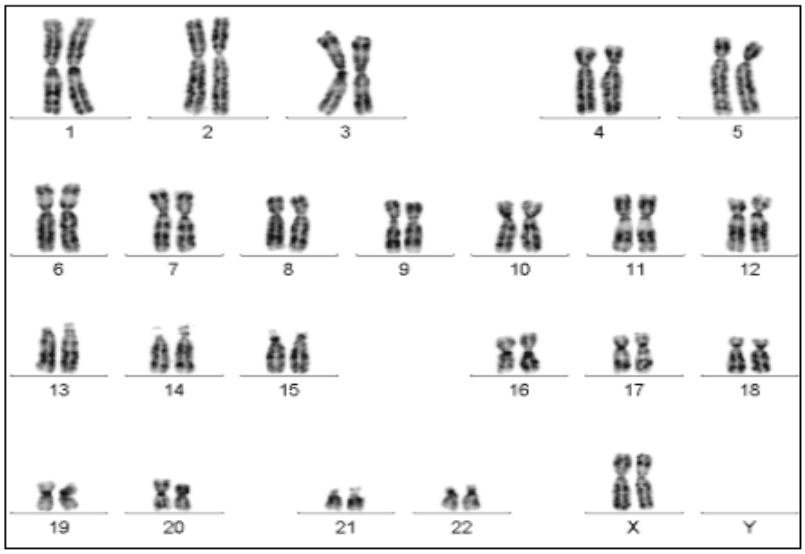
Quantitative AACh indexes we coordinated with ChA data rate. The latter also prevailed in combined pathology (CP and IDA) (Table 2).

Table 2: Frequency and types of chromosomal aberrations in children with complicated pneumonia combined with iron-deficiency anemia, M±m

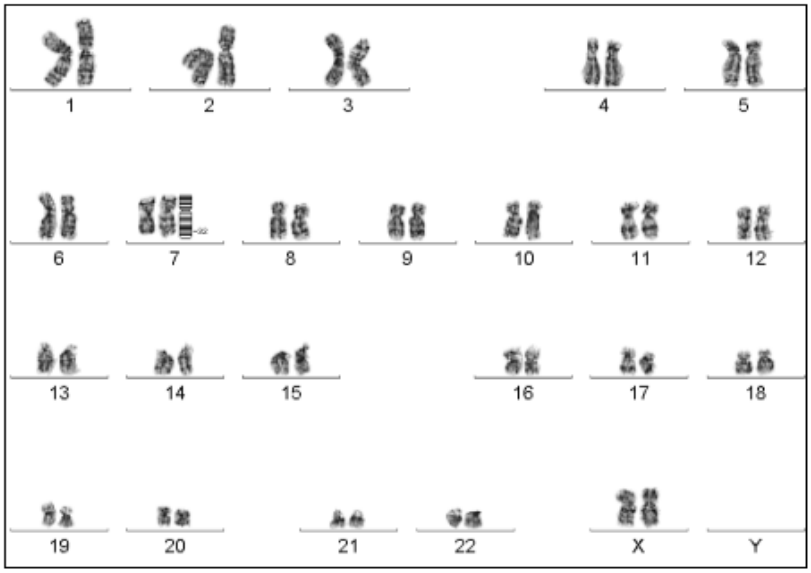
Examined groups, n=25	Chromosomal aberrations, %	Types of chromosomal aberrations, %		
		deletions, disruption	spaces	other
I, n=5	1,04±0,09	0,33±0,01	0,70±0,01	0,01±0,003
II, n=5	1,97±0,21	0,96±0,01*	0,91±0,02	0,10±0,001*
III, n=5	2,61±0,12*	1,48±0,04*	1,01±0,01	0,12±0,002*
IV, n=5	2,83±0,83*	1,67±0,02*	1,03±0,04	0,13±0,001*
V, n=5	3,46±0,15*	1,89±0,03*	1,12±0,02*	0,45±0,003*

Note * - trustworthy of difference from control indexes ($p< 0,05$).

In children with CP and IDA, the overall rate of ChA was higher than in children without IDA in 1.32 and 2.51 fold ($p<0.05$) compared with that in controls. The presence of chromosomal mutations was observed in all examined children, but with the increase in severity of iron deficiency anemia we noted changes in chromosome structure with greater probability. Average subgroup frequency of metaphases with ChaA in children with severe IDA prevailed the one in severe course of IDA in 1.33 times ($p<0,05$).



(a)



(b)

Fig 2: Karyotypes of a healthy girl (46, XX) from the control group (a) and of a girl suffering from pneumonia complicated with iron deficiency anemia of moderate severity , with deletion of the long arm of chromosome 7 (46, XX , del 7 q32). Coloring G method. Increase h1000.

Among the types of ChA chromatic single fragments have been identified that occurred with greater frequency in combined pathologies, especially in severe degree of IDA (see Table 2). Among chromosomal aberrations pair fragments were observed: in one child with CP and moderate IDA dicentric, fragment and one of the patients of the 5th group showed an abnormal monocentric one. Probable increase in frequency of chromosomal breaks and deletions has been found in children from the 2nd-5th clinical groups compared with controls (Table 2). The tendency to increasing the number of chromosome spaces depending on IDA severity has been found out.

Spaces are different from disruptions by the fact that there was no displacement between a fragment and chromosome; we observed connection between them.

All the above described changes in the chromosomal apparatus showed genotype instability that could lead to breach of genetic information and cell metabolism [7].

The given studies showed that in all groups of children with CP combined with IDA deletions of short arm of the fifth and sixth chromosomes and long arm of the fourth chromosome have been revealed. And in the 5th group deletions have been discovered in two children: of long arm of the fourth chromosome and short arm of the fifth chromosome. In addition, seven chromosome deletion has been identified in a girl with an average degree of IDA (Fig. 2).

4. Conclusions

1. We found an increase in the frequency of AACH and ChA in children with CP compared with the control and dependence of these indexes on the severity of IDA.
2. We established that the greatest ability to form associations was demonstrated by chromosomes 21, 15 and 14, the lowest one- by chromosomes 12 and 13 in each group of sick children.
3. In the spectrum of CHA advantage of deletions of long arm of the 4th chromosome and short arm of the fifth and sixth chromosome s have been proved.

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