

SCREENING ON PATIENTS WITH ALLERGIC BRONCHOPULMONARY DISEASES

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ABSTRACT

Objective: Half of the human population develops allergic symptoms to fungi during their lifetime. Knowing this fact, in the present study the screening for the presence of a specific *Aspergillus* IgG in patients with chronic bronchopulmonary and allergic diseases was investigated.

The aim of the study: Is to show the prevalence of aspergillosis in patients with allergic, bronchopulmonary pathology in various age groups in Karaganda of RK.

Materials and Methods: We examined 1147 patients with bronchopulmonary infections, allergic diseases. *Aspergillus* IgG were determined by ELISA using a set of reagents Vector-BEST (Russia). Statistical Analysis was performed using the STATISTICA-6 package.

Results: 32.6% of patients with bronchopulmonary, allergic diseases have a positive test for *Aspergillus*-Specific IgG immunoglobulins. The most often (43,28%) positive test was detected in children of 4-10 years old. These are children of primary school and preschool institutions. High percentages (40,72%) of positive tests were also noted in 0-3 age group. With increasing age, the number of positive results decreased. Analysis of patients with high values of antibodies also showed significantly high rates in children of 4-10 years (10,92%) compared with age groups 0-3 (5,39%), 21-50 3,8% and >50 years (4,17%) at $p=0,038$; 0,001 and 0,01 respectively. The most vulnerable age group is children 4-10 years old

Conclusion: Every third patient suffering from allergic or bronchopulmonary diseases has a positive test for the specific *Aspergillus* IgG. The most vulnerable age group is children 4-10 years old.

Keywords: *Aspergillus*-Specific IgG, ELISA, bronchopulmonary diseases, allergy.

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Introduction

According to the date of WHO, about 1.2 million people in the world suffer from CPA (chronic pulmonary aspergillosis) leading to pulmonary tuberculosis⁽¹⁾. Researchers believe that pulmonary tuberculosis (TB) appears to be the most significant driver for the global CPA problem, with an estimated 1.2 million CPA patients as a sequel to TB⁽²⁾.

Many species of *Aspergillus*, such as *Aspergillus fumigatus*, *Aspergillus flavus*, *Aspergillus niger* and *Aspergillus oryzae*, can be found on the allergens official website (www.allergen.org). The *Aspergillus* can induce and give rise to sensitization

in asthmatic patients as an allergen^(3, 4, 5). Mycoses in Kazakhstan are widespread as well as in Russia, Uzbekistan and Ukraine^(6, 7, 8, 9). At the same time, the prevalence of fungal diseases in Kazakhstan is not fully understood. A brief epidemiological analysis of fungal diseases is presented by Kemaykin VM at al.⁽⁶⁾. According to the analysis of the Ministry of Health and Social Development of the Republic of Kazakhstan in 2016 diseases caused by *Aspergillus* are widely represented in Kazakhstan: invasive aspergillosis-511, chronic lung aspergillosis-6231 and chronic allergic bronchopulmonary aspergillosis-306 cases⁽¹⁰⁾. We understand that these data do not reflect the true picture of the incidence of fungal

infections in Kazakhstan. Since mycological laboratories are not available in many regions. Where they are, modern informative diagnostic tests are not used. Kemaykin VM et al. consider, the incidence of fungal infections in Kazakhstan is not fully taken into account⁽⁶⁾. There are no scientific publications on the prevalence of aspergillosis and informative diagnostic tests of aspergillosis in the Karaganda region of Kazakhstan.

Materials and methods

This was a prospective study conducted between January 2017 and March 2018. We examined serum in 1147 patients for the presence of IgG immunoglobulins to Aspergillus. These were patients with allergic and bronchopulmonary diseases.

Patients were sent by pediatricians, pulmonologists, and immunologists from the clinic of the Karaganda region. IgG immunoglobulins to Aspergillus were determined in the Karaganda clinical diagnostic laboratory "Olymp".

All serum samples were stored at -20°C until processing. Aspergillus IgG were detected by ELISA. The ELISA was performed using a set of reagents Vector-BEST (Russia) for detecting specific Aspergillus IgG. ELISA results were evaluated by optical density (OD) using a Bio RAD microplate spectrophotometer. An OD index (coefficient of positivity) was calculated by OD of the sample to OD of a cutoff provided in the kit or critical OD. Critical optical density (COD) is equal to the half-sum of optical density values of two negative control samples plus a correction factor. In this series, the correction factor was 0.25. $COD = (OD_{-1} + OD_{-2})/2 + 0.25$. OD index values of ≥ 1 were considered positive.

Statistical analysis

Was performed using the STATISTICA-6 package.

The relative frequency (p) of the occurrence of an attribute was determined as follows:

$$p = \frac{k}{n}$$

k - Number of cases with the attribute of interest.
n - Sample size.

The attribute is defined as a specific characteristic or feature of a given subject p is calculated by sample, it reflects the population with some error:

$$m_p = \sqrt{\frac{p \times (1 - p)}{n}}$$

The confidence interval for the p is located within

$$p - t_{\alpha} \times \sqrt{\frac{p(1-p)}{n}} \text{ and } p + t_{\alpha} \times \sqrt{\frac{p(1-p)}{n}}$$

t_{α} - is the critical value of the bilateral t-criterion of the Student for a given α and $(n_1 + n_2 - 2)$ degrees of freedom. To compare the relative frequency of occurrence of an attribute in different independent sets, the criterion z was used:

$$z = \frac{p_1 - p_2}{\sqrt{m_{p1}^2 + m_{p2}^2}}$$

Differences were considered statistically significant at $p < 0.05$.

Results

We have identified Aspergillus IgG antibodies in 1147 patients with bronchopulmonary infections, allergic diseases. The age of patients ranged from 0 to 79 years (table 1). The distribution of patients by gender was as follows for women 642 (56%), men 525 (44%).

№	Age	n	%
1.	Upto 3 years	167	14,56
2.	4-10	238	20,75
3.	11-20	177	15,43
4.	21 -50	421	36,70
5.	>50 лет	144	12,55
6.	Total	1147	100

Table 1: The distribution of patients by age.

Patients with a positive result for the Aspergillus IgG with OD index ≥ 1 was 32.6%. The distribution of positive results in age groups is not the same (table 2).

Age years	*OD index - negative			OD index - positive		
	n	p%	95%CI	n	p%	95%CI
0-3	99	59,28	(49,59; 68,97)	68	40,72	(29; 52,44)
4-10	135	56,72	(48,35; 65,09)	103	43,28	(33, 68; 52,88)
11-20	115	64,97	(56,24; 73,7)	62	35,03	(23, 12; 46,94)
21-50	312	74,11	(69,24; 78,98)	109	25,89	(17, 64; 34,14)
>50	112	77,78	(70,07; 85,49)	32	22,22	(7,77; 36,67)
Total	773	67,4		374	32,6	

Table 2: Anti-Aspergillus IgG immunoglobulins of patients with bronchopulmonary, allergic diseases in different age groups.

*OD index (optical density index) or coefficient of positivity.

Seropositive results were significantly higher in the age groups 0-3 and 4-10 years old compared with age groups 21-50 and over 50 years old ($p < 0.01$).

Children 4-10 years old were the most vulnerable.

Patients of this age group were the leaders in the number of positive results for Aspergillus antibodies compared with children under the age of 3 years and 11-20 years. In general, the most often positive results for the Aspergillus IgG were recorded in children 0-10 years old, with increasing age, the number of positive results decreased. Analysis of Anti-Aspergillus IgG depending on the gradation of the OD index in patients of the 0-3 and 4-10 age groups showed the following: the proportion with OD index within (1-1.99) in these age groups was 23.35 % and 23.95% respectively (table 3).

Age	0-3 years			4-10 years		
*OD index grading	n	p%	95%CI	n	p%	95%CI
OD index - negative						
<1	99	59,28	(51,83;66,73)	135	56,72	(50,43;63,12)
OD index - positive						
1-1,99	39	23,35	(16,94;29,77)	57	23,95	(18,53;29,37)
2-2,99	16	9,58	(5,12;14,04)	15	6,3	(3,22;9,39)
3-3,99	4	2,4	(0,08;4,71)	5	2,1	(0,28;3,92)
≥4	9	5,39	(1,96;8,81)	26	10,92	(6,96;14,89)
Total	167	100		238	100	

Table 3: Level of the Anti-Aspergillus IgG immunoglobulins of children with bronchopulmonary, allergic diseases.

*OD index (optical density index) or coefficient of positivity.

Also, there was a uniform distribution of patients in these age groups with a coefficient of positivity (3–3.99) of 2.4% and 2.1%, respectively.

However, the frequency occurrence of patients with a fourfold increase in Aspergillus-specific IgG immunoglobulins was 2 times more in the age group 4-10 years. The age group 11-19 is the boundary between children and adults (Table 4).

Age	11-19 years		
OD index grading	n	p%	95%CI
OD index - negative			
<1	115	64,97	(57, 94; 72,00)
OD index - positive			
1-1,99	34	19,21	(13,41; 25,01)
2-2,99	12	6,78	(3,08; 10,48)
3-3,99	4	2,26	(0,07; 4,45)
≥4	12	6,78	(3,08; 10,48)
Total	177	100	

Table 4: Level of the anti-Aspergillus IgG immunoglobulins among middle school students and adolescent with bronchopulmonary, allergic diseases.

The OD index in the range of 1-1.99 was in 19.21%, in the range of 2-2.99, and more than ≥4 in 6.78%. The OD index of 3-3.99 was observed only 2.26% of patients in this age group.

Similar dynamics was observed in groups of children under 10 years of age. The frequency of occurrence of positivity index within 3-3.99 was the lowest among children and adolescent.

In the age groups of 20-50 and over 50 years, the frequency of positive results for the presence of specific Anti-Aspergillus IgG antibodies tended to decrease (Table 5).

Age	20-50 years			>50		
*OD index grading	n	p%	95%CI	n	p%	95%CI
OD index - negative						
<1	312	74,11	(69,92; 78, 29)	112	77,78	(70,99; 84,57)
OD index - positive						
1-1,99	62	14,73	(11,34; 18,11)	18	12,5	(7,1; 17,9)
2-2,99	24	5,7	(3,49; 7,92)	3	2,08	(0; 4,42)
3-3,99	7	1,66	(0,44; 2,88)	5	3,47	(0,48; 6,46)
≥4	16	3,8	(1,97; 5,63)	6	4,17	(0,9; 7,43)
Total	421	100		144	100	

Table 5: Level of the Anti-Aspergillus IgG immunoglobulins of adult with bronchopulmonary, allergic diseases.

The patients with an OD index of 1-1.99 were the most common 14.73% and 12.5% for the age groups of 20–50 and over 50 years.

Analysis of anti-Aspergillus IgG immunoglobulins with an OD of more than 4 showed that in the age group 4-10 years, the number of patients was significantly higher compared with other age groups (Fig. 1).

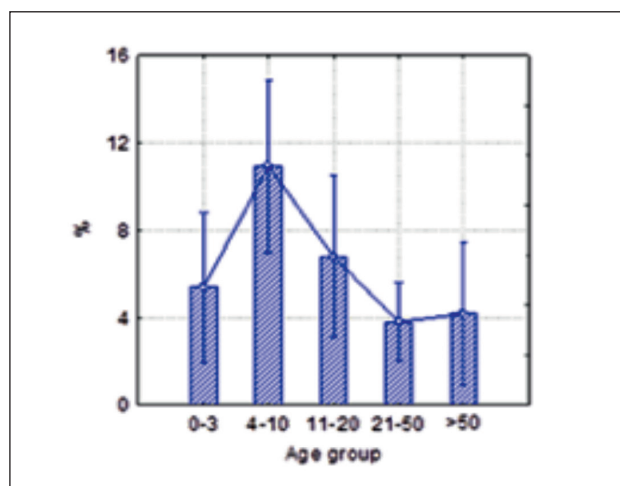


Figure 1: Percentage of persons with OD index ≥4 in different age groups.

Age group 4-10 was the most vulnerable. Significantly high antibody titer was compared to groups of 0-3 years, 21-50 years and > 50 years (Table 6).

Age (Years)	0-3			4-10				
OD index	n	p%	95%CI	n	p%	95%CI	z	p-level
≥4	9	5,39	(1,96; 8,81)	26	10,92	(6,96; 14,89)	-2,07	0,038975*
Total	167			238				
Age (Years)	11-20			4-10				
≥4	12	6,78	(3,08; 10,48)	26	10,92	(6,96; 14,89)	-1,49	0,134997
Total	177			238				
Age (Years)	21-50			4-10				
≥4	16	3,80	(1,97; 5,63)	26	10,92	(6,96; 14,89)	-3,19	0,001442*
Total	421			238				
Age (Years)	>50			4-10				
≥4	6	4,17	(0,9; 7,43)	26	10,92	(6,96; 14,89)	-2,57	0,01026*
Total	144			238				

Table 6: Anti-Aspergillus IgG immunoglobulins with an OD index ≥4 in patients with bronchopulmonary, allergic diseases of different age groups.

*Statistically significant differences in z - criterion.

Discussion

The problem of fungal diseases in Kazakhstan is relevant. Our studies have shown that 32.6% of patients with bronchopulmonary, allergic diseases have a positive test for Aspergillus antibodies. The most often (43, 28%) positive test was in children of 4–10 years old. These are children of primary school and preschool institutions. High percentages (40, 72%) of positive tests were also noted in 0-3 age group. Analysis of patients with high values of antibodies also showed significantly high rates in children of 4–10 years (10,92%) compared with age groups 0-3 (5,39%), 21-50 3,8% and >50 years (4,17%) at P=0,038; 0,001 and 0,01 respectively. With age, the number of positive results decreased, with the exception of the age groups 0-3 and 4-10 years, where on the contrary, the number of positive results was higher in children 4-10 years old. We attribute this to the fact that children under three years of age in Kazakhstan do not attend preschool institutions mainly and they have less contact with other children.

In our study, we touched the surface layer and studied specific Aspergillus antibodies only in patients with bronchopulmonary and allergic diseases. We have not investigated other patients susceptible to fungal infection, such as cancer, blood diseases, and tuberculosis. There is no doubt

that the data presented in the review on the incidence of aspergillosis in Kazakhstan are significantly underestimated.

We have shown on a large number of patients that the problem of aspergillosis in our region is relevant, especially in children. For its successful solution, the efforts of immunologists, mycologists, therapists and other specialists are needed. And also a more detailed study of the status of the issue is necessary.

Conclusion

Every third patient suffering from allergic or bronchopulmonary diseases has a positive test for the specific Aspergillus IgG. A positive test is more often found in children than in adults, The most vulnerable age group is children 4–10 years old.

Pediatricians, pulmonologists, allergists should examine children suffering from allergic and bronchopulmonary diseases for aspergillosis.

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