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ADENOVIRUS INFECTIONS

ADENOVIRUSNE INFEKCIJE

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Apstrakt

U petogodišnjem periodu serološki je pregledano 660 dece sa akutnim infekcijama gornjih delova respiratornog trakta, 260 dece sa znacima bronhitisa, 660 dece obolele od pneumonije, 560 dece sa mononukleoznim sindromom, 1170 osoba različite starosti sa limfadenopatijom i 780 osoba različite starosti sa gripoznim sindromom, kako bi se utvrdila učestalost raznih bolesti izazvanih adenovirusima u našoj sredini. Adenovirusi su bili uzročnici bolesti u 34,2% pregledane dece obolele od akutne infekcije gornjih delova respiratornog trakta, u 32,7% ispitanika sa bronhitisom, u 35,8% pregledane dece sa mononukleoznim sindromom i u 26,7% obolele dece sa pneumonijom. Isto tako, adenovirusi su bili uzročnici gripoznog sindroma i u vreme kada nije bio prisutan virus influenzae u populaciji (u 44,2% pregledanih), kao i u vreme kada je potvrđeno prisustvo virusa influenzae (u 40,0% slučajeva). Gripozni sindrom izazvan adenovirusima dokazan je podjednako često u svim starosnim grupama pregledanih, dok su limfadenopatije izazvane adenovirusima bile značajno češće u mladima od 20 godina.

INTRODUCTION

In recent times, we have been witnessing a continual increase in number of viral infections worldwide. Some of them are caused by new viruses (like SARS), others by viruses that are constantly changing and are very variable (like HIV), or constitute a permanent potential source of possible recombinations and mutations (like influenza A virus). Viruses are capable of avoiding immune system's defense mechanisms in various ways (by being latent in the cell, incorporating into the cell's DNA or episomal DNA, and by evading the immune system using latency and/or variability). Likewise, viruses can impair the immune system in order to avoid its defense mechanisms and survive inside the host organism. They can multiply in the immune cells, T and/or B lymphocytes, as well as in the antigen-presenting cells. Virus proteins can prevent secretion and function of certain cytokines, i.e. prevent cytokine's connection to specific receptors. Viruses can also stop the presentation of HLA molecules on the cell surface, where antigens (including viral antigens) are being presented to the immune system (1).

Viruses that have multiple types, which after the infection leave immunity only against their particular type, can cause frequent infections in the population (1). Children and

young adults are especially susceptible to these infections, particularly to viral infections spread by respiratory route in children in groups and crowds (1, 2, 3). This applies to adenovirus infections as well (3). Publication data indicate that Vojvodina ranks second in Europe in terms of frequency of adenovirus infections (10, 11).

Current virus classification divides viruses into 7 groups, primarily based on the viral genome and replication process. The Adenoviridae family is classified as group I, double-stranded DNA viruses. The family has two genera (genus Mastadenovirus – human adenoviruses, and genus Aviadenovirus – adenoviruses of birds and other animals). Adenoviruses are medium sized (70-90 nm) DNA viruses with icosahedral symmetry and viral genome in the shape of a linear double-stranded DNA. They don't have a second membrane around the nucleocapsid and are therefore resistant to ether and organic solvents. They have 252 capsomeres, 240 of which are hexagonal and located at the icosahedron's sides and edges. Capsomeres contain group-specific epitopes. There are 12 pentagonal capsomeres at the icosahedron's vertices, from where the 10-30 nm long fibers with attached hemagglutinin start. Pentagonal capsomeres contain dominant epitopes specific for the virus type (1, 4). Viral genome (one double-stranded DNA molecule) has a relative molecular mass of 20-30 million Daltons (DNA size

is 36-38 Kbp). Viruses have ten structural proteins whose relative molecular mass ranges from 5000-120000 Daltons. More than 90 types of these viruses are known, 51 of which are human types (1-51). Genus Mastadenovirus is divided into six subgenera (A-F).

Adenoviruses replicate in the cell nucleus, where they form typical intranuclear inclusions. They are released from the cell after the cell bursts open (1, 2, 4).

Adenoviruses were discovered in 1953. They were isolated from human adenoids (pharyngeal tonsils), from which their name is derived. Their initial name was AD viruses. Later on, they were given their current name – adenoviruses (1).

Nowadays adenoviruses are among the best researched viruses. In molecular biology, they are used as gene vectors, in vaccine preparation, and in gene therapy (4,5).

RESULTS

The aim of this paper was to determine the frequency of diseases caused by adenoviruses in our environment, as well as to determine the role of adenoviruses in the appearance of various infections (acute infections of the upper respiratory tract, influenza syndrome, bronchitis, pneumonia, febrile conditions followed by lymphadenopathy) in a sample of patients from Novi Sad and vicinity, primarily children under 15 years of age who constitute the most vulnerable population group in terms of adenovirus infections. The frequency of adenovirus infections was compared to the incidence of other virus infections in patients with the same diagnosis (parainfluenza virus, RS-virus and influenza virus, as well as Epstein Barr virus in patients with lymphadenopathy).

Serums of patients from Novi Sad and vicinity were analyzed: a) using the complement fixation test (CFT) to determine total antibodies (IgM and IgG) against the group adenovirus antigen, and/or b) using the ELISA test to determine specific IgM and/or IgG class antibodies against adenoviruses. Over a five-year period, patients (mainly children under 15) were referred to the Institute of Public Health of Vojvodina – Center for Virology for diagnostic virology testing. Among the tested individuals, there were 660 children

with acute upper respiratory tract infections, 260 children with bronchitis symptoms, 660 children with pneumonia, 560 children with mononucleosis syndrome, 1170 subjects of various ages with lymphadenopathy, and 780 subjects of various ages with influenza syndrome.

Adenovirus antigen used in the CF diagnostic test was derived from Vero cell culture (14, 15). Antigen preparation and titration was completed in the virology laboratories of the Institute of Public Health of Vojvodina. Antigen used in the ELISA test to detect IgM and/or IgG antibodies against adenoviruses was made by the Euroimmun Company. Test procedure and result interpretation were carried out according to manufacturer's instructions. ELISA test kits of the same manufacturer were used for diagnostics of infections caused by parainfluenza virus, RS-virus and Epstein Barr virus. CF test was used for diagnostics of influenza virus A and B (14, 15).

Statistical significance of the differences in frequency of adenovirus infections was determined using the χ^2 (chi-square) test. Yates' correction test was used for small samples.

Table 1 shows viral etiology of acute upper respiratory tract infections (pharyngitis, rhinopharyngitis, and tonsillopharyngitis) in 660 children under 15 years of age from Novi Sad and vicinity. Viral etiology of acute upper respiratory tract infections was confirmed in 511 of the tested individuals (77.4%). Statistically significant was the highest number of these infections (81.1%) determined in children aged 8-15 ($p=0.007$). Adenovirus infections were confirmed in 226 children (34.2%). Therefore, adenoviruses were found to be significantly the most frequent cause of acute viral infections of the upper respiratory tract in children, compared to parainfluenza virus (cause of disease in 113 children – 17.1%), RS-virus (78 children – 11.8%) and influenza virus A and B (94 patients – 14.3%). Adenovirus infections of the upper respiratory tract were most frequent in school age children (179 children – 35.4%). Adenoviruses

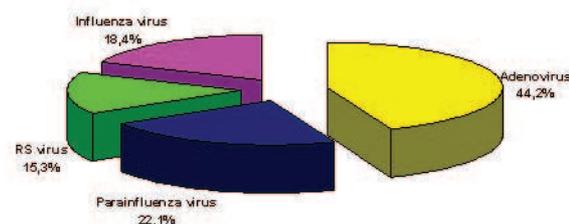


Table 1. Viral etiology of upper respiratory tract infections in 660 children of various ages

Age	Total number tested	Adeno virus	Parainfluenza virus	RS virus	Influenza virus		TOTAL
					type A	type B	
< 3 years	50	14 (28,0)	7 (14,0)	6 (12,0)	2 (4,0)	0 (0,0)	29 (58,0)
4-7 years	105	33 (31,4)	15 (14,3)	12 (11,4)	10 (9,6)	2 (1,9)	72 (68,6)
8-15 years	505	179 (35,4)	91 (18,0)	60 (11,9)	55 (10,9)	25 (4,9)	410 (81,1)
TOTAL	660	226 (34,2)	113 (17,1)	78 (11,81)	67 (10,2)	27 (4,1)	511 (77,4)
					94 (14,3)		

were significantly more common as etiological factors of upper respiratory tract infections in preschool and school children than in small children under 3 years of age ($p=0.005$).

Proportion of adenovirus infections and infections caused by other viruses in children with upper respiratory tract infections

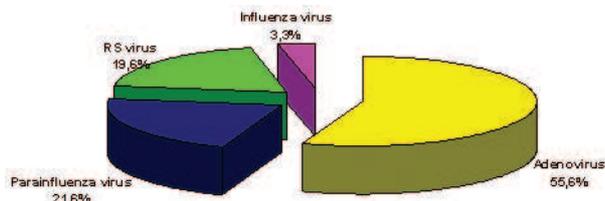


Table 2. Viral etiology of bronchitis in 260 children of various ages

Age	Total number tested	Adeno virus	Parainfluenza virus	RS virus	Influenza virus type		TOTAL
					A	B	
< 3 years	50	13 (26,0)	4 (8,0)	4 (8,0)	0 (0,0)	0 (0,0)	21 (42,0)
4-7 years	60	19 (31,6)	7 (11,6)	6 (10,0)	1 (1,6)	2 (1,9)	33 (54,6)
8-15 years	150	53 (35,3)	22 (14,7)	20 (13,3)	3 (2,0)	1 (0,7)	99 (66,0)
TOTAL	260	85 (32,7)	33 (12,7)	30 (11,5)	4 (1,5)	1 (0,4)	153 (58,8)
					5 (1,9)		

Table 2 shows viral etiology of bronchitis in 260 children of various ages from Novi Sad and vicinity. Viruses caused disease in 153 children with bronchitis (58.8%). Viral bronchitis was most frequently detected in school children (in 99 patients – 66.0%). Adenoviruses caused bronchitis in 85 children (32.7%). They were the most frequent etiological factor of bronchitis in children, more common than parainfluenza virus (confirmed as the cause of bronchitis in 33 children – 12.7%), RS-virus (30 children – 11.5%) and influenza virus (5 children with bronchitis – 1.9%). Adenoviral bronchitis was most common in school children (in 35.3% of the cases). In preschool and school children, bronchitis caused by adenoviruses was significantly more common than in small children under 3 years of age ($p=0.005$).

Proportion of adenovirus infections and infections caused by other viruses in children with bronchitis

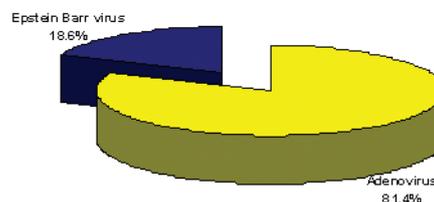
Table 3 shows viral etiology of febrile conditions followed by enlarged lymph glands (with or without enlarged liver and/or spleen). Viral etiology was confirmed in 247 of

the tested individuals (out of 560 children of various ages with the described clinical symptoms, who were referred for diagnostic virology testing). Accordingly, viral etiology of disease was confirmed in 44,1% of the tested children – significantly most frequently in school children (aged 8-15) – in 46.5% of the cases ($p=0.05$). Adenoviruses were significantly more common as the cause of disease, and were confirmed in 35.8 % of the tested children, compared to Epstein Barr virus (determined as etiological agent of disease in 8.2 % of the tested patients) – $p=0.05$. Adenovirus infections were significantly more common in preschool and school children than in children under 3 years of age – $p=0.05$.

Proportion of adenovirus infections and infections caused by E. Barr virus in children with lymphadenopathy

Table 4 presents the results of diagnostic testing for adenoviruses in individuals of various ages from Novi Sad and vicinity diagnosed with febrile conditions with enlarged

Table 3. Viral etiology of febrile conditions followed by lymphadenopathy in 560 children of various ages



Age	Total number tested	Adeno virus	Epstein Barr v.	TOTAL
< 3 years	35	10 (28,6)	0 (0,0)	10 (28,6)
4-7 years	100	36,0 (31,6)	3 (3,0)	39 (39,0)
8-15 years	425	155 (36,4)	43 (10,1)	198 (46,5)
TOTAL	560	201 (35,8)	46 (8,2)	247 (44,1)

Age	< 3 years	4-7 years	8-15 years	16-20 years	21-30 years	31-40 years	>40 years
Tested/Acute (% acute)	35/10 (28,6)	100/36 (36,0)	425/155 (36,4)	218/80 (36,7)	160/35 (21,96)	180/18 (10,0)	52/3 (5,7)
TOTAL	778/281 (36,1)				392/56 (14,3)		
	1170/337 (28,9)						

Table 4. Adenovirus infections in individuals of various ages with lymphadenopathy

lymph glands. It is evident that adenovirus infection was the cause of disease in 281 out of 778 (36.1%) of the tested patients under 20 years of age. In individuals over 20 years of age, adenoviral etiology was confirmed in 56 out of 392 of the tested individuals (14.3% of the cases). In patients aged 21-30, adenoviruses were confirmed in 21.9% of the cases, while in patients aged 31-40 only in 10.0% of the cases. In individuals over 40 years of age, adenovirus infections were determined in 5.7% of the cases. Therefore, adenoviruses caused febrile conditions with enlarged lymph glands in 28.9% of the tested individuals, significantly more

Table 5. Viral etiology of pneumonia in 660 children of various ages

Age	Total number tested	Adeno virus	Parainfluenza virus	RS virus	Influenza virus		TOTAL
					type A	type B	
< 3 years	247	58 (23,5)	23 (9,3)	78 (31,5)	2 (0,8)	0 (0,0)	161 (65,1)
4-7 years	184	60 (32,6)	12 (6,5)	3 (1,6)	9 (4,9)	1 (0,5)	85 (46,2)
8-15 years	229	58 (25,3)	2 (0,8)	1 (0,4)	5 (2,2)	2 (0,8)	680 (29,6)
TOTAL	660	176 (26,7)	37 (5,6)	82 (12,4)	16 (2,4)	37 (0,5)	314 (47,6)
					19 (2,9)		

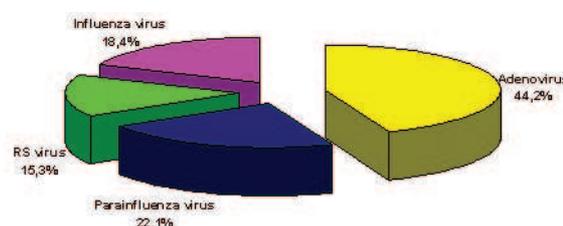
often in individuals under 20 years of age ($p=0.07$).

Table 5 shows viral etiology of pneumonia in 660 children of various ages from Novi Sad and vicinity. Viral etiology of pneumonia was confirmed in 314 patients (47.6%). Significantly the highest number of cases of viral pneumonia were detected in children under 3 years of age (65,1%) – $p=0.05$. In children under 3 years of age and preschool children (aged 4-7), there were significantly more cases of viral

Table 6. Adenoviral etiology of influenza syndrome in 520 individuals of various ages (during periods when influenza virus was not detected in the population)

Age	< 7 years	8-15 years	16-20 years	21-30 years	31-40 years	>40 years
Tested/Acute (% acute)	30/13 (43,3)	206/93 (45,1)	106/47 (44,1)	80/35 (43,7)	70/30 (42,8)	28/12 (42,8)
TOTAL	260/104 (40,0)					

pneumonia than in school children (where viral etiology was determined in 29.6% of the tested children) – $p=0.05$. Adenoviruses were more frequently confirmed as a cause of pneumonia (in 26.7% of the tested children) than parainfluenza virus (confirmed as etiological agent in 5.6% of the tested children), RS-virus (12.4%) and influenza virus (2.9%). Adenoviral pneumonia was most common in preschool children (aged 4-7), where adenoviruses caused disease in 32.6% of the tested individuals.



Proportion of adenovirus infections and other viral infections in children with pneumonia

Table 6 presents the results of diagnostic testing for adenoviruses in 520 individuals of various ages diagnosed with influenza syndrome. These tests were performed during periods when influenza virus was not detected in the population of Novi Sad and vicinity by the Epidemiology service which monitors incidence and frequency of diseases caused by influenza virus. Evidently, there were no significant dif-

ferences in percentage of adenovirus infections across all age groups of tested individuals diagnosed with influenza syndrome. The number of individuals with confirmed acute adenovirus infection was similar in all age groups. In the total sample of the tested individuals with influenza syndrome (outside of the epidemic season of influenza virus in Novi Sad and vicinity), adenoviruses caused disease in 44.2% of the cases.

Table 7 presents the results of diagnostic testing for adenoviruses in 260 individuals diagnosed with influenza syndrome. Patients of various ages were tested during periods when influenza virus was detected in high numbers of patients from Novi Sad and vicinity. It is evident that adenoviral etiology of disease was confirmed during these periods as well – in 40.0% of the cases, equally high in all age groups. However, although influenza virus was present in

Table 7. Adenoviral etiology of influenza syndrome in 260 individuals of various ages (during periods when influenza virus was confirmed in a significant number of patients)

Age	< 7 years	8-15 years	16-20 years	21-30 years	31-40 years	>40 years
<i>Tested/Acute (% acute)</i>	35/13 (37,1)	60/26 (43,3)	59/25 (42,3)	46/18 (39,1)	40/15 (37,5)	20/7 (35,0)
<i>TOTAL</i>	260/104 (40,0)					

the population of Novi Sad and vicinity during this period, it was not at the high epidemic level. These data indicate that influenza syndrome in our population is in large part caused by adenoviruses, regardless of patients' age or confirmed presence of influenza virus as the potential cause of disease in tested individuals.

DISCUSSION AND CONCLUSIONS

The results indicate that acute upper respiratory tract infections had viral etiology in 77.4% of the tested children. Bronchitis was caused by viruses in 58.8% of patients, pneumonia in 47.6%, and febrile condition with enlarged lymph glands in 44.1% of children. These percentages correspond to the numbers reported by various authors in their papers (11, 12, 13, 16). A great number of authors emphasize that acute respiratory infections (ARI), which account for two thirds of the diseases that require a doctor visit, usually have viral etiology (5, 6, 9, 17, 18, 19, 20). Mentioned publication data primarily refer to the ARI analysis in children. The fact that virus infections of the upper respiratory tract, acute virus bronchitis and febrile conditions with enlarged lymph glands (with or without enlarged liver and/or spleen) were most frequently detected in school children (aged 8-15), whereas virus pneumonia was most frequent in small children (under 3 years of age), also corresponds to publication data (16, 17, 18, 19, 20).

Adenoviruses were confirmed as significantly the most common cause of all mentioned clinical syndromes in children in our environment. These data correspond to publication data from numerous authors in Vojvodina, who point out the fact that Vojvodina ranks second in Europe in terms of frequency of adenovirus infections. The high incidence rate of adenovirus infections in Vojvodina, according to

these authors, may be attributed to the large number of adenovirus types which circulate in the Vojvodina population (1, 11). Convalescent immunity after the adenovirus infection is type-specific, relatively short-term and not particularly stable, which could also explain the high incidence of adenovirus diseases in our environment, particularly in children in groups and crowds. These data are confirmed by the presented diagnostic analysis of children from Novi Sad and vicinity. Adenoviruses, which caused acute upper respiratory tract infections in 34,2% of the tested children, were the most frequent etiological disease agents in school and preschool children. Acute bronchitis with adenoviral etiology (determined in 32.7% of the analyzed patients) was also most common in school and preschool children, as well as febrile conditions with lymphadenopathy (determined in 35.8% of the tested children). However, pneumonia caused by adenoviruses (confirmed in 26.7% of children) was most common in preschool age (in children aged 4-7 – in 32.6% of the cases). Pneumonia caused by RS-virus was most frequently diagnosed in children under 3 years of age, which also corresponds to the data in national and foreign publications (5, 17).

Obtained results demonstrate that adenovirus infections were significantly more frequent in individuals under 20 years of age diagnosed with febrile conditions with enlarged lymph glands (adenoviral etiology confirmed in 36.1% of the tested individuals under 20 years of age) than in patients over 20 years of age (adenoviruses confirmed in 14.3% of the cases). In older age groups, adenoviral etiology was increasingly rare (percentage decreased with the increase in age of the tested individual). Accordingly, in patients over 40 years of age, this percentage was only 5.7%. This should be taken into account. Diagnostic options should be extended to look for other possible causes of influenza syndrome in older patients.

Diagnostic testing for adenoviruses of patients with influenza syndrome during periods when influenza virus was not detected in the population, as well as periods when influenza virus was confirmed in high numbers of individuals from Novi Sad and vicinity, indicates that in both periods, adenoviruses frequently caused influenza syndrome in all age groups of the tested subjects. There were no significant differences in frequency of adenovirus infections between these periods, or between different age groups. This corresponds to the data obtained by American authors, who pointed out that during outbreaks caused by influenza virus, a great number of patients were diagnosed with influenza syndrome caused by other respiratory viruses (4, 5, 6). This leads to the conclusion that etiological diagnosis of disease cannot be made solely based on clinical symptoms. Clinical data, epidemiological knowledge and routine laboratory tests can direct clinicians towards a possible viral etiology of disease. However, a correct and precise etiological diagnosis can only be made with diagnostic virology testing (1, 2 14, 15, 17, 18, 20).

Abstract

Over a five-year period, serological testing was done on 660 children with acute upper respiratory tract infections, 260 children with bronchitis symptoms, 660 children with pneumonia, 560 children with mononucleosis syndrome, 1170 subjects of various ages with lymphadenopathy and 780 subjects of various ages with influenza syndrome, in order to determine the frequency of various diseases caused by adenoviruses in our environment. Adenoviruses caused disease in 34.2% of children with acute upper respiratory tract infections, 32.7% of patients with bronchitis, 35.8% of patients with mononucleosis syndrome and 26.7% of children with pneumonia. Likewise, adenoviruses caused influenza syndrome both during periods when influenza virus was not detected in the population (in 44.2% of the cases), as well as during periods when influenza virus was confirmed in the population (in 40.0% of the cases). Influenza syndrome caused by adenoviruses was determined in equal frequencies in all age groups of tested subjects, while lymphadenopathy caused by adenoviruses was significantly more common in young individuals under the age of 20.

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