



Identification of influenza, parainfluenza, adenovirus and respiratory syncytial virus during rhinopharyngitis in a group of Mexican children with asthma and wheezing

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RESUMEN

Antecedentes: las infecciones virales pueden promover la sensibilización alérgica en individuos genéticamente susceptibles. Además, son la principal causa de sibilancias en niños.

Objetivo: conocer la frecuencia con que los virus influenza, parainfluenza, adenovirus y sincitial respiratorio causan rinofaringitis en niños con asma y sibilancias.

Pacientes y métodos: Estudio prospectivo, longitudinal en una cohorte, en la que se incluyeron 168 niños con asma y sibilancias, que acudieron a la consulta externa del servicio de alergia del Instituto Nacional de Pediatría durante la primavera (de abril a junio) con signos y síntomas de rinofaringitis. Se tomaron muestras de secreciones nasales con hisopo largo y se identificaron los virus por inmunofluorescencia directa.

Resultados: se tomaron muestras de secreciones nasales a 100 niños con asma y a 68 con sibilancias, entre 2 y 17 años de edad. La identificación viral fue positiva en 75 y 44.1%, respectivamente. Por grupos de edad, predominó el virus sincitial respiratorio en niños preescolares; y el de la influenza A en niños escolares y adolescentes. Los virus influenza A, adenovirus y parainfluenza 3 se identificaron con mayor frecuencia en niños asmáticos, mientras que el de la influenza A y el sincitial respiratorio en los niños con sibilancias. Se encontró coinfección simultánea con dos virus en 17.1% de los niños con identificación viral positiva, la asociación más frecuente fue la de los virus parainfluenza 1 y 2.

Conclusiones: la identificación viral por inmunofluorescencia directa es una técnica rápida y no invasora que puede favorecer el inicio temprano de tratamientos antivirales en niños con asma y sibilancias.

Palabras clave: inmunofluorescencia directa, influenza, parainfluenza, adenovirus, virus sincitial respiratorio.

ABSTRACT

Background: Viral infections can promote allergic sensitization in genetically susceptible individuals. Besides, they are the main cause of wheezing in children.

Objective: To assess the frequency with which influenza, parainfluenza, adenovirus and respiratory syncytial virus (RSV) cause rhinopharyngitis in patients with asthma and wheezing.

Patients and methods: Longitudinal, prospective study in which 168 patients with asthma and wheezing, who attended the outpatient allergy clinic of the National Institute of Pediatrics, Mexico, during the spring (from April through June), with clinical signs and symptoms of rhinopharyngitis were included. Samples of nasal secretions were taken with a long swab, and identification of RSV, adenovirus, influenza A and B and parainfluenzae 1, 2 and 3 by means of direct immunofluorescence (DI) was carried out.

Results: Samples of nasal secretions were taken in 100 children with asthma and 68 with wheezing, aged 2-17 years. Viral identification was positive in 75% and 44.1% of the patients, respectively. By age group, RSV predominated among children in preschool age; and influenza A among children in school age and adolescents. Influenza A, adenovirus and parainfluenza 3 were identified more frequently in asthmatic children, whereas influenza A and RSV in patients with wheezing. Coinfection of two viruses was found in 17.1% of all patients with positive DI; the most frequent association was parainfluenza 1 and 2.

Conclusions: Viral identification by means of DI is a fast and non-invasive technique that could favor the early beginning of antiviral treatments in children with asthma and wheezing.

Key words: direct immunofluorescence, influenza, parainfluenza, adenovirus, respiratory syncytial virus.

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Viral infection is one of many risk factors that may sensitize a genetically susceptible individual, predisposing him to the development of allergic disease. Infections due to respiratory viruses, whose usual port of entry is the nasopharynx, are the main cause of wheezing in healthy children, and of acute asthmatic exacerbations in atopic children.¹ Transmission may occur via droplets spread while talking or coughing, by fomites, or by the orofecal route. There are at least 15 respiratory virus families, encompassing approximately 290 serotypes. They include DNA viruses (adenovirus) and RNA viruses (respiratory syncytial virus [RSV], parainfluenza, influenza).² It is estimated that an adult suffers an average of three episodes of respiratory infection in a year, whereas children suffer from six to eight.³ Among genetic and environmental factors that contribute to the development of wheezing or asthma in children are: being male, passive smoking, virus-induced inflammatory responses, a predisposition to produce IgE, alterations in airway function, size and dysregulation of airway tone.^{1,4} In allergic children, viruses provoke asthma attacks by additive or synergistic interactions with a preexisting allergic inflammation.⁵

Viral identification has increased due to development of more sensitive assays. Viral cultures have higher specificity, but they may take up to 4-8 weeks.⁶ One of the non-invasive and quick techniques for viral identification is direct immunofluorescence, that allows to visualize the viruses by electronic microscopy, thus having an immediate diagnosis. This test offers a high sensitivity and specificity, since monoclonal, high-reactivity marked antibodies are used.⁷

This prospective, longitudinal study in a cohort was designed to assess the incidence with which RSV, adenovirus, influenza and parainfluenza viruses cause rhinopharyngitis in children with asthma and wheezing in our Allergy department.

MATERIALS AND METHODS

During the spring (from April through June), samples of nasal secretions were taken in 168 children with signs or symptoms of rhinopharyngitis, attending the outpatient allergy clinic of the National Institute of Pediatrics, in Mexico City.

A diagnosis of rhinopharyngitis was considered when at least three symptoms in non-atopic children and five symptoms in atopic children (cough, sneezing, headache, fever or shivery, odynophagia, nasal obstruction, retropharynx hyperemia, hyaline rhinorrhea) were present. The samples were taken with long sterile swabs. Each swab was inserted 2-3 cm into the nostril and rotated thoroughly against the respiratory epithelial surface of the nasal cavity, within the first two days of signs and symptoms of upper respiratory tract infection. Nine nasal smears were made on three slides, leaving the samples to air dry. Direct immunofluorescence was carried out to identify RSV, adenovirus, influenza A and B and parainfluenza 1, 2 and 3 and was performed in two stages. First, the cells were fixed with acetone, and they were later covered with a conjugated of viral immunoglobulins and fluorescein isocyanate. Then, the slides were incubated in a wet chamber. In the second stage, the excess conjugate was removed and the slides were examined under a fluorescence microscope. Viral antigens were identified by their greenish yellow glow.⁶

Children having cough, dyspnea and/or wheezing and reversible airflow obstruction, during the previous year with positive skin prick tests for common antigens, were categorized as having asthma; and those with the same symptoms and signs with negative skin prick tests, were categorized as wheezing children.

Patients were considered as having allergic rhinitis if they presented with sneezing or nasal itching, watery rhinorrhea, and nasal congestion, and either eosinophilia in nasal discharge or positive skin prick test for common antigens. Skin tests were conducted with allergen extracts (Allerquim®) made of house-dust mites, molds, grasses, weeds, trees, cat and dog dander and cockroach. The skin tests were read after 20 minutes, and the results were considered positive if a wheal at least 2 mm larger than the control wheal was produced. A CT scan of paranasal sinuses was performed in all patients suspected of having sinusitis (nasal discharge, facial pain/pressure, headache, hiposmia, dizziness, cough, halitosis, swelling around the eyes, nasal blockage, postnasal discharge).

Statistical method: Data were analyzed using the statistical software kit Epi-Info 6.04. Sex, age and viruses identified were analyzed by means of parametrical tests.

RESULTS

Nasal swab samples were taken from 168 children, 100 with asthma and 68 with wheezing, ages ranging from 2 to 17 years old (mean 7.6 ± 3.4 years). Table 1 shows the frequency of viral identification by sex, age and condition. Viral identification was positive in 75 (75%) children with asthma and 30 (44.1%) with wheezing. The male:female ratio for asthma was 1.4:1, and for wheezing 1.5:1. The frequency of allergic rhinitis and chronic sinusitis in asthmatic patients were 69% and 9%, respectively; whereas in 13.2%⁹ out of the total of patients with wheezing there was a chronic sinusitis. The mean duration of the condition in the group with wheezing was 3.96 years, and in the group with asthma, 4.15 years. Forty nine percent of patients with asthma reported their condition as having lasted for two to three years.

Table 2 shows the frequency of viral identification by age group. In the pre-school age group, RSV predominated, followed by influenza A; in the school-age group,

influenza A was the most common virus, followed by adenoviruses; in adolescents, influenza A was the most frequent.

As to viral identification by group of conditions (Table 3), the rate of viral identification was higher in asthmatic children. It was found that influenza A was the most common virus in patients with asthma, and influenza A and RSV in patients with wheezing. In 10.7% of patients (18) there were coinfection of two viruses (Table 4), been the most frequent association parainfluenza 1 and 2. Viral coinfection was found in 15.2% (5/33) of preschoolers, 18% (11/61) of school aged, and 18.2% (2/11) of adolescents with positive identification. The female:male ratio for coinfection was 1.8:1.

Among asthmatic patients with positive DI (75), monosensitization was most frequent among preschoolers (9/16 = 56.2%), while polysensitization predominated in school-aged and adolescents (30/50 = 60% and 6/9 = 66.6%, respectively). Dust mites were the most frequent cause of sensitization, in all allergic conditions (69/75 = 92%) and in all age groups.

Table 1. Frequency of viral identification by sex, age group and condition. In parentheses, percentage of positive direct immunofluorescence (%DI+)

Positive DI/N Condition	N = 168	N (% DI+)	Preschoolers (33/57)		School aged (61/88)		Adolescents (11/23)	
			M	F	M	F	M	F
Asthma	29	14 (48.3)	1/3	1/2	8/9	2/8	2/5	0/2
Asthma and sinusitis	2	2 (100)	0/0	0/0	1/1	1/1	0/0	0/0
Asthma and allergic rhinitis	60	52 (86.7)	7/8	4/5	21/23	14/15	4/6	2/3
Asthma, allergic rhinitis and sinusitis	9	7 (77.8)	1/1	2/3	1/2	2/2	0/0	1/1
Wheezing	59	24 (40.7)	6/17	7/12	4/15	6/10	1/3	0/2
Wheezing and sinusitis	9	6 (66.7)	4/4	0/2	1/2	0/0	0/0	1/1

Table 2. Viral identification by age group

Age	Influenza A n (%)	Influenza B n (%)	P-I 1 n (%)	P-I 2 n (%)	P-I 3 n (%)	RSV n (%)	Adenovirus n (%)
2-6 y (n = 33)	7 (21.2)	5 (15.1)	4 (12.1)	4 (12.1)	5 (15.1)	8 (24.2)	5 (15.1)
6-12 y (n = 61)	20 (32.8)	5 (8.2)	4 (6.6)	4 (6.6)	12 (19.7)	12 (19.7)	15 (24.6)
> 12 y (n = 11)	4 (36.4)	0	1 (9)	1 (9)	3 (27.3)	3 (27.3)	1 (9)

Table 3. Viral identification by group of conditions

<i>Virus</i>	<i>Asthma 14 (14)</i>	<i>Asthma + aller- gic rhinitis 52 (52%)</i>	<i>Asthma + allergic rhinitis + sinusitis 7 (7%)</i>	<i>Asthma + sinusitis 2 (2%)</i>	<i>Wheezing 24 (35.3%)</i>	<i>Wheezing + sinusitis 6 (8.8%)</i>
Influenza A	4 (4)	15 (15)	2 (2)	0	6 (8.8)	4 (5.9)
Influenza B	1 (1)	5 (5)	1 (1)	0	3 (4.4)	0
Parainfluenza 1	1 (1)	3 (3)	1 (1)	1 (1)	3 (4.4)	0
Parainfluenza 2	1 (1)	3 (3)	1 (1)	1 (1)	3 (4.4)	0
Parainfluenza 3	4 (4)	11 (11)	2 (2)	1 (1)	2 (2.9)	0
RSV	2 (2)	12 (12)	2 (2)	0	6 (8.8)	1 (1.5)
Adenovirus	4 (4)	9 (9)	1 (1)	1 (1)	5 (7.4)	1 (1.5)

Table 4. Viral coinfection by group of conditions (n = 18)

<i>Condition</i>	<i>PI1 + PI2</i>	<i>Inf A + PI3</i>	<i>Inf A + RSV</i>	<i>Inf A + Adeno</i>	<i>PI 3 + Adeno</i>	<i>Inf B + Adeno</i>
Asthma	2	0	0	1	1	1
Asthma + allergic rhinitis	3	3	2	1	1	0
Wheezing	3	0	0	0	0	0

DISCUSSION

As criteria for population selection have been improved, and as methods for sample taking and virus identification and culture have been implemented in the last few years, the frequency with which viral infection is identified as the cause of wheezing and asthma exacerbations increases day to day.⁸ Nowadays, it is well known that viral infection is the main cause of wheezing in healthy children, and of acute asthma exacerbations in atopic children, besides being a risk factor for sensitization in genetically susceptible individuals.¹

Viral detection in upper respiratory tract infection is about 65% using PCR,^{8,9} and the highest detection rate (85%) is in episodes of wheeze.¹⁰ Moreover, it has been described that respiratory viruses can be detected even without symptoms of common cold.⁹ In this study, viral identification by DI was positive in 75 (75%) children with asthma and 30 (44.1%) children with wheezing. Although there are techniques with higher sensitivity, the authors selected this non-invasive test because the results could be obtained quickly making possible to initiate early antiviral treatment and then prevent an asthma attack.

The largest group of the sample was the one with asthma plus allergic rhinitis (69/168=41%). In accordance with data already published coexistence of both conditions may be found in up to 38% of patients with asthma.¹¹ We found allergic rhinitis in 69% of asthmatic children. As to the distribution of conditions by age group (Table 1), it was found that the percentage of patients with asthma was smaller in preschoolers (38.6%) than in school aged children (69.3%) and adolescents (73.9%), which could be explained by the latency period necessary for allergen sensitization.¹² Conversely, wheezing predominated in the pre-school group (61.4%, vs 30.7% and 26%, respectively), which is consistent with an increased frequency of respiratory viral infections in toddlers, associated with low levels of interferon-gamma and interleukin-12,¹³ and with attendance to day-care centers.¹³⁻¹⁴ As we mentioned, the mean duration of the condition in the group with wheezing was 3.96 years, and in the group with asthma, 4.15 years. Males predominated in all the groups under study (Table 1).

Prevalence of viruses associated to wheezing varies widely with the individual's age. Viruses that trigger wheezing in children younger than two years of age are RSV and parainfluenza 1; in the first three years of

age, parainfluenza 1, 2 and 3, and in children older than 5 years of age, influenza A and rhinovirus.¹⁵ Virtually every child is infected with RSV by the third birthday.¹⁶ Our percentages of isolation were in accordance with published reports. RSV predominated in preschoolers and influenza A in older children. The prevalence of viruses that cause upper airway infection varies seasonally. In the winter, RSV and influenza A predominate, whereas in the fall RSV; parainfluenza in the spring and summer, and adenovirus throughout the year.¹⁷⁻¹⁸ Then, a predominance of parainfluenza viruses 1, 2, and 3 were to be expected in the time of the year when the study was carried out (April to June). In our report, parainfluenza 3 was identified in 28.6% of subjects with asthma and positive DI (4/14) and in 21.2% (11/52) with asthma and allergic rhinitis, whereas parainfluenza 1 and 2 were positive in 26.7% of children with wheezing and positive DI (Table 3).

A characteristic feature of influenza is its striking contagiousness. As it has been described,¹⁹ influenza A was the virus most frequently identified in children with asthma and allergic rhinitis (15/52=28.8%) and among the school-age group (32.8%) and adolescents (36.4%) [Tables 2 and 3].

A high rate of viral coinfection (from 22% to 32%) has been described in respiratory tract infections,²⁰⁻²¹ we found it in 17.1% of all patients with positive DI (18/105). Although parainfluenza viruses 1 and 2 did not show a higher prevalence in either of the conditions under study, nor in any of the age groups; this association was found in 44.4% out of 18 cases with viral coinfection.

On the other hand, sinusitis has been linked to allergic diseases in 60% of patients with positive skin tests to common allergens,²² and 17% of children with wheezing.²³ In this study, out of 75 patients with asthma and positive DI, only 9 (12%) had sinusitis, and such association was more frequent in the wheezing group with positive DI (6/30= 20%).

It is well known that viral infection affect the paranasal sinuses leading to occlusion of the ostomeatal complex and damage to the epithelial layer, favoring recurrent sinusitis. In addition, disturbances of the cilia and mucus are present in allergic and wheezer children,

such as overproduction and poor clearance of mucus.²²⁻²⁴ Diagnosis of sinusitis in this study was established with paranasal sinuses CT scan and clinical features. With PCR, rhinovirus has been isolated in up to 50% of cases with rhinosinusitis.²⁴ We found a high rate of positive DI in all the patients with sinusitis (Table 1).

Finally, regarding positive skin tests to allergens there is evidence that viral infection may potentiate the sensitization to allergens.²⁵ Moreover, viruses have an additive or synergistic interactions with allergen exposure for provoke wheezing.^{5,26}

Dust mites are the main cause of sensitization in human beings, and are an important cause of asthma and allergic rhinitis.²⁷ Dust mites were the most frequent cause of sensitization, in all allergic conditions (69/75= 92%) and in all age groups. As it has been described,²⁶ the percentage of positive skin prick test increased with age, being 38.6% (22/57) in preschoolers; 69.3% (61/88) in school-aged and 73.9% (17/23) in adolescents. A high rate of monosensitized patients in preschoolers (56.2%) and of polysensitized patients in school-aged and adolescents (60% and 66.6%, respectively) was to be expected according to progressive sensitization with age.²⁶ The fact that influenza A was the virus most frequently identified in children with asthma and allergic rhinitis and among the school-age group and adolescents was an interesting finding since dust mites, by means of a serine-protease, enhance viral replication, protentiating the infectivity and propagation of influenza A.²⁸

As a conclusion, considering the high frequency of viruses involved in triggering wheezing and asthma, and taking into account their high sensitizing potential in patients with genetic predisposition to atopy, we think that it is crucial to implement preventive measures, such as breast feeding, avoiding contact with sick persons and attendance to day-care centers, and promote early vaccination in order to reduce the high incidence of viral infections in children.

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