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Original Communication

Identification of respiratory viruses in children with hyperreactivity or asthma in Mexico City

D. P. Rosete[1], Carlos Cabello[1], M. A. Galván[1], I. Calderón[1], R. Chapela[1], I. Páramo[1], A. M. Fernández[2], B. Muñoz[1], J. Villalba[1] and M. E. Manjarrez[1,*]

[1]Instituto Nacional de Enfermedades Respiratorias Ismael Cosio Villegas. Calzada de Tlalpan 4502, Sección XVI, CP 14080, Mexico D.F., Mexico, [2]Facultad de Medicina, UNAM, Av. Universidad 3000, Copilco el alto, CP 04510, Mexico D.F., Mexico

ABSTRACT

The prevalence of asthma and hyperreactivity have markedly increased in recent years. Evidences suggest that respiratory viruses frequently trigger exacerbations of asthma or hyperreactivity. This study was aimed at detecting and identifying those viruses that cause acute respiratory infections (ARI) in children with hiperreactivity or asthma, and determining their frequency. Besides, we evaluated if there is a correlation between the frequency of asthmatic crisis and viral infections. At National Institute of Respiratory Diseases Ismael Cosio Villegas, Mexico City, 79 children less than 15-year-old with hyperreactivity or asthma were studied. A nasopharyngeal swab sample was collected to detect viral antigens IA, IB, PI, AD and RSV. The viruses were isolated from cell cultures and their identification was by direct immunofluorescence. Blood samples were also taken for the detection of anti-viral antibodies. Forty-eight percent of the samples were positive for at least one of the studied viruses and of these, 47% were positives for RSV. In 57% of the children with hyperreactivity or asthma, antibodies against one or more viruses were detected. June through September was the period with the largest number of viral infection cases, which correlated with the highest frequency of asthma crisis in the study group. The results suggest that the presence of respiratory virus is a risk factor for the exacerbation of hyperreactivity or asthma and the risk was higher in the months of June through September.

KEYWORDS: asthma and hyperreactivity, acute respiratory infections (ARI), respiratory syncytial virus, adenovirus, influenza virus, parainfluenza virus

INTRODUCTION

Numerous studies have linked the viruses that cause acute respiratory infections (ARI) with the development of airway hyperreactivity, asthma and chronic obstructive pulmonary disease [1-10]. Asthma is the most frequent chronic bronchopulmonary disease in children [1]. Asthma exacerbations affect family and social life, with the consequent economic cost [11, 12]. Several factors have been associated with exacerbation of hyperreactivity and asthma but viral infections have been identified as one of the main causes [11], this has been demonstrated particularly in children under 3 years of age [13-17]. There are very few reports of respiratory infections due to virus in Mexico, and much less in relation with the frequency of respiratory viruses in hyperreactivity or asthma (H-A). In the National Institute of Respiratory Diseases Ismael Cosio Villegas (INER) the number of patients with asthma and with ARI is high. Therefore, the aim of this study

*Corresponding author:
e_manjarrez@yahoo.com

was to detect and to identify the viruses that cause ARI in children with H-A, and to determine the frequency of each of them, as well as to evaluate if there is a correlation between the frequency of the H-A and viral infections.

METHODS

Study population

79 patients between 0 to 15 years of age, diagnosed with H-A that were cared for in the INER at a probable early stage of viral infection. Patient selection was done after differential diagnosis. Patients with family antecedents of tobacco smoking were excluded. The control population was made up of 79 healthy subjects paired by age and gender, without antecedents of asthma, chronic obstructive pulmonary disease (COPD), or tobacco smoking in the family. Those with cardiovascular disease were excluded. Patients were divided into four groups according to age: group1 (under 1 year, up to 3 years of age); group 2 (4 to 7 years); group 3 (8 to 11 years); group 4 (12 to 15 years). The parents were invited and informed to participate in the project by signing the institutional written consent form. A physical exam was performed and a questionnaire was applied.

Sample for virus isolation

A nasopharyngeal exudate sample was taken from each patient to detect the following viruses: influenza virus (IA), influenza B (IB), parainfluenza (PI), adenovirus (AD) and respiratory syncytial virus (RSV). The sample was placed in 5 ml of L-15 Lebovitz medium (*In vitro*, Mexico, D.F.) with 0.5% bovine serum albumin (BSA) (Sigma) and antibiotics (100 UI/ml penicillin, 100 µg/ml streptomycin) (Sigma). Samples were centrifuged at 1500 rpm for 10 min at 4ºC, the supernatant was inoculated in a cell culture.

Virus isolation

HEp-2, Vero and MDCK cells were used. The cells were grown in 18×18 mm cover slips, placed in sterile Petri dishes, seven per dish, they were covered with minimum essential medium (MEM) (Sigma) supplemented with 10% fetal bovine serum (FBS) (Sigma) and antibiotics (penicillin/streptomycin). They were incubated at 37ºC in a humidified atmosphere with 5% CO_2, until a confluent mono-cellular layer was formed. The medium was eliminated followed by a wash with phosphate buffered saline (PBS) (0.8% NaCl, 0.1% $NaHPO_4$ and 0.2% $KHPO_4$, at pH 7.2). The supernatant of the nasopharyngeal sample was inoculated and incubated for 90 min to allow for adsorption, and medium was added (without FBS). The cultures were then incubated at 37ºC, followed up daily to detect cytopathic effects. The medium was changed twice weekly. After 10 days, the medium was collected from each sample, cells were washed with PBS and fixed with cold acetone for 5 min.

Virus identification

Virus IA, IB, PI, AD and RSV, identification was done through direct immunofluorescence. The cells fixed on the cover slips were washed with distilled water, eliminating any excess water, and covered with 15 µl of virus-specific monoclonal antibody conjugated with fluorescein isothiocyanate (Dako). Cells were incubated for 30 min at 37°C in a dark humid chamber. After which, they were washed with PBS several times to eliminate non-specifically adsorbed antibodies. Excess PBS was eliminated, and isopropidium (Sigma) was added for 5 min, followed by washings with PBS and 0.5% Tween (Sigma), dried and mounted with glycerol on glass slides. They were examined with an epifluorescence microscope. The positive controls were Vero cells infected with the different stock control viruses and the negative controls were uninfected Vero cells, both were processed in the same way as the samples.

Statistical analysis

For the analysis of infections in patients by the different viruses in the different groups by age and gender the Kruskal-Wallis H and Fisher's P were used. To compare the groups of healthy vs. H-A children and to compare the ranges between two viruses, the Mann Whitney test was applied.

Antibodies detection sample

A blood sample was obtained for the detection in serum of antibodies against the same viruses, this was achieved by using the complement fixation microtechnique. Soluble antigens were used (Bio-Whittaker), sheep erythrocytes, hemolysin and complements were obtained from guinea pigs. The test was done to 50% hemolysis [18].

RESULTS

Each group was formed by 79 children of either gender, and ages between 0 and 15 years. In the H-A groups, 42 were female and 37 were male. In the 79 children of the healthy groups, the same gender proportion was kept. In the H-A group, viruses were isolated and identified in 48% of the subjects, whereas in the healthy groups, viruses were isolated and identified in 7% (Figure 1A). Of the children with H-A, 61% presented wheezing during one respiratory infection in its early stages before being diagnosed with H-A.

In the H-A group, RSV was the most frequent virus found (47%) (Figure 1B). Only groups 1 and 3 showed a significant difference in viral infections with relation to gender, with more male children infected in group 1 than females ($p < 0.05$) (Table 1). The different viruses identified are shown in Figure 2. Viral infections were highest in group 3 (57%) and lowest in group 4 (42%). The highest incidence of infections was observed during the months of June to September (Figure 3), coinciding with the period with the highest number of H-A. RSV was the virus most frequently found ($p < 0.05$). The largest number of isolations of RSV was made in August (Figure 4).

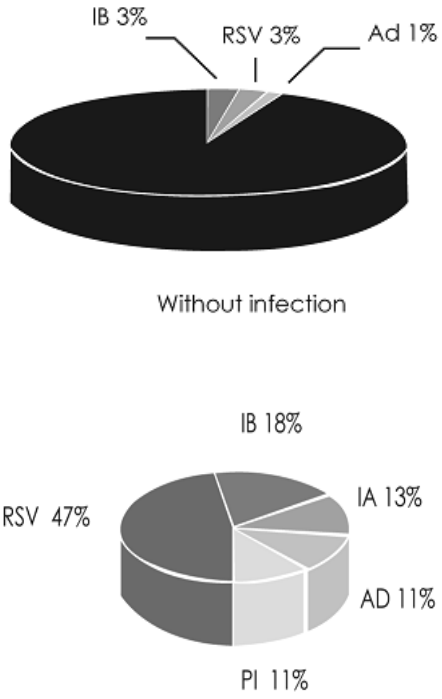

Figure 1. Viruses identified in children: A) healthy children, B) children with hyperreactivity or asthma crisis. IA: Influenza A; IB: Influenza B; Ad: Adenovirus; PI: parainfluenza; RSV: Respiratory syncytial virus.

Table 1. Viral infections in patients according to age group and gender.

Hyperreactivity or asthma patients						
Group	Age (Years)	Viral infection		Infection (%)	Family	Wheezing (%)
		Female	Male		Antecedents of asthma	
		Infected/without infection (%)				
1	<1-3	1/6	9/17	43	7(30)	18(78)
2	4-7	6/15	9/15	50	6(20)	10(33)
3	8-11	7/7	1/7	57	5(35)	12(86)
4	12-15	3/9	2/3	42	7(58)	9(75)
Total		17/37	21/42	48	25(32)	49(62)
Healthy controls						
1	<1-3	1/6	1/17	9		
2	4-7	1/15	1/15	7		
3	8-11	0/7	0/7	0		
4	12-15	1/9	0/3	8		
Total		3/37	2/42	7		

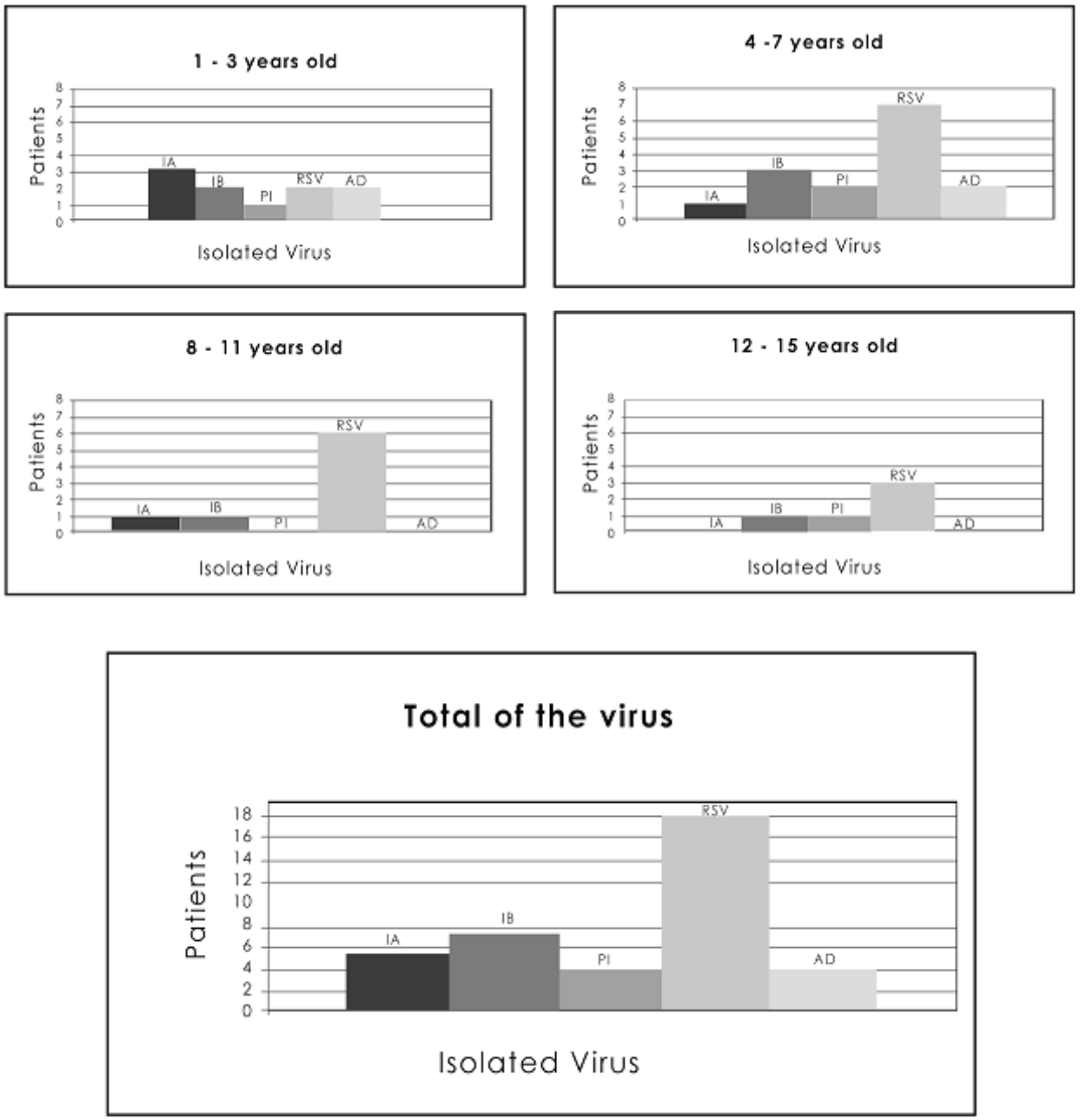

Figure 2. Isolated and identified viruses in children with hyperreactivity or asthma, per age group. IA: Influenza A; IB: Influenza B; Ad: Adenovirus; PI: parainfluenza; RSV: Respiratory syncytial virus.

To evaluate if the children with H-A had been in contact with one or more of the viruses under study, we searched for antibodies. We found that 57% of the H-A children had antibodies to one or more viruses (Table 2); of these, 75% had antibodies against PI, the most frequent PI was type 3 (Table 3).

DISCUSSION

Several studies reveal that seasonal distribution patterns of respiratory viral infections correlate with hospital admissions due to asthmatic crisis in children [2, 4, 7]. In Mexico, even though there is no reliable registry of the frequency of H-A, this is apparently high. Likewise, there are few studies

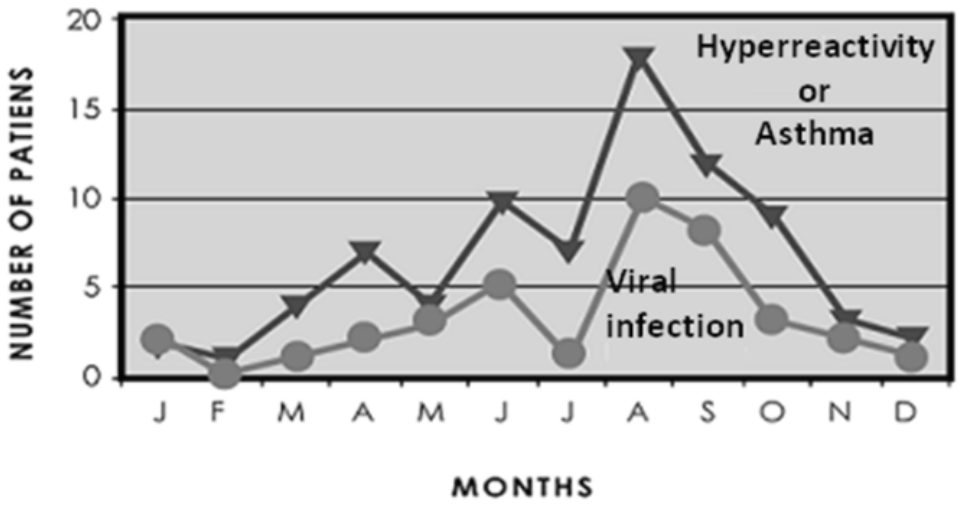

Figure 3. Correlation of hyperreactivity or asthma with viral infections in a year.

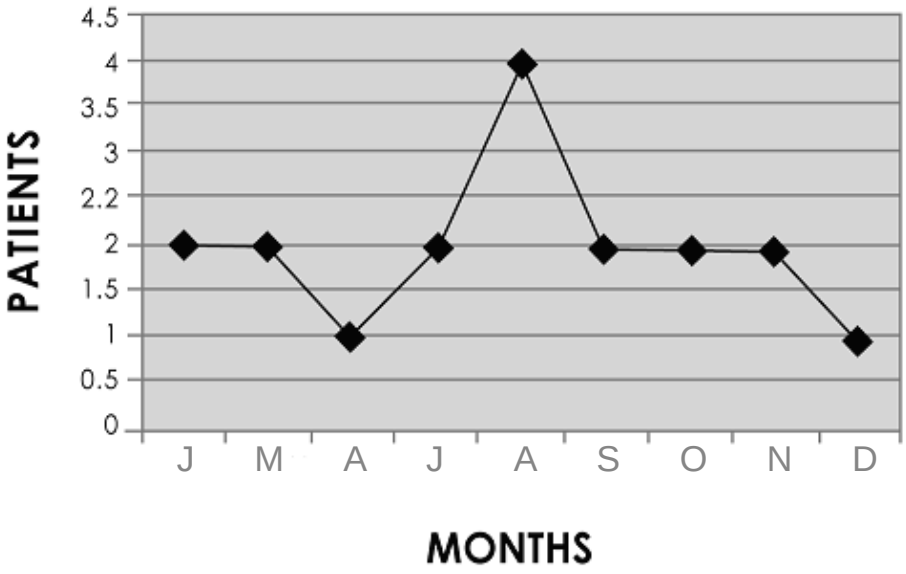

Figure 4. RSV isolation in children with hyperreactivity or asthma in a year.

Table 2. Detected antibodies in patients with hyperreactivity or asthma.

Virus type	Presence of antibodies (%)
Influenza A	23
Influenza B	9
Parainfluenza	75
Adenovirus	14
Syncytial respiratory	10

Table 3. Detected parainfluenza virus-1, -2, and -3 antibodies in patients with hyperreactivity or asthma.

Group (years)	PI-1	PI-2	PI-3	TOTAL	%
<1 to 3	2	2	7	11	46
4 to 7	1	7	7	15	62
8 to 11	0	1	1	2	8
12 to 15	0	1	3	4	17
TOTAL	3	11	18		
%	12	46	75		

about other related factors, such as the presence of respiratory viral infections. Therefore, one of the objectives of this study was to detect and identify the presence of respiratory viruses in a group of children with H-A in Mexico City, and find the frequency of each viral agent. We isolated at least one type of virus in 48% of the H-A patients. The most frequently found virus was RSV (47%), this finding correlates with other reports [4, 14]. Of the clinical data of the patients, two were relevant: 53% had familial antecedents of asthma and 61% showed wheezing before being diagnosed with asthma. Both are considered as risk factors for asthma [2, 14].

It has been reported that males are more sensitive to viral infections than women [2, 14], in this study, only group 1 showed more males infected than females (Table 1) ($p < 0.05$), whereas in group 3, the opposite was observed ($p < 0.05$). In relation to age, group 1 was found to have 43% of infected cases and all the different viruses were detected, the most frequent was IA, but this was not significant ($p > 0.05$).

IA was found in 13% of the subjects in groups 2 and 3. This virus is an important and frequent pathogen and the severity of the infection depends on the serotype of the infecting virus [2]; and it is also considered an important factor in relation to wheezing [7]. In a follow-up study performed in children infected by this virus at an early stage, wheezing or asthma, or both were observed at ages 6 to 11 years [17]. AD was not isolated in groups 3 and 4. These viruses can produce systemic manifestations and after a primary infection they can produce persistent and latent infections for long periods of time with an intermittent release of viruses, they induce a lasting immunity in relation to other viruses. It is possible that for this reason, older children are better protected. In ARI a higher frequency of AD has been found in children younger than 5 years old (5-10%) [19, 20]. In the case of PI, this virus is commonly detected in all age groups and is not confined to a particular season of the year. In this study, we found it in 11% of the cases, except in group 3. RSV was the most frequently found virus in all the age groups. It is well known that most children under 2 years of age have had infections by RSV and that early infection produces the first lesions that later on will cause obstruction of the airways [2]; insufficient lung and immune system maturity are apparently the reasons for this [2].

To find out if the children had been in previous contact with any of the viruses being studied, we searched for antibodies against these viruses. We found that 75% of the children had antibodies that recognized PI, and only 23% recognized IA. RSV was the most frequently isolated etiologic agent, however, the antibody detection was low (Table 2), probably because of one of the following factors: age, weak and short [21] or retarded immune response [17, 21, 22], evasion of the immune response, or, further more, higher neutralizing titers are needed for a more accurate detection [21-25].

An interesting finding regarding PI, is that it opposed the findings related to RSV, i.e., a lower percentage of isolation (11% PI vs. 47% RSV),

and a greater antibody response (57% PI vs. 10% RSV) (Table 2). Only serotype 3 of PI is considered responsible for 50% of the wheezing episodes in patients with asthma younger than 6 months. In this study, we observed a high frequency of antibodies against serotype 3 (Table 3). The titers and frequencies, as well as the ages, coincide with other reports [26-29].

We observed a correlation between the frequency of H-Ac and virus detection (Figure 3). In the months from June to September, this relationship was more notable, which coincides with the rainy season in Mexico City. The high humidity conditions favor the permanence of viruses and these conditions affect the H-A subjects which are prone to acquire a viral infection. The RSV was isolated mainly in August (Figure 4). Perhaps due to seasonal fluctuations, dates tend to shift, this has been seen with respect to other viruses, however, in the case of RSV this is a rare phenomenon, because it shows a very clear seasonality (spring and winter) particularly in temperate climates. In this study, we found that RSV behaved in a similar way as in a tropical or semitropical climate, where epidemics or greater incidence of viral infections are seen in the rainy seasons [17]. The risk for a severe disease with delayed consequences due to an RSV infection will depend on several factors from the host and the virus. Among those related to the host are age (> 6 months), premature birth (> 35 weeks of gestation), previous diseases (dysphasia, bronchopulmonar disease, congenital heart disease, immunodeficiency or immuno-suppresion, low birth weight, familial antecedents, etc.). Asthma is a multifactorial disease, with a very important inflammatory component. The study of these mechanisms will help us in the design of adequate therapies for the control or elimination of virus-induced acute exacerbations of H-A as well as to decrease H-A exacerbations.

COMPETING INTERESTS

None of the authors has a conflict of interest.

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