

Impact of Respiratory Infections by Influenza Viruses A and B in Pediatrics Patients from Federal University of Paraná, Brazil

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The objective of the present study was to determine the impact of influenza virus on pediatric hospitalized patients. We retrospectively reviewed records of children with laboratory diagnoses, by cell culture and/or indirect immunofluorescence assay, of influenza virus seen in a period of 6 years. A total of 1,033 samples were analyzed, 45 (4.3%) of them being reactive to influenza virus. Thirty-one samples were positive to influenza A virus and 14 to influenza B. The frequency of hospitalization in intensive care and medical emergency was found to be high. Three (8.6%) patients died, two of them due to respiratory failure. Low frequency of influenza virus infection was observed in the study. The data suggest the need of more efficient epidemiological surveillance measures in order to obtain reliable information to better assess the impact of the virus on our region and determine the need of preventive measures, such as immunization.

Key-Words: Respiratory infection, influenza virus, indirect immunofluorescence, children.

Influenza virus is a highly contagious respiratory agent that affects individuals of all ages and plays an important worldwide role by causing epidemics, mainly during autumn and winter [1,2]. It eventually leads to pandemics that are responsible for a large number of deaths [3]. According to data from the Centers for Disease Control and Prevention, influenza and its complications are the sixth cause of death among children younger than 4 years. In addition, 114,000 persons are hospitalized and 36,000 individuals die in the United States every year due to it [4].

The study of the severity and risk of influenza virus infection in children is fundamental for indication and adoption of control steps of this disease, such as immunization. Preventive measures are extremely important to contain influenza epidemics [5,6]. One of them that has shown a large impact is the multivalent vaccine formulated annually with the types and strains selected by the global influenza virus surveillance program. The vaccine has been found to be efficient and has been recommended for children older than 6 months; however, despite clear evidence that immunization reduces the risk of infection, the vaccine is rarely applied even to high risk children. Only 1 to 7% of these children receive the vaccine [7-11]. For patients who do not belong to the risk groups, a variety of strategies and interventions has been used to improve influenza vaccination rates encouraged by the Center for Disease Control [1]. It was reported that the majority of children hospitalized for influenza do not belong to the specific risk groups targeted by current recommendations, and that one third are younger than 6 months of age. Then, it was suggested that the target population for vaccination needs to be reexamined [12].

The objective of the present study was to determine the frequency and severity of influenza viruses A and B infections among pediatric patients hospitalized at General Hospital from Federal University, Paraná State (HC-UFPR), identify the risk factors and assessing the impact of the infection on patients under 6 months of age till 24 months considering the need of preventive measures, such as immunization.

Materials and Methods

We retrospectively analyzed the results of clinical samples of nasopharyngeal aspirates (NPA) or bronchoalveolar lavage (BAL) collected from pediatric patients hospitalized at HC-UFPR between January 1996 and December 2001, who showed clinical manifestations of respiratory diseases and respiratory viruses isolation and antigen determination had been requested. HC-UFPR is a hospital that attends patients from Curitiba city and metropolitan region with low socio-economic conditions. The pediatric unit is a reference that receives patients with severe diseases.

The records of patients with a positive result for respiratory influenza A or B virus were selected and the following data were obtained: date of hospitalization, age, gender, medical unit of hospitalization, present clinical manifestations, use of antibiotics, use of oxygen therapy, cultures with isolation of bacteria, number of deaths, and the time of hospitalization of each patient.

The collected samples were sent to the Laboratory of Virology and maintained under refrigeration until the time of processing for the determination of viral antigen. Approximately 1 mL of NPA was collected and fractionated for the determination of antigens from syncytial respiratory virus, influenza A and B virus, parainfluenza group, and adenovirus by indirect immunofluorescence assay (IFA) and cell culture as previously described [13,14]. Briefly, 500 µL of the sample were supplemented with 1 mL of transport medium (Hank's medium, 0.05% gelatin, pH 7.2) containing antibiotics. The sample was then inoculated into HEp-2 and MDCK cell lines and viral replication were monitored. Monolayer cells

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were collected and mounted on slides for the detection of viral antigen by IFA. Another aliquot of the material was transferred to a centrifuge tube, homogenized in 0.01 M phosphate-buffered saline (PBS), pH 7.2, and centrifugated. The pellet was suspended in PBS and mounted on a slide for IFA labeling, using monoclonal antibodies (Chemicon International, Inc. Temecula, USA).

Results

A total of 1,033 samples were analyzed for the presence of viral antigens. Forty-five (4.3%) were reactive to influenza virus, 31/45 (68.9%) to type A and 14/45 (31.1%) to type B. Influenza virus positivity showed a small predominance among girls (55.5%, 25 patients). The majority of children, 35/45 (78%), was younger than 12 months. Twenty-three 23/45 (51,1%) patients were younger than 6 months and 12/45 (26,7%) between 6 to 12 months. As shown in Table 1, 86.7% of the children were from medical emergency or intensive care unit beds. Table 2 summarizes the presence of respiratory signs and symptoms, with a higher frequency of fever, cough and dyspnea being observed. Risk factors were reported in 17/45 (37.7%) cases, with the main factors being prematurity (8.6%) and a history of previous pneumonias (11.4%). Clinical complications were observed for 28/45 (62.2%) patients, with acute tracheobronchitis (37.1%) and bronchopneumonia (34.3%) (Figure 1). Twelve 12/45 (26.6%) patients developed acute respiratory failure. The mean time of hospitalization was 9 days. Patient management consisted of antibiotic and oxygen therapy in 19/45 (42.2%) and 33/45 (73.3%) cases, respectively. Three 3/45 (6.6%) patients died, two due to respiratory failure.

Discussion

Previous study in our institution showed that 30% of tested samples were positive for the presence of respiratory viruses. The percentages of infection for influenza virus from outpatients and hospitalized, respectively, were 16.9% and 5.2%. It corresponds to the third main agent found [15]. Patients

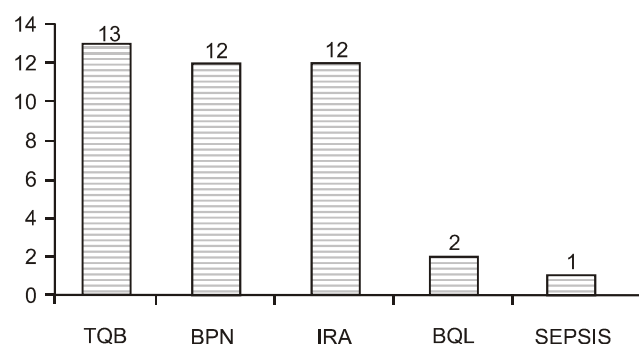
Table 1. General data by gender, age and health unit

Data	N	%
Gender		
Male	20	44.5
Female	25	55.5
Age		
≤06mo	23	51.1
06mo-2mo	12	26.7
12mo-24mo	4	08.9
≥24mo	6	13.3
Health unit		
Emergency unit	23	51.1
Intensive therapy	16	35.6
General pediatric	5	11.1
Neonatology	1	2.2

Table 2. Clinical manifestation observed in patients with Influenza A and B

Clinical manifestation	N	%
Rales	31	13.9
Fever	27	12.1
Cough	27	12.1
Dyspnea	18	8.1
Wheezing	15	6.8
Tachypnea	13	5.8
Decreased oxygen saturation	12	5.4
Nasal congestion	12	5.4
Vomiting	10	4.5
Lethargy	9	4.5
Anorexia	8	3.6
Febrile seizures	2	0.9

Figure 1. Complications observed in inpatients with viral infections.



TQB = traqueobronquitis BPN = broncopneumonia IRA = insf. acute resp.
BQL = bronquillitis SEPSIS = septicemy

with acute respiratory infection aged less than 5 years, followed for a period of 4 years and who had been hospitalized in emergency units or seen at outpatient clinics in the city of Rio de Janeiro, showed a low frequency of influenza A and B virus, with these viruses occupying third place among frequently isolated viruses. Influenza A virus was equally isolated from hospitalized and outpatient children, while influenza B virus predominated in the last one [16]. Few data regarding the prevalence of influenza virus infections in pediatric patients are available in Brazil. Recently, a surveillance system of these infections has been established for the Brazilian population. Samples collected by the Information System of Epidemiological Surveillance of Influenza Virus in Brazil (SIVEP-GRIPE) from patients with flu-like symptoms demonstrated a 19% frequency of influenza A virus and 1.5% frequency of influenza B in patients aged from 0 to 4 years, with the largest number of samples (36.5%) being collected from this age group [17]. Although the hospitalization for influenza is not high, it has significant consequence such as ICU admission, long time of

hospitalization, antibiotics use, oxygen therapy and death. The impact of influenza on children needs to be recognized in order to adopt preventive measures.

As reported previously, influenza virus, together with respiratory syncytial virus, is associated with the hospitalization of children due to lower respiratory tract infection, with respiratory syncytial virus being detected at a high frequency [18,19,20,21,22], especially in patients between the ages of 1 and 3 months [2,23]. The same was demonstrated in the present study in which the proportion of children aged from 1 to 6 months was 48.8%. The association between viral infectious agents and bacteria compromising the lower respiratory tract in pediatric patients has been reported, with the viruses detected in the samples possibly not being the only factors responsible for the complications observed. *Streptococcus pneumoniae*, *Staphylococcus aureus* and *Haemophilus influenzae* are among the pathogens commonly identified together with viral agents in the lower respiratory tract. Routine determination of these associated infections has been suggested to be performed in pediatric patients with lower respiratory tract infection [24]. In the present study, correlation between lower airway disease and presence of viruses and bacteria was not determined, and, thus, no conclusion could be drawn about whether these agents are solely and directly responsible for all alterations observed in the lower airways. The culture of bacteria in respiratory secretions is not effectuated routinely in our institution due to technical limitations, such as contamination. Coagulase-negative staphylococci were isolated in blood culture from 3 patients, a fact explained probably due to contamination during collection. Other potential pathogens such as other viruses, *Mycoplasma pneumoniae* and *Chlamydia pneumoniae* were not investigated.

Fever was the predominant finding at the beginning of infection and most patients with viral infections presented this symptom at least during some phase of the disease [25]. Studies monitoring fever in pediatric patients with infections caused by respiratory viruses have demonstrated a higher frequency and intensity of this symptom in patients infected with influenza A and B virus and adenovirus. However, the intensity and duration of this symptom were not suitable for the differentiation of bacterial and viral infection. Therefore, clinical assessment together with diagnostic tests should be the determining factor for the indication of antibiotics [13,26,27]. Particular attention should be paid to the causes of hospitalization. In the present study, tracheobronchitis (37.1%) and pneumonias (34.3%) were the most frequent complications, which, according to the literature, mainly affect children younger than 1 year, with the most severe manifestations being observed during the first months of life. About 94% of these children receive oxygen therapy, which is vital for this type of patient [23,28].

Vaccination against influenza virus is recommended for all age groups above 6 months [7]. However, it has been noted that influenza vaccination is routinely applied only to elderly individuals, while healthy children or even those considered to be at high risk are not routinely immunized, despite evidence that immunization markedly reduces the risk of infection, which is a predominant factor in children lives during the first year of age, when the first vaccination against this virus is usually performed [12]. Pre-school children, due to their contact with other children at day-care centers or pre-schools, show a high frequency of respiratory infections at this age [11,29], especially infections caused by influenza virus [19]. Influenza virus causes severe diseases in humans, mainly due to its antigen variability that can result in a benign phenotype or a potentially virulent strain [30,31]. Thus, the occurrence of a new pandemic is uncertain. Several reports have demonstrated the worldwide impact of the virus [3,31]. In January 2003, in Michigan, there were reports of a severe and incomprehensible disease causing the death of children and young adults with less than 21 years of age. Subsequently, 2 deaths were associated with influenza, and, in order to identify flu cases among healthy children and adolescents younger than 21 years, a more efficient influenza virus surveillance program was installed, permitting a better identification of the frequency of influenza-related severe complications in healthy children and a better assessment of the findings for incorporation into the children's immunization program [12].

In the present study, the prevalence of influenza virus was low among children hospitalized at our service, but was relatively high among patients occupying medical emergency or intensive care unit, with reports of clinical complications and cases of deaths. These data should be taken into account and children seen at outpatient clinics should be more carefully assessed in order to establish an efficient surveillance for influenza virus infection in Brazil, providing reliable data to encourage and adopt preventive measures, such as immunization. Important advances are currently being made in the development of vaccines and antiviral therapies, which might be of help to effectively prevent new pandemic.

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