ORIGINAL ARTICLE

Respiratory Viruses Detected in Hospitalised Paediatric Patients with Respiratory Infections

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Summary

Over 200 strains of respiratory viruses cause a variety of human infections ranging from common cold to life-threatening pneumonia. Respiratory viruses implicated in this study are respiratory syncytial viruses (RSV), adenovirus, influenza viruses and parainfluenza viruses. The objective of this study is to determine the epidemiology of respiratory viruses in paediatric patients with lower respiratory tract infection. The methods used were direct antigen detection method, shell vial culture method and conventional tube culture method. The samples included in this study are paediatric patients seen in Universiti Kebangsaan Malaysia Hospital, Kuala Lumpur with suspected acute viral respiratory infection, presenting with acute laryngotracheobronchitis (croup), bronchiolitis and pneumonia. Nasopharyngeal aspirates were collected and processed almost immediately. A total of 222 specimens were received during February 1999 to January 2000 showing a dual peak pattern in the months of April and December. The mean age of the patients was 13 months. Pneumonia (77.9%) was the most common clinical diagnosis in children with lower respiratory tract infection. This was followed by bronchiolitis (19.4%) and croup (2.7%). Viral aetiologies were confirmed in 23.4% of the patients. The most common respiratory virus isolated or detected was RSV, followed by parainfluenza viruses, influenza viruses and adenovirus.

Key Words: Respiratory Viruses, Respiratory Infections, Shell vial, RSV, Influenza virus, Parainfluenza virus, Adenovirus

Introduction

Respiratory viruses cause a wide range of diseases in children ranging from a mild flu-like illness to life threatening pneumonia. There are more than 200 strains known to infect humans. Among the common respiratory viruses are adenovirus, respiratory syncytial virus (RSV), influenza A and B viruses, parainfluenza viruses, rhinovirus and many others. They had caused enormous economic implication in the United States with regard to treatment cost, school missed and workdays missed in caring for sick children.

In Malaysia, no published data are available with regard to the epidemiology of respiratory viruses. This is probably due to the fact that it is not cost-effective to identify the causative agent routinely in patients with suspected viral respiratory infections. Thus, patients suffering from acute respiratory diseases are often treated without knowing the causative agents. The clinicians will treat their patients according to the best of their knowledge and guess, with guidance from literatures from abroad. In our study we attempt to determine the epidemiological characteristic of respiratory viruses in our local population. Hospitalised paediatric patients were chosen, as this group of patients will present with a more serious respiratory infection that demand intensive medical attention. Identifying the causative agent will give extra

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advantage to the managing clinicians. The use of unnecessary antibiotics can be discontinued, the need for other diagnostic tests can be reduced, isolation precaution can be carried out without prejudice and anti-viral agents can be instituted to certain viral infections.

Materials and Methods

The inclusion criterion was hospitalised paediatric patients admitted in the paediatric wards in Universiti Kebangsaan Malaysia Hospital, Kuala Lumpur from February 1999 to January 2000 who presented with suspected acute viral respiratory infections (pneumonia, bronchiolitis and acute laryngotracheobronchitis). Respiratory infections of more than seven days prior to admission and suspected nosocomial infections were excluded.

Nasopharyngeal aspirates were collected from the patients and sent to the virology laboratory within four hours. Three different techniques were used in order to maximise the detection of the viruses. They were direct antigen detection method (rapid test), shell vial culture method and conventional tube culture method.

Visible mucus was first discarded by using Pasteur pipette. The specimens were then centrifuged at 1000 g at room temperature. The sediments were smeared on glass slides for the rapid test while the supernatant were inoculated into the two culture systems. Indirect immunofluorescence tests were then performed immediately using commercial test kits (Light(tm) Diagnostic Respiratory Panel Indirect Fluorescent Antibody kit). This kit detects seven respiratory viruses namely, influenza A and B viruses, adenovirus, RSV and parainfluenza viruses (Type 1, 2 and Type 3). The results would be known after two hours.

With regard to the viral culture methods, two cell lines were used: Madin-Darby Canine Kidney (MDCK) cells and continuous human laryngeal carcinoma (HEp-2) cells. The former is suitable for the isolation of influenza viruses and parainfluenza viruses while the latter is suitable for RSV and adenovirus isolation. The cultures were kept in carbon dioxide incubators. They were observed daily for cytopathic effects (CPE). The shell vial cultures were processed after two days of incubation and indirect immunofluorescence test was done. The conventional tube cultures were incubated up to ten days and indirect immunofluorescence test would be done when CPE were evident.

Results

A total of 221 patients met the set criteria. In this study, boys outnumbered girls (63.1% and 36.9% respectively). The ages of the patients range from one month old to ten years old (mean 13.0 months, median 7 months). It is interesting to note that 73.9% of the patients were twelve months of age and below, and 87.4% were two years old and below.

A total of 222 specimens were sent to the laboratory during a period of twelve months. The distribution was shown in Figure 1. It virtually shows a dual peak pattern. The peak months seemed to be in April and December.

Pneumonia was clinically diagnosed in the majority (77.9%) of the patients. Bronchiolitis and croup contributed about 19.4% and 2.7% respectively. The average age of each group are shown in Table I. The mean age for children with bronchiolitis is about six months old and is statistically significant when compared to the mean age of other disease groups.

Throughout the period of twelve months, a total of 52 specimens (23.4% from the total specimens received) were positive either by direct antigen detection method or isolated by tissue culture. It also showed a dual peak pattern with peak months generally follow the trend of that in Figure 1.

Respiratory syncytial virus (52.8%) is the most common respiratory virus detected or isolated (Figure 2). The second most common virus detected or isolated is the parainfluenza virus group (18.9%) with predominance of parainfluenza virus Type 3 (10 of 13), followed by parainfluenza virus Type 1 (two patients) and parainfluenza virus Type 2 (one patient). Influenza viruses and adenovirus accounted for 13.2% and 9.4% respectively. No influenza B virus were detected or isolated.

The seasonal distribution of the individual viruses is shown in Figure 3. Generally, adenovirus, parainfluenza virus and influenza virus were found during the first half of the study period (February to July 1999) with a small cluster of parainfluenza virus in the early part of the second half. Respiratory syncytial virus was virtually present throughout the study period. It showed a dual peak pattern with peaks in March and November (almost similar to that of Figure 1).

Viruses were confirmed as aetiological agents in 22.5% (39 of 173) of all patients with pneumonia. Respiratory syncytial virus was either isolated or detected in 48.7% of them. The other aetiological agents included parainfluenza viruses (23.1%), influenza A virus (17.9%) and adenovirus (10.3%). In patients with bronchiolitis, 27.9% (12 of 43) of them were due to viruses. Respiratory syncytial virus was again the primary aetiological agent (75.0%). The rest were caused by parainfluenza virus Type 3 (16.7%) and adenovirus (8.3%). There were only two patients suffering from acute laryngotracheobronchitis who were virus-positive. The causative agents were identified as parainfluenza virus Type 2 and Type 3.

The mean age for RSV-positive patients with pneumonia was 12.3 months while the mean age for RSV-positive patients with bronchiolitis was 4.9 months (Table II). However, this is not statistically significant. Adenovirus and parainfluenza virus Type 3 also showed similar clinical picture.

The mean age for influenza A virus, parainfluenza viruses and RSV-positive patients were below 10 months old. Only adenovirus recorded an average age of 15.0 months old.

Table I: Mean and median of the disease groups

	Mean (months)	Median (months)	p value
Pneumonia	14.14	8.0	0.58
Bronchiolitis	6.2	6.0	<0.05
Croup	30.8	18.0	0.38

Table II: Comparison between the number of cases of pneumonia group and the bronchiolitis group with regard to the individual viruses

	Pneumonia	Bronchiolitis	p value
RSV	19 (12.3)	9 (4.9)	0.183
Adenovirus	4 (17.8)	1 (4.0)	0.479
Parainfluenza virus	9 (8.9)	2 (5.5)	0.434
Influenza virus	7 (5.3)	0	

Parenthesis denotes mean age in months.

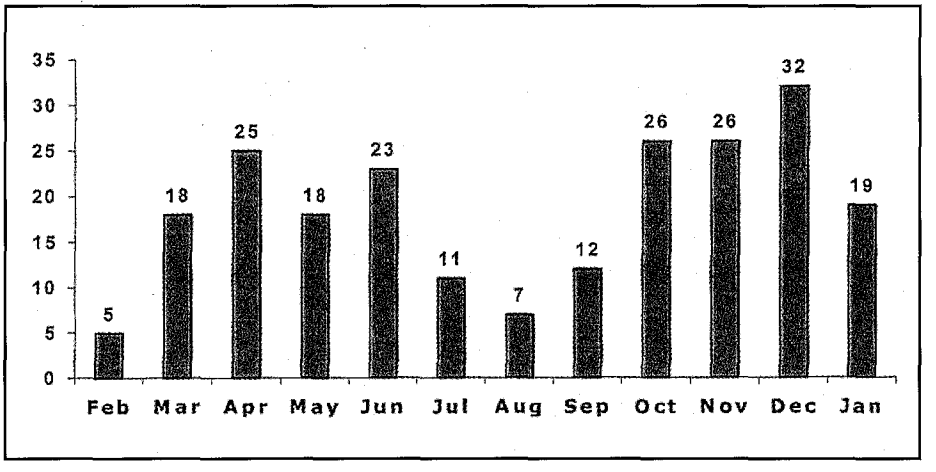

Fig 1: Total number of specimens received from February 1999 to January 2000

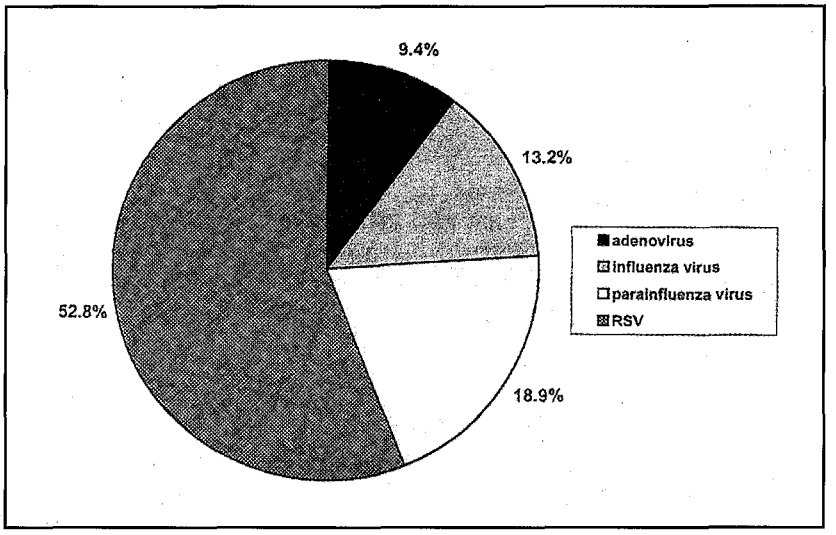

Fig 2: Distribution of respiratory viruses

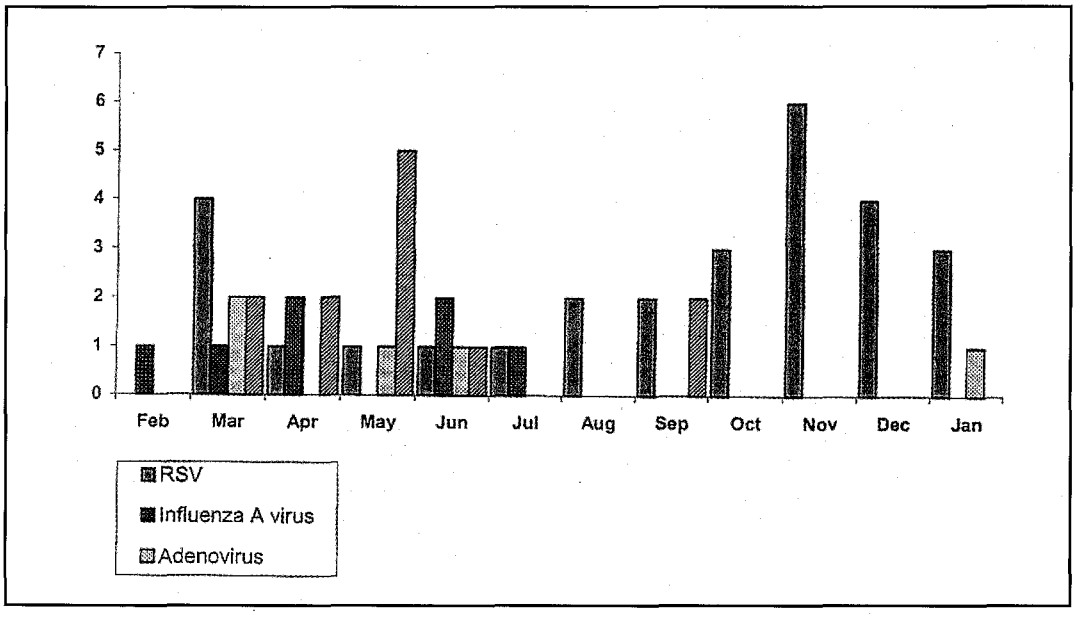

Fig 3: Monthly distribution of individual respiratory viruses

Discussion

Our study revealed the epidemiological characteristics of hospitalised paediatric patients with pneumonia, bronchiolitis and acute laryngotracheobronchitis in Universiti Kebangsaan Malaysia Hospital, Kuala Lumpur.

In our study, 87.4% of the patients were two years old and below. This illustrates the importance of acute lower respiratory tract infection among children of this age group. Studies on children with lower respiratory tract infection done in Argentina[1] and Bangladesh[2] also showed similar epidemiological picture. Slightly more than 75% of patients were clinically diagnosed as having pneumonia. This revealed pneumonia as the primary lower respiratory tract infection.

Viral aetiologies were confirmed in 23.4% of the patients. This figure is not much different from other studies. A Saudi study recorded 26.9% of their paediatric study population[3]. Studies in Pakistan and Singapore reported 38.8%[4] and 26.8%[5] respectively.

It is now confirmed that respiratory syncytial virus is still the major respiratory viral agent that causes concern in hospitalised paediatric patients locally. The fact that RSV accounted for more slightly more than 50% of the cases indicates that it is indeed an important agent. A big multi-centre study by the National Academy of Sciences, United States of America, done in ten developing countries in Africa, Oceania, Asia and Latin America also confirmed RSV as the most common respiratory virus affecting young children [6].

Parainfluenza viruses (18.9%) accounted for the second most common viral agents isolated or detected in this study. This is not surprising, as parainfluenza virus is known to be only second to RSV in causing lower respiratory tract infection in young children. In a 20-year surveillance study on otherwise healthy children, parainfluenza virus accounts for 17.4% of positive viral cultures in children with significant respiratory illness[7]. Parainfluenza virus Type 3 was the most commonly isolated virus (59%) followed by parainfluenza virus Type 1 (29%) and parainfluenza virus Type 2 (12%). The order of frequency of the various serotypes of parainfluenza virus isolated or detected is similar to our study and in a Thai Study[8]. Influenza virus was the third common (16%) viral agent isolated or detected and the rest of the 10% was adenovirus.

The monthly distribution of the respiratory viruses in this study was clearly influenced by RSV. Respiratory syncytial virus was endemic as it was found virtually throughout the study period. It peaked during the month of March and November. Respiratory syncytial virus is also endemic in Thailand but peaked in July and August, which is postulated to be associated with the rainy season [8]. In temperate countries, RSV usually causes epidemics occurring mostly in winter[1]. In hot climate countries such as the Saudi Arabia, RSV endemicity peaked in the cold season[3]. It is uncertain whether climate and humidity have a significant impact on the infectivity of the virus. However, it is observed to have very strong association with cold or rainy seasons as well as winter months. A proper controlled study is required to justify this hypothesis.

In general, parainfluenza virus Type 3 infections occur throughout the year but parainfluenza virus Type 1 and Type 2 infections occur as biennial epidemics in autumn[7]. However, this pattern is unlikely to be seen in this study, as the cohorts are only limited to those suffering from lower respiratory tract infection. In order to study the epidemiological pattern of parainfluenza virus infection, the cohort must also include those having upper respiratory tract infection as well as outpatients. In our study, parainfluenza virus was commonly found during the month of February to July with a peak in May. Parainfluenza virus infection occurred during the warmer months in Argentina[1]. However, the peak of parainfluenza virus infection was in February and March in Thailand[8]. Again, the association with climate and humidity remain something to be studied later.

In Thailand, influenza virus infection occurred all year round in paediatric patients[8]. In Singapore, the incidence of influenza showed a dual peak period, that is, during the months of May and June (the warmest months), and the months of December and January (the 'coolest' months)[9]. The incidence of influenza virus-infected children in the Saudi study is quite substantial (23.8%). It was also associated with the cold season [3].

In our study, the isolation of adenovirus was rather scattered throughout the study period. Both the Argentinean[1] and the Thai[8] studies reported respiratory adenovirus infection in paediatric patients to happen throughout the year. In a four-year retrospective study, 7% of hospitalised children with lower respiratory tract illness had adenovirus infection[10].

Generally, respiratory syncytial virus is commonly and classically associated with bronchiolitis. However, our study had shown that RSV-infected patients manifested with pneumonia more than bronchiolitis (about 2 fold). This figure is not totally surprising, as the number of patients with pneumonia greatly exceeded those with bronchiolitis. Respiratory syncytial virus was confirmed in 9 of 12 bronchiolitis patients. This proves that RSV is still the primary causative agent of bronchiolitis. All influenza virus-infected patients presented with pneumonia, which is of course the main pathology of this virus. Both viral-positive laryngotracheobronchitis patients were caused by parainfluenza virus infection, which is what parainfluenza virus is classically associated with.

Our study was designed to detect seven respiratory viruses on respiratory specimens. Some other common respiratory viruses such as the coronavirus or rhinovirus were not detected by the above methods. Not many literatures stressed on these viruses as they normally caused mild self-limiting disease that is not cost-effective with regard to laboratory investigations. Rhinovirus normally does not cause serious respiratory tract infection that warrant hospitalisation. It is rarely a problem with immunocompetent person. However, rhinovirus can cause pneumonia and acute obstructive bronchitis in children under 3 years old [11]. Coronavirus is very difficult to isolate. Like rhinovirus, coronavirus frequently causes mild upper respiratory illness. However, it can also cause pneumonia and other lower respiratory tract diseases in children. Enteroviruses also cause respiratory infections and could be detected by as much as 15%[12].

Blind staining of specimens of conventional tube culture method with no visible CPE after ten days' incubation of were not done simply due to cost factor. If blind staining were done, there might be some specimens that gave positive result. As much as 16% of the conventional tube culture would be positive when blind staining was done after two weeks [13].

In order to get better epidemiological characteristic of respiratory viruses infection in paediatric patients, this study should be extended to at least another one or two years. Knowing the causative agents of hospitalised paediatric patients give several advantages with regard to the management of the patients. This is complimented by the availability of two rapid methods used in our study, namely the direct antigen detection test (results available within 2 hours) and shell vial culture method (results available within 2 days). The duration of hospitalisation can be shortened, the use of unnecessary antibiotics can be discontinued and the need for other diagnostic tests can be reduced. Patients identified with RSV or influenza virus infection can be given antiviral agents and can also be isolated from other patients. Isolation of RSV-infected patients is important because a study in Canada reported that 6% of their RSV-positive patients were acquired through nosocomial means[14]. Problem will arise when children with risk factors for severe disease may have prolonged stay and excess mortality.

In centres where isolation of patients with respiratory infection is a common practice, patients' overisolation is a problem[15]. With the introduction of rapid diagnosis utilising the two methods mentioned earlier, early discontinuation of isolation is possible and hence resulted in the use of fewer barriers (i.e. masks, gowns) and thus decreased cost, more freedom for patients and visitors, reduced staff workload associated with maintaining isolation and increased bed availability.

In conclusion, acute respiratory viral infections occur frequently in paediatric patients of two years and below. The vast majority of the patients presented with pneumonia. Viral aetiology was diagnosed in 23.4% of hospitalised paediatric patients. Respiratory syncytial virus remains the most common causative pathogen followed by influenza virus, parainfluenza virus and adenovirus.

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