Original Articles

Prevalence of parainfluenza and influenza viruses amongst children with upper respiratory tract infection

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ABSTRACT

Objectives: The objective of this study was to determine the prevalence of Parainfluenza and Influenza causing upper respiratory tract infections and to evaluate shell vial culture assay and direct immunofluorescence assay.

Methods: A retrospective study during the period between November 1997 and May 1998. A total of 350 nasopharyngeal aspirates were obtained from children suffering from respiratory tract infections. Nasopharyngeal aspirates were investigated for the presence of Parainfluenza 1, 2 and 3, Influenza A and B using shell vial culture assay, conventional culture assay and direct immunofluorescence assay.

Results: Parainfluenza 1 were identified in 3%, Parainfluenza 2 in 5% and Parainfluenza 3 in 6%. Influenza A were identified in 4% and Influenza B in 2%. Parainfluenza 1, 2 and 3 were isolated in children less than 5 years old. Most of Parainfluenza cases were associated with other upper respiratory infections. Shell vial assay showed a sensitivity of 90-93% and specificity of 99-100% for detecting Parainfluenza 1, 2 and 3.

Conclusion: These results emphasize that shell vial assay is important for the diagnosis of Parainfluenza and Influenza, although direct immunofluorescence assay is the superior diagnositic assay.

Keywords: Parainfluenza, influenza, shell vial, conventional culture, immunofluorescence.

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Acute respiratory tract infections (RTI) accounts for nearly one third of all deaths among children below 5 years of age in many countries.[1,2] RTI kills four million children every year in developing countries and most of these deaths are caused by pneumonia.[3] In these countries, the rate of infection is considerably higher particularly during infancy and early childhood.[4]

The majority of viruses causing RTI and upper respiratory tract infections (URTI) are ortho-myxoviruses (Influenza A and B),[5,6] paramyxoviruses (respiratory syncytial virus (RSV),[7,8] and Parainfluenza viruses types 1, 2 and 3).[9,10] Viruses such as Influenza and Parainfluenza do not establish persistent infection (latency), and isolation almost indicates active viral disease.

Parainfluenza combined with respiratory syncytial virus represent the most significant upper respiratory viruses in infants and young children,[11] while Parainfluenza was the major cause of croup laryngitis.[9] As there is no effective antiviral agent or vaccines or both for Parainfluenza infections, then the best method for limitation of its spread is the proper hygiene state.[12]

Influenza causes highly contagious respiratory disease, which typically results in epidemic infection.[13,14] Influenza is short lived and a relatively mild infection in healthy individuals, but large number of patients involved in an epidemic can include significant number of deaths.[5]

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Influenza virus is responsible for 14% of childhood fever with RTI severe enough to warrant physician's attention.[13] Children experience myositis, otitis media, and neonatal infection may result in unexplained fever,[15] and it is potentially fatal.[16]

Influenza A and B cause essentially the same spectrum of disease. However, hospitalization due to type A is more than those of type B.[15] Influenza virus is well known for the changes in the antigenicity due to genetic drift and shift. This explains why influenza continues to be a major epidemic disease and frequently produces worldwide pandemics.[13] Many studies conducted in the United States indicated that Influenza A infection peak is usually in the winter[17] and involves around 10% of the population especially among children while Influenza B usually accounts for less than 3% of clinical influenza cases.[15]

Amantadine drugs are used to reduce the duration of symptoms of type A Influenza, and is moderately effective medicine.[18] However, new medicines designed to treat the influenza by halting viral replication in humans, can also serve as a novel kind of prevention.[19] There is no fully protective influenza vaccine. However, the subunit vaccines with different variants of influenza virus glycoproteins could be successful if it includes current viral strains, although, a new live attenuated influenza vaccine is promising.[20]

Methods.

Patients. During the period between November 1997 and May 1998, a total of 350 children aged less than 13 years old suffering from respiratory infections were admitted to the Respiratory Disease Unit at Princess Rahma Hospital, northern Jordan. It is the only pediatric hospital in northern Jordan. NPAs were obtained from all patients. Patients with congenital and persistent respiratory disease were not included in this study. A special form was used for each patient recording clinical data, age, sex, length of hospitalization and use of antibiotics.

Specimen collection and processing. NPAs were collected[7] and processed[21] from the patients.

Preparation of cell pellet for immunofluorescence staining. The cell suspension was prepared from the pellet obtained after specimen centrifugation by washing the pellet twice in PBS, pH 7.4 for 10 minutes to decrease the mucus viscosity. The sediment was resuspended in 0.5 ml PBS and 20ul of cell suspension was spotted per well of an 8 multi-well slide (5 mm diameter). The slide was dried by hotplate at 40°C, fixed with cold acetone at 4°C for 10 minutes, and stored in an air-tight box at -70°C until used for staining.

Direct immunofluorescence staining. 10ul of mouse monoclonal isothiocyanate-labelled anti-RSV antibody (CHEMICON, USA) was added to each well. The slide was incubated at 37°C for 30 minutes in a humid chamber, then rinsed for 10-15 seconds in PBS, pH 7.4, and mounted in phosphate-buffered glycerol (20%-80%, v/v). The slide was read using a fluorescence microscope (Nikon, Japan) at X 20-40 magnification. Specimens yielding less than an average of 10 columnar epithelial cells per well were rejected. RSV infected and uninfected human epithelial cells (HEp-2) were stained as positive and negative controls, in each trial. The presence of one positive columnar epithelial cell was required to consider a specimen positive for RSV antigen.

Conventional culture assay. The specimen inoculation procedure was carried out according to the clinical microbiology procedure.[21] Culture tubes were examined daily for evidence of viral replication and development of CPE. If CPE developed, the incubation was stopped and the cell culture harvested and prepared for immunofluorescence staining.

Shell vial culture assay. Shell vial (SV) assay was performed.[22] SV tubes were examined daily for toxic effect or contamination. At the end of the incubation period (regardless the presence of CPE), the HEp-2 monolayer was harvested from the bottom of the SV tubes by scraping into small volumes of PBS, pH 7.4 by a rubber policeman, and 20ul of scraped cells were spotted onto each well of an 8-well slide and prepared for immunofluorescence staining.

Data analysis. Data was collated and analyzed by an IBM PC computer. The comparisons between groups were carried out by the chi-square **(χ^2)** test.

Results.

Table 1 shows the number of positive Parainfluenza and Influenza for any of the assays used. Parainfluenza 3 was equally recovered in both SV and conventional culture (CC), 15 of 16 (94%) cases for each. Parainfluenza 2 was detected more by SV 14/17(82%). However DFA detected all positive cases of Influenza A and B, but Influenza A and B were poorly detected with HEp-2 cell line in shell vial; 4/15 (27%) cases of Influenza A and 2/7 (29%) cases of Influenza B. Similar results were obtained using HEp-2 cell line in conventional culture; 1/15 (7%) cases of Influenza A, and 1/7 (14%) cases of Influenza B.

Table 2 shows the age and sex distribution of the positive and negative cases for Parainfluenza and Influenza. The age of children under study was ranged from 1 month to 13 years old with a mean of 7 months. Data showed that Parainfluenza were associated more with patients aged between 1-<5 years old ($P<0.001$).

Table 3 shows the observation in 67 children positive with Parainfluenza and Influenza. The majority of Parainfluenza cases is significantly associated with other RTI (croup, upper respiratory tract, bronchitis, and wheezy chest) ($P<0.01$). This study shows that 87% and 91% of hospitalized children with Parainfluenza and Influenza have received at least one type of antibiotic.

Table 4 shows the frequency of signs and

Table 1 - Number of positive parainfluenza 1, 2 and 3 and Influenza A and B by any of the SV, DFA and CC assays in NPAs of 350 children.

Positive virus (no./%)	No. (%) of specimen positive by:		
	SV	CC	DFA
Parainfluenza 1 (12/3)	10 (83)	10 (83)	7 (58)
Parainfluenza 2 (17/5)	14 (82)	12 (71)	10 (59)
Parainfluenza 3 (16/5)	15 (94)	15 (94)	6 (37.5)
Influenza A (15/4)	4 (27)	1 (7)	15 (100)
Influenza B (7/2)	2 (29)	1 (14)	7 (100)

SV: shell vial, CC: conventional culture, DFA: direct immunofluorescence assay

Table 2 - Age and sex distribution of children positive for Parainfluenza 1, 2 and 3 and Influenza A and B by any of SC, CC or DFA assays.

Age (years)	No. of children (M, F)*	%	No. of positive children (M, F)* (para/infl)	% (para/infl)
<1	236 (126, 110)	67	22 (8,6)/(9,8)	9.3/7.2
1-<5	102 (60, 42)	29	23 (10,9)/5 (3,2)	22.5**/4.9
5-13	17 (11,6)	5	0/0	0/0
Total	**350**		**35/22**	

*M: Male, F: Female, **Significant at P<0.001

Table 3 - Observations in 67 children positive for Parainfluenza and Infuenza by and of SC, CC or DFA assays.

Observations	No of positive children, % (para/infl)
Clinical diagnosis	
Bronchiolitis	7 (16)/3 (14)
Pneumonia	10 (22)/9 (41)
Bronchopneumonia	9 (20)/7 (32)
Other respiratory infection (Bronchitis, croup and upper respiratory infection)	19* (42)/3 (14)
Duration of hospitalization (days)	
1-3	26 (58)/13 (59)
4-6	14 (31)/ 7 (32)
7-9	4 (9)/ 2 (9)
>9	1 (2)/ 0
No. of different antibiotics received	
0	6 (13)/ 2 (9)
1	26 (58)/13 (59)
2	11 (24)/ 5 (23)
3	2 (4)/ 2 (9)

*Significant at P<0.01

symptoms of 45 children positive for Parainfluenza. Cough and wheezing were the most prominent features for children under the study.

Tables 5, 6 and 7 show the comparison between SV, CC and DFA for isolation of Parainfluenza.

Discussion.

This is the first study from Jordan to examine the role of Parainfluenza 1, 2, and 3 and Influenza A and B as the etiological agent of URTI in children under 13 years old. NPAs were collected during the winter season (November, 1997 - May, 1998), as URTI is more prevalent during this period in the northern part of Jordan. In this study, the clinical data and the isolation of Parainfluenza and Influenza viruses by SV, CC, and DFA were evaluated.

Parainfluenza viruses are one of the common causes of URTI in childhood, while the distribution of Parainfluenza viruses among hospitalized children varies in different developing countries ranging from 7% to 13%.[9,20,22-25]

Some studies indicated that the infection rate of Parainfluenza virus 2 was less than Parainfluenza virus 1 and 3.[26,27] On contrary, this paper showed that the infection rate of Parainfluenza virus 2 (5%) was slightly higher than Parainfluenza viruses 1 (4%) and 3 (5%). Our results are in parallel with a study carried out in Newcastle[22] which showed that the incidence rate of Parainfluenza 2 (7%) alone among children was more than Parainfluenza virus 1 (3%) and 3 (5%) infection rates.

Variation in the overall infection rate of Parainfluenza viruses in literature may be due to annual and seasonal variation, type of specimen used, or the method used for diagnosis. Influenza viruses cause a high contagious respiratory disease.[13]

Table 4 - The frequency of signs and symptoms for 45 children with Parainfluenza infection.

Signs and symptoms (%)
Conjunctivitis (1)
Diarrhea (2)
Vomiting (3)
Cynosis (4)
Grunting (6)
Fever (10)
Tachpnea (13)
Crepitation (17)
Retraction (21)
Wheezing (35)
Cough (37)

Table 5 - Comparison SV and CC assays for the diagnosis of Parinfluenza 1, 2 and 3 in NPAs of 350 children.

Virus (No. of positive isolates)	SV+ CC+	SV+ CC-	SV- CC+	SV- CC-
Parainfluenza 1 (12)	9	1	1	339
Parainfluenza 2 (17)	11	3	1	335
Parainfluenza 3 (16)	14	1	1	334

SV: shell vial, CC: conventional culture

Table 6 - Comparison SV and DFA assays for the diagnosis of Parinfluenza 1, 2 and 3 in NPAs of 350 children.

Virus (No. of positive isolates)	SV+ DFA+	SV+ DFA-	SV- DFA+	SV- DFA-
Parainfluenza 1 (11)	5	4	3	338
Parainfluenza 2 (15)	7	5	2	336
Parainfluenza 3 (16)	6	9	0	335

SV: shell vial, DFA: direct immunofluorescence assay

Influenza viruses have worldwide distribution and occur in epidemic pattern. The distribution of Influenza virus A and B among hospitalized children in developing countries ranged from 2-5% in one study.[21] Our findings indicate that the majority of Parainfluenza cases are associated with children between 1-<5 years of age (P <0.001) as was seen in another worldwide study.[9,26]

A peak age was evident in Parainfluenza viruses, highest (35%) among infants, 23%-27% among children less then 10 years, while a drop to 5% appears in children 10-15 years old and zero % from 15 year olds above (Personal communication from Professor Sami E. Fathalla about URTI viruses in Egypt).

Although Influenza viruses are isolated more frequently from older children and adults, many studies documented that these viruses produce illness in many children who are less than 5 years of age.[15,28] Our study indicates that Influenza viruses occur mainly in children under 1 year old (7%) more than children between 1-<5 years (5%) but this association is not statistically significant due to the limited number of our trial. Parainfluenza viruses were the major cause of croup and produce a spectrum of illnesses ranging from mild upper respiratory tract infection to more severe and prolong lower respiratory tract infection including pneumonia.[9] Our findings indicates that the majority of Parainfluenza cases are significantly associated with other URTI (croup, upper respiratory tract, bronchitis, and wheezy chest) (P<0.01).

HEp-2 cells have been well demonstrated to be very sensitive cell line for isolation of Parainfluenza 1, 2, and 3, that is why we used it for this study, while it is not the media of choice in influenza viruses.[31] In this study, the total number of Parainfluenza viruses detected by both SV and CC assays was 34 (76%) out of 45 positive samples by any of SV, CC, and DFA. Our findings showed that the sensitivity and specificity of SV assay for detection of Parainfluenza viruses were 90%-93% and 99%-100%. It was found that all Parainfluenza cases detected by CC were recovered by SV[29] and the result of SV assay for detection Parainfluenza viruses was correlated with CC.[30] We reported here a high SV sensitivity (ranged from 90% to 93%) and specificity (99%-100%) for Parainfluenza viruses. Our results regarding SV sensitivity and specificity for Parainfluenza viruses were agreed with others,[29] but higher than what was reported in some studies that reported 80% sensitivity and 89% specificity of SV for Parainfluenza viruses.[30] Because SV is sensitive, highly specific, has high predictive values, cost effective, and rapid (results obtain within 2-4 days using SV compared to 6-14 days using CC), SV does not need daily monitoring CPE or hemadsorption as CC, and since both methods relay on immunofluorescence staining for definitive diagnosis, we strongly believe as others,[31] that SV assay is alternative for conventional culture for isolation of Parainfluenza virus 1,2, and 3. Others reported that DFA sensitivity was 64% and specificity was 98% for Parainfluenza virus 1, and 31% and 100% for Parainfluenza virus 3.[22] Recent studies show that the sensitivity of DFA for detection Parainfluenza virus 3 was 66%, while DFA specificity was 100% for Parainfluenza virus 3.[31] Our DFA results regarding Parainfluenza virus 1 (sensitivity 56%, specificity 100%), Parainfluenza virus 2 (sensitivity 58%, specificity 99%), and Parainfluenza virus 3 (sensitivity 40%, specificity

Table 7 - Comparison CC and DFA assays for the diagnosis of Parinfluenza 1, 2 and 3 in NPAs of 350 children.

Virus (No. of positive isolates)	CC+ DFA+	CC+ DFA-	CC- DFA+	CC- DFA-
Parainfluenza 1 (11)	6	4	1	339
Parainfluenza 2 (15)	7	4	3	334
Parainfluenza 3 (16)	6	9	0	335

CC: conventional culture, DFA: direct immunofluorescence assay

100%) are correlated with those reported previously for each virus. The difference in sensitivity between our results and those reported by other researchers for the same virus can be due to variations in availability and quality of the reagents, the type of the collected specimens, way of specimen collection, transportation and processing,[32,33] and may be due to the way of interpretation of DFA results using the fluorescence microscope.[18,32]

If the laboratory is equipped with an adequate fluorescent microscope, we believe that the use of DFA for rapid diagnosis of Parainfluenza and Influenza is convenient, time saving, and potentially cost saving, especially when compared with labor - consuming virus isolation techniques.

In the present study, we were able to detect only 4 of 15 Influenza A cases and 2 of 7 cases of Influenza B using HEp-2 cell culture. So we recommend using other types of cell line for diagnosis of these two viruses. MDCK or PMK is recommended by many researchers for the isolation of Influenza A and B.[6]

This limited study demonstrates that the laboratory diagnosis of viral infection is important in the management of hospitalized children with URTI. This leads to decreased use of unnecessary and costly antibiotic therapy and reduces the length of hospital stay for patients. Further advanced tests for detection and typing of Parainfluenza and Influenza using PCR for example, is advisable for better judgements, drawing conclusions and recommendations.

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