Validity of Immunofluorescence Test for the Detection of Respiratory Viruses Causing Acute Lower Respiratory Tract Infection Among Under Five Children

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Abstract: Background: Respiratory viruses cause a variety of human infections, ranging from the common cold to life-threatening pneumonia. Over 200 strains of virus can cause respiratory disease. The majority of severe viral respiratory infections are caused by relatively few viruses, primarily parainfluenza virus types 1, 2 and 3, respiratory syncytial virus (RSV), influenza A and B viruses, and adenovirus. Objective: The purpose of this study was to see the validity of Immunofluorescence test for the detection of Respiratory viruses causing acute lower respiratory tract infection among under five children. Methodology: This cross sectional study was conducted in the Department of Virology at Bangabandhu Sheikh Mujib Medical University, Dhaka from July 2002 to June 2003 for a period of one year. The children with the age group of below five (5) years presented with the clinical manifestations of acute lower respiratory tract infection (ALRI) who were visited or were admitted to Dhaka Medical College Hospital (DMCH), Dhaka were selected as study population. Nasopharyngeal aspirates were collected. Viruses were detected by cell line culture and direct Immunoflorescence (DFA) method. Result: The study was carried out among 100 children aged from new born to 60 months with acute lower respiratory tract infection (ALRI). Highest rate (85.7%) of isolation was obtained among children between 0 to 24 months of age. There was a significant reduction in the number of cases in older children in 25 to 60 months of age group. The most common virus isolated from the under five children was respiratory syncytial virus which was 20(95.2%). Adenovirus was isolated in only 1(4.8%) case. No other viruses were found in this study. DFA method typically more rapid than the cell culture and also detect virus which has lost viability in transit. Culture methods on the other hand, are more favorable for detecting low titer of viable virus. In this study 17 samples are positive by cell culture and these are also positive by DFA. Total 21 samples are positive by DFA and among them 4 samples are negative. Conclusion: DFA is highly sensitive and specific for detection of respiratory viruses among the under-five children. Furthermore the accuracy of this test is also very high. Therefore it is recommended that the DFA test can be used for the detection of respiratory virus from the children presented with respiratory tract infection.

Keywords: Respiratory Viruses, Acute Lower Respiratory Tract Infection, Under Five Children, Cell Culture, Immunoflourescence, Test Validity

1. Introduction

Acute respiratory tract infections are the leading cause of death among children throughout the world. An estimated 6.5 million children below 5 years of age die each year due to acute respiratory tract infections [1]. The majority of deaths due to respiratory tract infections are caused by acute lower respiratory tract infection (ALRI) [2]. It has been estimated that four million children in developing countries die each year from pneumonia [3]. In rural areas of Bangladesh, a child below 5 years experiences two to three episodes of ALRI each year. ALRI is also the principal cause of hospitalization among children in both urban and rural areas [4].

Respiratory viruses cause a variety of human infections, ranging from the common cold to life-threatening pneumonia. Over 200 strains of virus can cause respiratory disease [5]. The majority of severe viral respiratory infections are caused by relatively few viruses, primarily parainfluenza virus types 1, 2 and 3, respiratory syncytial virus (RSV), influenza A and B viruses, and adenovirus [6].

Cell culture method is sensitive and can detect a broad spectrum of viruses [7]. Numerous rapid antigen detection systems are also very sensitive and specific and available for the diagnosis of respiratory viruses [8]. The purpose of the present study was to see the comparison between immunoflorescence and cell line culture of viruses causing acute lower respiratory tract infection among fewer than five children.

2. Methodology

This cross-sectional study was conducted in the Department of Virology at Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka form July 2002 to June 2003 for a period of one (01) year. Children with both sexes with the age group of fewer than 5 years of age presented with the clinical manifestations of acute lower respiratory tract infection (ALRI) who were visited or were admitted to Dhaka Medical College Hospital (DMCH), Dhaka were enrolled in the study. Informed verbal consent was taken from the parents. Detailed history was recorded in a preformed questionnaire. Nasopharyngeal aspirates were collected from the pediatric in-patient and outpatient department of Dhaka Medical College Hospital (DMCH) Dhaka, Bangladesh. The laboratory works were performed in the department of Virology at BSMMU and the cell line culture of respiratory viruses were performed in the Institute of Public Health (IPH), Mohakhali, Dhaka. Children accepted for this study were <5 years old and had symptoms of cough and one or more of the following: Respiratory rate more than 50/min, chest retraction, wheezing, stridor, cyanosis, rales, fever, inability to feed, history of illness less than 7 days. Isolation of respiratory viruses was performed by inoculation of specimen in HEp-2 cell line and identification of respiratory viruses by direct fluorescence antibody technique. The numerical data obtained from the study were analyzed and significance of difference was estimated by using the statistical methods. Data were expressed in percentage as applicable. Comparison between groups was done by Chi -square test. Probability less than 0.05 was considered as significant.

3. Results

The study was carried out among 100 children aged from new born to 60 months with acute lower respiratory tract infection (ALRI). Highest rate (85.7%) of isolation was obtained among children between 0 to 24 months of age. There was a significant reduction in the number of cases in older children in 25 to 60 months of age group. Difference between the rate of isolation of respiratory viruses in the 0 to 24 months age group and that in age group 25 to 60 months was significant (P <0.05). Viral infection was more common in 0 to 24 months of age group (Table 1).

Table 1. *Distribution of Infected Children with Respiratory Viruses According to Their Age Group (n=100).*

Age	Virus detection		Total	P value
	Positive	Negative		
0 to 24 months	18(85.7%)	60(75.9%)	78(78%)	
25 to 60 months	3(14.3%)	19(24.1%)	22(22%)	
Total	21(100%)	79(100%)	100(100%)	

*Chi-square test was performed corrected by Fisher's Exact Test.

The most common virus was respiratory syncytial virus 20(95.2%) isolated from the under five children. Adenovirus was isolated in only 1(4.8%) case. No other viruses were found in this study (table 2).

Table 2. *Distribution of Virus According To Clinical Manifestation (n=21).*

Virus Name	Frequency	Percentage
RSV	20	95.2
Influenza virus	0	0.0
Parainfluenza virus	0	0.0
Adenovirus	1	4.8
Total	**21**	**100.0**

A total of 21 samples were positive by Immunofluorescence and viruses were isolated from 17 (17%) of the 100 patients (Table 3).

Table 3. *Shows the frequency of different viruses which were isolated by cell culture method and by Immunofluorescence from nasopharyngeal aspirate.*

DFA	Cell Line Culture		Total
	Positive	Negative	
Positive	17(100.0%)	4(4.82%)	21(21%)
Negative	0(0.0%)	79(95.18%)	79(79%)
Total	17(100.0%)	83(100.0%)	100(100.0%)

The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy were 100.0%, 95.2%, 80.9% (95% CI 60 to 92.33%), 100.0% (95% CI 95.4 to 100.0%) and 96.0% respectively.

***Table 4.** Diagnostic Validity of the DFA test.*

Values	Value	95%CI
Sensitivity	100.0%	80.49 to 100.00%
Specificity	95.2%	88.12 to 98.67%
PPV	80.9%	60 to 92.33%
NPV	100.0%	95.4 to 100.0%
Accuracy	96.0%	90.07 to 98.90%

positive predictive value=PPV; negative predictive value=NPV

4. Discussion

Viral respiratory tract infections are the most common diseases affecting humans throughout the world. More than 5 million children under the age of 5 years experience lower respiratory infections. Respiratory syncytial virus (RSV), influenza A and B viruses and human parainfluenza virus (HPIV) type 1, 2 and 3 cause about 80.0 to 90.0% of viral LRIs [10]. Respiratory syncytial virus (RSV) is a leading cause of acute lower respiratory tract infections in infants and young children and is responsible for nearly 50% of all bronchiolitis and 25.0% of all pneumonia cases during early months of life. It occurs at a frequency of more than four times compared to other respiratory viruses [11].

In the current study of 100 children of less than 5 years old, 78% were in 0 to 24 months age group. Viral infection was more common in this group than 25 to 60 months. Thus among the 78 patients in this age group of (0 to 24 months) respiratory viruses were detected about 18 (23.07%) while respiratory viruses were detected only in 3(13.63%) of 22 children who were aged 25 to 60 months. Similar observation was been reported by Huq et al [1]. They reported that the rates of viral shedding in children aged 0 to 24 months and 25 to 60 months was 24.5% and 12.5% respectively.

In the present study, respiratory viruses were detected by Immunofluorescent test in 21% out of 100 patients under 5 years age. A comparable rate of detection of respiratory viruses was reported from a study conducted in New Haven, USA, where respiratory viruses were detected in 367(23.97%) out of 1531 children with LRTI11. Ruutu et al [9] reported 51.9% prevalence of respiratory virus in Filipino children. Respiratory syncytial virus is the most frequent agent of acute lower respiratory tract disease in young infants19. In the present study RSV was responsible for highest number of viral infections. Thus, among the total 21 respiratory isolated viruses, RSV was detected in 20(95.23%) cases. Similar finding was reported by Huq et al [1] from a study conducted in Dhaka where they have detected 103(78%) RSV among the total 132 isolated respiratory viruses. Hijazi et al [12] also detected 168(73%) RSV among the total 230 respiratory viruses isolated from children with LRTI from a study in Kuwait. There were some studies of conducted among children in a number of countries had shown a rate of detection of respiratory virus ranged from 19 to 51.9%. Forgie et al [13] reported 19% prevalence of respiratory virus in rural children in Gambia. John et al [14] reported 49% prevalence of respiratory virus in Southern India and Shann et al [15] reported 29.0% prevalence from a study in children in New

Guinea. In the present study the rate of respiratory virus isolation from children of acute respiratory tract infection was 21.0%. Thus it was evident that prevalence found in the present study was also within the range of prevalence from these studies. This study included only children who were brought to the hospital, the sample biased toward more severe cases of ALRI and was not representative of all cases in the community. The most important outcome of this study was the detection of viruses in children with ALRI.

Viral disease diagnosis has traditionally relied on the isolation of viral pathogens in cell cultures. Although this approach is often slow and requires considerable technical expertise, it has been regarded for decades as the gold standard for the laboratory diagnosis of viral disease [16]. Cell culture studies provide a valuable complement to in vivo experiments, allowing for a more controlled manipulation of cellular functions and processes. For decades, cell lines have played a critical role in scientific advancements, yet researchers have become increasingly cautious when interpreting data generated from cell lines only [17].

Immunofluorescence a method of determining the location of antigen or antibody in a tissue section or smear using a specific antibody or antigen labeled with a Flurochrome [16]. There are two major types of immunofluorescence techniques, both based on the antigen—antibody reaction in which the antibody attaches itself to a specific antigen. In the direct fluorescent antibody (DFA) method, the antibody coats the antigen and cannot be easily removed by washing. The antibody remains attached to the cell after all non-antibody globulin has been washed away. The antibody has been rendered fluorescent by conjugation with fluorescein or another dye. In indirect fluorescent antibody (IFA) method, the specific antibody is allowed to react with the antigen. The non-antibody globulin is then washed off. This is then treated with a labeled antibody to the specific antibody. Fluorescent antibody studies have been used in the detection of numerous bacterial, viral, fungal, and protozoan infections and in the identification of many microscopic tissue constituents [18].

DFA method typically more rapid than the cell culture and also detect virus which has lost viability in transit [19]. Culture methods on the other hand, are more favorable for detecting low titer of viable virus. In this study 17 samples are positive by cell culture and these are also positive by DFA. Total 21 samples are positive by DFA and among them 4 samples are negative. True positive cases are 21 and false positive are 4 in number. In this study false negative is 0(0.0%) and total negative are 79 in number. Therefore total positive are 21 and total negative are 79 in number.

Data were extracted to construct two-by-two tables, which were used to calculate the sensitivity and specificity of the rapid DFA tests. In this study sensitivity 100.0%, specificity 95.2%, and accuracy 96.0%. Another study was done in Columbus and they compared with culture with DFA kit and had a sensitivity and specificity of 73.0% and 92.0% respectively [20].

5. Conclusion

In conclusion the DFA is highly sensitive and specific for detection of respiratory viruses among the under-five children. Furthermore the accuracy of this test is also very high. Therefore it is recommended that the DFA test can be used for the detection of respiratory virus from the children presented with respiratory tract infection.

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Conflict of Interest

There was no conflict of interest to any of the authors.

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