RESPIRATORY SYNCYTIAL VIRUS IN SAUDI PATIENTS ADMITTED TO HOSPITAL WITH BRONCHIOLITIS: USE OF DIRECT FLUORESCENT ANTIBODY TESTS AS A RAPID DIAGNOSTIC TOOL

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Bronchiolitis is a major cause of morbidity and occasional mortality in young infants and children all over the world. Respiratory syncytial virus (RSV) is the major etiologic agent responsible for this condition. The illness is more severe in patients with bronchopulmonary dysplasia and congenital heart diseases, especially with pulmonary hypertension and preterm infants. Early treatment with ribavirin is recommended in infants with underlying immunodeficiency and complicated heart disease (including pulmonary hypertension) and should be considered in infants who are severely ill with $PaCO_2$ less than 65 mmHg (American Academy of Pediatrics).[1] Recently, RSV-specific immunoglobulin (RSVIG) was found to be effective in ameliorating RSV infections when used prophylactically in patients with bronchopulmonary dysplasia (BPD), congenital heart disease and prematurity.[2,3] RSVIG may also have therapeutic effectiveness in those high-risk infants.[1] Early diagnosis is also required to institute infection control measures. It is thus desirable that early and accurate diagnosis of RSV infection be made if these therapeutic modalities are to be employed early to obtain maximum benefit. In this study, we compared the efficacy of direct fluorescent antibody (DFA) technique as a rapid and reliable diagnostic tool to isolation of the virus in cell culture. We also describe the magnitude of bronchiolitis as experienced in a pediatric hospital in Saudi Arabia, as well as the significance of RSV as its main etiologic agent compared to the Western experiences.

Patients and Methods

During the year 1413H, 485 patients were admitted to Suleimania Children's Hospital in Riyadh City with the clinical diagnosis of bronchiolitis. During the study period of the winter season of the same year, from 19.4.1413H to 8.8.1413H, which is considered the peak season for RSV infection in this part of the world,[4] all patients with bronchiolitis were screened for eligibility to be enrolled in the study, as per the following criteria: 1) age below two years; 2) presence of wheezing—audible and/or on auscultation; 3) no previous history of wheezing; 4) no chronic pulmonary disease such as cystic fibrosis or BPD; 5) no congenital heart disease; 6) no significant radiological consolidation. The diagnosis was made on the history of cough and/or wheezes, tachypnea, retractions, and wheezing and crackles on auscultation. As the diagnosis of bronchiolitis is essentially clinical, based on history and clinical findings, we chose the above diagnostic criteria. Hyperinflation on the chest film is also an important pointer to the diagnosis, but it was not done in all patients. The eligibility criteria were devised to exclude, as much as possible, other causes of wheezing in infancy to avoid the error of overdiagnosing bronchiolitis. Patients with radiologic consolidation were excluded because young children with pneumonia may also wheeze, thus questioning the diagnosis of bronchiolitis in those patients. The hospital scientific committee approved the project and informed consent was taken from the parents.

Specimen collection and laboratory method: Nasopharyngeal aspirates (NPA) were collected by mechanical suction using a #8 infant tube with 2 mL of virus transport medium (VTM) for virological examinations. The material was divided into two parts: one for detection of antigen by direct fluorescent antibody (DFA) technique and one for virus culture. The samples for viral culture were immediately transferred to the virology laboratory of King Khalid University Hospital. The time required between sample collection and processing for culture was less than two hours.

DFA: The aspirate part of DFA was diluted in 10 mL of phosphate-buffered saline (PBS) and centrifuged for 10 minutes at 1000 Gs. The pellet was resuspended and washed three times in PBS, adjusted to an optimum concentration (5-8 cells/high-power field), deposited on

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Teflon-coated slides and fixed in cold acetone. Staining and identification respiratory viruses such as respiratory syncytial virus, parainfluenza viruses 1, 2 and 3, influenza A and B and adenoviruses, were carried out as per the standard procedure of direct immunofluorescent technique employing fluorescent-conjugated antibodies for the above-mentioned viruses from DAKO Diagnostics LTD., Denmark. The specimen was considered positive for a particular virus if at least four fluorescing cells per field were seen.

Virus culture: The following two cell lines obtained from laboratories (UK) were used for isolation of viruses: HEp-2 (a continuous epithelial cell line derived from a human carcinoma of the larynx) and MDCK (a continuous epithelial cell line derived from a dog kidney). MDCK was used because of its susceptibility to influenza viruses, especially in the presence of trypsin.[5] Virus isolation was according to recommended tissue culture technique.[6] Cultures were observed for up to two weeks for cytopathic effect. Early detection of influenza A and B was also attempted blindly on MDCK cells using the shell vial technique.[7]

Results

The total number of patients admitted with a clinical diagnosis of bronchiolitis in the year 1413H was 485, which is 5.2% of all hospital admissions that year. During a three-and-one-half-month winter study period of the same year, 89 patients were considered eligible to be included in the study, as per predetermined eligibility criteria. Naso-pharyngeal aspirates were sent for all 89 patients for DFA and culture, but 15 samples were inadequate because the aspirated material did not contain enough cells. Out of 74 specimens, 52 were positive for RSV either by DFA and/or culture techniques, giving RSV a positive rate of 70%. Both the tests were done in 48 patients (Table 1). No other viral agents were isolated, although we looked for parainfluenza types 1, 2 and 3, influenza type A and B and adenovirus. The sensitivity and specificity of DFA were 92% and 82% respectively, compared to cell culture, whereas the positive predictive value (PPV) and negative predictive value (NPV) were 94% and 74% respectively.

TABLE 1. *Virological studies (RSV culture and DFA results in 48 patients where both tests were done).*

	Culture positive	Culture negative	Total
DFA positive	34	2	36
DFA negative	3	9	12
Total	37	11	48

DFA=direct fluorescent antibody.

Discussion

Bronchiolitis is a major cause of morbidity and occasionally mortality in young infants and children. Chanock et al. estimated that 1% of all children will require hospitalization for bronchiolitis in the first year of life and 40% of those cases will be due to RSV infection.[8] A similar trend was also suggested by Handerson et al. in North American urban pediatric practice.[9] In more recent Canadian studies, RSV was found to be responsible in 27% to 61% of cases of bronchiolitis.[10-13] It has been observed that the severity of illness is markedly increased in a certain group of patients having BPD or congenital heart disease, especially with pulmonary hypertension and prematurity with or without BPD. It has been suggested that these groups of high-risk patients should be treated with ribavirin, making an early and accurate diagnosis of RSV infection extremely desirable. Also, early diagnosis is important if effective infection control measures need to be implemented. Recently, Groothuis et al. has shown that RSVIG in doses of 750 mg/kg intravenously monthly during the winter RSV season prevents or significantly ameliorates the severity of the disease in patients with BPD, congenital heart disease and prematurity.[3] It is also possible that RSVIG may have a therapeutic role in these high-risk groups.[1] In the absence of any safe and effective active immunization strategy for RSV, prompt and accurate diagnosis and early initiation of treatment is assuming a more practical and important role in the proper management of RSV infection, especially in the above-mentioned high-risk group of the pediatric population, which is steadily increasing due to advances in perinatal care. In our study, we looked for the etiologic importance of RSV in bronchiolitis in an urban pediatric hospital in the Kingdom of Saudi Arabia. We also assessed the reliability of DFA for early and accurate diagnosis of RSV infections, as suggested in various other studies. DFA is a simple and rapid diagnostic test where results can be obtained in hours whereas culture methods require between three and five days, depending on the techniques. We found that bronchiolitis is a major illness in Saudi infants, responsible for 5.2% of all clinical diagnoses made during hospital admissions in one particular year. This percentage was similar to that seen in Hong Kong, which was 6.6%.[14] In 70% of our patients admitted with bronchiolitis, RSV was found to be the etiologic agent. Out of all those with the admission diagnosis of bronchiolitis, we selected a group of patients according to predetermined criteria for virological studies during the peak bronchiolitis period. Our findings are compatible with the findings of other studies which show sensitivity and specificity of DFA in the diagnosis of RSV in the range of 82% to 98% and 84% to 94% respectively.[14-18] The specificity and sensitivity of DFA compared to culture in our study were 92% and 82%

respectively. So DFA techniques have excellent sensitivity and specificity compared to culture methods. Primary cell lines were not used in this study and so the low sensitivity of the cell culture system used may account for the lack of detection of other respiratory viruses.

We conclude that bronchiolitis is a common illness in Saudi infants, being a significant cause of hospitalization, and the major etiologic agent is respiratory syncytial virus. Direct fluorescent antibody test as a rapid diagnostic method has excellent sensitivity and specificity compared to culture and is recommended as the diagnostic method of choice in suspected RSV infection.

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