World Journal of Medical Sciences 6 (1): 27-32, 2011
ISSN 1817-3055

Bacterial and Respiratory Viral Infection among Hospitalized Children

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Abstract: Respiratory tract infection is a frequent cause of pediatric morbidity and mortality and a common reason for admission in acute care hospitals and outpatients visits. The present study included 87 children with lower respiratory tract infection who were examined, (43.7%) of cases were proved to be due to bacterial causes, while (56.3%) were attributed to viral infection. *Streptococcus pneumonia*, *Streptococcus pyogenes*, *Staphylococcus aureua*, *E. coli*, *Pseudomonas aeurginosa*, *Haemophililus influenza* and *Proteu mirabilis* were the most isolated organisms. The age of children was ranged from 2 months to 12 years old. Using Direct Immunoflourescent assay (IF) and Enzyme Linked Immunosorbent assay (ELISA) for detection of viral antigen in the examined samples, RSV (51% and 46.9%) respectively, adenovirus (24.5%), Influenza virus (18.4%) and parainfluenza (10.2%). RSV was statistically significantly among children 1- < 5 years old. Different manifestations were investigated in this study, Croup was the most prevalent manifestation among (34.7%), bronchiolitis (32.7%), broncho-pneumonia (20.4%) and lastly pneumonia (12.2%).On detection of viral infection association to seasonal variation winter season was the most significant season for infection. There were no significant relation between type of infection and physical or X-ray finding.. The validity of IF in comparison to ELISA sensitivity (84%) specifity (91.7%) and accuracy (87.75 %). IF and ELISA easy, rapid and reliable methods of LRI diagnosis.

Key words: Respiratory Bacterial Infection · Respiratory Viral infection Hospitalized Children · ELISA · IF

INTRODUCTION

Lower respiratory infections (LRIs) continue to threaten the health of children worldwide and are exacerbated by global environmental problems such as air pollution. In the developing world where nutrition remains poor and access to healthcare is scarce, LRIs are the most common cause of illness and death in children. Outcomes of LRI illness are far better in developed countries, but the overall morbidity of LRI is still high and may exceed that of other age groups [1, 2].

The respiratory tract in children and adult is a target for many micro-organisms. Although bacterial causes may be important pathogens (Gram positive cocci, *Streptococcal pneumonia*, *Staphylococcus aureus* and Gram negative bacilli as *E. coli* and *Pseudomonous oreganos*) viral causes may be also recorded [3, 4].

Respiratory syncytial virus (RSV) is a major cause of acute respiratory illness in infants and young children being responsible for 50% of all bronchiolits cases and 25% of all pneumonia cases during first months of life. It is also the most common cause of nosocomial infection in pediatric wards and morbidity death from RSV occurring in children less than 2 years of age [5].

Yang *et al.* [6] reported that viral etiology of 43.7% of 882 examined random samples, at least one of 9 different respiratory viruses was detected. Among these viral isolates, seasonal influenza A virus (67.3%), influenza B virus (27.8%) and human parainfluenza virus (PHIV) 1, 2, or 3 (1.3%). In addition, 2 cases (0.5%) of each adenovirus, HSV-1, enterovirus and RSV were also found in the samples. Co-infections with more than one virus were revealed in (2.1%) of the tested samples [6].

Other respiratory viral infection may account for considerable morbidity and many admissions to hospitals among infants and children. Worldwide, it is estimated that more than 4 million children die each year of acute lower respiratory infection. RSV and PIV type 1, 2, 3 accounted for most virus infection [7, 8].

This study aimed to the incidence and associated with acute lower respiratory infection in young children to LRT infection.

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MATERIALS AND METHODS

Subjects: Eighty-seven children with lower respiratory tract infection attending Pediatric Department, King Abdulaziz University were included in this study. Their age ranged from few months to12 years old. All of them had primary acute lower respiratory infection in the form of bronchopneumonia, acute bronchiolitis, croup, wheezing bronchitis and pneumonia. Informed consent was obtained from all participant parents. All participant parents were free to withdraw their children from the study at any time. If any adverse effects had occurred, the experiment would have been stopped, with this being announced to the Human Subjects Review Board. However, no adverse effects occurred and so the data of all the participants were available for analysis. This study was approved by the Scientific Research Ethical Committee, Faculty of Applied Sciences, King Abdulaziz University.

All the patients were subjected to complete history: age, sex, previous episodes of LRT infection, vaccination and complaint .Also, clinical examination, both general chest in addition to chest x-ray of both posterior-anterior and lateral views were done for all subjects. Complete blood picture, sputum samples and coagulated blood were evaluated for all participants.

Sample: Sputum samples and nasopharyngeal swabs were collected in sterilized clean container. The sputum specimens were Gram stained, streaked on nutrient, blood, chocolate, MacConkey and agar plates (Bio Merieux-France and incubated at 37°C. [10].

Blood Culture: Blood culture was done for each patient according to Granguli *et al.* blood was withdraw and immediately inoculated into Castaneda blood culture bottle (Croma test, laboratories Knicboker,) every 24 into blood chocolat, MacConkey and if no growth was obtained blood culture negative.

Processing for Viruses Identification: The samples were diluted with virus transport medium (0.5% gelatin hanks balance salt solution with penicillin, streptomycin) upon arrival to centrifugation was performed at 3000 R.P.M. for 30 minutes at 4°C. The deposit resuspended is PBS and mixed well, centrifuged at 2000 R.P.M. for 10 minutes, was three times [12].

Immunofluorescent Examination: For detection of influenza A, B and respiratory syncytial viruses kit from Bio Merieux Vitk Inc. The deposit was spotted on coated slides, air dried and fixed with cold acetone for 10 minutes and then was stained by using anti- RSV FITC conjugate monoclonal antibody counter stain for and by using monoclonal antibody labeled with fluorescent isolate (FITC) specific for influenza A and B . The slides were incubated for 15 minutes at 37°C in a moist chamber and dried. Detection of fluorescent cell was performed using 40X objective fluorescent microscope [13].

Abbott Test Pack RSV enzyme immunoassay (EIA) and Directigen FLU-A for rapid diagnosis of RSV and parainfluenza using (Virotechsystem diagnostic GmbH) .For detection of adenovirus Adenocolone EIA diagnostic kit (Cambridge bioscience) [14].

The methods were used according to instruction manufacturers. In adenovirus performed by using monoclonal antibodies against the group reactive hexone antigen shared by all 41 human adenovirus types in a solid phase sandwiched between solid phase and antibody conjugated to horseradish peroxidase enzyme after addition of enzyme substrate (urea peroxidase and chromogen). The enzyme conjugate in the wells converted the colorless substrate to blue color which measure at OD 450 nm.

Statistical Analysis: The chi-square and t-test were used for statistical analysis contrast. Sensitivity, specificity, positive and negative predicitive values were estimated. Sensitivity = no. of false negative. Specificity =no. of false positive.

RESULTS

Results of this study revealed that *Streptococcus pneumonia*, *Streptococcus pyogenes*, *Staphylococcus aureua*, *E.coli*, *Pseudomonas aeurginosa*, *Haemophililus influenza* and *Proteu mirabilis* were the most prevalent organisms among investigated children (Table 1). age and sex distribution among different viral infection, RSV was statistically significant among 1-<5 years old (Table 2). Also, clinical findings showed that bronchiolitis and croup were the most prevalent manifestations detected among examined children (Table 3).

Table 1: Different bacteria

Isolated organisms	No. (38)	%
Streptococcus pneumonia	9	23.7
Streptococcus pyogenes	7	18.4
Staphylococcus aureua	6	15,8
E. coli	6	15.8
Pseudomonas aeurginosa	4	10.5
Haemophililus influenza	3	7.9
Proteu mirabilis	3	7.9

Table 2: Age and Sex positive viral infection cases

						Sex Males		Females	
Viral infection	No	%	Age < 1 year	1- <5 years	>5 years	No.	%	No.	%
RSV	23	46.9	0	21*	2	9	41	14	51.9
Adenovirus	12	24.5	2	8	2	5	22.7	7	25.9
Influenza	9	18.4	1	3	5	5	22.7	4	14.8
Parainfluenza	5	10.2	0	4	1	3	13.6	2	7.4
Total	49	100	3	36	10	22	44.9	27	55.1
X2		11.59			1.23				
P value		0.008*	Sig.						

Table 3: Association of viral infection with finding.

	Bronchiolitis (16)		Pneumonia (6)		Bronch pneumonia (10)		Croup (17)			
Different clinical finding	No.	%	No.	%	No.	%	No.	%	P value	X2
Nasal discharge	7	43.8	0	0	6	60	7	41.2	0.06	NS
Fever	10	62.5	4	66.7	5	50	11	64.7	0.075	NS
Cough	13	81.5	4	66.7	5	50	9	52.9	0.3	NS
Expectoration	6	37.5	2	33.3	5	50	8	47	0.88	NS
Dyspnea	9	56.3	2	33.3	1	10	5	29.4	0.15	NS
Vomiting	3	18.8	0	0	0	0	4	23.5	0.54	NS
Diarrhea	2	12.5	0	0	1	10	3	17.6	0.42	NS
Irritability	5	31.3	0	0	0	0	7	41.2	0.12	NS

NS= Non significant S= Significant

Table 4: Relation of virus infection x- ray and finding.

	RSV(23)		Adeno. (12)		Influ. (9)		Parainflu.(5)			
	No.	%	No.	%	No.	%	No.	%	X2	P
Temperature										
> 39°C	5	21.7	4	33.3	2	22.2	1	20	2.27	N S
38-39°C	6	26.1	5	41.7	5	55.6	2	40	2.64	NS
38-< 37°C	12	52.2	3	25	2	22.2	3	60	4.52	NS
Chest X-ray										
Normal	10	43.5	5	41.7	6	66.7	2	40	1.75	NS
Interistial	8	34.8	4	33.3	2	22.2	2	40	0.62	NS
Change										
Hyperinflation	5	21.7	3	25	1	11.1	1		0.66	NS

NS= Non significant S= Significant

Table 5: Seasonal variation associated with virus infection

	RSV (23)		Adeno (12)		Influenza (9)		Parainfluenza (5)		Total (49)			
Season	No.	%	No.	%	No.	%	No.	%	No.	%	X2	P
Winter	10	43	6	50	4	44.4	2	40	22	44.9	17.29	0.001*
Autumn	6	26	3	25	2	22.2	1	20	12	24.5	0.11	0.99
Spring	4	17	2	16.7	-	-	1	20	7	14.3	1.87	0.59
Summer	3	13	1	8.3	3	33.4	1	20	8	16.3	2.7	0.44

NS= Non significant S= Significant

Table 6: Association of different clinical manifestation with ICU

Clinical Manifestation	Total number (49) No.	%	ICU (6) No.	%	X±SD hospital Admission in days	
Croup	17	34.7	1	16.7	4.5	+2.2
Acute bronchiolitis	16	32.7	1	16.7	3.6	2*
Wheezing bronchitis	12	24.5	1	16.7	6.5	3.6
Viral pneumonia	6	12.2	1	16.7	8.5	2.5
Broncho -pneumonia	10	20.4	2	33.2	7.2	2.3
F = 5.58	P<0.001**					

Table 7: Validity test of ELISA in comparison to IF for diagnosis of RSV infection.

		ELISA (23)		
		Positive	Negative	Total
IF (25)	Positive	21	2	23
	Negative	4	22	26
	Total	25	24	49

Sensitivity (84%) Specifity (91.7%)
Accuracy was (87.75) Positive predictive (91.3 %)
Negative predictive (84.6%)

Results of the present study showed no significant association between virus infection to x- ray and physical finding (Table 4). Where there was a highly significant association between virus infection and winter season (Table 5). Also, there was a high significant association between different manifestation and ICU admission (Table 6). While, the results of validity test of ELISA in comparison to IF for diagnosis of RSV were positive predictive (91.3 %) versus negative predictive (84.6%) (Table 7).

49 samples were tested by both ELISA and IF techniques for RSV detection. 23 samples were positive by ELISA and 26 gave negative results, while by IF, 25 samples were positive and 24 gave negative results. There was agreement for positivity in 21 samples. 2 samples positive by IF were false negative by ELISA (sensitivity ; 84 %), on the other hand 4 samples were positive by IF were false negative by ELISA test(specificity

DISCUSSION

Viral lower respiratory tract infections in infants and children are an important medical and socioeconomic problem worldwide. Viral lower respiratory tract infections are mild and self limiting in most cases. Particular patient groups are at risk of a severe course of disease, previously healthy infants with a viral lower respiratory tract infection may also develop severe disease [15].

Treatment moderate viral lower respiratory tract infection is mainly supportive. Lack of means to control viral lower respiratory tract infection has led to great variation in management worldwide Development of practical guidelines and educational programmes in both clinical and outpatient settings may be helpful and cost saving in the control of viral lower respiratory tract infection in infants and children [1].

Our results declared that (43.7%) of cases were proved to be due to bacterial causes *Streptococcus pneumonia, Streptococcus pyogenes, Staphylococcus aureua, E. coli, Pseudomonas aeurginosa, Haemophililus influenza* and *Proteua mirabilis* were the most isolated organisms. Our results in agreement with Macfarlane *et al.* who found that Over 50% of patients have direct and/or indirect evidence of infection, most commonly bacterial *Streptococcus pneumoniae, Haemophilus influenzae, Moraxella catarrhalisatypical* organisms, *Chlamydia pneumoniae, Mycoplasma pneumoniae*. Also, Hare *et al.* [16], found that Streptococcus pneumoniae, nontypable Haemophilus influenzae and Moraxella catarrhalis, were cause of lower airway infection. Our results correlated with Malekshahi *et al.* [5] and Kim *et al.* [17], who those viral infections were more prevalent among young children.

RSV correlation to age group, our study revealed that 1-< 5 years old was statistically significant. Seasonal was significantly proved to be associated with winter season.

Our results in agreement with Pingsheng *et al.* [18], reported that among children studied during five winters, from birth through early childhood, to the winter virus peak.

Also, Satpathy *et al.* [19] and Van Woensel *et al.* [20]. Declared that virtually all children have been exposed to the virus by the age of 5 years. Different manifestations were investigated in this study, Croup was the most prevalent manifestation (34.7%), bronchiolitis (32.7%), bronchio-pneumonia (20.4%) and lastly pneumonia (12.2%) In our study the frequency of detected respiratory viruses infection was (56.3%) of cases RSV (46.9%) of cases manifested with bronchiolitis, while most of PI manifested with croup. Further more adenovirus was associated with wheezy bronchitis, Croup and pneumonia. Our results in accordance with different other studies

Hamzé *et al.* [21], they revealed that respiratory infection attributed to RSV (26.7%) was significantly associated with the presence of rhinorrhoea and dyspnoea and the absence of pneumonia signs on chest X-ray and bronchitis or bronchiolitis

Van Woensel *et al.* [20] reported that is by far the most common cause of viral lower respiratory tract infection in infants and young children. Virtually all children have developed antibodies to by the age of 3 years. In addition, an estimated 75% of all admissions for bronchiolitis in children under 5 years of age are related to [22].

Our results detected that normal physical finding recorded among investigated children, temperature <37°C was recorded among most investigated cases also normal Xray finding was detected among most investigated case, while few cases were recorded with interstial changes and hyperinflation. Six cases with LRT infection were admitted to intensive care unite (ICU). The higher percentage of them were attributed to broncho- pneumonia (33.3%) Our results in agreement with Elaine *et al.* found that all viral cases with LRI admitted to ICU because of pneumonia and broncho-pneumonia [29].

Direct Immunoflourescent assay (IF) and ELISA detect RSV (51% and 46.9%) respectively, adenovirus (24.5%), Influenza virus (18.4%) and parainfluenza (10.2 %). Our finding nearly the same as that detected by Deng *et al.* found that viral pathogens identified by immunofluorescence technique (43.7%). Among these viral isolates, (67.3%) were seasonal influenza A virus, (27.8%) were influenza B virus and (1.3%) were human parainfluenza virus 1, 2, or 3.

Our result not in accordance with Reis *et al.* [23], a total of 316 samples of nasopharyngeal aspirate from infants up to two years of age with acute respiratory-tract illnesses for detection of RSV using three different techniques: viral isolation, direct immunofluorescence and PCR (11.4%) were positive for RSV, considering the three techniques. Our results also confirmed with others as Freymuth *et al.* [24], Immunofluorescence assay (IFA). Our results were greater than that recorded by Ahn *et al.* [25] as overall isolation rate was 22.1%. The viral pathogens identified were adenovirus (12.7%), influenza virus type A (21.1%), -type B (13.9%), parainfluenza virus type 1 (13.5%), -type 2 (1.3%), -type 3 (16.0%) and respiratory syncytial virus (21.5%). The occurrence of ALRIs was highest in the first year of life, although parainfluenza virus type 1 infection occurred predominantly in the second year of life and influenza virus caused illnesses in all age groups.

In the study, the validity test of IF in comparison to ELISA the sensitivity (84%), the specifity (91.7%) positive predictive (91.3 %), negative predictive (84.6%) and the accuracy (87.75 %).

In conclusion virus infection was more prevalent among children investigated with LRI RSV, PI, Adenovirus were the most detected viruses. Most of cases of viral infection were less than 5 years old and manifested by bronchiolitis while PI, influenza and adenovirus associated with croup, wheezy bronchitis and ELISA methods are reliable methods for detecting LRI.

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