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ORIGINAL ARTICLE

Comparison of Reverse Transcription-PCR and Viral Culture for detection of Respiratory Syncytial Virus in Young Children: Relation to Epidemiological and Clinical Findings

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ABSTRACT

Background: *RSV is the most frequently identified agent responsible for LRTIs especially bronchiolitis worldwide. Multiple methods are used for the laboratory diagnosis of viral infections, including viral culture, antigen detection, nucleic acid detection, and serology. Morbidity and mortality of RSV infection are higher in infants suffering from concomitant disease as prematurity, congenital heart disease and others.* ***Objectives:*** *To compare the value of Reverse-Transcription Polymerase Chain Reaction (RT-PCR) and Conventional Viral Culture in diagnosis of RSV, to determine the incidence of RSV in pediatric respiratory illness and patients' findings and to determine the mortality rate of RSV infection with concomitant disease.* ***Methodology:*** *This study was conducted on Ninety seven children (30 females and 67 males aged from 0 month up to 5 Years and were presenting with clinical pictures suggesting of LRTI (acute bronchiolitis or pneumonia). All patients were subjected to history taking, complete medical examination and thorough laboratory and radiological investigations. Tissue culture and RT-PCR were done on nasopharyngeal aspirates collected from all of these patients and the results of the two methods were compared with patient's demographic data, clinical and radiological findings to determine the best method for detection of RSV.* ***Results:*** *RSV was detected in 68.04% and 30.9% of LRTI cases by using RT-PCR and tissue culture respectively and there was a highly significant statistical difference (P value= 0.0001).The highest isolation rate of RSV (56.7% and 40.9%) was in children with age from 0-6m by tissue culture and RT-PCR respectively. The incidence of RSV was decreasing by increasing the age of the patients. By both techniques for RSV isolation, RSV was higher in males than females but there was no statistically significant difference. Sixty–six positive RSV cases detected by RT-PCR were distributed as the following in both seasons; 43.94% in January, 31.82%, in December, 22.73% in February and only 1.51%, in November. RSV was isolated from 92.7% of 61 cases of bronchiolitis by RT-PCR and the presence of hyperinflated chest is significantly higher among positive RSV cases (P value=0.001). Mortality rate in positive RSV patients with comorbidities was 25%.* ***Conclusion:*** *RT-PCR was more sensitive than tissue culture in diagnosis of RSV, especially in older age group cases. Mortality rate was higher among positive RSV who had concomitant diseases.*

INTRODUCTION

RSV is a globally ubiquitous respiratory pathogen of the *Paramyxoviridae* family. It is a major cause of hospitalizations, morbidity and mortality among children due to lower acute respiratory tract infections that manifested as bronchiolitis or pneumonia [1]. Acute bronchiolitis is characterized by tachypnea, wheezing, suprasternal or intercostal retraction, cyanosis or apnea, and even respiratory failure. Risk factors for severe bronchiolitis include prematurity, cardiovascular disease, chronic pulmonary disease and immunodeficiency [2].

RSV is a highly communicable but humans are the only known reservoir. Its incubation period varies from two to eight days. The virus spreads from respiratory secretions through close contact with infected persons *via* respiratory droplets or contact with contaminated surfaces or objects. Infection by RSV may confer partial immunity so individuals may be infected repeatedly with the same or different strains of RSV [3].

Infection with RSV is a clearly identified as a winter virus, usually occurring within the period from October to March with most infections occurring in a relatively short epidemic of about six weeks [4]. In 'high-risk' children (especially having cardiac abnormalities and multiple co-morbidities), the mortality rate of RSV

infected children is about 3%[2]. RSV-associated mortality is highest in developing countries, but RSV can have a significant burden on the cost of care and the economy of all countries [5].

Multiple methods are used for the laboratory diagnosis of RSV infections, including viral culture, antigen detection, nucleic acid detection, and serology [6]. For faster diagnosis, the isolation of RSV in cell culture has been replaced by antigen detection-based assays and new sensitive molecular techniques such as RT-PCR [7]. However, cell culture method continues to be valuable because it allows the amplification of small amounts of virus that are present in a specimen, providing isolates for subtyping and further analysis. Moreover, it is less likely to diagnose false epidemics and permits the recovery of several additional agents that may be present in a specimen [8].

In this study, we aimed to assess the value of RT-PCR and conventional viral culture in diagnosis of RSV, to study the epidemiology of RSV in pediatric respiratory illness and patients' findings and to determine the mortality rate of RSV infection with concomitant disease in our locality.

METHODOLOGY

Study population and selection of patients

This study was conducted at the Microbiology and Immunology Department, Faculty of Medicine, Menoufia University in collaboration with the Pediatric Department, Faculty of Medicine, Menoufia University during the period from October 2013 to April 2014 (season 1) and from October 2014 to April 2015 (season 2).

Ninety seven children (30 females and 67 males aged from 0 month up to 5 Years were presenting with clinical pictures suggesting of LRTI (acute bronchiolitis or pneumonia).

Medical records were reviewed for detailed demographic, clinical, and laboratory data, radiographic images and underlying conditions of the patients. All the clinical symptoms and signs were recorded on a standardized form on hospitalization of the patients.

The study protocol was approved by local ethics committee of the Menoufia University. Informed consent was obtained from parents of patients before the beginning of the study.

Specimen collection

Nasopharyngeal aspirate specimens were collected by inserting a tube attached to a mucus extractor into one nostril and applying gentle suction by suction apparatus. These aspirated contents were suspended in virus transport medium "Dulbecco′ s minimal essential medium (DMEM)". The specimens were divided into two aliquots; one of them was transported to Microbiology laboratory to be inoculated on tissue culture for RSV detection without delay and the other one was kept at -80°C for RT-PCR testing.

RSV inoculation and isolation in cell culture: (Nunes and Moura, 2006) [9]

The specimens received in DMEM were vortexed for 30 seconds, 8 drops of antibiotic solution were added to samples with a Pasteur pipette before the growth medium was aspirated and 0.2ml sample inoculum was inoculated into duplicate tubes of HEp-2 cells with confluent monolayer. After addition of maintenance medium, the tubes were incubated at 37°C. Four uninoculated cell culture tubes were included as cell culture controls.

Microscopic examinations for cytopathic effect (CPE) were performed daily for 10- 14 days using inverted microscope. The culture was considered positive when giant cells and syncytia were observed (fig.1). When CPE reached third (+3) or fourth degree (+4) the cell culture tubes were removed from the incubator and the cell associated virus was released by repeated freezing and thawing for three times. Then, the suspension was diluted 1/1000 and amplified by another two successive passages. Samples were followed up for 2 weeks before they were considered as negative.

Reversed- transcription polymerase chain reaction (RT-PCR):

(A) RNA Extraction:

This was carried out by the use of the **Thermo Scientific Gene JET Viral DNA and RNA Purification Kit # K0821** supplied by Thermo Fisher Scientific Inc, California, USA for the extraction and purification of genomic RNA from viral strains. This was achieved through the following steps;

1. The sample was lysed by incubation with lysis solution and proteinase K (they inactivate both RNases and DNases, ensuring protection of viral nucleic acids against degradation) under denaturating conditions at elevated temperatures (56°C).
2. The lysed sample was transferred to a spin column where released viral nucleic acids immediately bind to the silica-based filter in the presence of chaotropic salts. The remaining lysate was removed by centrifugation.
3. The remaining contaminants were removed by using Wash Buffer 1 and 2, whereas pure nucleic acids remain bound to the membrane.
4. Pure viral nucleic acids were released from the spin column filter using Eluent. The purified nucleic acids were ready for subsequent use in downstream applications.

On starting a new procedure, we always used a freshly prepared mixture of carrier RNA and lysis solution. To calculate the correct quantity of carrier RNA and lysis solution required to process multiple specimens, the table supplied in the extraction kit was used.

(B) cDNA synthesis, amplification and detection: (Nikfar et al., 2013) [10]

PCR was done using RT-PCR kit according to the following protocol:

1st step PCR was done by using (Perkin-Elmer Gene Amp PCR System 9600 thermal cycler): 5ul of extracted RNA was combined with 1 ul of G1 and G2 primers, 1.25 ul of RT buffer, 0.5 ul of verso enzyme mix and volume was completed to 25ul by DEPC water.

1st step RT-PCR thermal cycling program:

	Temp	*Time*	*Number of cycles*
cDNA synthesis	50°C	15 min	1 cycle
Verso inactivation	95°C	2 min	1 cycle
Denaturation	95°C	20 sec	35-45 cycles
Annealing	56°C	30 sec	
Extension	72°C	1 min	
Final extension	72°C	5 min	1 cycle

2nd cycle RT- PCR: 5 ul of 1st cycle product was combined with 12.5 ul of Master mix, 1 ul of G3 andG4 primers and volume was completed to25 ul. The PCR was performed for 35 cycles ,initially for 5 min at 95°C followed by, 1 min at 95°C, followed by 1 min at 72°C, 1 min at 95°C and finally 5 min at 72 °C for one cycle. The expected final PCR product was 326bp (fig. 2).

Primers (Qiagen Germany):

For 1st cycle

G1: CCA TTC TGG CAA TGA TAA TCT C

G2: GTT TTT TGT TTG GTA TTC TTT TGC GA

For 2nd cycle

G3: CGG CAA ACC ACA AAG TCA CAC

G4: GGG TAC AAA GTT AAA CAC TTC

Statistical analysis

Computer SPSS program version 17 was used. The results were expressed by applying Chi-square test. $p < 0.05$ was considered to be significant.

RESULTS

This study was carried out on 97 children (patient group); 67 males and 30 females, their ages ranged from 0-5 years (mean±SD;9.3±2.31 years old).They were admitted to Pediatrics Department, Faculty of Medicine, Menoufia University during the period from October 2013 to April 2014 and the period from October 2014 to April 2015. They were diagnosed at the time of admission as lower respiratory tract infections. Nasopharyngeal aspirate specimens were collected and transported to Microbiology laboratory to be immediately inoculated on tissue culture for RSV detection and for RT-PCR testing (Fig. 1&2).

RSV was detected in 30.9% and 68.04% of cases by using tissue culture and RT-PCR respectively. There was a highly significant ($p<0.0001$) difference between the two methods for detection of RSV (Table 1)**.** The specificity and sensitivity of tissue culture method were calculated in relation to PCR as a gold standard method used; Tissue culture showed sensitivity of 41%, specificity 90.0%, positive predictive value 90.0%, negative predictive value 42.0% (Table 2)**.**

The highest isolation rate of RSV (56.7% and 40.9%) was in children with age from 0-6 months by tissue culture and RT-PCR respectively. The incidence of RSV by tissue culture and RT-PCR was decreasing by increasing the age of the patients but without significant difference. By both techniques for RSV isolation, RSV was higher in males than females but there was no significant difference between positive and negative RSV patients by both tissue culture and RT-PCR as regarding the gender (Table 3)**.** Fifty specimens were collected in the 1st season (winter 2013-2014) and 47 in the 2nd season (winter 2014-2015, where sixty–six positive RSV cases were detected by RT-PCR were distributed as the following in both seasons; 29 (43.94%) in January, 21(31.82%), in December, 15 (22.73%) in February and only 1(1.51%), in November (Fig. 3)**.**

The severity of symptoms was decreasing with increasing age of the patients because higher number of patients suffering from clinical symptoms was at younger age < 1 year compared to number of patients at older age > 1 year (Table 4)**.**

In positive RSV cases by RT-PCR, cough was the most predominant symptom (72.7%), followed by wheezing (66.75), rhinorrhea (57.6%), fever (42.4%) and cyanosis (9.5%). On the other hand, in the positive RSV cases by tissue culture, rhinorrhea and wheezing were the predominant symptoms (85.7%), followed by cough in (66.7%) and fever (60 %). As regard respiratory distress; grade I was the most predominant in RSV positive cases by RT-PCR (74.2%) and tissue culture (80%) (Table 5)**.** The clinical diagnosis of the positive cases for RSV; the virus was isolated from 61 cases of bronchiolitis (92.4%) by RT-PCR and from 28 cases (93.3%) by tissue culture, while 7.6% and 6.7 % of positive RSV cases had pneumonia that was detected by RT-PCR and tissue culture respectively (Table 5)**.**

Table (6) showed that the presence of hyper inflated chest was significantly higher among positive RSV cases (P value=0.001) while there was non-significant difference between positive RSV and negative RSV cases regarding increased bronchovascular markings.

As shown in table (7), 16 out of 97 patients of LRTIs had comorbidities, all of them had RSV detected by RT-PCR. Mortality rate in these patients was 25% (4 patients). Percentage of death was higher among positive RSV who had comorbid congenital heart disease 60% (3 patients) followed by prematurity 25% (one patient).

Table 1: Distribution of RSV among 97 LRTI patients by using tissue culture and RT-PCR.

Method of detection	*Positive RSV*	*Percentage*	*Negative RSV*	*Percentage*	*P value*
Tissue culture	30	30.9	67	69.1	
RT-PCR	66	68.04	31	31.9	0.0001

Table 2: Validity of tissue culture in relation to PCR results.

	Sensitivity	*Specificity*	*PPV*	*NPV*	*Accuracy*
Tissue culture:	41%	90.0%	90.0%	42.0%	57.0%

Table 3: Age and gender-related RSV among 97 LRTI patients by using tissue culture and RT-PCR.

Demographic data	*RSV-positive by tissue culture*		*RSV-positive by RT-PCR*		*P value*
	No. (30)	**%**	**No. (66)**	**%**	
Age					
- **0-6 months**	17	56.7%	27	40.9%	
- **6-12 months**	10	33.3%	19	28.8%	
- **12-18months**	2	6.7%	10	15.15%	0.28
- **18-24 months**	1	3.3%	8	12.12%	
- **>24 months up to 5 years**	0	0%	2	3.03%	
Gender					
- **Males**	23	76.7%	51	77.3%	0.84
- **Females**	7	23.3%	15	22.7%	

Table 4: Clinical symptoms associated RSV-positive LRTI patients by RT-PCR according to age.

Symptom / *Age group Months*	*Cough*		*Rhinorrhea*		*Fever*		*Wheezing*		*Cyanosis*		*Cerpitations*		*Respiratory distress*	
	+	-	+	-	+	-	+	-	+	-	+	-	+	-
0-6 months	20	7	24	3	20	7	22	5	4	23	4	23	26	1
6-12 months	15	4	13	6	7	12	15	4	1	18	4	15	18	1
12-18months	5	5	1	10	2	8	5	5	1	9	4	6	9	1
18-24 months	3	5	0	8	1	7	3	5	0	8	1	7	6	2
>24 months up to 5 years	0	2	0	2	0	2	1	1	0	2	2	0	2	0

Table 5: Comparison between positive RSV cases by RT-PCR and tissue culture regarding clinical symptoms.

	Positive RSV by RT-PCR (66)		*Positive RSV by Tissue culture (30)*	
	No.	**%**	**No.**	**%**
• **Symptoms**				
- **Cough**	48	(72.7%)	20	(66.7%)
- **Rhinorrhea**	38	(57.6%)	26	(85.7%)
- **Fever**	28	(42.4%)	18	(60%)
- **Wheezing**	44	(66.7%)	26	(85.7%)
- **Cyanosis**	6	(9.5%)	0	(0%)
• **Observed signs**				
Respiratory distress				
- **Grade I (Tachypnea)**	49	74.2%	24	80%
- **Grade II (chest wall retraction)**	12	18.18%	6	20%
- **Coarse Crepitation**	15	22.72%	1	3.33 %
Clinical diagnosis				
- **Bronchiolitis**	61	92.4%	28	93.3%
- **Pneumonia**	5	7.6%	2	6.7%

Table 6: Comparison between radiological findings among cases of LRTI positive and negative RSV by RT-PCR.

Item	*RSV Positive (n=66)*		*RSV Negative (n=31)*		*Total*		*P-value*
	No.	**%**	**No.**	**%**	**No.**	**%**	
Hyperinflated chest:							
Present	45	68.3	7	21.1	52	60	0.001
Absent	21	31.7	24	78.9	45	40	
Increased bronchovascular markings:							
Present	63	95.1	31	100	94	96.7	0.83
Absent	3	4.9	0	0	3	3.3	

Table (7): Comparison between alive and dead cases regarding the presence of co -morbidities.

Co - morbidities:	*Total (97)*	*Alive (No.=93)*		*Dead (No.=4)*		*Chi square*	*P value*
		No.	**%**	**No.**	**%**		
Present:	16	12	12.9	4	100.0	21.12	0.0001
• **Prematurity**	4	3	75	1	25		
• **Congenital heart disease**	5	2	40	3	60		
• **Down "s syndrome**	2	2	100	0	0		
• **Chronic lung disease**	2	2	100	0	0		
• **Malignant disease**	3	3	100	0	0		
Absent:	81	81	87.1	0	0.0		

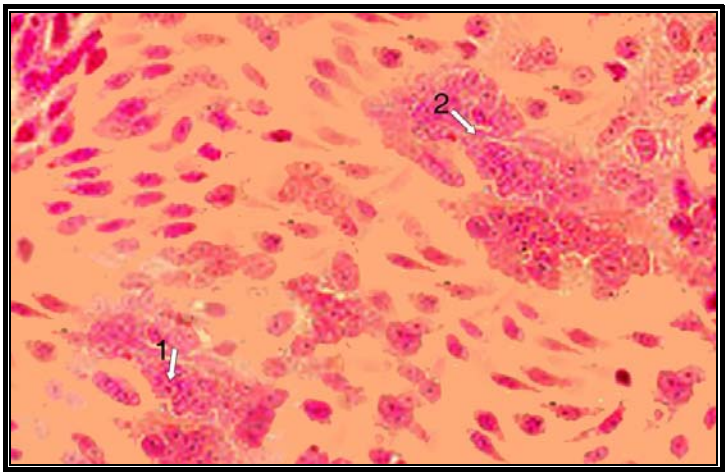

Fig. 1: HEp-2 cell culture showing characteristic RSV infection CPE
(giant cells and syncytia formation: Arrows 1&2) x400 magnification and stained by safranin T.

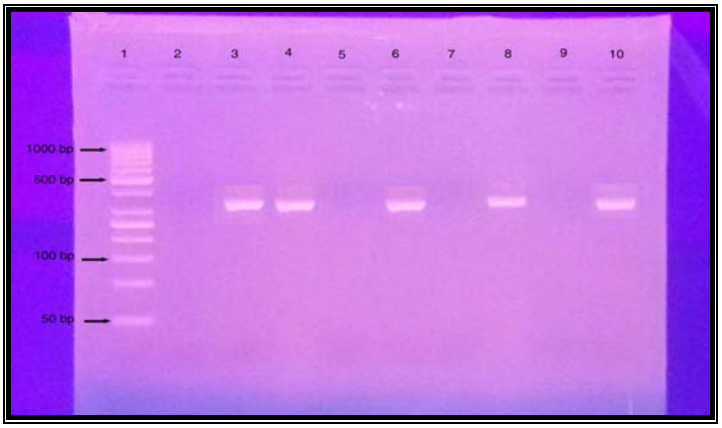

Fig.2: Agarose gel electrophoresis analysis of RT-PCR amplification products:
Lane 1: molecular weight marker
Lanes 3, 4, 6, 8 and 10 were positive samples (RSV appeared as a band at 326 b.p.)
Lanes 2, 5, 7 and 9 were negative samples.

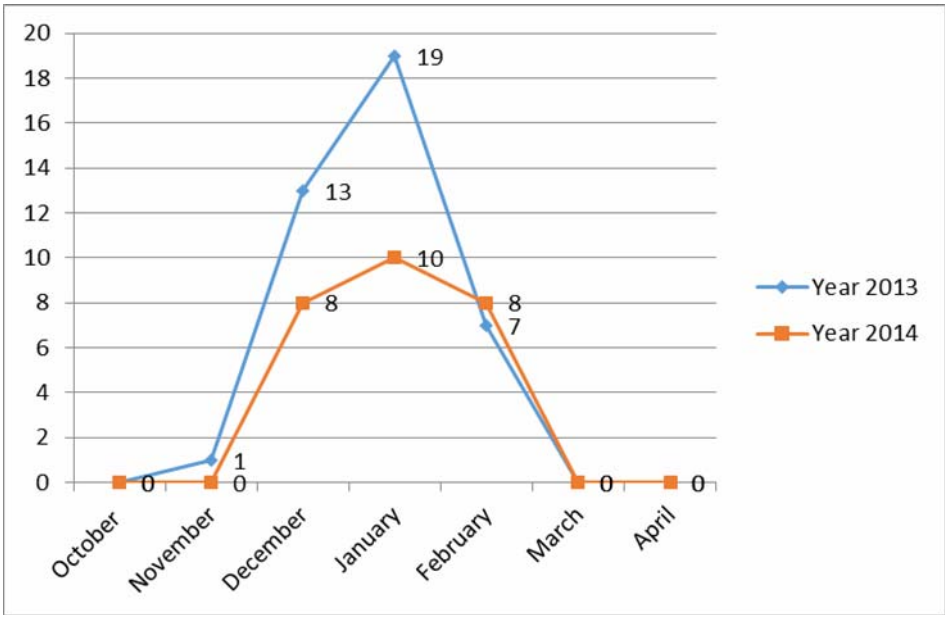

Fig. 3: The distribution of 66 isolated positive cases for RSV by RT-PCR during the 2 seasons of 2013-2014 and 2014-2015

DISCUSSION

RSV is recognized as the most frequent agent responsible for LRTIs in children, where bronchiolitis is the signal illness for RSV [11]. In infants and young children, RSV infections progress from upper to lower respiratory tract infections in approximately 40% of infected children [12]. RSV infections are associated with significant diseases burden in children in term of hospitalization, related complications, and even mortality [13].

In this study, there was a significant ($p<0.0001$) difference between two previous methods for detection of RSV. Thirty out of 97 (30.9%) from nasopharyngeal samples showed RSV-like CPE (syncytia formation) in HEP-2 cells, while 68.04% of them (66 specimens) were positive for RSV by RT-PCR. RSV viability may be lost during processing of specimens or infectivity may be compromised by the presence of secretory antibodies in respiratory secretions capable of blocking virus adsorption [14].

Similar results were observed in other studies that were done by Falsey et al [15, 16] in (2002) and in (2003) as they found that 37% and 34% of nasopharyngeal samples were positive for RSV by tissue culture respectively. A higher result of RSV detection by tissue culture was reported by Gueudin et al.,[17] (45.3%) and Popow-Kraupp and Aberle [18] (46%). On the other hand, lower results of positive RSV by tissue culture were demonstrated by many authors. Torres and vicente [19] found that only 142 out of 776 (18%) nasopharyngeal aspirates of children were positive for RSV, Templeton et al.,[20] found that the incidence of RSV was 19%. Also, Jonathan [14] found that 11 out of 100 specimens (11%) were positive for RSV by tissue culture. The lowest result (9.8%) was obtained by Khalil et al., [21] as 22 samples out of 224 showed RSV- like CPE in HEP-2 cells.

Viral culture procedure is a slow method for diagnosis of RSV, as it takes 3 to 10 days from inoculation on HEp-2 cells to produce results. Therefore, the majority of cases may be discharged from the hospital before the culture results became available [6].

In the present study, RT-PCR was done as a confirmatory test for viral culture. Nested RT-PCR is a diagnostic test as it was proved to be more reliable regarding time, sensitivity and specificity [22]. Another advantage is the avoidance of viral neutralization or inactivation by antiviral products within respiratory secretions that can inhibit viral growth in culture [23]. Unfortunately, the applicability of RT-PCR on wide scale will be difficult due to its high costs [22]. Studies comparing molecular diagnostic assays to virus isolation in cell culture and to antigen detection assays have demonstrated superior sensitivity for nucleic acid tests which leads to an approximately two-fold increase of detection rates in infants with respiratory illness [1].

Similar results were obtained by Tabatabai et al., [4] as RSV infection was detected by RT-PCR in 134 out of 242 samples (55.4%) that were collected from hospitalized LRTIs children. Also, Azkur et al., [2] detected that RSV was the most common virus (45.5%) identified by RT-PCR in patients of bronchiolitis. A higher result was demonstrated in a study done in Saudi Arabia, by Akhter et al., [24] where RSV was identified in 83% of LRTIs patients. On the other hand, lower results were reported by many researchers; Fattouh et al., [25] in

Egypt, (16.4%), Popow- Kraupp and Aberle [18] (22%), Nikfar et al., [10] in Iran, (9%), Yadav et al., [23] in Delhi, in India (12.3%). Also, Khalil et al., [21] in Sudan reported that 19.6% were positive for RSV (44 out of 224 patients) and Yadav et al., [13] noticed that RSV was detected in only 10.8% of their patients. Low results in the previous studies might be attributed to the lack of going off some cases for specialized clinics because of their mild symptoms and signs and considerable distance between villages and hospitals [21]. Moreover, the difference in RSV epidemiology in the world may be related to differences in climate conditions, environmental factors and severity of its epidemics from one year to another [10].

Primary RSV infection commonly occurs within the first year of life [26] and the risk of RSV infection decreases with increasing age [4]. This fact has been confirmed in this research as the highest percentage of positive RSV by RT-PCR and tissue culture was in age < 1 year (69.7% and 90% respectively). In the study of Tabatabai et al.,[4] age group analysis revealed that infants below one year of age had the highest RSV infection rates (77.6 %). Also, Khalil et al.,[21] noted reduction in the number of children infected with RSV with increasing age as RSV infection was 68.2% in children < 2years and 31.8 % in children 2-5 years. Moreover, Yadav et al.,[13] showed in their study that RSV was demonstrated in much higher percentage (72%) in age group 2-6 months.

In this study, higher age group cases were better diagnosed by RT-PCR rather than by tissue culture where; 20 cases (30.30%) and 3 cases (10%) of the positive RSV patients aged from 1 year to 5 years were positive for RSV by RT-PCR and tissue culture respectively. In accordance of this result, Popow-Kraupp and Aberle, [18] showed that the best method for detection of RSV was RT-PCR in children older than 1 year. Previous studies demonstrated that there was a reduction in the efficacy of RSV detection with increasing age of the patient [27, 28, 29, 30]. The better detection of RSV by RT-PCR in children of older age group can be explained as young children experiencing primary infection usually shed large quantities of RSV over a prolonged period of time. With the increasing number of subsequent infections, virus is shed in lower quantities for shorter times due to the presence of secretory and humoral virus-specific antibodies influencing the degree of viral replication [18, 31].

As regards to gender, predominance of males was evident in positive RSV group (77.3% by RT-PCR and 76.7% by tissue culture) with a significant difference between males and females who had RSV infection (p=0.01). Male sex was reported in some studies as a risk factor for RSV infection [32]. RSV infection was confirmed in 47 male vs. 44 female and 57 male vs. 43 female by Savić et al.,[33] and Nikfar et al.,[10] respectively. In the study of Tabatabai et al., [4], males were representing 61.9% and females were 38.1% of positive RSV cases. Also, Khalil et al., [21] reported that RSV was detected in 12.9% male and 6.7% female patients, using RT-PCR technique and there was no significant difference.

Infection with RSV occurs in seasonal outbreaks, which arise annually, peaking during the winter in temperate climates and during the rainy season in warmer climates, although exceptions to this pattern occasionally occur [10]. In the United States, the annual epidemics usually begin in November, peak in January or February, and end in May [34]. This study confirmed the seasonal character of RSV infections with an increasing in hospitalization rate of LRTIs and an increased isolation rate of RSV from November to February. The incidence of positive RSV cases in this study was 1.51%, 31.82%, 43.94% and 22.73% in November, December, January and February respectively in both seasons (2013-2014 and 2014-2015). This result was in agreement with that reported by Cui et al., [35] in China and Savic et al., [33] in Serbia. Also, Yadav et al., [13] observed a rising in RSV infection towards winter season, where 13 positive RSV cases were detected from November to February months compared to only one case in August and October. In contrast, Breiman et al., [36] in Kenya and Oladokun et al., [5] in South Africa showed that RSV cases were recorded from January until October with most cases occurring in May.

RSV infection in children almost always causes clinical manifestations that can vary widely in severity, depending on the patient's age, co morbidities, environmental exposures, and history of previous infections. Typically, the infection starts with signs and symptoms of mucosal inflammation and irritation of upper respiratory tract (congestion, rhinorrhea and sneezing). After few days, lower respiratory tract clinical manifestations appear in the form of cough and increased work of breathing [22]. In this study, evidence of respiratory tract infection in positive RSV was cough (72.7%), wheezing (66.7%), rhinorrhea (57.6%), fever (42.4%) tachypnea (74.2%) and chest wall retraction (18.18%). These findings were similar to that reported by Nikfar et al., [10] who found that cough was present in 77.7% of cases, followed by fever in 55.5% of cases. Those authors observed that 100%, 88.8%, and 22.2% of their positive RSV cases had tachypnea, wheezing and cyanosis respectively.

In the present study, the clinical symptoms were more severe among positive RSV cases which were detected by tissue culture than by RT-PCR; as rhinorrhea (%85.7), wheezing (%85.7), cough (%66.7), and fever (%70). These results agreed with the fact that, as far as RSV is concerned higher viral loads seem to correspond with a more severe clinical course of the disease [18]. Also, Buckingham et al., [37] and DeVincenzo et al., [38] demonstrated that the higher quantities of the RSV titres or loads in nasal aspirates correlated with more severe symptoms and signs in their patients.

Bronchiolitis is the most prominent sign for clinical diagnosis of patients with RSV infection especially in infants [39]. The present study further supported that finding and showed that positive RSV cases detected by RT-PCR and tissue culture had bronchiolitis with a higher prevalence (92.4% and 93.3% respectively).This was in agreement with Nikfar et al., [10]; as 100% of positive RSV children whose ages less than one year had bronchiolitis. Also Tabatabai et al., [4] showed that, the majority of positive RSV children presented with bronchiolitis.

In this study, only 7.2% and 6.7% of positive RSV case detected by RT-PCR and tissue culture respectively had pneumonia. In contrast, a higher result was reported by Goto-Sugai et al., [39] (33.3%) and Khalil et al., [21] (53.3%) This variation may be due to the fact that, children with acute bronchiolitis are at higher risk of developing pneumonia [21].

Radiological findings of positive RSV positive patients were obtained and compared with negative RSV patients, where hyperinflated chest was more significantly ($p<0.001$) present in 68.3%. There was no significant difference between positive and negative RSV cases regarding increased bronchovascular markings. These findings were similar to that reported by Aboul –Ftouh et al., [40], as hyperinflated chest was found in 68% of RSV positive with bronchiolitis. Also, Savic et al., [33] showed that hyperinflation was the most common radiographic finding, while infiltrations and consolidations were less prevalent.

Morbidity and mortality rates in RSV infections are higher in premature infants and in those with chronic lung disease (e.g., bronchopulmonary dysplasia, cystic fibrosis, and interstitial lung diseases) or hemodynamically significant congenital heart disease [12]. In the present study, all of the 16 cases had different concomitant diseases were positive for RSV by RT-PCR, four out of them died (25%). One child (25%) was premature and 3 (75%) were suffering from congenital heart disease. No death occurred in positive RSV patients without co-morbidities. Savic et al., [33] and Azkur et al., [2] noted that the mortality rate in positive RSV with concomitant diseases was 16.7% and 3.2% respectively. Increasing in cases of death in children infected with RSV in presence of other concomitant disease may be the result of complications and greater length of hospital staying [2]. Therefore, in most countries with developed healthcare system passive immunization with palivizumab, recombinant monoclonal antibody is performed in specially defined groups [41].

CONCLUSIONS

RT-PCR was more sensitive than tissue culture in diagnosis of RSV with increasing age. RSV was found to be the major cause of LRTI in infants and young children. RSV was the major cause of LRTI in infants and young children especially those below 6 months. The incidence was decreasing by increasing the age. RSV was a main cause of acute bronchiolitis from November to February. Mortality rate was 25% among positive RSV who had concomitant diseases.

RECOMMENDATION

Passive immunization with specific monoclonal antibody (palivizumab) during RSV season should be considered especially in children with concomitant diseases.

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