GLOBAL JOURNAL OF BIO-SCIENCE AND BIOTECHNOLOGY

www.scienceandnature.org

DETECTION OF RESPIRATORY SYNCYTIAL VIRUS IN HOSPITALIZED CHILDREN WITH ACUTE RESPIRATORY TRACT INFECTIONS IN KURDISTAN REGION OF IRAQ

Tariq A.G. Aziz[1], Zainab A. Hamid[2] Ali H. Bayati[3]
[1]Department of Microbiology, School of Medicine, Sulaimani University. Sulaimani city, Kurdistan Region/Iraq.
[2]Department of Microbiology, Baghdad Medical College, Baghdad, Iraq ,[3]Community Health Department, Technical College of Health, Sulaimani Polytechnic University, Sulaimani city, Kurdistan Region/Iraq.

ABSTRACT
Human respiratory syncytial virus (hRSV) is an important viral pathogen of respiratory tract infections among children. The aim of this study was to detect hRSV infections among hospitalized children with respiratory tract infections in Suleimani governorate and to compare different diagnostic techniques for hRSV. Nasopharyngeal and throat swabs were obtained from 300 hospitalized children with acute respiratory infections under 5 years of age between April 2011 and March 2012. Each sample was used for hMPV detection by conventional reverse transcriptase (RT – PCR), direct fluorescent assay (DFA), and immunochromatography (ICG). A questionnaire was designed to collect data about the hospitalized children. The percentage of hRSV infections was 27 % by RT – PCR, 24.7% by DFA, and 24.3% by immunochromatography. The peak of hRSV infection was in January. hRSV is a common cause of RTIs in hospitalized children in Sulaimani city. RT-PCR is more sensitive than antigen detection methods for the diagnosis of hRSV viruses.

KEY WORDS: human respiratory syncytial virus, hospitalized children, direct fluorescent assay, RT-PCR.

INTRODUCTION

Human respiratory syncytial virus (hRSV) is a globally ubiquitous respiratory pathogen of the *Paramyxoviridae family*. *It* is a major cause of hospitalizations, morbidity and mortality among children due to acute respiratory tract infections (ARTIs) manifested as bronchiolitis or pneumonia [1]. The current study was designed to examine the frequency of hRSV infections among hospitalized children less than 5 years of age in Kurdistan Region using RT-PCR, direct fluorescent assay and immunochromatography, also to evaluate different diagnostic methods for detection of hRSV infections, and assessing the clinical characteristics of this virus.

MATERIALS & METHODS

A. Study design:
Cross sectional, hospital – based study.

B. Patients:
Three hundred hospitalized children less than five years of age were enrolled in this study; they were admitted to Pediatric Teaching Hospital in Suleimani city due to respiratory tract infections of undiagnosed etiology. The specimen collection started in April 2011 to March 2012. A signed informed consent or verbally agreement was obtained from the legal guardians of children participating in this study.

C. Questionnaire:
The patients' group was subjected to a questionnaire that included demographical and clinical data related to respiratory infections.

D. Specimens:
Two nasopharyngeal swabs (NPSs) and two throat swabs (TSs) were taken from each child.

E. Methods:
Immunochromatography **(**ICT), direct fluorescent assay (DFA), and reverse transcriptase- polymerase chain reaction (RT – PCR) were used for detection of hRSV in swabs from children in the study.

ICT: This test is ready to use strip test (RSV-Respi strips) for detection of hRSV in respiratory specimens (Coris BioConcept Company, Belgium).

DFA: DFA was performed according to manufacturer instructions (Light Diagnostics, Millipore, USA).

Molecular Technique: The conventional RT-PCR was the molecular technique for detection of hRSV. The technique targeted the L gene of hRSV genome by using primers designed in this study (table1) and was taken from NCBI Reference Sequence: NC_001781.1.

TABLE 1: PCR primers of the current study

Gene	Polarity of primer	Nucleotide position of primer	Primer sequence (5' 3')	Amplicon size
hRSV	*F*	*13319 – 13343*	*AGCAGAATTCACAGTTT GCCCTTGG*	*601*
L gene	*R*	*13919 – 13897*	*ATGATGCCAAGGAAGCA TGCAGT*	

The technique was performed in the following sequence:
1. Extraction of viral RNA form NPS by using viral DNA/RNA extraction kit (Intron Biotechnology Company/South Korea) and was performed according to manufacturer instruction.
2. Reverse transcription: this was done with two – steps reverse transcriptase – PCR kit in thermal cycler (TC312/ Techne Company/United Kingdom) for the synthesis of first strand cDNA and was performed according to manufacturer instruction.
3. PCR amplification: The PCR amplification protocol, which is mentioned in table 2, was used in this study. Ready to use positive control was used to check the third step.

TABLE 2: The protocol for PCR amplification

Component	Volume
Master mix	12.5 µl
Forward primers	3 µl
Reverse primers	3 µl
CDNA	6.5µl

The components are added in one microcentrifuge tube for a total of 25 µl reaction volume. Positive control is added in a separate tube with its primers.

4. Horizontal gel electrophoresis (Phero – Sub 1010-E/ Biotec – Fischer Company / USA) was used for detection PCR products and the amplicons were verified using DNA ladder, then the amplicons were viewed in Gel documentation (Uvido/ Uvitec Company/UK).

RESULTS

The results of ICT showed that 73 (24.3%) of the NPS and 69 (24%) of the TS were positive for RSV. The difference between NPS and TS statistically was not significant (P= 0.7733). DFA technique showed that 74 (24.7%) of the NPS and 72 (24%) of the TS were positive for RSV, again the difference between the two results statistically was not significant (P= 0.9242).

Figure (1) illustrates the infected pseudostratified ciliated columnar epithelium using NP swab under a fluorescent microscope, while figure (2) demonstrates the negative cases, comparable results for stratified squamous epithelium of throat swabs are seen in figures (3) and (4) respectively.

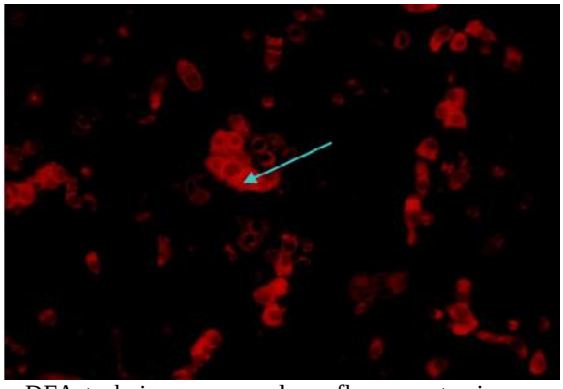

FIGURE 1: Positive result of the DFA technique seen under a fluorescent microscope for the detection of the hRSV antigen in nasopharyngeal swab using Rhodamine stain. The psuedostratified columnar epithelial cells of the nasopharynx show prominent red fluorescence in cytoplasm (arrow) while the nuclear region is pale.

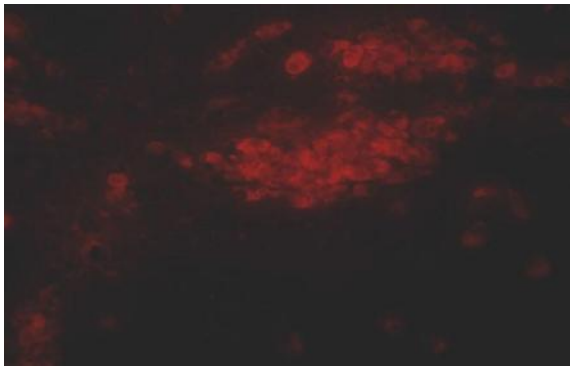

FIGURE 2: Negative result of DFA technique seen under a fluorescent microscope for the detection of HRSV antigens in nasopharyngeal swab using Rhodamine stain. Both cytoplasm and nucleus for each psuedostratified columnar epithelial cell of nasopharynx are red pale non-fluorescent in appearance.

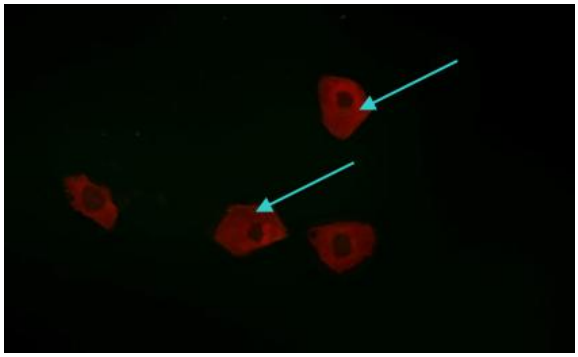

FIGURE 3: Positive result of DFA technique seen under a fluorescent microscope for the detection of hRSV antigen in throat swab using Rhodamine stain. The stratified squamous epithelial cells of oropharynx are showing prominent red fluorescence in cytoplasm (arrows) while the nuclear region is pale.

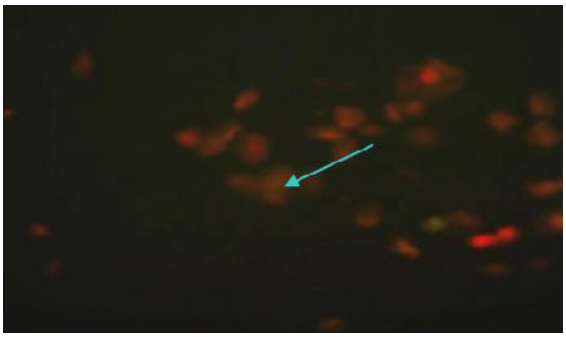

FIGURE 4: Negative result of DFA technique seen under a fluorescent microscope for the detection of hRSV antigen in throat swab using Rhodamine stain. Both cytoplasm (arrow) and nucleus for each stratified squamous epithelial cell are non-fluorescent faint red in appearance.

The results of RT-PCR technique showed that 81 (27%) patients were infected with hRSV while the remaining 219 (73%) patients were negative.

All children were divided into five groups, <1, 1 to < 2, 2 to < 3, 3 to < 4, and 4 to < 5; the highest frequency of hRSV infections by RT-PCR was found in the age group 1 to < 2 years old, while the lowest frequency was in age group 3 to < 4 years.

Demographic and clinical characteristics of hRSV infections among children in this study are summarized in table-3.

TABLE 3: Demographic and clinical data of hRSV – positive group and hRSV – negative group.

	Frequency of hRSV – positive patients	Frequency of hRSV – negative patients	P value
Sex			
Male	50	143	0.5888
Female	31	76	
Residency			
City	32	96	
District	41	74	0.0086*
Village	8	49	
Clinical features			
Fever	71	157	0.0037*
Dyspnoea	57	169	0.2307
Cough	59	182	0.0513
Sputum	26	61	0.4768
Wheezing	71	88	< 0.0001*
Tachypnoea	9	17	0.3615
Runny nose	68	67	< 0.0001*
Abnormal breathing sounds	58	102	0.0001*
Added breathing sounds	47	69	0.0001*
Cyanosis	7	29	0.3224
Mean hospital stay (days)	4.5	3	0.0411*
Clinical illness			
Bronchiolitis	37	42	< 0.0001*
Pneumonia	22	59	
Chest x-ray			

Normal	26	133	< 0.0001*
Abnormal	55	86	
Condition at discharge			
Complete recovery	9	13	
Discharged with medication	72	204	0.1419
Death	0	2	

*Significant differences among results

The highest frequency of hRSV infections was observed in January (21 cases [25.9%]), (figure 5).

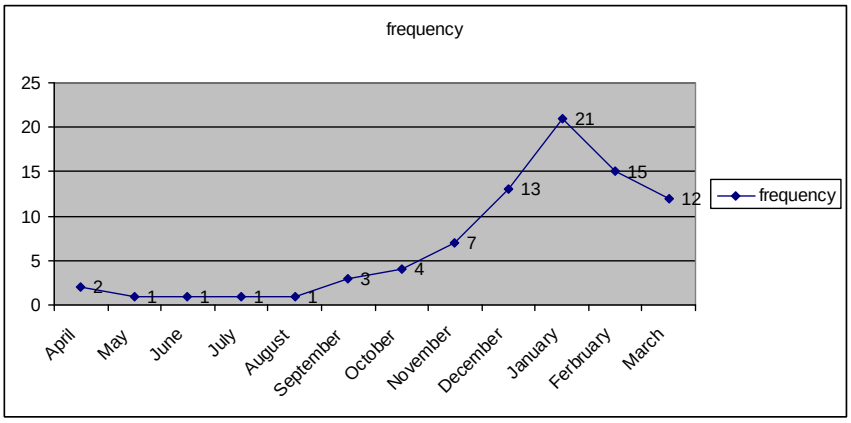

FIGURE 5: Seasonal distributions of hRSV infections among hospitalized children in the study group.

DISCUSSION

In this study, hRSV was detected in 81 (27%) out of 300 hospitalized children using a conventional multiplex RT-PCR technique in a year round –base; this frequency reflects the high incidence of hRSV among children who were hospitalized due to RTIs. Two previous studies in Iraq studied the frequency of hRSV among children using the antigen detection methods; the frequency of hRSV in the first one was 37.6%[2], while in second study was 45% [3].The prevalence of hRSV infections in the nearby countries showed variations in frequency. An Iranian study found that 45.6% of children fewer than 5 years were infected with hRSV[4], in Jordan two studies showed different frequencies, 64% [5], and 25.46% [6]. In a Saudi Arabian study, 54.4% of children were infected by hRSV [7]. In the current study, ICG was of less sensitivity than other techniques, this result is in accordance results of Borek, *et al.* [8]. The ICG and DFA techniques were more sensitive when NPS was the specimen compared to TS, though the differences were not statistically significant. Similar results were obtained by Curi Kim, et al. [9]. Some studies used nasopharyngeal aspirate as a specimen for the detection of hRSV and it was more sensitive though more invasive [10]. The DFA technique has a higher sensitivity and represents a better alternative to ICG. Several studies have found that for hRSV infection, tissue culture and molecular techniques do not add significantly to the number of infections detected compared to DFA testing [11]. In the current study, RT-PCR was used as the gold standard technique in the detection of hRSV. It had a better sensitivity than all other techniques used in this study, but it is time consuming, has higher cost, and technically more difficult to be performed as it needs many equipments, diverse materials, and well trained laboratory personnel. Many other studies showed comparable results [12]. The percentage of hRSV – positive children with bronchiolitis (45.7%) was higher than hRSV – negative children. These results reflect the importance of hRSV as a causative pathogen among children having bronchiolitis. Milder E, and Stempel HE reported similar results as bronchiolitis was detected in 43%–74% of cases [13, 14]. Twenty two (27%) of children were diagnosed as having hRSV pneumonia, which is less than hRSV bronchiolitis. Many other studies recorded hRSV as the most frequent identified virus in children with pneumonia [15]. According to this study, 1 to < 2 years is the age group mostly infected with hRSV which might be due to the decline of maternal antibodies in child blood which are protective against hRSV. Other studies noticed that infants younger than 1 year were mainly infected [16], a result which differs from findings in the current study**.** Although male to female ratio was nearly 2:1, gender was not identified as a significant risk factor for hRSV infection when compared to the hRSV – negative children. This is in agreement with other published studies [17]. Nevertheless, some studies have shown a male predominance when compared to hRSV – negative group, as reported by D'Elia *et al.* [18]. It is possible that peoples' stay indoors in winter will increase the risk of hRSV transmission and especially among children who catch their infections from older people.

Most other studies from different regions all over the world showed winter season predominance of hRSV infections. In Japan, Pakistan and Jordan similar result were recorded [19-21]; though in Thailand and in similar climate countries, the annual peak of hRSV hospitalization among children is occurring between July and October which are the rainfall months in this country [22].

CONCLUSION

hRSV was a common respiratory viral pathogen in hospitalized children, and RT-PCR was more sensitive than antigen or antibody detection methods for the diagnosis of hRSV, while DFA and

immunochromatography were a good alternative technique. hRSV bronchiolitis was more common than hRSV pneumonia, and autumn – winter period was the most frequent time for hRSV.

REFERENCES

[1]. Peng, D., D. Zhao, J. Liu, X. Wang, K. Yang, H. Xicheng, Y. Li and F. Wang. "*Multipathogen infections in hospitalized children with acute respiratory infections*" Virology Journal, Vol. (6), pp.155. (2009).

[2]. Albargish, K. A. and H. J. Hasony. "*Respiratory syncytial virus infection among young children with acute respiratory tract infection in Iraq*" Eastern Mediterranean Health Journal,Vol. (5), No.5, pp.941-948. (1999).

[3]. Shony M. Odisho, A. S. A.-B., and Nahi Y. Yaassen. *"Detection of Respiratory syncytial virus infection in a sample of infants in Iraq"* Iraqi Journal of Medical Scince, Vol.(7), No. 4, 9. (2009).

[4]. Zahra Chavoshzadeh, B. A., Alireza Fahimzad, Hamid Reza Samakosh, Ghamar Tag Khanbabaei, and Seyed Ahmad Tabatabaei. *"Molecular Study of Respiratory Syncytial Virus, Human Rhinovirus and Human Metapneumovirus, Detected in Children With Acute Wheezing"* Archives of Pediatric Infectious Diseases, Vol. (1), No.1, pp. 14-17. (2012).

[5]. Ali, S. A., J. V. Williams, Q. Chen, S. Faori, A. Shehabi, E. A. Jundi, N. Khuri-Bulos, and N. Halasa. *"Human metapneumovirus in hospitalized children in Amman, Jordan"* Journal of Medical Virology, Vol. (82), No. 6, pp.1012-1016. (2010).

[6]. Bdour, S. *"Respiratory syncytial virus subgroup A in hospitalized children in Zarqa, Jordan."* Annals of Tropical Paediatrics, Vol (21), No. 3, pp. 253-261. (2001).

[7]. Meqdam, M. M. and S. H. Subaih. *"Rapid detection and clinical features of infants and young children with acute lower respiratory tract infection due to respiratory syncytial virus"* FEMS immunology and medical microbiology**,** Vol. (47), No.1, pp.129-133. (2006).

[8]. Anita P. Borek, Susan H. Clemens, Valerie K. Gaskins, Deborah Z. Aird and Alexandra Valsamakis. *"Respiratory syncytial virus detection by Remel Xpect, Binax Now RSV, direct immunofluorescent staining, and tissue culture"*. Journal of Clinical Microbiology, Vol. (44), No.3, pp. 1105–1107. (2006).

[9]. Kim, C., J. A. Ahmed, R. B. Eidex, R. Nyoka, L. W. Waiboci, D. Erdman, A. Tepo, A. S. Mahamud, W. Kabura, M. Nguhi, P. Muthoka, W. Burton, R. F. Breiman, M. K. Njenga and M. A. Katz. "*Comparison of nasopharyngeal and oropharyngeal swabs for the diagnosis of eight respiratory viruses by real-time reverse transcription-PCR assays0"* PLoS One Vol. 6, No.6, e21610. (2011).

[10]. Stensballe, L. G., P. E. Kofoed, E. J. Nante, M. Sambo, I. P. Jensen and P. Aaby. "*Duration of secretory IgM and IgA antibodies to respiratory syncytial virus in a community study in Guinea-Bissau.*" Acta Paediatrica, Vol. (89), No. 4, pp. 421-426. (2000).

[11]. Gregson, D., T. Lloyd, S. Buchan and D. Church. "*Comparison of the RSV respi-strip with direct fluorescent-antigen detection for diagnosis of respiratory syncytial virus infection in pediatric patients*" Journal of Clinical Microbiology, Vol. (43)**,** No.11, pp. 5782-5783. (2005).

[12]. Caram, L. B., J. Chen, E. W. Taggart, D. R. Hillyard, R. She, C. R. Polage, J. Twersky, K. Schmader, C. A. Petti and C. W. Woods. *"Respiratory syncytial virus outbreak in a long-term care facility detected using reverse transcriptase polymerase chain reaction: an argument for real-time detection methods*" Journal of American Geriatric Sociaty, Vol. (57), No. 3, pp. 482-485. (2009).

[13]. Milder, E. and J. C. Arnold. *"Human metapneumovirus and human bocavirus in children."* Pediatric Research Vol. (65), No. 5, pp 78-83. (2009).

[14]. Stempel, H. E., E. T. Martin, J. Kuypers, J. A. Englund and D. M. Zerr. "*Multiple viral respiratory pathogens in children with bronchiolitis*" Acta Paediatrica, Vol. (98), No.1, pp.123-126. (2009).

[15]. Hammitt, L. L., S. Kazungu, S. C. Morpeth, D. G. Gibson, B. Mvera, A. J. Brent, S. Mwarumba, C. O. Onyango, A. Bett, D. O. Akech, D. R. Murdoch, D. J. Nokes and J. A. Scott. *"A preliminary study of pneumonia etiology among hospitalized children in Kenya"* Clinical Infectious Diseases, Vol. 54, No. 2, p190-199. (2012).

[16]. Papenburg, J., M. E. Hamelin, N. Ouhoummane, J. Carbonneau, M. Ouakki, F. Raymond, L. Robitaille, J. Corbeil, G. Caouette, L. Frenette, G. De Serres and G. Boivin. "*Comparison of risk factors for human metapneumovirus and respiratory syncytial virus disease severity in young children.*" The Journal of Infectious Diseases Vol. (206), No. 2, pp. 178-189. (2012)

[17]. Riccetto, A. G., J. D. Ribeiro, M. T. Silva, R. S. Almeida, C. W. Arns and E. C. Baracat. *"Respiratory syncytial virus (RSV) in infants hospitalized for acute lower respiratory tract disease: incidence and associated risks"* The Brazilian Journal of Infectious Diseases, Vol. (10), No. 5, pp. 357-361. (2006).

[18]. D'Elia, C., M. M. Siqueira, S. A. Portes and C. C. Sant'Anna. *"Respiratory syncytial virus -- associated lower respiratory tract infections in hospitalized infants"* Revista da Sociedade Brasileira de Medicina Tropical Journal, Vol.(38), No. 1, pp. 7-10. (2005).

[19]. Sato, M., R. Saito, T. Sakai, Y. Sano, M. Nishikawa, A. Sasaki, Y. Shobugawa, F. Gejyo and H. Suzuki. "*Molecular epidemiology of respiratory syncytial virus infections among children with acute respiratory symptoms in a community over three seasons.*" Journal of Clinical Microbiology, Vol. (43), No. 1, pp. 36-40. (2005).

[20]. Tariq, W. U., T. Waqar, S. Ali and E. Ghani. "*Winter peak of respiratory syncytial virus in Islamabad*" Tropical Doctor**,** Vol. (35), No.1, pp. 28-29. (2005).

[21]. Al-Toum, R., S. Bdour and H. Ayyash. *"Epidemiology and clinical characteristics of respiratory syncytial virus infections in Jordan"* Journal of Tropical Pediatrics, Vol. (52), No. 4, pp. 282-287. (2006).

[22]. Fry, A. M., M. Chittaganpitch, H. C. Baggett, T. C. Peret, R. K. Dare, P. Sawatwong, S. Thamthitiwat, P. Areerat, W. Sanasuttipun, J. Fischer, S. A. Maloney, D. D. Erdman and S. J. Olsen. "*The burden of hospitalized lower respiratory tract infection due to respiratory syncytial virus in rural Thailand*." PLoS One, Vol. (5), No.11, e15098. (2010).