

Detection of Respiratory Syncytial Virus and Human Metapneumovirus in Children with Respiratory Tract Infection by Use Real Time Polymerase Chain Reaction

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Abstract

Background: Respiratory tract illness is a major cause of morbidity and mortality among children, elderly and immunocompromised patients worldwide.

Objective: To determine the infection rate of respiratory syncytial virus and human metapneumovirus among children with respiratory tract infection in Baghdad city.

Patients and Methods: A cross sectional study was based on the processing of nasopharyngeal swabs from 150 children with acute respiratory tract infections, (81) males and (69) females; aged under five years old, who was admitted to Al-Imamin Al-Kadhimin Medical City and Pediatrics Protection Hospital in Baghdad during the period from December 2017 till April 2018. Nasopharyngeal swabs were collected from each participant and stored as frozen at -70 °C to use for RNA extraction and real time- polymerase chain reaction.

Results: Out of all these samples, 54 samples were positive for respiratory syncytial virus (36%) and human metapneumovirus (1.33%). The infection rate of respiratory syncytial virus is more common in males (57.41%) than females and in children \leq one year (37.04%) also high frequency were noticed among patients live in an urban area (72.22%) (50%) respectively and winter. According to different clinical feature, fever, cough, and wheezing were more common.

Conclusion: The infection rate of respiratory syncytial virus was more than human metapneumovirus in children with respiratory tract infection using real time-PCR technique and the clinical manifestations were more common during respiratory syncytial virus and human metapneumovirus infection are cough, fever, wheezing.

Key words: Respiratory tract infection, respiratory syncytial virus, human metapneumovirus, real time-PCR.

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Introduction

Acute respiratory infection (ARI) is the major cause of morbidity and mortality. In young children, the elderly, and immunocompromised individuals throughout the world [1]. Several types of viral families

such as Paramyxoviridae, Orthomyxoviridae, Picornaviridae, Adenoviridae, and Coronaviridae which considered the major viral etiological agents of ARI in all age groups [2].

Respiratory syncytial virus (RSV) and human metapneumovirus (hMPV) component of a single strand, negative sense RNA genome, helical nucleocapsid, and they are enveloped [3]. Respiratory syncytial infection and hMPV are believed to transmit through close contact with a person who has an active infection or direct contact with infectious secretions on environmental surfaces such as droplets, saliva, or large particle aerosols [4]. Despite the fact that fomites may be a source of contamination [5].

The human metapneumo virus is closely associated with respiratory syncytial virus, by far the most essential airway virus influencing children worldwide [6]. It has been accounted that the RSV and hMPV infections in children may be quite similar [7]. But there is also some confirmation that RSV causes more serious ailment than HMPV. Risk factors for extreme RSV infection are young age, prematurity, chronic lung illness, chronic heart illness and serious neurological disabilities [8].

Respiratory syncytial virus and hMPV infection are blurred distinguishable, both they cause upper respiratory infection which begins with flu-like illness, fever; headache; sneezing, and then progresses down into the lower respiratory tract to cause bronchiolitis, pneumonia, and they implicated with allergy and asthma exacerbation [9]. Respiratory syncytial virus constitutes more than 60% of respiratory tract infection, while human metapneumovirus around 12% [10]. Re infection in RSV and hMPV happens with

comparable strains in spite of normal infection stimulating high levels of antibody against conserved antigens [11].

The diagnosis of RSV and hMPV infections can be made by several techniques, including culture, nucleic acid amplification tests (NAAT), antigen detection and serology test, but detection of viral RNA by NAAT such as reverse transcriptase-PCR (RT-PCR) assay is the most sensitive method for diagnosis of RSV and hMPV infections [12].

In Iraq, several studies have been conducted in various provinces to identify these viruses such as study done by Al-Mola et al (2013) in Hilla city[13], Aziz (2015) in Sulaimani city[14], Al-Ameedy (2016) in Najaf city[15], Al-Charrakh et al (2016) in Wasit province[16], Al-Mossawi et al (2016) in Al-Amarah City[17] and finally Atyah et al (2017) in Baghdad city[18]. This study design to investigate of co infection between RSV and hMPV in children under five year's age.

Patients and Methods

Study population and selection of patients

A cross sectional study was based on the processing of nasopharyngeal from one hundred fifty (69 females and 81 males) under five years old who was admitted to Al-Imamin Al-Kadhimin Medical City and Pediatrics Protection Hospital in Baghdad during the period from December 2017 till April 2018. Data were collected by interview with each parent of a participant or relevant through structural questionnaire which include age, gender, season, residence, fever, cough, wheezing, history of

asthma, nasal discharge, type of infection and diarrhea.

Nasopharyngeal swab received in viral transport media (Sigma Virocult Company, UK) without antibiotics [19]. And transportation of swabs by a cool box to the virology unit at the National Central Public Health Laboratory. Then were vortexed for 15 seconds to dislodge material on the swab into the transport medium. On completion of routine investigations for microbial causes of respiratory tract infection all residual nasopharyngeal aspirate samples were divided into aliquots, labelled and stored at 2°C to 8°C for no longer than 24 hours or frozen at -70°C for prolonged storage until the time of analysis.

RSV and hMPV detection

Viral RNA was extracted from nasopharyngeal swab using RNA extraction kit quick protocol kit (Cat. No.52906 - Qiagen - Germany), according to the manufacturer's instructions In this study, we used a real-time RT-PCR assay for the detection of RSV and hMPV in a 7500 fast Applied Bio- systems instrument.

For the RSV F gene detection, the amplification done by using forward primer sequence (5-GGC AAA TAT GGA AAC ATA CGT GAA-3), with reverse primer (5-TCT TTTTCT AGG ACA TTG TAY TGA ACA G-3) and probe (5-CTG TGT ATG TGG AGC CTT CGT GAA GCT-3). The probe was labeled with 5' reporter dye FAM and the 3' at a non-fluorescent dye BHQ1 [20].

While one pair of specific primers are tested to reverse transcribe and amplify the human metapneumovirus highly conserved F genes. Primers were highly specific for the F capsid genes of the HMPV, the forward sequence primer was (5'- CAA GTG TGA CAT TGC TGA YCT RAA -'3), reverse primer (5'- ACTGCCGCACAACAACATTTAGRAA-'3) and Probe (5'- TGGCYGTYAGCTTCAGTCAATTCAAC AGA -'3). The probe for hMPV uses FAM dye on the 5' reporter end and a non-fluorescent dye on the 3' quencher end [21].

The master mix in use for the one step RT-PCR of RSV and Hmpv detection was Ag path-ID™ one step RT-PCR kit (P/N AM1005,437424 and 4387391, Applied Biosystem, USA). were added to 12.5µl of 2x RT-PCR buffer, 1 µl of 25x RT-PCR enzyme Mix, 0.5 µl pmol conc. of each primer and 0.5 µl pmol conc of the probe, 5 µl of Nuclease free-water, 5 µl of RNA templates, the total of reaction mixture 25 µl. Amplification and detection were done with an Applied Biosystem 7500. Briefly, one cycle for 1 min at 45°C and 10 min at 95°C, followed by 45 cycles for 15 s at 95°C and 1 min at 55°C.

Results

The rate of respiratory syncytial virus infection among children under five years age was 36% (54 out of 150) samples and human metapneumovirus was 1.33% (2 out

of 150) samples by Real time PCR as shown in Table (1) and Figures (1), (2).

Table (1): Infection rate of RSV and hMPV in children with respiratory tract infection by real time PCR.

Viruses	Examined No.	Positivity (%)
Respiratory syncytial virus	150	54(36%)
Human metapneumo virus	150	2(1.33%)

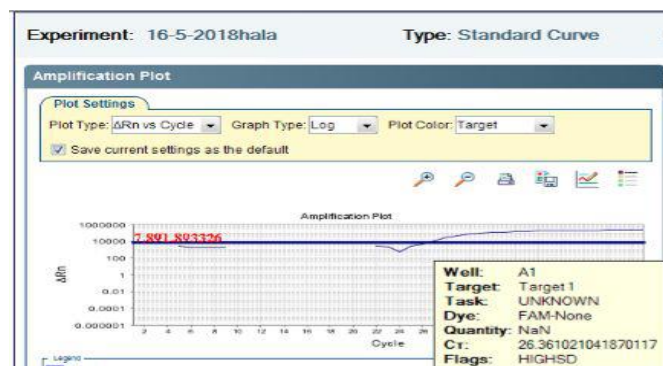


Figure (1): Real time PCR of human respiratory syncytial virus.

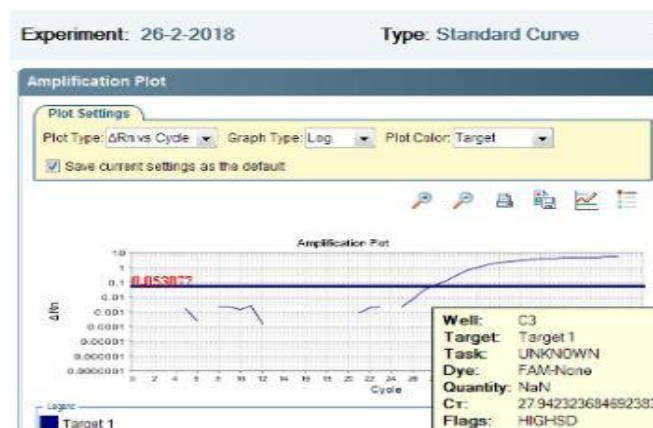


Figure (2): Real time PCR of human metapneumovirus.

Infection with respiratory syncytial virus was more common in males 31(57.41%) than females 23 (42.59%). The highest infection rates (37.04%) were noticed in age group (1-12 month) followed by (13-24 months). Most infections occurred in urban area 72.22%.

While human metapneumo virus infection had equally percentage regarding gender and residence as well as equal present 50% in the age group 1-12 month, and 13-24 month, also a high percentage of infection were recorded in urban area as shown in the Table (2).

Table (2): Distribution of RSV and hMPV in patients with RTI according to demographic features using real time - PCR.

Variable factors		RSV No. Samples (%)	HMPV No. Samples (%)
Gender type	Male	31 (57.41%)	1(50%)
	Female	23 (42.59%)	1(50%)
Age groups	(1-12) months	20(37.04%)	1(50%)
	(13-24) months	15 (27.78%)	1(50%)
	(25-36) months	10(18.52%)	
	(37-48) months	5 (9.26%)	
	(49-60) months	4 (7.41%)	
Residence	Rural	15 (27.78%)	1(50%)
	Urban	39 (72.22%)	1(50%)
Seasons	Winter	42 (77.78%)	1(50%)
	Spring	12 (22.22%)	1(50%)

Distribution of RSV infection, according to the clinical features were detailed in Table (3), 35 (64.81%) children were suffering from lower respiratory tract infection, 53(98.15%) had a cough while 49(90.74%) had fever, 43(79.63%) were suffering from wheezing, 27.78% of children had a history of asthma. Nasal

discharge are present in 68.58% and 42.59% were suffering from diarrhea. On the other hand relation between human metapneumo virus infection and clinical features showed that all children had a lower respiratory tract infection, cough, fever and wheezing while 50% of children had a history of asthma, nasal discharge and diarrhea.

Table (3): Distribution of RSV and hMPV in patients with RTI according to clinical features using real time PCR.

Variable factors		RSV No. Samples (%)	hMPV No. Samples (%)
RTI	Upper	19(35.19%)	0
	Lowe	35(64.81%)	2(100%)
Cough	Yes	53(98.15%)	2(100%)
	Lower	1(1.85%)	
Fever	Yes	49(90.74%)	2(100%)
	No	5(9.26%)	
Wheezing	Yes	43(79.63%)	2(100%)
	No	11(20.37%)	0
History of asthma	Yes	15(27.78%)	1(50%)
	No	39(72.22%)	1(50%)
Nasal discharge	Yes	37(68.51 %)	1(50%)
	No	17(31.49%)	1(50%)
Diarrhea	Yes	23(42.59%)	1(50%)
	No	31(57.41%)	1(50%)

Discussion

The infection rate of the current study was 36% of RSV infections and 1.33% of hMPV infections by real time polymerase chain reaction. The infection rate of RSV use real time-PCR is comparable with several studies conducted in different area such as Khadadah et al. (2010) reports that the percentage of infections is 36.8% in hospitalized children with respiratory tract infection in Kuwait [22] and with a study done by Parsania et al., (2016) shows that 31.1% of RSV infections among Iranian children < 5 years of age [23].

The infection rate of RSV in this study is relatively low compared with data reported

in Baghdad by Odisho et al. (2009), the percentage is reached to 79% among the children who have a respiratory tract infection [24]. In this study the infection rate seems to be lower with than other studies such as study done by Atyah et al., 2017 who recorded (6.6%) in children under 15 years old in Baghdad [18]. Another study done by Al-Charrakh et al., (2016) recorded (18.75%) in asthmatic patients used real time polymerase chain reaction in Wasit city [16]. The positive rate of hMPV in the current study was (1.33%) comparable to results was obtained by Zhang et al., (2018) detected in 2% (103 out of 5133) of ARI

patients in Southern China [25]. Also, this result consistent with that reported by Mohamed et al.,(2014) showed 4% in Egypt [26] and Essa et al., (2015) found 5.3% in Kuwait [27]. Other studies found different percentages ranging from (1.5%-18.2%) [28][29][30]. These differences in frequency in the reports might reveal changed epidemiological outlines of RSV and hMPV infections in various countries, which in turn might be linked to environmental features, geographical factors, differences in host genetic predisposition, immune status, size of the sample, method for detection, and different viral strains going into various geographical zones [31]. According to gender, it has been found that RSV infection in males more than females seems to be similar to those who participated in other studies such as, Zahran et al., (2017) in Egypt [12] and Hassan et al., (2018) in Iraq [32]. While the current study is inconsistent with a study conducted by Reina et al., (2008) which revealed that the gender, females (53.2%) was higher than males among children infected with hMPV[33]. And with Zuo, et al., (2009) found no significant differences regarding sex in children positive to hMPV[34]. Regarding the age, it shows that infections with RSV were more common in age group <1 year, 20(37.04%) use a real time PCR technique, when compared to older children. This result was comparable to that Chen et al.,(2010) in china [35] and Khalil et al., (2015) in Egypt [36], The incidence of severe RSV infections was highest among infants and young

toddlers and peaked in the 1 to 2 month-old infants.

Data obtained from this study indicate that most positive patients of RSV from urban area and exactly 39(72.22%), (Table 2), this result which agreed to similar study that has reported increased infections in Kurdistan region of Iraq [32]. In contrast, this result disagreed with a study done by Aziz (2015) to detect hMPV [14]. The reason of high prevalence in the urban residence could be related to crowded and bad hygiene in the large cities.

In the current study, seasonal peaks of the RSV and hMPV were circulated primarily during the winter and early spring in Iraq, this result in agreement with Al-Shami *et al.*, (2009) who mentioned that ratio was higher in January and February in Iraq [37]. And with another study done by Banerjee et al., (2011) hMPV circulated predominantly during the winter-spring period of 2010-2011 [38]. Different type of clinical feature was studied in this study, It has been shown that LRTI may be associated with higher RSV and hMPV viral loads than URTI that is agreement study done by Bosis *et al.*, (2008) [39] and Zhang *et al.*, (2018) [25].

Fever is generally more widespread among children with respiratory tract infection, this result in agreement with previous study done by Aziz *et al.*, (2015) reported fever and wheezing were more common clinical sign in children with hMPV [14]. While Ji Wang, *et al.*, (2009) found fever, cough, and rhinorrhea were more common than other signs. This might reveal the wide-ranging

signs during viral infection [40]. The most frequent symptoms of hMPV are fever and cough, in accordance with a study done by Zhou *et al.*, (2017) among hMPV positive cases fever was 33(82.50%) [30]. And Zeng *et al.*, (2015) [6]. Concerning the cough and wheezing, Ali *et al.*, (2010) in reported the clinical signs associated with hMPV infection were similar to those of other respiratory viruses, except children with hMPV were more likely to present with fever than children not infected with hMPV, also Cough 100% and Wheezing 89% [41]. Zahran *et al.*, (2017) found wheezing (85.7%), cough (66.7%), and fever (70%) [12]. The results of the present study showed (27.78%) of RSV and (50%) of hMPV infection may be associated with asthma exacerbation which are in agreement study done by Da silva *et al* (2013) [42] and with Wu *et al.*, (2008) who mentioned at winter, the virus peak had an increased risk of bronchiolitis in infancy and of asthma during childhood in USA [43]. Concerning diarrhea, this study showed that 23 cases (42.59%) RSV-positive children were suffering from diarrhea. which is in agreement with previous reports done by Hassan *et al.*, (2018) reported 13 out of 20 (6.4%) cases had diarrhea in hospitalized children from the Kurdistan region [32] other study found that diarrhoea was more common in patients with hMPV plus other pathogens detected (22.2%, 4/18), compared with patients with hMPV only (3.6%, 3/83) (Zhang *et al.*, 2018) [25].

Conclusions

The infection rate of respiratory syncytial virus was more than human metapneumovirus in children with respiratory tract infection using real time-PCR technique and the clinical manifestations were more common during respiratory syncytial virus and human metapneumovirus infection are cough, fever, wheezing. Further study with large sample size are needed to clarify this issue as well as study role of other viruses in co-infection.

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