Study Role of Respiratory Syncytial Virus Associated with Infection of Respiratory System of Children in AL-Miqdadiya/ Diyala

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Abstract

Human Respiratory Syncytial virus was major cause of viral infection of lower respiratory tract infection among infants and young children less 2 years old. HRSV was an enveloped virus with negative sense, single Stranded RNA, belonging to the family of *pneumoviridae* and genes *Orthopneumovirus*. This study was aimed to detection prevalence of respiratory syncytial virus in children with respiratory tract infection using Real time - PCR .This study included 50 sample from both gender and ages less than 10 years old, sample were collected from respiratory system patient from 40 patients and 10 controls. Results of current study showed that infection percentage of HRSV was 35% and all controls were un infect. The infection percentage of virus was more common in males 47% than females and in children, < one year 37.9% also high frequency were noticed among children who depended on artificial feeding.

***Keywords**: Respiratory syncytial virus, real time-PCR.*

Introduction

Respiratory virus infection was the world's leading cause of morbidity, hospitalization and mortality. This affected people of all ages especially infant, elderly and individuals with weakened immune systems [(1)]. Upper respiratory tract infections URTIs and lower respiratory tract infections LRTIs was often caused by a wide variety of viruses such as Human respiratory syncytial virus, Human parainfluenza viruses and Human Adenoviruses were the most significant respiratory viral infections [(2)]. The prevalence rate of respiratory viral infections globally was very high viruses were reported as causes of pediatric acute tract infections in up to 95% of cases[(3)]. In Iraq, studies reported that the prevalence rate were 1% to 36%[(4)]. It was estimated to case 30 million acute respiratory infection and more than 60.000 children deaths would wide each years. It was estimated that RSV in the USA causes 17,358 deaths per years [(5)]. Human Respiratory Syncytial virus was an enveloped virus with negative sense, single Stranded RNA, belonging to the family of *pneumoviridae* and genes *Orthopneumovirus*. HRSV was categorized in two major antigenic subgroup A and B based on the sequence and antigenic differences [(6)]. Most children were infected at least once in the first two years of age and its responsible for a quarter of all cases of pneumonia in the first months of life globally, classified as the second common cause of past-neonatal infant death after malaria. HRSV was still causing annual outbreaks with no safe and successful vaccine developed yet [(7)]. HRSV constitutes over 60% of respiratory tract infection [(8)]. Laboratory methods ready for the detection of RSV include, virus isolation in cell culture, detection of viral antigens by direct or indirect immunofluorescent (IF) staining (DFA / IFA) or by enzyme- linked immunosorbent assays (ELISA) and the detection of viral nucleic acid by amplification assays [(9)]. Most reverse transcription polymerase chain reaction

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(RT - PCR) and real time polymerase chain which represent rapid and sensitive methods for detection of RSV [10].

Materials and Method

Collection of Sample

The research was conducted in AL-Muqdadiyah district /Diyala governorate, in Zahraa Hospital for women and children extending from October 2020 to February 2021.

The current study included 40 swabs were taken from nasopharyngeal, included 17 females and 23 males, and the control group was composed of 10 people 5 females and 5 males. Samples were collected from children admitted in hospitalizes with respiratory infection from 1 month to 10 years, depending on the clinical diagnosis. Data were collected through an interview with parents or relative of each participant through a structural questionnaire that included gender, age and breast feeding to obtain samples for research purposes .Nasopharyngeal Swabs arrived in viral transport media without antibiotics. And stored at -70c until the time of analysis.

HRSV detection

Viral RNA was extracted from nasopharyngeal swab using ready kit Ribovirus cat. No 52906 RNA extraction kit quick protocol /Italy according to manufacturer's instructions. In this study, we used a real time RT-PCR assay for detection of RSV in a 7500 fast Applied Bio-systems instrument.

Estimation RNA concentration

Measuring the concentration of RNA in the sample using Quintus florometer, which is used to estimate the concentration of extracted RNA. 1mL of RNA is mixed with 99mL of diluted Quant flour dya after incubation for 5 minutes. The size of the RNA concentration was measured.

Primer Preparation

The primer preparation by Microgen company/ korea in lyophilized form, and used in previous studies (11). Lyophilized primers were dissolved in a nuclease free water to give a final concentration of 100 pmol/ml as a stock solution. A working solution of these primers was prepared by adding 10 mL of primer stock solution (stored at freezer -20c) to 90 mL of nuclease solution of 10 pmol/ml.

Table (1) show sequence of primers that used in the Real Time PCR of the respiratory syncytial virus.

Primer name	Sequence 3-5	Size bp	Source
RSV-F	5′-GGCAAATATGGAAACATACGTGAA-3′	300	11
RSV-R	5′-TCTTTTTCTAGGACATTGTAYTGAACAG-3′	300	

Reaction setup and Thermal cycling protocol

The master mix in use for one step RT-PCR of RSV detection was used(Go Taq R-step RT-9PCR/promega/ USA).Were added to5 ml qPCR master mix ,0.25 ml of RT mix ,0.25 ml of Mg cl 2 ,0.5 ml of Forward primer ,0.5 ml of Reverse primer and 3.5 ml of RNA .Total volume 10 ml. Briefly, one cycle for 15min at 37 c◦ and 5min at 95 c◦ , followed by 40 cycles for 20s at 95 c◦ and 20 min at 63 c◦.

Statistical Analysis

Using SPSS (package or social science) statistical program .The significant value was chosen at the level (p=0.05).

Results

The results showed that viral infection in 14 children with respiratory system infection from 40 patients with percentage 35 % with a statistical significance p-v =0.001

Table (2) Show the numbers of positive and Negative Samples by RT-PCR

Group	Positive		Negative		Total		P- Value
	No	%	No	%	No	%	
Patients	14	35	26	65	40	100	0.001
Control	0	0.0	10	100	10	100	
Total	14	28	36	72	50	100	

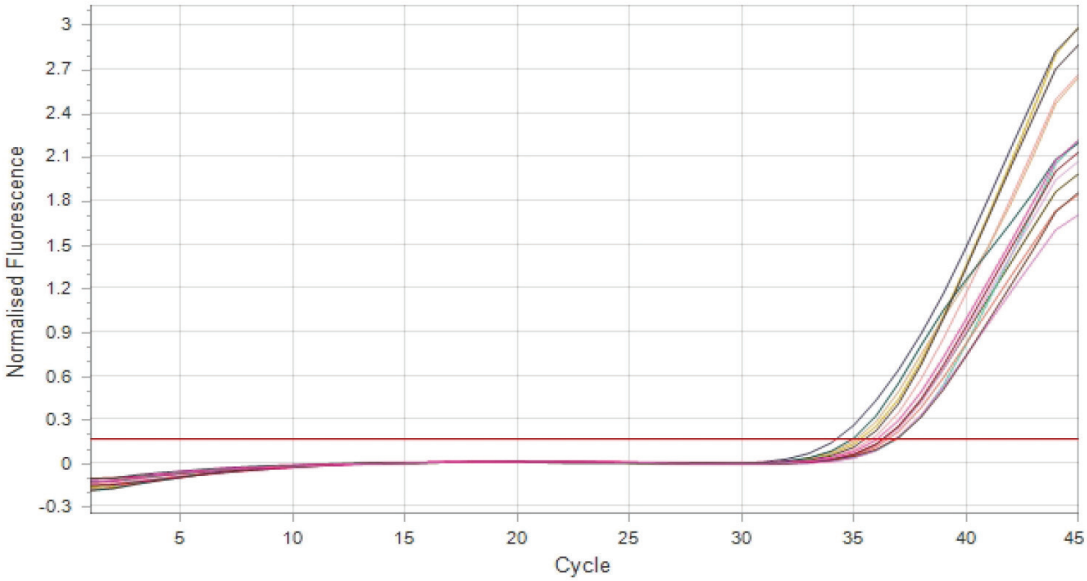

Figure (1) positive result for detection of the RSV by RT-PCR

Viral infection according gender

The results of the current study appeared no statistical evidence of a positive rate of HRSV in both sexes, male with recorded 47% and female was recorded 26%, as showed in table 3.

Table (3) viral infection according gender

Gendar	Positive		Negative		Total		P. Value
	No	%	No	%	No	%	
Female	6	26	17	73.9	23	57.5	P=0.343
Male	8	47	9	52.9	17	37.7	
Total	14	35	26	65	40	100	

Viral infection according age group

The results of the current study showed an increase in the positive rate in the age group 1month-1years, 11(37.9%) followed the age group 2-4 year which 3 (27.2%).As for the rest of the age groups no positive rate was recorded with significant, as shown in table 4.

Table (4) Viral infection according age group

Age Group	Positive		Negative		Total		P. Value
	No	%	No	%	No	%	
1month - 1year	11	37.9	18	62.0	29	72.5	P=0.004
2 - 4 year	3	27.2	8	72.7	11	27.5	
5 - 7 year	0	0.0	0	0.0	0	0	
6 - 10 year	0	0.0	0	0.0	0	0	
Total	14	35	26	65	40	100	

Viral infection according type of feeding

The results of the current study showed a high positive of HRSV with artificial feeding 61.5% followed by breast feeding were 44.4% and mixed 11.1% with no a statistical significance as shown in the table 5.

Table (5) Viral infection according type of feeding

Typed feeding	Positive		Negative		Total		P. Value
	No	%	No	%	No	%	
Breast feeding	4	44.4	5	55.5	9	22.5	P=8.893
Artificial	8	61.5	5	38.4	13	32.5	
Mixed	2	11.1	16	88.8	18	45	
Total	14	35	26	65	40	100	

Discussion

The results of this study was agreement with the study conducted in 2018 in Diyala governorate, where the positive rate for the virus 36% [11]. Also agreement with study conducted in Turkey and the positive rat 37.9% [12]. Corresponds to other study conducted in Kuwait and the rate was positive 36.8% [13]. These variation in occurrence between studies may be due to different epidemiological trends of RSV infection in different countries, which may be due to environmental factors geographical factors, difference in host genetic susceptibility, Sampling size, immune status and detection technique and / or various viral strains circulating in different parts of the world. Environmental factors such

as high temperature and high relative humidity are the first and most important cause [14]. According to gender, it was found that infection with HRSV in males more than females, appears to be similar to those who participated in other studies performed by Hassan *et al*., (2018) in Iraq [4] and Zahran *et al*., [15] in Egypt (15) and Khuri-Bulos *et al*., [16] in Jordan. The reason therefore seems to be of anatomic natural that have shorter and narrower airways and more like to develop bronchical obstruction in case of RSV infection[17]. According to age the results of this study were agreement with the study conducted in Diyala showed the positive age group 1 month - 1 years (37.04%) followed by the age group 2-4 years at a rate of (27.78%) (11). Non-agreement with study conducted in Baghdad the positive age group < 6 months (56.82%), 6-12months (27.27%) and 12 - 24 months (15.91%) [18]. Samples collected from children under 5 years suggesting that burden of occurrence of respiratory infections is higher in this groups [19]. The results of the current study found that individuals infected with HRSV was among children less than 5 year, indicating that the rate of HRSV infection decreases with old ages compared to young ages [20]. The other explanation for the result between age groups: RSV infection was higher among infants and young children and peaked in children 1 years old. This was in line with a study done by Hall *et al*., [21], the highest rate is observed infants. According to type of feeding it was found artificial feeding more than breast feeding, appears to be similar to those who participated in other studies conducted in Iraq [22]. And outside Iraq such as in Bangladesh and Korea [23,24]. The role of breast feeding was preventing RSV disease and hospitalization for RSV was undisputed. However data about the specific protective effect of breast feeding on RSV infection were conflicting. The reason for its protective effect is attributed to RSV-IgA and Lactoferrin in the breast mile and to the fact breast milk promotes maturation through the influence of prolactin. [25].

Conclusion

The infection rate of virus was high in children with respiratory tract infection using real time –PCR technique in Al-Muqdadiyah district .The highest infection rate was in children less than two years ,RSV infection in males is higher than females .RSV is seen that the children who are exclusively breast feed suffer less likely from bronchiolitis

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