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Molecular Study for Respiratory Syncytial Virus in Thi-Qar Province, Iraq

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ABSTRACT

Human respiratory syncytial virus (HRSV) is an important cause of acute respiratory infection (ARI) and mortality in humans; especially among children, elders and immune-compromised individuals around the world. This study aims to determine the infection rate of respiratory syncytial virus in patients with respiratory tract infection and identify the genotyping in Different cities in Thi-Qar province During the period from February to May. A total of 100 throat swabs samples were collected from patients having flu-like illnesses from age range between (6-65) years from both sex . Result showed 38 % positive for RSV , 24% was in genotype A and 14% in genotype B ,.The conclusion of this study revealed that respiratory syncytial virus A infections were more frequent than RSV B among patients with acute respiratory tract infection, and The frequency of female patients infected with RSV were higher than their male.

Introduction

Human Respiratory syncytial virus (HRSV) is a major cause of acute respiratory infection (ARI) in children worldwide (Kim *et al*., 2000; Tsolia et al., 2003; Kwofie *et al*., 2012). It is responsible for seasonal outbreaks (Madhi *et al*., 2006; Bloom Feshbach *et al*., 2013) and is associated with substantial morbidity and mortality rates, especially in people with some underlying conditions (Anderson *et al*., 2016). Every year, HRSV is a source of significant hospital admissions in young children with a considerable rate of mortality in high-risk groups (Szabo *et al*., 2013; Reeves *et al*., 2017). H ARI has a major impact on healthcare resources and costs (Forbes *et al*., 2010; Anderson *et al*., 2017). Furthermore, there is growing evidence that HRSV ARI in childhood is linked to long-term impaired lung function that can manifest as recurrent wheezing or asthma (Fauroux *et al*., 2017).

Respiratory syncytial virus particles are pleomorphic with both spherical and filamentous particles of different sizes; which comprise of a nucleocapsid bundled in a lipid envelope got from the host cell plasma membrane (Collins *et al*., 2013)It is a cytoplasmic with linear, negative sense, ssRNA genome of approximately 15,000 nucleotides that is classified in the Pneumovirus genus of the Paramyxoviridae family. The viral genome encodes 11 proteins. Of these, the G and F-proteins are the major surface antigens of RSV, which is involved in virus attachment to cell receptors and the mediation of cell membrane fusion, respectively. Both G- and F proteins are accessible to neutralizing antibodies, however, only the G-protein is known to accumulate mutations in response to host immunological pressures (King AM *et al*., 2012) .

RSV virus, classified according to antigenic differences in virion structural proteins into 2 subtypes, A and B (Mufson *et al*., 1985). Both subtypes usually circulate during epidemic seasons, following an irregular, alternating prevalence pattern, subtype A having a cumulative higher prevalence than subtype B (Bont L *et al*., 2016). Although RSV-A is thought to have a more severe clinical course (Tabarani *et al*., 2013), several papers reported no significant differences in disease severity between the 2 subtypes (Comas *et al*., 2018) or found that RSV-B causes more severe disease than RSV-A (Espinosa *et al*., 2017).This inconsistency could result from bias due to inclusion in these studies of infants with different respiratory diseases, differing in age, coming from different climates, or other confounding factors. Equally important, a different disease course may also reflect different RSV genotypes (Griffiths *et al*., 2017).

Materials and Methods:

Methods

Samples collections:

A total of 100 throat swab samples were collected from patients by using sterile nylon swabs (Regular Flocked swab, Cat. No. 520CS01, Copan Diagnostics Inc., Murrieta, Calif, USA) in 1 ml of transport media (UTM-RT, Cat. No. 92562, Copan Diagnostics Inc., Murrieta, Calif, USA). Samples collected were transported on ice at the same day of collection and stored at −80°C until processed..

RNA extraction

RNA was extracted from throat swab samples using QIAamp Viral RNA Mini Kit according to manufacturer's instructions.

Polymerase Chain Reaction

RT-PCR was performed by using Respiratory Syncytial Virus (RSV) RT-PCR Kit (Shanghai ZJ Bio – Tech Co., ltd) according to manufacturer's instructions.

RT- PCR Thermocycler conditions:

RT- PCR thermocycler conditions were done in table (1) :

Table-1: RT-PCR Thermo Cycler Conditions

PCR step	Temp.	Time	Repeat
Initial Denaturation	94ºC	5min.	1
Denaturation	94 ºC	30sec.	40 cycle
Annealing	63 ºC	30sec	
Extension	72 ºC	2min.	
Final extension	72 ºC	5min.	1
Hold	4 ºC	Forever	-

cDNA synthesis

This was accomplished by adding 0.1-1.0 ng / µl template RNA and RNase/DNase free water into the Maxime RT Pre-Mix tubes (Oligo dT15 or Random Primer) to a total volume of 20µl (iNtRON Biotechnology, Inc., Korea). cDNA synthesis that was carried off at 45°C for 60 minutes, and followed by Reverse transcriptase (RTase) inactivation at 95°C for 5 minutes.

Nested RT-PCR for RSV-A

The primer sequences of RSV-A (RSV-A External PCR RSVA-G513-F: AGTGTTCAACTTTGTACCCTGC, RSVAF131 R:CTGCACTGCATGTTGATTGAT, and for Nested PCR RSVAG606-F: AACCACCACCAAGCCCACAA,RSVA-F22-:CAACTCCATTGTTATTTGCC) (Uzma *et al*,. 2013), Optimized PCR reaction for RSV cDNA amplification was performed as follows, 3µl of RNA extract was added to 5 µl PCR premix (Maxime PCR premix kit (i-Tag)) containing i-Tag TM DNA polymerase, dNTP mixture and reaction buffer. Two µl of primers and 15µl of distilled water were then added to the PCR premix. The PCR program consisted of 94°C for 5 min, followed by 40 cycles of PCR, consisting of 30 s at 94°C, 30 s at 58°C, and 1 min at 72°C, with a final extension step of 10 min at 72°C. The expected productsof583bpamplicon were analyzed by electrophoresis on a 1.5% agarose gel; the product was visualized by staining with 0.15% Ethidium bromide using UV gel documentation system INGeNius (Analytika Jena, Germany). For the nested PCR the cycling protocol was the same as for the external PCR, except for the annealing temperature, which was 53°C. The nested amplicons of 391 bp were visualized by agarose gel electrophoresis as mentioned above.

RT-PCR for RSV-B

Conventional RT-PCR Primers for RSV-B (BGFSeq.1: A G A G A C C C A A A A A C A C Y A G C C A A , B G R S e q . 2 :ACAGGGAACGAAGTTGAACACTTCA) (uzma et al ,. 2013),were used Optimized nPCR reaction for RSV cDNA amplification was performed as follow,3µl of RNA extract was added to 5 µl PCR premix [Maxime PCR premix kit (i-Tag)] containing i-Tag TM DNA polymerase, dNTP mixture and reaction buffer. Two µl of primers and 15µl of distilled water were then added to PCR premix. The PCR program consisted of the following protocol; 94°C for 15 min; 40 cycles of 94°C for 30 s, 63°C for 1 min, and 72°C for 1 min, and final extension step at 72°C for 10 min. The expected amplified amplicon of 772 bp were analyzed by

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electrophoresis and visualized as described above to PCR and nested PCR.

Results

Distribution Respiratory Syncytial virus detected in throat swab Specimens according to genotype.

one hundred outpatients had been suffered from respiratory illness, the throat swab of them were undergone to laboratory examination, the positive samples with Respiratory Syncytial virus were 38(38%), According to genotype of the virus as in Table (2) 24 (24%) were encountered with the RSV-A , and 14 (14%) were detected with RSV- B , This results showed there was statistical significant differences ($p<0.01$) as in table 2.

Table- 2 Distribution of Enteric Viruses Detected in throat swab Specimens of patients Group .

Type Of Virus	Patients Group	
	No. of +ve cases/ No. of tested cases	% Positive
RSV- A	24/100	24
RSV-B	14/100	14

The nested amplicons of 391 bp were visualized by agarose gel electrophoresis as mentioned above as in figure 1.

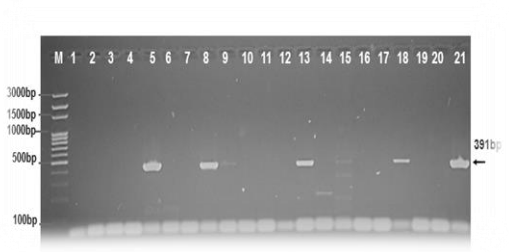

Figure -1: Agarose gel electrophoresis image that showed Nested PCR product analysis for of attachment glycoprotein (G) gene in respiratory syncytial virus genotype A (RSVA). M (Marker ladder 3000-100bp). Lane (1-21) showed some positive RSVA at 391bp product size.

.

The expected amplified amplicon of 772 bp were analyzed by electrophoresis
and visualized as described above to PCR and nested PCR as in figure 2.

Figure - 2: Agarose gel electrophoresis image that showed RT-PCR product analysis for of attachment glycoprotein (G) gene in respiratory syncytial virus genotype B (RSVB). M (Marker ladder 3000-100bp). Lane (1-22) showed some positive RSVB at 772bp product size.

Distribution of RSV infection among patients groups according to six:

Table - 3 demonstrate of RSV - positive in patients group according to gender. The study found that 100 patients with respiratory illness, , the percentage of female infected with RSV-B is higher than male . The patients group by RSV show that (45%) of them are males and (55%) are females This result shows there is statistical significant differences between male and female ($P<0.01$)

Table-3: Distribution of RSV infection among patients groups according to six.

Variable	RSV- Group	
Sex groups	No. of +ve cases	% of +ve cases
Male	17	45
Female	21	55
Total	38	100

Discussion

According to the results of the current study, the infection rate of RSV was 38% using conventional PCR. This result is comparable with several studies conducted in different area such as study done by Al-Charrakh *et al*. (2016) recorded (18.75%) in patients used real time polymerase chain reaction in Wasit city and with Hassan *et al*. (2018) revealed a seroconversion rate of RSV was (20.4%) among patients in the Kurdistan region . The results in the present study are higher than those obtained by Abduljabbar *et al*. (2019) who are indicated that among 150 samples, 26 % positive for RSV infections, and lower compared with data reported in Baghdad by Odisho *et al*. (2009) the percentage is reached to 79% among the patients who have respiratory tract infection.

According to genotybe This study found a remarkably higher rate of RSV subgroup A (24. %) than RSV subgroup B(14%). This is of no surprise because similar results were found in other countries as well Zlateva, *et al*. (2007) , Oliveira *et. al*. (2008) and Fieldhouse et al., (2018).

On the other hand, this result was disagreement with other studies such as a study done by an Al-Mossawi *et al*. (2016) who recorded 8% and 14% of respiratory syncytial virus type A and B respectively, among children suffering from respiratory tract in Al-Amarah city and with study done by a Kenmoe *et al*. (2018) found RSV group A and group B co-circulated in this population at 17.4 and 82.6%, respectively.

This contradiction could be attributed to difference in study design and population, definition of disease severity, the distribution of RSV subtypes (Hirsh *et al*., 2014) And the interplay between host and virus factors, including RSV load (Fodha *et al*., 2007) Also a possible explanation for these alterations is the development of specific immunity against a specific RSV type that is prevalent in the country in the preceding year.

For that, subgroups shift of RSV from year to year that may affect the immunity acquired against the previously circulating viruses (Iwane *et al*., 2013).

According to sex The prevalence of respiratory syncytial virus in the present study among females is (55%) cases are higher than those in males (45%) cases with significant differences between them.This finding corresponds with other previous studies by Reina et al., (2008) which revealed that the gender, females (53.2%) was higher than male and the results match also with Yaser Arjeyni *et al*. (2017) in Iran (62.5%) were female and the rest (37.5%) were males and While the current study is inconsistent with a study conducted by Rodriguez-Fernandez *et al*. (2017) in Texas Zahran *et al*. (2017) in Egypt ,Hassan *et al*. (2018) in Iraq , And Jepsen *et al*. (2018) male were at higher risk of severe RSV infection as compared to female in Denmark.

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