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Detection of human metapneumovirus RNA sequence in nasopharyngeal SWAP sample from children with acute respiratory tract infections in Najaf Province, Iraq

Wurood Kadhim Abed ✉; Humam Kasem Hussein

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Detection of Human Metapneumovirus RNA Sequence In Nasopharyngeal Swap Sample from Children with Acute Respiratory Tract Infections in Najaf Province, Iraq.

Wurood Kadhim Abed[1,a)], Humam Kasem Hussein[2,b)]

[1] *Department of Biology, College faculty of Science, Kufa University, Najaf, Iraq*
[2] *Technical Institute of AL-Najaf, AL-Furat AL-Awsat Technical university, AL-Kufa, Iraq.*

[a)] Corresponding author email: worrod@sadiq.edu.iq
[b)] kuh,hum@atu.edu.iq

Abstract: The purpose of this subject is to identify what is being studied in the article, which is the involvement of human Metapneumovirus in children with respiratory illnesses. During the period November 2020 to February 2021, 100 patients with respiratory tract infections were admitted to Al Zahra Teaching Hospital and AL-Forat AL-Awsat Teaching Hospital in Najaf Governorate. Nasopharyngeal swabs were collected from patients for molecular diagnosis of human metapneumovirus using Real-Time-PCR. The patients were distributed based on age into five groups as follows (Less than one, 1-2, 2-3, 3-4, and 4-5 years), and twenty samples of healthy individuals were approved as a control group without any clinical signs of infection. the children of age group less than one year more vulnerable to infections, their rate significant difference was recorded between boys and girls according to sex distribution ($P<0.005$). There were more boys than girls as a percentage. 53% of the virus was determined to be present, according to this analysis, and the genes in the virus and those discovered in the National Center for Biotechnology Information.

Keywords: Respiratory tract infections, human metapneumovirus, f gene direct in RT-PCR, Sequencing.

INTRODUCTION

hMPV of the paramyxoviridae family and the pneumovirinae subfamily, the human metapneumovirus (hMPV) is an encapsulated single-stranded negative-sense RNA virus (1). Children and elderly patients contract the infection by direct contact with secretions, including saliva and droplet (2). Symptoms begin after the secretion of RNA from 5-14 days, and during the acute phase of infection appear on the patients (fever, wheezing, cough, pneumonia, nasal congestion, sore, purulent cough bronchitis, otitis media, dyspnoea) (3). Van den Hoogen discovered the hMPV in the Netherlands, however, the virus was initially isolated from preserved nasopharyngeal specimens (4). Patients during the presence of the virus have symptoms that may be ambiguous but are consistent with acute bronchitis, older people, and children, especially in cases where they had a chronic illness, should be taken seriously. The virus has a three to the six-day incubation period. (5). There are mainly two major techniques for the detection of hMPV RT-PCR and immunology techniques (6). The purpose of this study is to identify the epidemiological traits of hMPV infections among hospitalized children from Iraq's Al-Najaf who have acute respiratory tract infections (ARTI).

MATERIALS AND METHODS

100 samples were taken from patients with upper and lower respiratory tract infections who were hospitalized at Al-Zahra Teaching Hospital and Al-Furat Al-Awsat Teaching Hospital in the province of Al-Najaf. The average age

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of the patients with respiratory tract infections was 5 years, and there were (37) girls and (63) boys. between February 2021 to November 2020.

Collection of Specimens

Nasopharyngeal swabs were obtained following clinical diagnosis by the specialist physician and stored in container tubes on the virus transport media were transported to the hospital laboratory and kept at the temperature of -80C until the tests.

Nasopharyngeal swabs were obtained for diagnosis by RT-PCR.

Control: Twenty specimens were obtained from healthy children subject to serve as a control group that is without respiratory infections (healthy)

Real-Time PCR Technique

The primers in a table (1), are required for RT-PCR diagnosis of human metapneuom virus Viral RNA was extracted using an ELK Biotechnology viral RNA clean kit by the manufacturer's instructions. The Macro-genre corporation offered one pair of RT-PCR primers for the Fusion gene human Metapneumovirus, which was employed in the amplification of RT-PCR.

TABLE 1. Set primer used RT-PCR fusion gene for hMPV.

Gene of Hmpv	Polarity of primer	Nucleotide position of primer	Product size	sequence size
hMPV f gene	F	3796-3815	465 bp	ATGTTGGAGAACCGTGCGTA
	R	4260-4241		CCCTACTCTGTTGCTGCCAA

Conventional-PCR for Sequencing Analysis

Used Conventional-PCR for detection of gene sequencing from two positive specimens, used (f) gene of hMPV using primer designed in this study to found out how well the specimens are compatible with isolates in the gene bank, table (2) shows instrument conditions used with Conventional-PCR.

TABLE 2: program the conventional-PCR instrument conditions.

Step	Temperature	Time
1- cDNA synthesis	42◦C	5min
2- inactivate RT	95◦C	4min
3- Denature	95◦C	2min
4- Annealing	58◦C	30sec
5- Extend	72◦C	1min

RESULT

Demographic Study

Distribution According to Age:

The study's analysis of 100 patients with acute respiratory tract infections revealed a significant difference between those who had positive and negative hMPV cases ($P < 0.005$). The samples are separated into five categories: 0–1, 1–2, 2–3, 3–4, and 4–5 years).

TABLE 3. The association between the infection with hMPV and age groups

Age (years)	N0.	hMPV positive children PCR	hMPV negative children PCR	Total %	P- Value
>1 Year	Count	20	20	40	0.904 no. sig.
	%	37.7%	42.6%	40%	
(1-2) Years	Count	9	9	18	
	%	17%	%19	18%	
(2-3) years	Count	9	5	14	
	%	17%	10%	14%	
(3-4) years	Count	6	6	12	
	%	11%	12%	12%	
(4-5) years	Count	9	7	16	
	%	17%	14%	16%	
	p-value	0.005*	0.005*		

*statistical signification

Distribution According to Gender:

In this study, the distribution according to gender with hMPV infected cases were including positive children infection hMPV, and negative infection children hMPV.

TABLE 4. The association between the infection with hMPV and gender

Gender	No.	hMPV Positive (PCR+)	hMPV negative (PCR -)	Total %	P- Value
Boys	Count	32	29	61	0.005
	%	60%	61%	62%	
Girls	Count	21	18	39	
	%	40%	38%	38%	
	P-Value	0.005*	0.001*		

Detection of Human Metapneumovirus (hMPV) by Real-Time PCR:

Total of 100 specimens of nasopharyngeal swabs from suspected patients with human Metapneumovirus(hMPV) suffered from upper and lower respiratory tract infections (bronchiolitis and pneumonia), specimens were examined by RT-PCR from which (53%) specimens were 53% positive, and (47%) were100 specimens were the negative result for this test as illustrated in figures (1) and (2).

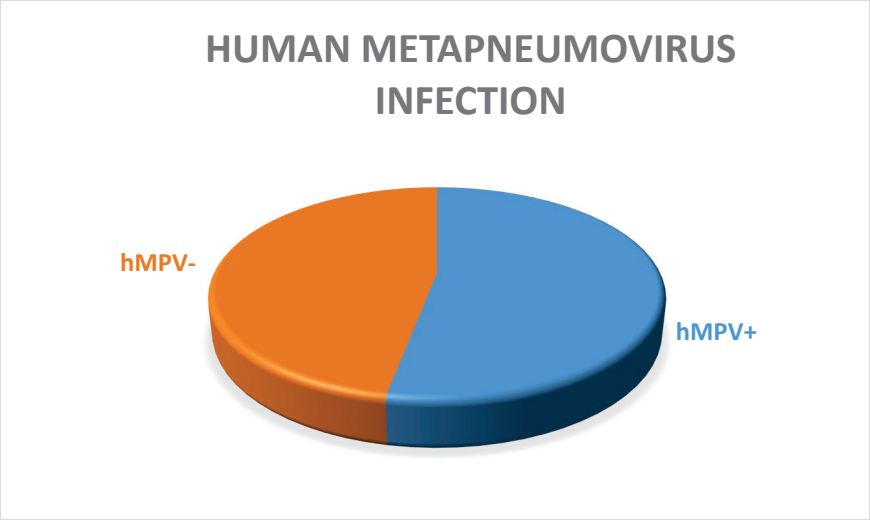

FIGURE 1. Distribution of patients infected with hMPV according to RT-PCR.

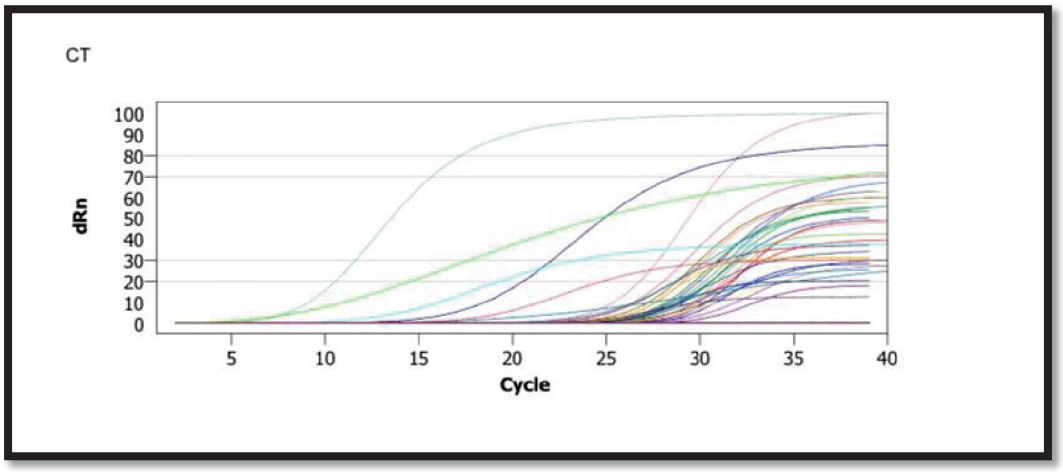

FIGURE 2. RT-PCR amplification plot of hMPV from nasopharyngeal swabs specimens. where the tested specimens were the positive reaction

Sequence Analysis

RNA sequencing was performed for detection of human Metapneumovirus in two positive nasopharyngeal swab specimens by conventional PCR after sequencing of the 465pb PCR product fig (3), F gene was intended for the macro-gene company in South Korea for processing RNA sequencing by applied biosystem in national center bioinformatics as a shown appendix (1,2).

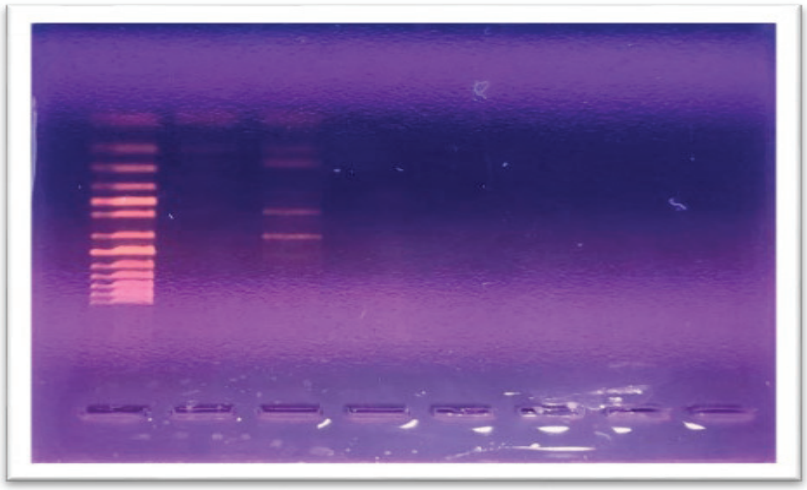

FIGURE (3): Sequence of (f) primer under UV light, which indicates the 465bp (shows the two positive specimens used Conventional-PCR).

Identification of Sequence Analysis:

Table (5): identification Results of Nucleic acid *Fusion* primer (f) which were two local isolates by *human Metapneumovirus* gene sequencing and alignment with other global isolates in GenBank.

TABLE (5): Identification sequence of fusion primer

Isolate NO.	Max Score	Total Score	Id%	Submission number
(1) s-Sf	92.4	92.4	98.1%	SUB9732745
(2) s-sR	92.4	92.4	98.1%	SUB9732990

DISCUSSION

This finding was similar to that of Vicente et al research (7), who found a substantial difference between the positive and negative groups when it came to hMPV. According to the findings, the percentage of hMPV-positive cases was greater in the group of babies aged less than one year, as shown in table (4); this percentage was consistent with work of Edwards et al (8).

The reasons may be that infants are more affected than other groups due to an immature immunity and the failure of the respiratory system and lungs to reach full maturity (9). The reason for the significant difference in hMPV infection between the 24 months and 5-year groups, according to (10), is that the axis of the bronchioles in an infant is much narrower than in older children, making it more susceptible to obstruction, which leads to difficulty breathing and immune system maturation. The risk factors for infection with respiratory infections include poor nutrition, as well as lack of personal hygiene or, maybe due to infection through contact with children or even adults infected with one of the respiratory viruses, in addition to their exposure to tobacco smoke and not to get vaccinations regularly (11).

This finding was comparable to that of Schuster et al (12), who detected substantial differences between both groups of hMPV positive and negative children.

The reason for this appears may be anatomical: males' airways are shorter and narrower, making them more susceptible to acquiring bronchial blockage as a result of hMPV infections (13). In the Canadian PICNIC trial, a boy's sex was found to be a substantial and independent risk factor for hMPV, and RSV-related hospitalization (14,15). In the may opinion that boys were more than females due to their lack of the X chromosome, which aids in the development of respiratory disorders, while females have an additional X chromosome that carries genes that give

immunity and play a role in respiratory tract development. In Iraq, children infected with hMPV accounted for 40% of upper and lower respiratory tract infections when evaluated by RT-PCR, according to a study conducted in 2017 (16). The N gene was found to be more conserved in the whole genome sequence of four strains of hMPV, followed by the F gene. Most genotypes were affected by changes in the sequence of the G and SH genes, and the regions between the G, SH, and I genes were extremely long (200nm), indicating that the N gene does not have a specific function but rather indicates a broad biological role in the hMPV genome (17). This result explains why the f gene was chosen in the study. The RT-PCR method is regarded as the best approach currently available for diagnosing viruses, particularly the hMPV virus. Because it is a sensitive and accurate method, it was employed by introducing the F gene into hMPV (18). N-linked carbohydrates, which are frequently necessary for protein folding or control of fusion, are changed on the trimeric type I integral membrane protein known as F protein. Additionally, precursors of fusogenically inert F proteins are produced, and these precursors are eventually cleaved to generate a physiologically active (19). f protein is included in the development stage of a vaccine against hMPV and has distinctive properties that add to the distinct functional and immunological differences between hMPV proteins (20).

CONCLUSION

1. The gene sequence of F primer was similar to global isolates and there were no isolates recorded for NCBI in Iraq.
2. The age group less than one year was more susceptible to the infection, while the severity of the infection decreased as the age increased.
3. In this study that there is a significant difference based on sex distribution while there is no significant difference in geographical distribution.

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