Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 8341 - 8349
Received 25 April 2021; Accepted 08 May 2021.

Molecular Study of Human *Metapneumovirus* in Children among Respiratory Tract Infection in Najaf Province, Iraq

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Abstract:

humanMetapneumovirus(hMPV) is a paramyxovirus that is an uncommon cause of bronchiolitis and pneumonia in children, elderly and immunocompromised patients. This cross-sectional study was conducted in the Najaf province throughout from November 2020, to February 2021, a total of 100 patients suspected of having RTIs, this study including ages from less than one to five years. One type of respiratory sample was collected from nasopharyngeal swabs, Real-Time-PCR assay was used for molecular detection of hMPV by Fusion (F) gene primer, the result revealed that out of 100 specimens, 53% specimens were positive hMPV, the specimens were divided based on age groups, gender, geographic distribution, and feeding pattern, after that the ages were divided into five categories, less than one, (1-2),(2-3),(3-4), and (4-5) years, and the no significant difference were found age distribution and the (P>0.005), the highest rate of infection with hMPV was the group less than one year, which was (37.7%), compared to other groups. The results were also shown according to the gender distribution of the boys were more infected with the hMPV compared to girls, where was their (60.4%), and there was a significant difference (p<0.005). however, there were no significant differences between the geographical distribution and feeding pattern where (p>0.005).

Keywords: human Metapneumovirus, F gene direct in RT-PCR, Respiratory tract infections.

1-Introduction:

humanmetapneumovirus(hMPV) is enveloped single-stranded negative-sense RNA virus is a member of the paramyxoviridae family and subfamily pneumovirinae (1). The children and elderly patients infected transmitted by direct contact with secretions, involving droplets and saliva (2). Symptoms begin after the secretion of RNA from 5-14 days, during the acute phase of infection appear on the patients (fever, wheezing, cough, pneumonia, nasal congestion, sore,

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purulent cough bronchitis, otitis media, dyspnoea)(3). The hMPV was identified in Netherland by van den Hoogen however the virus was first isolated from stored nasopharyngeal (4). Three to six days incubation period of the virus, while persons and adults during the presence of the virus have symptoms may be unclear but the typical symptoms of acute bronchitis, persons older and children serious especially for cases as they had the chronic disease(5). There are mainly two major techniques for the detection of hMPV RT-PCR and immunology techniques(6). This study aims to determine the epidemiological characteristics of hMPV infections among hospitalized children with acute respiratory tract infections(ARTI) from AL-Najaf of Iraq

2-Material and methods:-

2-1-Specimens collection:-

One hundred clinical samples were randomly collected from nasopharyngeal swabs in AL-Zhara Teaching Hospital and Middle Euphrates Teaching Hospital from different areas of Al-Najaf city, samples were collected during a period from (6 November 2020 to 2 February 2021). Population groups subject groups were distributed into five groups this distribution was made depending on age and clinical status of both gender sixty-one male while female thirty-nine cases.

2-2-Real-Time PCR technique:-

Diagnosis of *human Metapneuomvirus* by RT-PCR design relying on primers in a table (1). RNA extraction viral RNA was extracted by using a viral RNA clean kit (ELK biotechnology) according to the manufacturer's protocol. One set of RT-PCR primers for Fusion gene *human Metapneumovirus*have been used in the amplification in RT-PCR, the primer was provided by Macro-genre company.

Table (1):-Set primer used RT-PCR Fusion protein gene for hMPV(7)

Gene	Polarity of primer	Nucleotide position of primer	Amplicon size	Primer sequence
hMPV f gene	F	3796-3815	465	ATGTTGGAGAACCGTGCGAT
	R	4260-4241		CCCTACTCTGTTGCTGCCAA

3-RESULT:-

3-1- Detection of human Metapneumovirus (hMPV) by Real-time PCR

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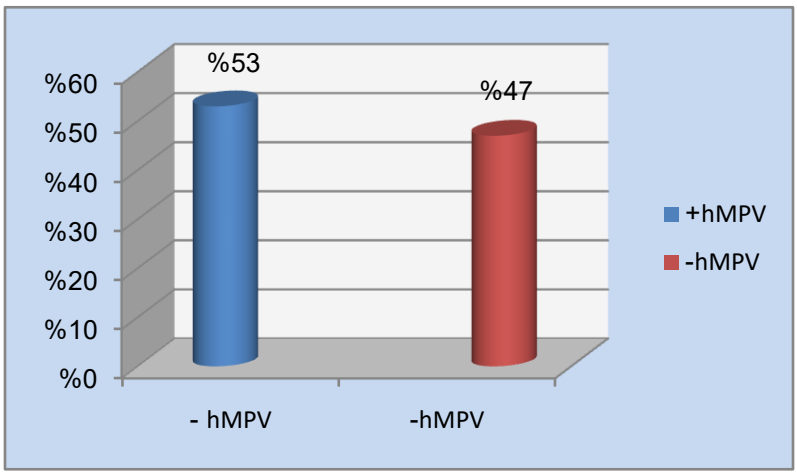
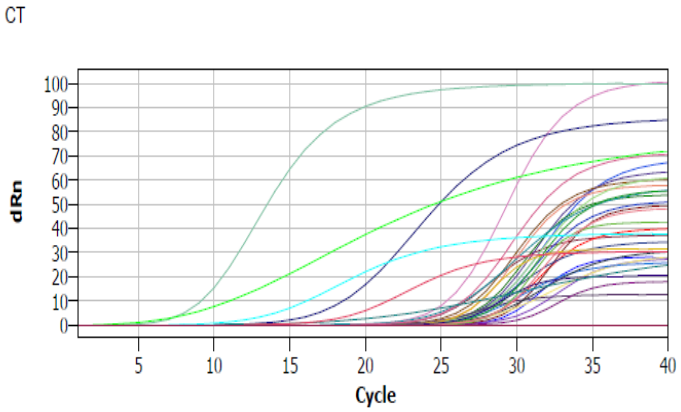
The results revealed a total of 100 sample nasopharyngeal swabs of suspected human Metapneumovirus(hMPV) infected upper and lower respiratory tract infections (bronchiolitis and pneumonia) patients were subjected to fusion protein(F) in RT-PCR from which 53% gave a positive hMPV, and (47%) negative hMPV, figure(1), and figure (2) appears to show study samples according to RT-PCR.



Fig(1):- the distribution of patients according to RT-PCR.



Fig(2):- RT-PCR amplification plot of hMPV from nasopharyngeal swabs sample where the tested samples were the positive reaction

3-2-Age distribution:- A total of 100 patients with upper and lower respiratory tract infection enrolled in the study showed that a significant difference was observed between patient's positive and negative cases hMPV ($P<0.005$), as in table (2) shown the age distribution.

Table(2):- The association between the infection with hMPV and the age.

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Age groups	hMPV+ children	hMPV- children	Total	p- value
<1 year	20 (37.7%)	20 (42%)	40 (40%)	
(1-2) years	9 (17%)	9 (19.1%)	18 (18%)	0.904
(2-3) years	9 (17%)	5 (10.6%)	14 (14%)	no.sig
(3-4) years	6 (11.3%)	6 (12.8%)	12 (12%)	
(4-5) years or equal 5 years	9 (17%)	7 (14.9%)	16 (16%)	
p-value	0.002*	0.003 *		
* statistical significance				

3-3- Sex distribution:-

In this study, the sex distribution with hMPV infected cases was including boys (60.4%), and girls(39.6%), as in table (3). statistical difference between sex distribution of inpatients with hMPV positive and negative children($P<0.05$).

Table(3):- The association between the infection with hMPV and the sex

Gender	hMPV+ children	hMPV- children	Total	P-value
Boys	32 (60.4%)	29 (61.7)	61 (61%)	0.892
Girls	21 (39.6%)	18 (38.3%)	39 (39%)	no.sig
P-value	0.005*	0.001*		
* statistical significance				

3-4- Geographical distribution:-

The geographical distribution for 100 patients suffers from respiratory tract infection(upper or lower respiratory tract infections) including in this study explained in the table (4). Depending on the home address, children with hMPV were in urban (54.7%) while in rural were (45.3%). There was no statistically significant difference regarding geographical distribution for hMPV

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positive and negative cases (P>0.005). but there was a significant difference between the negative cases (p<0.005).

Table(4):- The association between the infection with hMPV and the geographical.

Residence	hMPV+ children	hMPV- children	Total	P-value
Urban	29 (54%)	33 (70%)	62 (62%)	0.111 no.sig
Rural	24 (45.3%)	14 (29.8%)	38 (38%)	
P-value	0.492 no.sig	0.001 *		

3-5-Feeding practice distribution:-The study found that the groups of hMPV-positive and negative children, found statistical difference feeding practice (p<0.005) as in table(5).

Table (5):- The association between the infection with hMPV and feeding practice.

Feeding practice	hMPV+ children N=53	hMPV- children N=47	Total
breastfeeding	7 (13.2%)	11 (23.4%)	18 (18%)
Formula-feeding	16 (30.2%)	14 (29.8%)	30 (30%)
Breast-formula-feeding	9 (17%)	6 (12.8%)	15 (15%)
Family feeding	21 (39.6%)	16 (34%)	37 (37%)
P-value	0.002 *	1.8 no.sig	
* statistical significance			

4- Discussion:-

The importance of this study surely from its in AL-Najaf province and its comprehensiveness as it covers the most non-common viruses causing RTIs that are detected by recent molecular techniques. annual specific precautions had usually adopted against RTIs during the winter season that necessitates preparedness of health authorities before adequate time among exacerbating factors is the family crowdedness plus the majority of Iraqi families still use the

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kerosene heaters as a home warming system(8). These heaters are sharply elevating the home temperature as it lacks sensory control plus the expulsion of irritant gases for the respiratory tract(9).Furthermore, viral RTIs are frequently exacerbated by superimposed bacterial pathogens that may progress to LRTIs and pneumonia Additional worsening factors include the presence of displaced and refugee peoples who complain about bad living conditions besides the general neglect of their health status. In this regard(10).

Regarding the age at infection, that the majority of children less than 5 years in Europe and North America have been infected with hMPV, probably because they are highly susceptible to infections with this virus additionally, the current results are consistent with most previous studies suggesting that the infection with hMPV was a highest among children <5 years old(11). Decreased gradually with increased age, even though some of these studies were reported a few cases of hMPV infection in other age groups(12).

As for the sex distribution, therefore, seems to be anatomic that boys have shorter and narrower airways and are more likely to develop a bronchial obstruction in the case of hMPV infections the percentage of boys was higher than girls, and the reason is attributed(13). Who found that boys were more than female, also the reason it is due to their lack of the X chromosome, which helps in the development of respiratory diseases in them, and the female have an additional X chromosome which carries genes that provide them immunity and play the role of development of the respiratory tract(14).

There was been an increase in the percentage of children with hMPV infection in urban because one of the reasons for the increase in the number of children infected with viruses in the respiratory tract in the urban the infected children were together in the nursery and kindergarten(15). Pollution of an environment may be the reason for increasing their infection children rate with hMPV Exposure to indoor air pollution has 2.3 times increased risk of respiratory infections predominantly, LRTI(16).

About the feeding pattern of children, the results of this study were that children who depend on formula-feeding were more susceptible to infection than other groups Breastfeeding is included among the protective factors for respiratory infections in infants. The protective role of breastfeeding against respiratory infections has been repeatedly demonstrated for children living

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in developing countries although breastfeeding is described as protective also in industrialized countries(17). Infants hospitalized with hMPV bronchiolitis often have decreased nutritional intake due to respiratory distress and tachypnea with increased insensible losses and will need fluid and nutritional support. Continued oral feeding in the presence of significant tachypnea and respiratory distress is known to increase the risk of aspiration(18). Breastfeeding has numerous benefits for both infant and maternal health with formula-fed infants at increased risk for gastrointestinal, respiratory, and ear infections, obesity, and atopic disease(19). Risk of hospitalization for bronchiolitis. Breastfeeding, even in association with formula milk, reduces the risk of hospitalization for bronchiolitis during the first year of life. Encouraging breastfeeding might be an effective/inexpensive measure of prevention of lower respiratory tract infections in infancy(20).

Conclusion:-

1- The study concluded that hMPV has an important role as a viral cause of the high rate of RTIs in the Najaf community, especially among children.

2- The variation of hMPVcan be observed in age groups where the highest infection was less than years, in this study was the percentage of boys was higher than girls.

3- in this study we found the effect of infection on the geographical distribution of patients, as well as there was an effect of breastfeeding on hMPV infection.

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