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Molecular study of human metapneumovirus among children under 5 years with acute respiratory tract infection in Basrah city, Iraq

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ABSTRACT

Human metapneumovirus (hMPV) is a major global factors that contributes to children getting acute respiratory tract infections (ARTI). This virus is frequently present in paediatric patients, with children under the age of two being particularly vulnerable. However, they are very common from early childhood until age five. Two hundred oropharyngeal swabs were collected from children with respiratory tract infections (RTIs), between December 2020 to December 2021 and were tested for hMPV. The database registered in the work included the sex, age, the season in which the specimen was obtained, feeding style, and the mother's education of infected children as well as major clinical respiratory tract infection symptoms. A molecular method was used to identify the presence of hMPV, and the results showed that 13% of the 200 samples tested positive for the virus. The samples were further broken down based on age groups, sex, season, feeding style, and mothers' education. According to age, the infection rate was noticed high in the age group (2-24 months) which was (13.7%) compared with (25-60 months) age group (12.4%) and there is no significant difference. The results were also shown according to the sex distribution, the infection with hMPV was more in males (14.8%) rather than females (9.7%). But this difference was not significant ($P > 0.05$). The majority of cases of hMPV occur in the winter and spring with a rate (15.8%), followed by (11.1%) in autumn. While 0 (0%) in summer. There was no noticeable difference ($P > 0.05$). The majority of hMPV infections have been identified with a rate (18.2%) in mixed feeding, followed by (13.0%) in bottle feeding and (10%) in breastfeeding. There was no significant difference ($P > 0.05$). while the distribution of infection hMPV against mother's education was as follows: 13.2% in 7-12 years and 12.9% in ≤6 years and 12.8% in ≥ 13 years.

Keywords: *Factors, Infections, Work, Results*

INTRODUCTION

Viral respiratory infection (VRI) describes the group of clinical conditions caused by a spectrum of viruses infecting the respiratory tract and are ubiquitous in the community (Heikkinen and Järvinen, 2003). The viruses most common factor of illness in children, and infants as well as major public health issues. Because of their widespread prevalence, transmission simplicity, and high morbidity and mortality rates (Tregoning and Schwarze, 2010; Mahony et al., 2011). Human metapneumovirus (hMPV) is one of the main global factors that contributes to children getting acute respiratory tract infections (ARTI) (Divarathna et al., 2021). This virus is frequently presentin paediatric patients, with children unde r the age of two being particularly vulnerable (Boivin et al., 2002). However, they are very common from early childhood until age five (Gálvez et al., 2021). The hMPV genus belongs to the genus Metapneumovirus, subfamily Pneumovirinae and family Paramyxoviridae (Fouquet et al., 2006). The hMPV is negative-stranded, and non-segmented and the viral RNA is around 13 kb long (Ji et al., 2021; Kaida et al., 2006). The typical shape of the hMPV is spherical, with an envelope having a diameter of 150–200 nm (Peret et al., 2002). The hMPV is an important viral agent associated with severe bronchiolitis and pneumonia in children (Zhang et al., 2018). Patients experience symptoms between 5 and 14 days after the release of RNA, which happens during the acute phase of the infection (fever, wheezing, cough, nasal congestion, sore throat) (Hall et al., 2011). In recent years, the polymerase chain reaction (PCR) has been used more and more in molecular diagnostics to the point where it is now regarded as the gold standard for detecting nucleic acids from various sources and is indispensable tool in the research lab. Also, the Real-time PCR has become more popular due to its enhanced reproducibility, speed, sensitivity, and low risk of carry-over contamination (Almayahi et al., 2022; Mackay et al., 2002). The objective of this study was to analyze the frequency of hMPV infection among children under 5 years of age in Basrah city south of Iraq.

MATERIALS AND METHODS

Patients and study design

A total of two hundred oropharyngeal swabs were collected from children who have been suffering from respiratory tract infection (RTI), the database of those infected children has been registered in the present work, involving the patient's name, their age, sex as well as season and major clinical RTI symptoms, like the cough, fever, sore throat. The selected patient's age was below five years old both sexes, from Basrah Maternity and Childre Hospital in Basrah city between December 2020 to December 2021. Every patient's information has been obtained according to a questionnaire format after obtaining the consent of the parents.

Samples collection

Clinical samples have been oropharyngeal swabs, obtained once from every one of the infected children. The samples were collected by inserting fine stick sterile plastic with vials containing viral transport media (VTM), which were made especially for this, were given to the participants. These vials were then transported to the lab and stored there at 70°C until use.

RNA extraction

Viral RNA was extracted from oropharyngeal swabs using QiaAmp Viral Mini Kit (Qiagen) and prepared depending on the manufacturer ’s instruction.

Reverse-transcription polymerase chain reaction (RT-qPCR)

RT-qPCR was performed for the diagnose of human metapneumovirus based on the F gene and this technique was carried out according to the method described by the manufacturer's instructions. RNA specimens after extraction were then tested by RT-qPCR for the presence of hMPV. RT reactions were performed with QuantiTect Reverse Transcription Kit (Qiagen, Germany), according to the manufacturer’s specifications. Each cDNA was subsequently tested by real time-PCR with add SYBR Master (Add bio, Korea), according to the manufacturer's specification. The primer used in the testing of the respiratory specimens was provided by (Scientific research. Company, Iraq) in the following table (1).

TABLE 1: Primers used for the real-time polymerase chain reaction.

Virus	Gene	Primer sequence (5′ to 3′)	Reference
HMPV	F	F: CAAGTGTGACATTGCTGAYCTRAA R: ACTGCCGCACAACATTTAGRAA	(Zhou et al., 2018).

The conditions of the real-time PCR thermocycler are shown in table (2).

TABLE 2: Real-time PCR thermocycler conditions.

The qPCR steps	Temperature	Time	Repeated cycle
Initial Denaturation	95 °C	10 min	1
Denaturation	95 °C	10 sec	40
Annealing\Extension Detection(scan)	55 °C	30 sec	

RESULTS

Detection of HMPV from children with RTI

From December 2020 to December 2021, a total of 200 oropharyngeal samples were collected from children with respiratory infections, the hMPV was identified in 26 (13%) samples by using molecular methods.

Age and sex distribution of HMPV infection

Table (3), shows the distribution of hMPV infection according to the sex and age of the patients. Infection with hMPV was more in males (14.8%) than females (9.7%). The infection rate was noticed high in the age group (2-24 months) which was (13.7%) compared with (25-60 months) age group (12.4%). But this difference was not significant (P > 0.05).

TABLE 3: Distribution of human metapneumoviruses in children with RTIs according to sex age groups.

Variable factors		Total No.	No. of positive	%	P-value
Gender type	Male	128	19	14.8	0.301
	Female	72	7	9.7	
	Total	200	26	13	
Age groups	2-24	95	13	13.7	0.784
	25-60	105	13	12.4	
	Total	200	26	13	

HMPV infection seasonal distribution

A relationship between the infection of the hMPV and seasons was shown in table (4). The large percentage of hMPV infections occurred during the winter and spring seasons with rate (15.8%), followed by (11.1%) in autumn. While 0 (0%) in summer. There was no significant difference (P > 0.05).

TABLE 4: Distribution of hMPV infection in children with RTI according to seasons.

Season	Total No.	No. of + ve	%	P- value
Winter	76	12	15.8	0.227
Spring	57	9	15.8	
Summer	22	0	0	
Autumn	45	5	11.1	
Total	200	26	13	

Distribution of HMPV-positive cases against feeding style

Association between feeding pattern and hMPV infection was done on infected age group (2-24) months as seen in table (5), and found that hMPV has been identified with rate (18.2%) in mixed feeding, (13.0%) in bottle feeding and (10%) in breastfeeding. There was no significant difference (P > 0.05).

TABLE 5. Distribution of hMPV infection in children with RTI according to feeding style.

Style of feeding	Total No.	No. of + ve	%	P- value
Breast	30	3	10	0.690
Bottle	46	6	13.0	
Mixed	22	4	18.2	
Total	98	13	13.3	

Distribution of HMPV positive cases against Mother's education

Table (6) indicates the relationship between education and HMPV infection in children with RTI. As seen in table (4.) the distribution of infection hMPV was as follows: 13.2% in 7-12 years and 12.9% in ≤6 years and 12.8% in ≥ 13 years of eduction.

TABLE 6: Distribution of hMPV infection in children with RTI according to parents' education.

Mother's years of education	Total No.	No. of + ve	%	P- value
≤6 years	85	11	12.9	0.997
7-12 years	68	9	13.2	
≥ 13 years	47	6	12.8	
Total	200	26	13	

4. DISCUSSION

The importance of this study aimed to describe the detection of hMPV infection in children under 5 years old who were suffering from RTIs in Basrah city by recent molecular techniques. hMPV was identified in 26 (13%) cases of clinical RTIs. However, in the current study, the frequency of sole infections was lower than (28.20%) in Al-Najaf city by Al-Hisnawi et al. (2022), and higher than another study from Saudi Arabia, by Alsuheel et al. (2019), who revealed a detection rate of HMPV (9.9%). While, our result is similar to rate in Kurdistan (13.4%) from hospitalized children (Hassan et al., 2018). The wide difference in detection rates in the literatures, probably due to heterogeneity in study designs, type of respiratory specimens and detection protocols (Hassan, 2015). the ratio of the hMPV infection in males was more than females in our study but this finding was not statistically significant. Also, there is a report from Latin America, in 2011, they observed no difference in the prevalence among the sexes (Garcia et al., 2012). In some studies, more patients with hMPV infection were found in boys than in girls (Van den Hoogen et al., 2003; Peiris et al., 2003), whereas others have not (Boivin et al., 2003). Children 2 to 24 months old have a higher rate of hMPV infection than older children, according to our research. Our findings are equivalent to Freymuth et al. (2003) and Esper et al. (2003), who examined the hMPV in respiratory specimens from children who were negative for other respiratory viruses. In both of those studies, more than half of the hMPV cases involved infants aged 3 to 24 months. The most detectable samples of hMPV came from children under the age of two in a study by Van Den Hoogen et al. (2003) from The Netherlands. Based on statistical analysis, there was no significant association between hMPV infection and age. Usually, hMPV infections are reported during winter-spring and autumn but didn't detect in summer. A similar observation was made in the study by Ramaekers et al. (2017). While Hassan et al. (2018) showed that the peak season for respiratory viruses in Kurdistan, late autumn to early spring, coincided with the majority of

respiratory infections in children were recorded. In Iran, Moattari et al. (2010) found that the rate of hMPV cases was rising in the winter and spring. The prevalence of HPMV was high in children who depend on mixed feeding. But there is no significant difference between the type of feeding and viral infection. These findings in agreement with the results found by Al-bahadily et al. (2017) and Etiler et al. (2002) found that there was no statistical association between ARI and late weaning time. While a study by Kadhim Al-Jaferi (2014) concluded that early weaning from breastfeeding before 6 months, the use of weaning food, and formula feeding are all significantly linked to ALRTI. 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 (Lanari et al., 2013). Moreover, the rate of infection in our study for HPMV was higher in children whose mothers had 7-12 years level of education and there was no significant difference between viral infection and mothers' education. As the level of education increases, one expects that the person will adopt a positive attitude toward protecting and preventing their children from respiratory viral infection by taking for example seasonal influenza vaccine and this agreement with the study by Hameed and Jubair (2021) in Iraq (Hila city), found there was no significant relationship between parents' attitude and educational level of parents. The study by Salem et al. (2002) found that parents with low levels of education were linked to wheezing, but the findings were not statistically significant. But, Hassan et al. (2001) recorded low parental education, as significant risk factors were younger age (2–6 months). According to the study's findings, concluded that hMPV has an important role as a viral cause of the high rate of RTIs in Basrah city, especially among children. No variation of hMPV can be observed in age groups, where, in this study, the percentage of boys was higher than girls. In this study, the seasonal variation of hMPV was seen. While mothers' low educational level and style of feeding were associated with a viral infection, the results were not statistically significant.

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