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Molecular detection of Human metapneumovirus in children with RTI in Al-najaf city

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ABSTRACT

Human metapneumovirus is one of the respiratory viruses with increasing importance in clinical field. The present study does focus on the incidence of human metapneumovirus (HMPV) among hospitalized pediatric patients suffering from respiratory tract infection. The patients were recruited from inpatients of Al zahraa hospital, Najaf City, Iraq. Studies conducted in various countries all over the world has verified that the human metapneumovirus (HPMV) is the most etiological agent of respiratory infections among children. In this context, this study encompassed 156 children suffered from respiratory infections with clinical manifestations like cough, wheezing, shortness of breath, fever, rhinorrhea, and nasal congestion. The recruited patients were those routinely do visit the external clinics of Al zahraa hospital, Najaf City from September 2020 to April 2021. The presumptive diagnosis by the physician was viral respiratory infection with human metapneumovirus (HPMV). Data of participants including clinical symptoms, age and family history were collected by using a questionnaire specially designed for this purpose. Molecular detection and genotyping of HMPV from patient with respiratory tract infections in AL-zahraa hospital, Najaf city, Iraq. One hundred fifty six nasopharyngeal swabs samples were suspected of having respiratory tract infections of different ages were enrolled, Real-time PCR assay was used for molecular detection of HMPV, and one PCR primer set for Fusion (F)gene of HMPV has been used in order to get PCR products used in the sequencing method for genotyping of the virus and phylogenetic tree analysis. Statistical analysis for all data was done using Statistical Package of Social Sciences (SPSS), version 27, (Inc., Chicago, IL, USA) computer software. Statistical comparison between study groups analyzed using chi-square test and T test. $P<0.05$ was regarded as statistically significant (Al-Ukaelii and Al-Shaeb, 1998). Molecularly, the results revealed that out of 156 specimens, 44(28.20%) specimens were positive for HMPV, with higher in ages less than 1 year 24 (54.5%). clinically, Dyspnea 40(90.9%) and cough 30(68.1%) were associated with HMPV. Genotypically, 9 HMPV positive samples were showed good (F) gene sequences that submitted to NCBI. A phylogenetic tree was constructed to portray the genetic relatedness among the nucleotides sequences of fusion protein F isolated from the nine strains of human metapneumovirus (HPMV). The constructed Neighbor joining tree reflected the speciation of the nine strains of human metapneumovirus (HPMV) into five groups namely 1-5. Group1 included human metapneumovirus (HPMV) –HW-4 and human

metapneumovirus (HPMV)-HW-8. Group 2 encompassed human metapneumovirus (HPMV)-HW-2 and human metapneumovirus (HPMV)-HW-7. Group 3 included human metapneumovirus (HPMV)-HW-1 and human metapneumovirus (HPMV)-HW-3. However, group 4 included human metapneumovirus (HPMV)-HW-6 and human metapneumovirus (HPMV)-9. The group 5 included human metapneumovirus (HPMV)-HW-5. Only HW-4 could be genotyped as genotype A2, whilst, the other four remaining strains HW-1, HW-2, HW-7, and HW-9 could not be genotyped. Despite the potential of fusion protein F in the infection process of human metapneumovirus (HMPV) as reported by several studies, four strains namely HW-3, HW-5, HW-6, and HW-8 proved to harbor non-functional fusion protein. These results would necessitate further confirmation by repeated sequencing through at least three runs to verify the non-functionality of fusion protein in these human metapneumovirus (HMPV) strains. Otherwise, a conclusion addressing the presence of other proteins or factors contributes greatly in the infectivity and attachment-entry of the virus inside host cells might be outlined. Another approach to unveil the mystery of the presence of non-functional fusion proteins among virulent Human metapneumovirus (HMPV) strains isolated from cases with acute symptoms is monitoring the expression of the fusion protein in these strains on both transcriptional and translational level in further studies.

1. INTRODUCTION

HMPV is a Pneumoviridae virus that is negative-stranded and non-segmented. Furthermore, the viral RNA is around 13 kb long and consists of eight genes (P, N, F, M, SH, M2, L, and G) that code for nine different proteins. HMPV is divided into genotypes B and A, each of which has two sub-types (B1 and B2; and A1 and A2), according to an evolutionary analysis regarding several genes. The major antigen of hMPV is the fusion protein which is encoded via F gene, and it is commonly utilized for typing. Multiple sub-types of HMPV might exist at the same time, while the dominant types might be changing over time [1].

HMPV was assigned to the Mononegavirales order within the Paramyxoviridae family. Which is categorized to 2 sub-families; which include the Paramyxovirinae and Pneumovirinae of which the HMPV belongs to Metapneumovirus genus [2].

HMPV is most commonly transferred from infected individual to others via secretions from sneezing and coughing, close personal contact, like shaking hands, and touching surfaces or objects which have the viruses on them before touching the eyes, nose, or mouth. HMPV is circulating throughout the United States in various annual seasons. The circulation of HMPV starts begins in the winter and continues till or through spring [3].

Human metapneumovirus (hMPV) is one of the major causes of acute respiratory tract infections (ARTI) in childrenworldwide. hMPV has two major types, A and B and four subtypes, A1, A2, B1, and B2.hMPV A2 is further divided into three sub- genotypes, A2a, A2b and A2c. This study was undertaken to determine the distribution, seasonality and subtypes of hMPV circulating in children [4].

The most severe symptoms associated with hMPV infections are often reported in infants between younger than one year old, but are highly prevalent during early childhood up to five years old. High-risk factors in infants include asthma, preterm birth, and previous infections with other respiratory viruses, such as the human respiratory syncytial virus (hRSV, recently renamed human orthopneumovirus), and these predispose infants to a more severe disease manifestation after an hMPV infection [5].

2. Materials and Methods

2.1 Patients and study design

A total of one hundred fifty six inpatients of children who have been suffering from the respiratory tract infection (RTI), the data-base of those patients has been registered in the present work, involving the patient's name, their age, gender as well as major clinical RTI symptoms, like the cough, fever, nasal discharges (i.e. rhinorrhea), sneezing, and asthma attacks which have been principally evaluated, by pediatricians consultant through taking main clinical characteristics of asthma, encompassing (dyspnea and wheezing), and 50 healthy pediatric control. The selected patients' age was from 6 months to 12 years old of both genders, from Hospitals at Al najaf city between Dec. 2020 and Apr. 2021. Every patient's information has been obtained according to a questionnaire format.

2.2 Samples collections

Clinical samples have been nasopharyngeal swab, obtained once from every one of the patients through inserting fine stick sterile plastic, provided with a VTM (i.e. viral transport medium), to nasopharynx, to the point where a stick is challenging, after that, the stick has been gently withdrawn and put afterwards on VTM that has been prepared for that purpose, after that, samples have been transported with ice bag from hospital to department of the blood bank to be stored under a temperature degree of -70^{o}C.

2.3 Statistical Analysis

Statistical analysis for all data was done using Statistical Package of Social Sciences (SPSS), version 27, (Inc., Chicago, IL, USA) computer software. Statistical comparison between study groups analyzed using chi-square test and T test. $P<0.05$ was regarded as statistically significant (Al-Ukaelii and Al-Shaeb, 1998).

2.4 Primers

These primers were considered by using the complete sequence of fusion protein (F) gene in hMPV by (reference; almossawi et al., 2016) and that used in PCR technique and DNA sequencer for hMPV genotyping study. The primers were delivered by (Bioneer. Company, Korea) as following in the table 1:

Table (1): Primers sequences of genes:

Genes	Primer sequence (5′-3′)		amplicon
F gene primer	F	5′-TGATGTTGGAGAACCGTGCA-3′	428bpbp
	R	5′-GTAGCAAGCAACCAAAGCCC-3′	

F: Foreword, R: Reverse

3. Result

This study encompassed 156 children suffered from respiratory infections with clinical manifestations like cough, shortness of breath, fever, rhinorrhea, and nasal congestion. The recruited patients were those routinely do visit the external clinics of Al zahraa hospital, Najaf City from September 2020 to April 2021.

Fourty four out of one hundred fifty six nasal swab samples proved to have positive results (Figure 1) regarding the human metapneumovirus (HPMV) by molecular detection of the gene encoding fusion protein (F) by PCR using gene primer specific primer set, a protein mediates the fusion of the viral envelop to the cellular membranes to establish the infection. The amplified DNA fragment encoding the fusion protein F was 428 bp.

Present finding regarding the Incidence of human metapneumovirus (HMPV) in Al zahraa hospital, Najaf City, Iraq revealed a frequency of occurrence of 28.20% (44/156=28.20%): with significant increase of infection rate.

Table (2) distribution of viral positive samples in patients and control

Groups	**No.**	**Positive samples**	**%**
Control	50	0	0
Patients	156	44	28.20
X^2	17.93*		
P value	0		

*Significant difference at P<0.05

Table 3: Distribution of hMPV based on age group

Age group	**HMPV**
Less than 1 year	24 (54.5%)
1-3 year	14 (31.8%)
4-7	4 (9.1%)
8-12	2 (4.5 %)

The highest percentage was 24 (54.5%) of children Under 1 year in whom have hMPV infection.

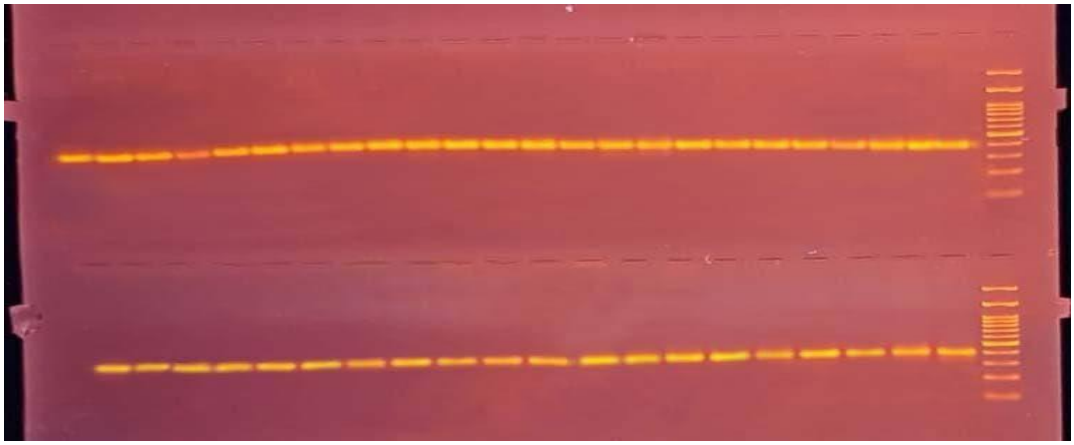

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Figure 1: 1% agarose gel electrophoresis showing PCR amplification of the fusion protein F from representative samples. Lane M: DNA ladder.

To get further confirmation about the identity of the PCR product, assuming to be a partial fragment of the fusion protein F from human metapneumovirus (HPMV), nine PCR products were sent to DNA sequencing.

The nine sequences were deposited in the GenBank under the accession number: MZ221975.1, MZ221976.1, MZ221977.1, MZ221978.1, MZ221979.1, MZ221980.1, MZ221981.1, MZ221982.1, and MZ221983.1. The non-function fusion protein F had the following accession numbers MZ221977.1, MZ221979.1, MZ221980.1, and MZ221982.1. According to the guidelines of the submission in GenBank, the viral isolates were assigned to a designation namely HW-1 to HW-9.

A phylogenetic tree was constructed to portray the genetic relatedness among the nucleotides sequences of fusion protein F isolated from the nine strains of human metapneumovirus (HPMV) (Figure 4). The constructed Neighbor joining tree reflected the speciation of the nine strains of human metapneumovirus (HPMV) into five groups namely 1-5. Group1 included human metapneumovirus (HPMV) –HW-4 and human metapneumovirus (HPMV)-HW-8. Group 2 encompassed human metapneumovirus (HPMV)-HW-2 and human metapneumovirus (HPMV)-HW-7. Group 3 included human metapneumovirus (HPMV)-HW-1 and human metapneumovirus (HPMV)-HW-3. However, group 4 included human metapneumovirus (HPMV)-HW-6 and human metapneumovirus (HPMV)-9. The group 5 included human metapneumovirus (HPMV)-HW-5.

The five genotypes of Human metapneumovirus retrieved from GenPet were genotype A1, A2, A2b, B, B1, and B2 as shown in Figure 3.4. Only HW-4 could be genotyped as genotype A2, whilst, the other four remaining strains HW-1, HW- 2, HW-7, and HW-9 could not be genotyped as shown in Figure 3.4.

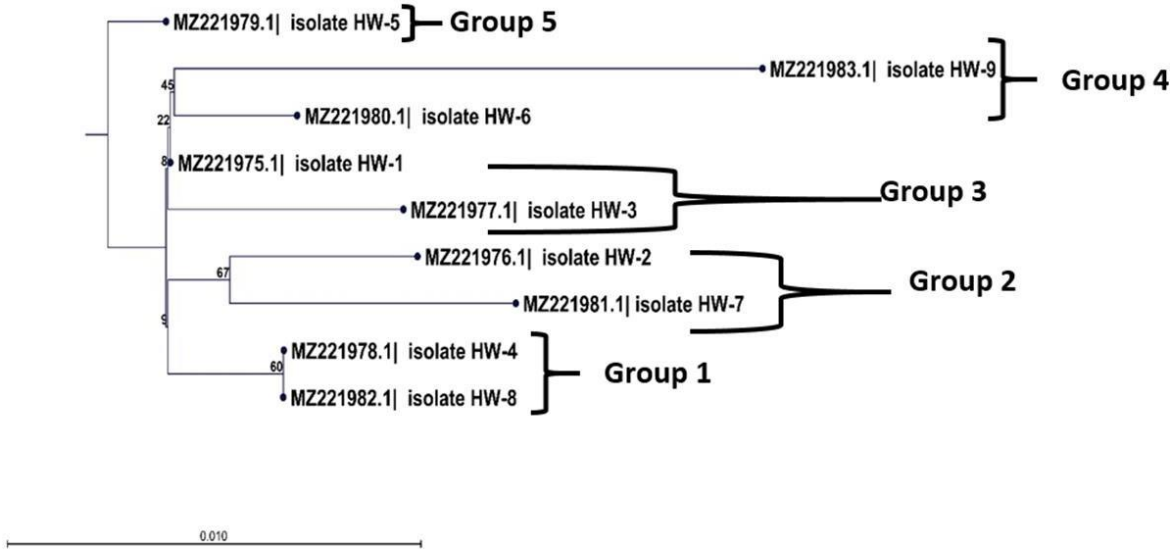

Figure 3.4: Neighbor –joining method performed by CLC Sequence Viewer 8.0 program showing the genetic relatedness among 9 nucleotides sequences encoding Human metapneumovirus fusion protein from nine isolates. Numbers on branches represent the bootstrapping values of 1000 re-samplings.

4. Discussion

The present finding regarding the Incidence of human metapneumovirus (HMPV) in Iraq revealed a frequency of occurrence of 28.20% (44/156=28.20%). The frequency of occurrence of human

metapneumovirus (HMPV) was variable from country to another. Previous studies conducted in Iraq revealed the prevalence of human metapneumovirus (HMPV) among children was 9.3% (30/323=9.3%) in Diyala Province in 2020 [6], 16% (48/400=16%) in [7], 1.33% (1.99/150=1.33%) in Baghdad from 2017-2018 [8], and 9.9% (9.9/100=9.9%) in Baghdad [9]. Other study conducted in Southern India revealed a frequency of occurrence for human metapneumovirus (HMPV) of 5% (23/447=5%) [10]. A retrospective study conducted on immune-compromised hosts (hematopoietic SCT (HSCT) recipients) showed 7.2% (11/153=7.2%) had acquired human metapneumovirus (HMPV) infection during the study period (6.4% in 2001, 4.7% in 2002 and 11.1% in 2003) [11]. Other retrospective study conducted in Germany revealed that the frequency of occurrence of human metapneumovirus (HMPV) was 3 and 11.9 % among outpatients with influenza like illness and hospitalized children, respectively between 2000-2001and 2009-2010 [12]. In Iran, a previous study inferred a frequency of occurrence of 10% (20/200=10%) among children patients suffering from respiratory tract infections [13].

Multiple sequence alignment on a nucleotide level revealed that there were some point mutations scattered allover the whole length of the partially amplified fragment of fusion protein F, as shown in Figure 3A & B). At the nucleotide position 17-19, three point mutations were detected AGA in MZ221983.1 whilst, the other remaining eight sequences were GAG. Another detected two point mutations were localized at position of 54 and 55. At these two positions, the wild type were GC, where the mutant type was CG represented in only one sequence namely MZ221982.1 while, the remaining sequences had the wild type GC. Other two point mutations were localized at positions 241 and 242. The wild type alleles were GA existed in all sequences except the sequence MZ221982.1 harbored the mutant alleles AG. The above-mentioned point mutations allover the nine nucleotide sequences for fusion protein F were for instance not exclusively.

A phylogenetic tree was constructed to portray the genetic relatedness among the nucleotides sequences of fusion protein F isolated from the nine strains of human metapneumovirus (HPMV) (Figure 4). The constructed Neighbor joining tree reflected the speciation of the nine strains of human metapneumovirus (HPMV) into five groups namely 1-5. Group1 included human metapneumovirus (HPMV) –HW-4 and human metapneumovirus (HPMV)-HW-8. Group 2 encompassed human metapneumovirus (HPMV)-HW-2 and human metapneumovirus (HPMV)-HW-7. Group 3 included human metapneumovirus (HPMV)-HW-1 and human metapneumovirus (HPMV)-HW-3. However, group 4 included human metapneumovirus (HPMV)-HW-6 and human metapneumovirus (HPMV)-9. The group 5 included human metapneumovirus (HPMV)-HW-5.

In order to investigate the effect of these point mutations on the functionality of the fusion protein F, these nine nucleotide sequences were screened by the ORF Finder server of NCBI to detect the presence of open reading frame (ORF) in these sequences. Results of OFR Finder (Open Reading Frame) revealed that only five out of nine sequences were encoded for partial sequence of coding sequence of fusion protein F of human metapneumovirus (HPMV). However, the remaining four sequences were not encoding for open reading frame (ORF). Consequently, they were considered as non-functional fusion protein F. Conversely, the functional fusion protein F sequences were assigned the following accession numbers in GenPet database: QZA01143.1, QZA01142.1, QZA01141.1, QZA01140.1, and QZA01139.1.

Phylogeny studies conducted on human metapneumovirus (HMPV), isolated from different countries either from outpatients or inpatients, have revealed that there exists currently two lineage genotypes A and B with their four subgenotypes A1, A2, B1, and B2 co-circulating among patients infected with human metapneumovirus (HMPV). However, the prevalence of occurrence of these genotypes and their sublineages is variable from country to another depending on age group, detection method, and seasonal variations. Jallow

and his co-workers inferred that the predominate genotypes and sublineages in their studies from 2012-2016 in Sengal were confined to A2, B1, and B2 [14]. Moreover, the same genotypes and their sublineages A2, B1, and B2 were reported in other countries such as Egypt [15], Kenya [16], [17], Saudi Arabia [18], Croatia [19], and Panama [20]. In contrast, William et al. proved in their studies the co-circulating of the 4 subgenotypes of human metapneumovirus (HMPV) [21]. Similarly, Reiche and his co-workers verified the co-circulating of the four subgroups of human metapneumovirus (HMPV) [22].

The distribution of Human metapneumovirus (hMPV) based on the age groups. The highest percentage was 24 (54.5%) of children Under 1 year in whom have hMPV infection. Several studies reported roughly percentage for hMPV with the age groups (4-7 years old) like Nandhini et al., showed 23 (5%) of 447, out of all, 8 (34.7%) in children with (1-5 yrs.) [10], other study which worked by they displayed in 2000 outpatients with age (1-5) years old, only 12% of cases have shown hMPV [23]. The different results regarding prevalence of hMPV among different geographical area described in different studies may be attributed to the number of factors such as distinct approaches used to accomplish the detections, locations of study, study's duration, seasonal variation, the age of the participated patients, types of the collected specimen and the numbers of specimens.

5. Conclusion

Human metapneumovirus has recently been identified as the etiologic agent of respiratory tract infection in Al Zahraa hospital in Najaf City, especially in children. As molecular technique testing get more advanced, additional viruses will be identified, The incidence rate of human metapneumovirus (HMPV) in this study among children patients suffering from respiratory tract infection was 28.20%, and The genotype A with the sub-lineage A2 was detected in this study.

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