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Molecular Study of Human Metapneumovirus, Human Respiratory Syncytial Virus and Human Parainfluenza Virus Type 1, 3 among Patients with Respiratory Tract Infections in Diyala Governorate, Iraq

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Chapter One

1. Introduction

1.1. Overview:

In the global term, respiratory viral infections are a leading cause of morbidity, hospitalization, and mortality affecting peoples of all ages particularly, infants, young children, the elderly, and immunocompromised individuals (Hijano *et al*., 2018; Li *et al*., 2019). Upper respiratory tract infections (URTIs) and lower respiratory tract infections (LRTIs) are frequently caused by a wide spectrum of viruses causing various clinical syndromes with variable outcomes ranging from common colds, pharyngitis, croup (laryngotracheobronchitis), otitis media, bronchiolitis, and viral pneumonia (Tregoning and Schwarze, 2010; Das *et al*., 2018; Gottlieb, 2019). The most important respiratory viral infections are influenza viruses type A and B, Human Respiratory Syncytial Virus (HRSV), Human Parainfluenza viruses (HPIVs), and Human Adenoviruses (HADVs), plus a number of newly discovered human respiratory viruses for the first time including Human Metapneumovirus (HMPV), Severe Acute Respiratory Syndrome Coronavirus (SARS), Middle East Respiratory Coronaviruses (MERS-CoV), Human Bocavirus, and Human Rhinovirus (Berry et al., 2015; Hasan *et al*., 2018; Tambyah *et al*., 2019; Bradley and Bryan, 2019).

Worldwide, the prevalence rate of respiratory viral infections is very high since viruses were identified as causes of the pediatric acute respiratory tract infections (ARIs) in up to (95%) of cases (Linden *et al*., 2019). Moreover, viruses are the main cause of (90%) of URTIs, and

about (30%) of LRTIs (Korsman *et al*., 2012). In 2013, WHO estimated that ARIs accounted for more than (8%) of all deaths in the Eastern Mediterranean Region (WHO, 2013). HMPV, HRSV and HPIVs are the most common and important causes of LRTIs in infants and children, while, in older children and adults, it causes recurrent-infections that are in the most cases are mild and self-limited in healthy individuals, but can be exacerbated causing serious or fatal diseases in elderly, persons with cardiopulmonary illnesses and immunocompromised patients (Chow *et al*., 2016; Williams *et al*., 2017).

Human Metapneumovirus is a single-stranded RNA virus, belongs to *Metapneumovirus* genus within Pneumoviridae family. Since the first isolation of this virus from (28) Netherlands' children in 2001; HMPV has appeared as one of the important causative agents of ARIs in all ages worldwide. The clinical manifestations of acute and severe RTIs that caused by HMPV are similar and undistinguishable from those caused by of HRSV, which are mostly characterized by mild symptoms to severe cough, bronchiolitis and pneumonia (van den Hoogen *et al*., 2001; Taniguchi *et al*., 2019; Peña *et al*., 2019). HMPV is transmitted by close or direct contact with infected secretions like; droplets, aerosols and fomites. Viral shedding occurs after incubation period of 7-9 days (Vinci *et al*., 2018). Genetic analysis and sequence variability of HMPV (G and F) genes, identified two main genotypes or major genetic sub-groups, designated (A and B), each with two minor sub-lineages (A1, A2, B1 and B2) (Schuster and Williams, 2018). HMPV is responsible for up to 10% of viral respiratory infections and most children under the age of 5 years have already been infected with the virus, the prevalence rate of HMPV infection in different epidemiological studies varies between (5-25%),

HMPV positivity rate was detected among nasal sinus swabs with a statistically significant difference compared to nasopharyngeal and throat swabs (96.7%, P=0.0001). HRSV positivity rate was significantly higher in the first season compare to the second season (93.3% versus 6.7%, P=0.004), March compared to other months of the year shows a highest detection rate (P=0.012), HRSV was detected in (53.3%) of throat swabs which were significantly higher compared to nasopharyngeal swabs (40.0%) and nasal sinus swabs (6.7%) (P=0.003). HPIV-3 positivity rate during the season I was higher compared to season II was (64.7% versus 35.3%) (P=0.525) and November showed the highest detection rate (29.4%, P=0.340); however, the difference was failed to reach the statistical significance for both. Additionally, throat and nasal swabs proved effectiveness in showing the highest positive rate of the HPIV-3 (64.7%, 35.3%) respectively. Regarding the age-related positivity of HMPV, HRSV and HPIV-3 the infections were highest among children under 5 years of age (86.7%, 53.3%, 82.4%) respectively. Clinical pictures most closely related to the higher positivity rate of HMPV and HPIV-3 were Bronchiolitis/bronchitis (86.7%, 88.2%) with a statistically significant difference (P=0.001, 0.003).

Phylogenetic analysis of HMPV attachment glycoprotein (G) gene showed that of the 28 Iraqi isolates collected during the second season, 15 of these isolates belong and cluster within genotype (B), sub-lineages (B2) circulated in this region. These isolates were registered with the (GeneBank) at the National Center for Biotechnology Information (NCBI) and a global accession number was obtained. Multiple sequence

suggesting that HMPV is a ubiquitous and globally distributed virus (Schuster and Williams, 2019; Tambyah *et al*., 2019).

Human Respiratory Syncytial Virus is an enveloped virus with negative sense, single-stranded RNA, belongs to *Orthopneumovirus* genus, Pneumoviridae family. Based on the sequence and antigenic differences, HRSV was classified in two main antigenic subgroups A and B (Shi *et al*., 2017). HRSV is one of the most prevalent viruses in the world, infecting children and frequently identified as a major pathogen in adults, particularly the elderly. The clinical picture most closely related to HRSV infection is an upper respiratory infection, but in young children, the bronchiolitis and lower respiratory disease usually appear with obstruction in the small airway, and pneumonia may rarely develop, which leads to respiratory failure, and consequently apnea and death (Sun *et al*., 2019). HRSV is still causing annual outbreaks with no safe and effective vaccine developed yet. Most children are infected at least once in the first two years of age, and it is responsible for a quarter of all pneumonia cases in the first months of life worldwide, ranking as the second common cause of post-neonatal infant death following malaria (Cody Meissner, 2018; Perk and Ozdil, 2018).

Human Parainfluenza Viruses (HPIVs) include several closely related viruses of enveloped single-stranded negative sense RNA viruses belonging to *Respirovirus* genus Paramyxoviridae family, causes many respiratory diseases ranging from the common cold to flu-like syndrome or pneumonia; croup is the most obvious severe clinical manifestations (Branche and Falsey, 2016). By genetic analysis, HPIVs are grouped into four serotypes that are highly transmissible and responsible for up to 10%

of all hospitalized children under 5 years of age due to an acute respiratory infection (Linster *et al*., 2018; Zaki, and Keating, 2018). HPIV-1 and HPIV-3 is the second after HRSV as the main cause of severe respiratory tract illnesses. HPIV-3 infects most of infants during the first year of age, causing seasonal outbreaks leading to a significant burden of illness in children and responsible for 40% of pediatric hospitalizations for pneumonia and bronchiolitis, while, HPIV-1 is responsible of 75% of croup cases. Due to incomplete immunity during childhood, reinfection with HPIV can occur in adults accounting for (15%) of respiratory diseases (Branche and Falsey, 2016; Burrell *et al*., 2017).

Molecular techniques have greatly improved the diagnosis of respiratory pathogens, and are the new gold standard. The developed multiplex PCR amplification techniques and the current increasing use of it for detection of respiratory pathogens in URTIs and LRTIs have provided new data on the epidemiology and genetic diversity of these respiratory pathogens and have shown that most of hospitalized children with ARIs often infected with multiple viruses. On the other hand, it supplied the best understanding of the seasonal distribution of these pathogens and their association with particular clinical manifestations. The prevalence and genetic diversity of these viruses may vary depending on various factors such as geographical location, health and genetic factor of the community, climatic conditions as well as the impact of health reality. Epidemiological studies by using molecular techniques around the world have confirmed the prevalent of HMPV, HRSV and HPIV-1, 3 (Stover and Litwin, 2014; Das *et al*., 2018).

countries through the multiple and pairwise alignment of these isolates with international isolates available in the GeneBank database.

Three hundred and twenty-three patients from those clinically suspected as having respiratory tract infections RTIs (children, adults and elderly) were included. The study was extended over two seasons; 185 patients were included in the first season (January, February, March, April, May) and 138 in the second season (November and December). Three different respiratory specimens were collected including nasopharyngeal swabs, nasal swabs and throat swabs. Human privacy was respected through obtaining official written approval from the Research Ethics Committee in Diyala Directory of Health and specimen's collection was done under the direct supervision of specialized doctors and practitioners after approval of the study participants or their parents. Specimens were analyzed by using real-time PCR (RT-PCR/qPCR) assay for (HMPV, HRSV and HPIV-1/HPIV-3) and conventional PCR assay for (HMPV and HRSV). Genotyping for the positive samples of HMPV and genetic subgrouping of HRSV was performed.

The results showed that HMPV single infections was detected in 30(9.3%) and a co-infection with HPIV-3 was (n = 1) that had the same clinical manifestations and single infection with the HRSV was detected in 15(4.6%) and a co-infection with HPIV-3 was (n = 1), while HPIV-3 alone was detected in 17 (5.3%) and no HPIV-1 infections were detected. The highest positivity rates of HMPV appeared in specimens collected during November and December, season II with a significant difference compared to other months (50%, P= 0.0001). Obviously, the highest

In Iraq, studies reported that the prevalence rates of HMPV infection ranged from (1.33% to 29.74%) (Abduljabbar *et al*., 2018; Hassan *et al*., 2018), HRSV prevalence rates were (1% to 36%) (AL-Bashar *et al*., 2017; Abduljabbar *et al*., 2018; Hassan *et al*., 2018), while, the prevalence rate of HPIV-1 and 3 was (32.17% and 13.21%) (Kadim, 2016). However, virological data concerning the seasonal distribution patterns and circulating genotypes of HMPV, HRSV and HPIV-1, 3 in the Iraqi community and for all age group are currently limited, although many comprehensive studies have investigated the phylogenetic analysis of HMPV isolates, in particular, has been published in many other countries.