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Detection and Genotyping of Respiratory Syncytial Virus and Human Metapneumovirus Among Children with Respiratory Tract Infection

A Thesis

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بسم الله الرحمن الرحيم

يَرْفَعِ اللَّهُ الَّذِينَ آمَنُوا مِنْكُمْ وَالَّذِينَ أُوتُوا الْعِلْمَ دَرَجَاتٍ وَاللَّهُ بِمَا تَعْمَلُونَ خَبِيرٌ ۝

صدق الله العظيم

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Dedication

To the light of my life.... Father and Mother

For their endless love, support and encouragement.

To that who gave me support to overcome all difficulties in life...

My husband

To the Dears....My Brothers and Sister

To my soul .... My sweet daughter Haya

To everyone who helped and supported me in my study.

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SUMMARY

Respiratory viruses are the leading cause of serious respiratory tract infections in children, elderly and immunecompromised, throughout the world. However, information about the genotyping of respiratory syncytial virus (RSV) and human metapneumovirus (hMPV) is limited in Iraq.

The aims of this study are to determine the rate of respiratory syncytial virus and human metapneumovirus infections in children with acute respiratory tract infection and the correlation between these viruses and different parameters such as age, gender, month of infection, residence, cough, fever, wheezing, history of asthma, nasal discharge and neurological manifestation and to identify human respiratory syncytial virus and genotyping among study population.

This cross sectional study was based on the processing of nasopharyngeal swab from 150 children with acute respiratory tract infections, males 81(54%) and females 69(46%) aged under five years old, who were admitted to Al-Imamin Al-Kadhimin Medical City and Pediatrics Protection Hospital in Baghdad during the period from December 2017 till April 2018. These swabs were collected from each participant and stored as frozen at -70 °C until use for RNA extraction and real time- polymerase chain reaction and nested polymerase chain reaction to detect of respiratory syncytial virus and human metapneumovirus in study population.

The results showed that out of all these samples, 54 samples were positive for RSV 54(36%) and 2 for hMPV 2(1.33%). The infection rate of RSV was more common in males (57.41%) than females and in children ≤ one year (37.04%) also high frequency was noticed among patients live in urban area 72.22% and 50% respectively also in winter. According to different clinical feature, fever, cough, and wheezing were more common.

The sequence conducted for some RSV- positive samples, 11 respiratory syncytial virus isolates were in genotype B and 1 in genotype A. The sequence of RSV B the locally detected samples were closed to Argentina and Tailwind isolates while in genotype A detected samples were closed to isolates from different regions included Saudi Arabia, German and India isolates.

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Table of Abbreviations

Abbreviation	Meaning
ARI	Acute respiratory infection
ART	Acute respiratory tract
bp	base pair
CD4+	Cluster of differentiation 4
CD8+	Cluster of differentiation 8
CDC	Centers for disease control and prevention
CPE	Cytopathic effect
DFA	Direct immunoflourescent assay
DNA	Deoxy ribose nucleic acid
EIA	Enzyme immunosorbent assays
F	fusion glycoprotein
G	Attachment glycoprotein
GAGs	Glycol amino glycan
HCMV	Human cytomegalovirus
hMPV	Human metapneumovirus
HVR	Hyper variable regions
IFNAR	Type I Interferon receptor
Ig	Immunoglobulin
IL	interleukin
INF	Interferons
JAK1	Janus kinase
LLC-MK2	Rhesus monkey kidney cells
M	Matrix protein
ml	Mille letter
mRNA	Messenger RNA
N	Nucleoprotein

NF-κB	Nuclear factor-kappa B
NK	Natural killer cell
NPS	Nasopharyngeal swab
NS	Nonstructural protein
ORF	open reading frame
P	phosphoprotein
PAMPs	Pathogen-associated molecular patterns
pH	Power of hydrogen
PRRs	Pattern recognition receptors
qPCR	quantative polymerase chain reaction
RIG-I	retinoic acid-inducible gene I
RNA	Ribonucleic acid
RNP	Ribonucleoprotein
RSV	Respiratory syncytial virus
RT-PCR	Reverse transcriptase polymerase chain reaction
SH	Small hydrophobic glycoprotein
SPSS	Statistical package for social sciences
ssRNA	Single strand ribonucleic acid
Th1	T helper 1
TLRs	Toll-like receptors
TMK	Tertiary monkey kidney
TNF-α	Tumor necrosis factor alpha
TRM	Tissue-resident memory
TYK2	Tyrosine kinase 2
URT	Upper respiratory tract
UV	Ultra violet
VTM	Viral transport media
WHO	World health organization

Chapter One
Introduction

1. 1 Introduction.

Acute respiratory infection (ARI) is the major cause of morbidity and mortality. In young children, the elderly, and immunocompromised individuals throughout the world (Zhang *et al*., 2009). Several types of viral families such as *Paramyxoviridae, Orthomyxoviridae, Picornaviridae, Adenoviridae*, and *Coronaviridae* are considered the major viral etiological agents of ARI in all age groups (Bertino *et al*., 2002).

Respiratory syncytial virus (RSV) and human metapneumovirus (hMPV) compose of a single strand, negative sense RNA, helical capsid, and they are enveloped (Nagakumar and Doull, 2012). Regarding to the genetic analysis of both viruses, they were separated into two genotypes each, RSV-A, RSV-B; hMPV(A and B), and the later can be subdivided into A1; A2, B1 and B2 (Wang *et al*., 2014; Thongpan *et al*., 2017). Transmission of these viruses through close contact with a person who has an active infection or direct contact with infectious secretions on environmental surfaces such as droplets, saliva, or large particle aerosols (Haas *et al*., 2013). Although fomites may also be source of contamination (Tollefson *et al*., 2010).

Human metapneumo virus is nearly associated with respiratory syncytial virus, when they were considered as essential airway viruses influencing children throughout the world (Zeng *et al*., 2015). It has been suggested that the infection of RSV and hMPV in children may be very comparable (Adams *et al*., 2015). There is also some confirmation that RSV caused more serious ailment than hMPV, according to number of factors were statistically associated with a higher rate of severity as opposed to mild infections. The most important of these were prematurity, the presence of bronchopulmonary dysplasia, chronic heart illness and serious neurological disabilities (Papenburg *et al*., 2012; Shi *et al*., 2015; De Wall *et al*., 2018).

Although, it has not been studied to the same extent, it seems that some of these factors may also increase the risk of severe hMPV infection, with some exceptions (Schuster *et al*., 2015; Pancham *et al*., 2016). Respiratory syncytial virus and hMPV infection are blurred distinguishable, both they cause upper respiratory infection which begin with flu-like illness such as fever, headache, sneezing, and then progress down into the lower respiratory tract to cause bronchiolitis, pneumonia, and they implicated with allergy and asthma exacerbation (Rezaee *et al*., 2017). Respiratory syncytial virus constitutes over 60% of respiratory tract infection, while hMPV about 12% (Kahn, 2006).

Reinfection with similar strains of RSV occurs despite natural infection inducing high levels of antibody against conserved antigens. The immunomodulatory mechanisms of RSV are thus highly effective in inhibiting long-term protection, with disturbance in type I interferon signaling, antigen presentation and chemokine-induced inflammation. These lead to widespread effects on adaptive immunity with impaired B cell memory and reduced T cell generation and functionality (Ascough *et al*., 2018).

Molecular methods such as reverse transcriptase-polymerase chain reaction (RT-PCR) are the preferred diagnostic modality for detecting RSV and hMPV. Few vaccine candidates have been shown to be effective in preventing clinical disease, but none are yet commercially available (Panda *et al*., 2014; Zahran *et al*., 2017).

In Iraq, several studies have been conducted in various governorates to identify these viruses. All of these studies focused on infection rate such as study done by Al-Mola *et al* (2013) in Hilla city, Aziz (2015) in Sulaimani city, Al-Ameedy (2016) in Najaf city, Al-Charrakh *et al* (2016) in Wasit province, Al-Mossawi *et al* (2016) in Al-Amarah City and recently Atyah *et al* (2017) in Baghdad city.

1.2 Aims of study.

1. Determining the infection rate and genotyping of respiratory syncytial virus and human metapneumovirus in children with acute respiratory tract infection.

2. Investigating the correlation among respiratory syncytial virus, human metapneumovirus and patients descriptive data such as age, gender, residence, month of infection, cough, fever, wheezing, history of asthma, nasal discharge, and neurological manifestation.