**Ministry of Higher Education and Scientific Research**
**University of Al-Qadisiayah**
**College of Medicine**
**Department of Medical Microbiology**

# Molecular Investigation of *Human metapneumovirus* and *Respiratory syncytial virus* in Children in Al-Amarah City

**A thesis**
**Submitted to the Council of the College of Medicine / University of Al-Qadisiayah in Partial Fulfillment of the Requirements for the Degree of Doctorate of Philosophy in Medical Microbiology**

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بسم الله الرحمن الرحيم

وَمَا تُقَدِّمُوا لِأَنْفُسِكُمْ مِنْ خَيْرٍ تَجِدُوهُ عِنْدَ

اللَّهِ إِنَّ اللَّهَ بِمَا تَعْمَلُونَ بَصِيرٌ ۝

صدق الله العلي العظيم

سورة البقرة – الاية 110

الأهداء

الى أمي وأبي ..... تغمدهما الله برحمته الواسعة

الى كل من ابدى لي العون وشد على أزري في اظهار هذا الجهد

الذي امل ان ينتفع به.

## Summary

The current study has targeted for detection of two important pathogenic viruses which responsible for respiratory tract illness (RTI) in children that involved *Human metapneumovirus* (hMPV) and *Respiratory syncytial viruses* (RSV), is to establish the basic step of the database and archives of molecular study and investigation of a particular viruses, the clinical specimen which was preferred in this study was nasopharyngeal (NP) swab that are taken from 230 children who experiencing from the respiratory tract illness, who were frequent to the Al-Sader Teaching Hospital in Al-Amara city during the period from December 2014 to April 2015, the collected specimens are put on the viral transport media (VTM) which furnished for this purpose and then transported by an ice bag from the hospital into the pharmacy department for storage in -70˚C, after that, the molecular techniques were used in this study which are comprised quantitative real time PCR (qRT-PCR) for investigation and diagnostic of particular viruses and Livak and Schmittgen equation, (fold change= $2^{-\Delta\Delta CT}$), for estimating the gene expression of hMPV (G glycoprotein & M2-2) and chemokines gene expression which are induced by RSV, (TARC & MDC).

The results that attained from this study are summarized in the following points:

1. Out of 230 children suffering from respiratory tract infection, only 7 (7%) were encountered with the *Human metapneumovirus*, 8 (8%) and 14 (14%) were detected with the *Respiratory syncytial virus* type A and B respectively.
2. The distribution of *hMPV* and RSV-A, RSV-B based on age groups, and it represents the highest percentage was 4(57.1 %) of children (4-7) years old in whom have hMPV infection, and highest percentage, 6(75 %) in

age group (<1) and 5(35.7 %) for (<1) in whom have encountered RSV-A and B respectively.

3. The main clinical symptoms of upper respiratory tract (sneezing, rhinorrhea, croup) and lower respiratory (cough, wheezing, dyspnea, and crepitation) infection that caused by hMPV, of this symptomatic' group of patients studied, about (5, 71.4%) feverish; (5, 71.4%) coughing; (4, 57.1%) wheezing, (3, 42.8%) sneezing, (2, 28.5%) rhinorrhea; (4, 57.1%) dyspnea; (2, 28.5%) crepitation; (1, 14.2%) croup.
4. The main clinical features caused by RSV-A, B, cough was reported as the highest frequently, 7 (87%); 12 (85.7%) respectively, after that, fever, wheezing and dyspnea are less, 6 (75%) for all these signs of the serotype A, while, of serotype B were 10 (71.4%) for fever and 11 (78.5%) for wheezing and dyspnea, it is worth noting the signs of the upper respiratory illness, sneezing and rhinorrhea are the least one of that, they were 2 (25%); 3 (37.5%) for serotype A, and 4 (28.5%) for both signs of serotype B. The others signs such as crepitation and croup are also reported for both serotypes of RSV, 3 (37.5%) for both signs of serotype A and 7 (50%), 5 (35.7%) for crepitation and croup of serotype B respectively.
5. The genetic diversity of hMPV is so substantial in this study and the F-fusion protein was done sequencing for describing the prevalent isolate which is disclosed two genotypes A and B, of hMPV, genotype A1 was found in (1/5, 20%), A2 was not existing in the current study, and B1 was present in (3/5, 60%) and B2 (1/5, 20%).
6. The homology of local current isolates were 100% of hMPV-S1(KY441445),hMPV-S3(KY441447),, at the nucleotides sequencing level with the KM408076.1, KJI96323.1respectively, and 99% of hMPV-S4 (KY441448), with JQ041689.1 strain, three of five HMPV-S2

(KY441446),,S3, and hMPV-S5 (KY441449), were identical (100%) so, the genotype B is predominant (80%).

7. The bioinformatics tools has been performed on the nitrogenous bases and amino acid sequencing to predict the secondary structure of F fusion protein of hMPV through discloses the hydrophobicity and hydrophobicity properties of a particular protein, as well as revealing the domains and loops with study the types of strips that assembly through amino acids organization, all these tasks were done by several pocked programs which prepared for this duty.
8. All positive results of hMPV are folding change expression of G-protein gene, with a higher level range from 13.642 into 50.213 folds.
9. The results of M2-2 gene expression of hMPV which reveal higher level of M2-2 ranging from 78.793 to 6.543 folds that represent the hyperactivity of hMPV in the airway epithelial cells.
10. The expression level of TARC gene, which induced by RSV-A that ranging from the high level 40.786 to lower level 19.160 folds.
11. The expression level of TARC gene, which induced by RSV-B that ranging from the high level 64.000 to lower level 9.646 folds.
12. The expression level of MDC gene, which induced by RSV-A that ranging from the high level 37.792 to lower level 7.727 folds.
13. The expression level of MDC gene, which induced by RSV-B that ranging from the high level 48.840 to lower level 6.063folds.
14. Last, the IL-8 has been estimated and the result was 4(4%) are positive, out of 7 of hMPV, 3(42.8%) are IL-8 positive, while there's only 1(7.14%) are IL-8 positive for RSV-A.

The major conclusions of current study are both of these viruses tend to cause strictness lower respiratory illness and asthma exacerbation, as well as the sublineage hMPV, A1; A2; B1; and B2 are widespread different, and produce recurrent infection beside that, the genetic diversity of both groups of RSV is as similar as hMPV, which can co-circulate with various level of prevalent rate.

## List of contents

## List of tables

## List of figures

## List of abbreviations

| Abb. | Meaning |
|---|---|
| ANN | The Artificial Neural Network |
| APV | Avian Metapneumovirus type C |
| ARDS | Acute Respiratory Distress Syndrome |
| BPV | bovine pneumovirus |
| cDNA | complementary DNA |
| COPD | Chronic Obstructive Pulmonary Disease |
| CPE | Cytopathic Effect |
| CRFK | Crandell Rees feline kidney cell line |
| DEPC | Diethylpyrocarbonate |
| GOR | Garnier, Osguthorpe & Robson |
| HEp-2 | Human epithelial type 2 cells |
| HMM | Hidden Markov model |
| hMPV | Human metapneumonia infection. |
| HRP | Avidin-Horseradish peroxidase |
| HVR | Hypervariabiltly |
| IL-8 | Interleukin 8 |
| LLC-MK2 | Rhesus Monkey Kidney Cells |
| MAVS | Mitochondrial antiviral-signaling protein |
| MDC | Monocyte Derived Chemokine |
| moDC | monocytes-derived DC (moDC) |
| MPV | Mice Pneumonia Virus |
| NASBA | Nucleic Acid Sequence-Based Amplification |
| NSP | Non-Structural Protein |

| | |
|---|---|
| ORF | Open Reading Fragment |
| ORSV | ovine RSV |
| pDC | plasmacytoid DC |
| qPCR | Quantitative real-time PCR |
| rhMPV-ΔG | Lacking the G protein hMPV. |
| RIG-I | Retinoic acid-inducible gene I |
| RSV | Respiratory Syncytial virus |
| SP | Structural Protein |
| Taq | *Thermus aquaticus* |
| TARC | Thymus and Activation Regulated Chemokine |
| TEM | Transmission Electron Micrograph |
| Vero E6 | Cell lines *verda reno* Epithelial cell. |
| VSV | Vesicular Stomatitis Virus |
| VTM | Viral Transport Medium |

# 1. Introduction and literature review

## 1.1. Introduction

The respiratory tract infection (RTI) in children is caused by several pathogenic microorganisms such as bacteria, viruses, parasites and fungi, but the pathogenic viruses particularly *Respiratory syncytial virus* (RSV), *Human metapneumovirus* (hMPV) are the most frequent cause RTI than other pathogenic, (Claesson *et al*., 1989; Juven *et al*., 2000; Simoes *et al*., 2006), children are most susceptible to respiratory tract infection which are attributed to several features that attendant to physiological, immunological and anatomical characteristics (Lanata *et al*., 1998). Acute viral respiratory tract infection constitutes about 30-40% (Cilla *et al*., 2008). *Respiratory syncytial virus* constitutes over 60% of respiratory tract infection, while *human metapneumovirus* about (12%), (Crowe, 2004; Williams *et al*., 2004; Kahn, 2006; Principi *et al*., 2006;) these viruses RSV and hMPV, are candidate as a material in this study due to these viruses causes the most viruses that upper respiratory tract illness such as flu-like, rhinitis, sinusitis and they cause bronchiolitis, croup, pneumonia, as well as may be responsibility to stimulate an allergy and asthma exacerbation, all these complications are considered worse cases particularly in children (Chano *et al*., 2005;Kahn, 2006; Klein *et al*., 2006; Ordás *et al*., 2006; Sloote *et al*., 2008; Stensballe *et al*., 2008). Isolation and identification of these viruses, RSV and hMPV, assistant to govern the clinical management of patient and consequently it is much needed to grow best technique to enhance the diagnosis of pathogen. *Respiratory syncytial virus* and hMPV are strictly related; they incorporated under the same family, *Paramyxoviridae*, and they divergent into two subfamily, *Paramyxovirinae* for RSV, and *Pneumovirinea* for hMPV, indeed, hMPV is very similar to the *Avian metapneumovirus* (AMPV), they have shared the gene order, which involved the 3′ F-M2-SH-G-L 5′, (van den Hoogen *et al*., 2001; Boivin *et al*., 2002), they

have a zoonotic source, it established via serological techniques and bioinformatics studies (de Graaf *et al*., 2008). *Respiratory syncytial virus* and hMPV compose of a single strand, negative sense RNA, helical capsid, and they are enveloped, although they have the same gene order, the genome of hMPV deficits two genes that encoding for the non-structural (NS1 and NS2) proteins, which they do work together to interact with the immune response of the exposed host, as a consequence, they have proficiency to stimulate variant immune response products (Bitko *et al*., 2007; Elliott *et al*., 2007; Moore *et al*., 2008; Ling *et al*., 2009). Rely on the genetic analysis of both viruses, RSV and hMPV, separated into two genotypes, RSV-A, RSV-B; hMPV (A and B), and the later can be subdivided into A1; A2, B1 and B2 (Bastien *et al*., 2004; Ishiguro *et al*., 2004).

Genetic diversity is a hard sign of positive selection and upsets the competence of virus to ongoing circulating in population (Herbert *et al*., 2005). Undeniably, the genotypes of hMPV reveal fluctuations circle in a different social requests and in the same society repetitively, that is believed to happen depend on development of acquired immunity to infection with this particular strain (Carneiro *et al*., 2009; Aberle *et al*., 2010; Williams *et al*., 2010). Clinically, RSV and hMPV infection are blurred distinguishable, both they cause upper respiratory infection which begin with flu-like illness, fever; headache; sneezing, and then progress down into the lower respiratory tract to cause bronchiolitis, pneumonia, and they implicated with allergy and asthma exacerbation (Mary, 2014), indeed, severity of RSV and hMPV infection may slightly variant (Jesse *et al*., 2012). As above declared, hMPV genome includes nine gene for encode protein, P, Phosphoprotein; G, Glycoprotein (attachment protein); SH, hydrophobic protein; F, fusion protein, L, large polymerase protein; N,

nucleoprotein; M2, matrix protein (overlapping M2-1, M2-2) (van den Hoogen *et al*., 2001; van den Hoogen *et al*., 2002). Pneumovirus can encode two critical glycoproteins on their surface, F and G; that contribute to fuse viruses with the specific receptor on the cell membrane (Walsh *et al*., 1983), however, the G glycoprotein of hMPV serves as a virulence factor which interrupts the mitochondrial signals in the epithelial cells of the respiratory tract, so, it inhibits the airway epithelial cells for production of chemical mediators like cytokines, chemokines and type I interferon (Bao *et al*., 2008), also it disorders the signals that depend on the TLR4 in monocyte-derived dendritic cells (Kolli *et al*., 2011). M2-2 glycoprotein of hMPV plays an essential role for competent of transcription and replication of particular virus, also it blocks the innate immunity of the host (Ren *et al*., 2012).

*Respiratory syncytial virus* infection particularly in an early life of children is more expected to produce asthma overstimulation (Singurs *et al*., 2000), RSV induces both Th1 and Th2 to produce several chemokines, which transported into the site of inflammation to recruit and traffic the other inflammatory cells (Tripp *et al*., 2005; Kristjansson *et al*., 2005; Becker *et al*., 2006). Several studies exhibited the viral respiratory infection such as RSV, hMPV, *parainfluenza virus* and coronavirus infection in linking with 85% of the asthma exacerbation (Busse *et al*., 2010). Asthma is a chronic disease which mediated by an immune response that characterized by Th2 $CD4^+$ predominant which expressed chemokine (CC motif) receptors 4 (CCR4) for binding certain chemokines specifically Thymus and Activation-Regulated Chemokine (TARC/CCL17) and Macrophage-Derived Chemokine (MDC/CCL22), TARC is a chemotactic factor for immature dendritic cells and T lymphocytes and expressed in alveolar cells of human, it has been concerned in human atopic

asthma, and increased level on the apical surface of respiratory epithelial cells, TARC is inducible by RSV infection (Sekiya *et al*., 2000). Macrophage-Derived Chemokine (MDC/CCL22) is another important chemotactic agent which produced from several cells like bronchial epithelial cells, natural killer (NK) cells, macrophage and monocyte-derived dendritic cells (Hirata *et al*., 2003; Monick *et al*., 2007). Respiratory syncytial virus indirectly stimulates epithelial cells to secrete too much of TARC and MDC via Th2 cytokines such as IL-4 (Hirata *et al*., 2003).

Bioinformatics is a tool interconnect the several sciences such as mathematics, statistics approaches and computer skill on the biology and medicine, for analysis and annotation of the whole genome of organism, determine biological evaluation through has known phylogenetic tree and expectation of protein structure **(**Jackson and Steen, 2005; Campell *et al*., 2007). The topics of bioinformatics encompass multiple tracks for instance splitting coding and non-coding districts, recognition and evaluation gene production by using DNA material, while the multiple sequence alignment and appreciate of preservative sequence motifs and prediction of secondary, tertiary construction, also involved known the three dimensional structure of protein with measurement the protein geometry, all these applications are performance on protein elements, in other word, bioinformatics allows the clarification of job from the DNA/RNA sequence that is considered the corner to all fields of contemporary biology (Luscombe *et al*.; 2001; Cann, 2005). However, bioinformatics tactic is the acquirement, packing, organization, documentation, analysis, and declaration of information associated to biology. The term has been invented in 1990 with the practice of computers in DNA sequence analysis (Hanrahan *et al*.; 2015). Anyway, the analysis data inquiry, sequence comparison, linkage analysis and

phylogenetic understanding, of the bioinformatics devices are accomplished by different numerous programs which intended for these tasks, and the subsequent lines taken on the role of clarify the techniques and its requests of bioinformatics systems (Rastogi *et al*., 2008).

After giving this layout about the current study, it would look at the determination of certain of the molecular features of hMPV, and concern of RSV which are accomplished by the following objectives:

1- Identification of human metapneumovirus and both genetic groups of respiratory syncytial virus and compare between patients infected with hMPV and RSV, through the age groups; clinical features and prevalence rate of these viruses from children who have respiratory tract infection.
2- Studying the genetic diversity of sublineage groups of hMPV through analyze the F-glycoprotein fusion and align these sequences with other sequence that available in the GenBank database, and then determine which has more prevalent in a particular season.
3- Constructing the phylogenetic tree of hMPV and determine the homology of the local isolates as well as, another bioinformatics tools have been submitted to analysis and prediction model of F fusion protein of hMPV.
4- Recognizing whether hMPV is truly infecting or no, through the determination of gene expression of virus virulence factor, G-protein, and M2-2 protein which do as a preventing and reducing the immune response to hMPV.
5- Establishing the relationship between RSV and allergy exaggeration through determination the host gene expression (TARC and MDC) which induced by RSV infection.

6- Estimating interleukin 8 (IL-8) levels and states the relatedness with undertaken consideration viruses, RSV and hMPV.

## 1.2. Literature Review

### 1.2.1. Human Metapneumovirus

*Human metapneumovirus* (hMPV) is recently isolated and separated, in 2001, by specialists from the Netherlands, van Hoogen and his colleagues who depicted this infection when they observed a distinct (CPE) in cell culture in tertiary monkey kidney cells (van den Hoogen *et al*., 2001) effect culture from the nasopharyngeal aspirate of children got respiratory syndromes as common cold, bronchiolitis, and asthma exacerbation (Boivin *et al*., 2002; Hamelin *et al*., 2004, Bosis *et al*., 2005).Dutch examiners found an obscure infection in respiratory discharges gathered from young with lower respiratory disease. Infection contaminated cell supernatants were analyzed by electron microscopy and found to contain pleomorphic infectious particles measuring 150 to 600 nm, with spike-like enveloped projections of 13 to 17 nm (Schildgen *et al*., 2011).

*Human metapneumovirus* is an omnipresent respiratory pathogen that has been going in human population undetected for a considerable length of time. The first report distinguished hMPV-particular antibodies in human sera was during from the 1950s (van Hoogen, 2001) and hMPV has been identified by RT-PCR in samples from 1976 (Williams *et al*., 2004). Phylogenetic examination of several hMPV gene sequences proposes that hMPV deviated from *Avian metapneumovirus* (AMPV) between 200–300 years ago (de Graaf *et al*., 2008, Yang *et al*., 2009). It is a main source of lower respiratory infection in infants and children overall (Williams *et al*., 2006, Boivin *et al*., 2003) it is otherwise related to extreme infection in immunocompromised hosts or persons with concealed disorders (Mahdi, *et al*., 2003). This virus causes a clinical range of disease from upper respiratory system infection to serious lower respiratory tract illnesses (e.g., bronchiolitis and pneumonia) (Brodzinski, *et al*., 2009).

Its' pathogenesis is resemble hRSV and causes exacerbation, slaughtering and necrosis of the bronchiolar epithelium (Loughlin and Moscona, 2006). Exploratory studies in nonhuman primates and little creature models (hamsters, cotton rats, and mice) show that hMPV replications in the upper and lower respiratory tract epithelium and exhibit no proof of viral scattering, demonstrating a particular tissue tropism for hMPV which is predictable with clinical disease has been founding among human infection (Kuiken *et al*., 2004; Hamelin *et al*., 2016). With the broad studies and examination by utilizing molecular systems, for example, polymerase chain reaction (PCR) and electronic microscopy to distinguish the hMPV.

*Human Metapneumonia virus* has been contracted a kick out of the chance to intense respiratory disorder in people of all ages. In children, hMPV symptomology as RSV though in young children, hMPV more often than not causes influenza like disease (Boivin *et al.,* 2002)**.**

Depending on the structure and morphology of hMPV virion and organization of genome, hMPV was considered as the first member of *Human metapneumovirus* genus, with the *Pneumovirinea* subfamily of the *Paramyxoviruses* family**,** the following is the taxonomy of hMPV (Fouquet *et al*., 2006).

**Order:** *Mononegavirales*

**Family:** *Paramyxoviridae*

**Subfamily**: *Paramyxovirinae*; Genus: *Respirovirus*; Species: *Human Parainfluenza* type 1 and 3

**Genus**1: *Rubulavirus*; Species: *Human Parainfluenza* type 2 and 4; *Mumps virus*

**Genus2**: *Morbillivirus*; Species: *Measles virus*

**Genus3**: *Henipavirus*; Species: *Hendra virus and Nipah virus*

**Subfamily**: *Pneumovirinea*

**Genus:** *Pneumovirus*

**Species**: *Respiratory Syncytial virus*; subgroup: A & B

**Genus**: *Metapneumovirus*: **species:** *Human metapneumovirus*; subgroup: A & B

### 1.2.2. Virological properties of *Human metapneumovirus* (hMPV)

*Human metapneumovirus* is an enveloped, single-strand, negative sense RNA virus belonging to the family *Paramyxoviridae*, subfamily *Pneumovirinea*, and genus *Metapneumovirus*, it belongs two genotypes, A and B, the nucleotides and amino acid sequence identities between the hMPV groups are 80% and 90%, respectively, each of them has two siblings $A_1$(NL 00-1 like) , $A_2$ (NL 93-1 like); $B_1$(NL 99-1 like); $B_2$ (NL 94-1 like) (Kaida *et al*., 2006). The sequencing of amino acid and association of the genome of *Avian pneumovirus* is looking like to the hMPV, in this way, hMPV was characterized in *Metapneumovirus* class of the order *Mononegavirales*, consequently the hMPV is closely identified with the avian pneumovirus, few studies have been carried out using serological tests, denoted to particular hMPV antibodies that evidence the probable zoonotic prospect is more likely to occur happen not before 1958 (van den Hoogen *et al*., 2001).

*Human metapneumovirus*, as other members of the *Paramyxoviridae*, is a typically spherical, enveloped with a diameter of 150- 200 nm, others shape could be present such as filamentous with diameter of 100-400 nm and pleomorphic, the virus particles are surrounded by a lipid envelope that derived from the plasma membrane of the infected cells, the lipid layer of the hMPV is very susceptible to organic chemical agents (Peret *et al*., 2002 ), there are three virus glycoproteins on the viral envelope, glycoprotein G for attachment, F for

fusion and small hydrophobic protein (SH), are inserted in the envelop of virion as in the figure (1-1) (Collins and motet, 1993).

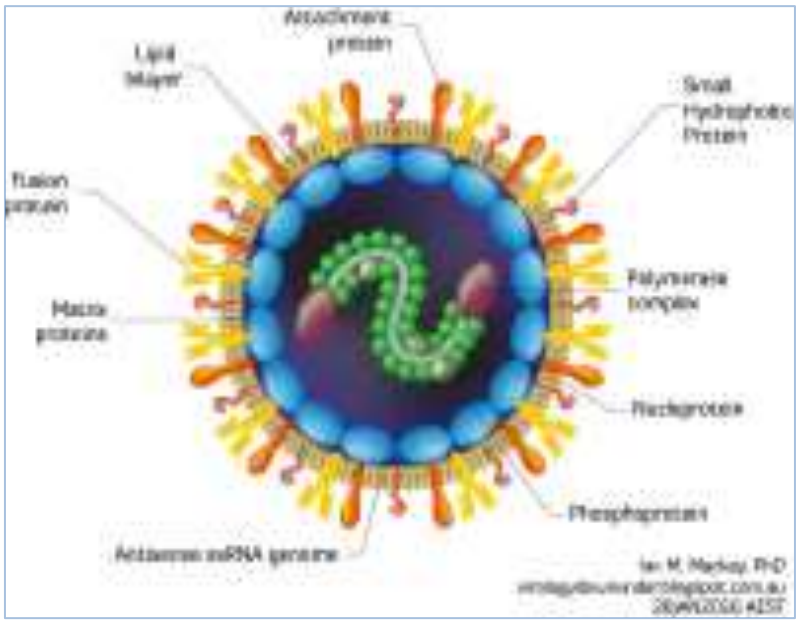


**Figure (1-1): Model structure of hMPV (Mackay, 2016).**

Glycoprotein G with internal amino acid and external carboxy-terminal components, while F protein with the amino acid terminus located outside the virus particle and a short cytoplasmic carboxy-terminal positioned inside. The G and F proteins consist of 10- to 14-nm spikes on the virion surface, therefore the virus is strongly related to the *Avian metapneumovirus* type C (AMPV), formerly *Turkey rhinotracheitis virus*, and these two viruses have been separated by taxonomists into a separated genus, metapneumovirus. The genomic RNA of hMPV is complexed with the viral glycoprotein (N), large proteins (L) and the phosphoprotein (P) to produce the helical nucleocapsid which is situated within the M-protein and forms a link between the nucleocapsid and envelope of the virion, the M2-1 transcriptional enhancer protein is also thought to be related with the nucleocapsid (Easton *et al*., 2004). The consistent amino acid

arrangement of the G protein contains a lonely hydrophobic locale that is sited close to the N end and is thought to serve as both an uncleaved signal peptide and a film stay. The C-terminal three-fourths of the atom are thought to be extracellular. The hMPV G protein has a high substance of serine and threonine deposits, which are potential acceptor locales for O-connected glycosylation and a high substance of proline, build ups elements imparted to vigorously glycosylated mucin-like structures (van den Hoogen, 2002). The expected supplementary elements of the G protein were affirmed by examinations of the biosynthesis, glycosylation, intracellular transport, and cell surface articulation of the G protein (Liu, 2007).

It has been planned that cell glycosaminoglycan, including heparin sulfate-like molecules, are included in the coupling of the G protein to the host cell (Weigl, 2007). Recombinant infections without the G protein have the capacity to reproduce *in vitro* and *in vivo*, representative that assembly by means of the G protein is not required for consequent strides in the replication cycle (Biacchesi, 2005). The support association of the hMPV F protein is like that of other viral class I combination proteins, where the F protein is incorporated as a F0 portent protein that requires cleavage by proteases to yield the actuated disulfide-connected F1 and F2 subunits (Lamb, 2007). The F protein seems to require exogenous protease enactment, as it is not cut intracellularly (Smith, 2009). Membrane combination by paramyxovirus F proteins for the most part happens at the plasma layer of the host cell at impartial pH (Ryder, 2010), which diverges from the case for infections picking up passage through a pH-subordinate endocytic progression. Fascinatingly, it has been demonstrated that syncytium arrangement for hMPV strain Can97-83 is progressive by the hMPV F protein at low pH, signifying one of a kind instrument of activating combination among the *Paramyxoviruses* F proteins (Schowalter, 2006).

### 1.2.3. Genetic characterization of *Human metapneumovirus* (hMPV)

The length sequences of hMPV genomes have been reported (Herfst *et al*., 2004; Biacchesi *et al*., 2003). The RNA genome of the hMPV is about 13 kb in length, single-stranded, negative sense (Easton *et al*., 2004). The hMPV genome encode nine proteins in the order SH-G-F-M2-L-5; aMPV, 3-N-P-M-F-M2-SH-G-L-5) (Collins *et al*., 1986; Ling *et al*., 1991; Yu *et al*., 1992).The M2 gene encodes 2 proteins, M2-1 and M2-2, using overlapping open reading frames, as in RSV (van den Hoogen *et al*., 2002).

*Human metapneumovirus* genome do not have nonstructural proteins NS1 and NS2 at the 3' end of the viral genome and standing of F closely adjacent to M, the F gene was shown quite well-conserved by sequence analysis technique Fig.1-2.

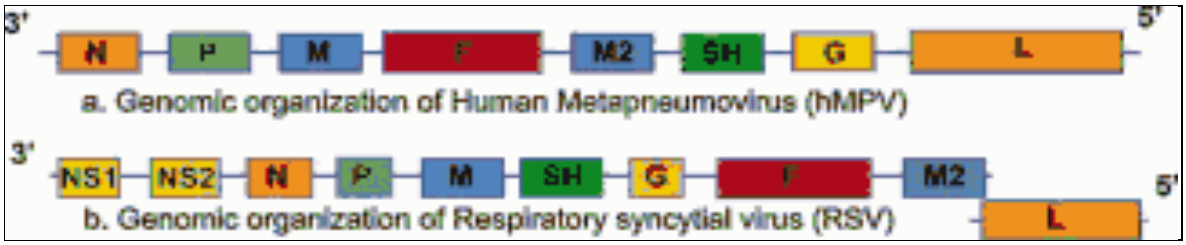



**Figure (1-2): Genomic organization of (a) human metapneumovirus (hMPV) and (b) respiratory syncytial virus (RSV), showing the important differences between the two viruses (Bastein *et al*., 2003).**

The N gene that codes for N protein, 394-amino acid, is the first gene in the hMPV's genomic analysis and its length is identical to the length of the N of APV-C and less than those of other paramyxoviruses. Based on the sequencing of amino acid of N protein, it has revealed 88% similarities with the APV-C while only 7-11% with other paramyxoviruses (Barr *et al*., 1991). The phosphoprotein ORF gene in the hMPV's genome is the second ORF that codes

for 294-amino acid which identity with 68% with P protein of APV-C and 22-24% with the P protein of RSV, and is similar to P of many other paramyxoviruses (Sedlmeier and Neubert, 1998).

The matrix protein ORF consist of 254-amino acid which is encoded by the third ORF of the hMPV's genomic map, the length of M ORFs is the same size as the M ORFs of other metapneumovirus and the amino acid sequencing of the M-ORF is higher (76%-87%) than other metapneumovirus and it is lower identity than RSV and MPV (37%-38%) and 10% similarities with other paramyxoviruses. The fusion protein ORF is the fourth protein, which composed of 539-amino acids and it is encoded by the F ORF which is situated nearby to the M ORF, the identity of the sequencing of amino acids of F ORF protein with APV-C, APC-A and paramyxoviruses is 81%; 67%; and 10-18% respectively (van den Hoogen *et al*,.2001).The F fusion protein is an essential viral fusion protein, which has non-furin F1/F2 cleavage position adjacent a SH protein and two heptad repeats in the extracellular site that enable fusion with cell cytoplasmic membrane, The F protein of paramyxoviruses is a viral fusion protein class I and main goal of the response to neutralizing antibody (Lamb and Parks, 2007). Firstly, the folds of the F protein are metastable, changeable pre-fusion (Yin *et al*., 2005; Lamb *et al*., 2007) which based on activation, undertakes extensive refolding (Yin *et al*., 2005) attached to membrane receptor. As well as the main antigenic place of hMPV F protein has been recognized (Ulbrandt *et al*., 2008).

The assumed F ORF of hMPV is situated neighboring to the supposed M ORF, which is distinctive for members of the *Metapneumovirus* genus. The F gene of hMPV encodes a 539-amino acid, which are 2 amino acid residues lengthier than F of APV-C. Amino acid sequence has 81% identity with APV-C, 67% with

APV-A and -B, 33–38% with other pneumovirus F proteins, and only 10–18% with other paramyxoviruses. One of the preserved structures amongst F proteins of paramyxoviruses and also realized in hMPV is the scattering of cysteine residues (Yu *et al.*, 1991). *Metapneumovirus* part 12 cysteine residues in F1 (7 are preserved amongst all *paramyxoviruses*) and 2 in F2 (1 is conserved among all paramyxoviruses), of the three latent N-linked glycosylation spots existing in the F ORF of hMPV, none are common with RSV and two (positions 66 and 389) are shared with APV. The third, exclusive, potential N-linked glycosylation location for hMPV is located at position 206. Notwithstanding the comparatively low percentage of sequence distinctiveness with other *Paramyxoviruses*, F proteins of *Paramyxoviridae* members are manufactured as indolent ancestors (F0) that are sliced by proteases of host cell which create N-terminal F2 subunits and large C-terminal F1 subunits. The cleavage site of hMPV comprises the residues RQSR. Together arginine residues are common with APV and RSV, while the glutamine and serine residues are shared with other paramyxoviruses such as human parainfluenza virus type 1, *Sendai virus*, and *Morbilliviruses* (van den Hoogen *et al*.; 2002). The F fusion proteins of other members' pneumovirus such as *Avian pneumovirus* (APV) and other *Paramyxoviruses* are deficit the sequence between the two cleavage sites (Gonza´ lez-Reyes *et al.*, 2001; Zimmer *et al.*, 2001). Nearby to the fusion peptide and transmembrane piece are two sections that have heptad duplications, HRA and HRB, which are fairly poor in glycine, comprise no helix-breaking proline, and have charged amino acid side chains in all heptad positions (Russell *et al.*, 2001).

The G protein owns a glycosylated type II mucin-like protein properties, but it doesn't contain the conserved cysteine group as in the RSV and APV G protein, therefore it is called "cysteine noose" but it is highly variable as G gene in the

RSV, it may be due to the immune response of a host and can see the schematic diagram of hMPV particle and its ribonucleoprotein (RNP) complex in figure (1-3) (Crowe, 2004).

### 1.2.4. Molecular Epidemiology of human metapneumovirus (hMPV)

*Human metapneumovirus* is an omnipresent respiratory pathogen that has been coursing in human populations undetected for a considerable length of time. The first report recognized hMPV-particular antibodies in chronicled human sera from the 1950s (van den Hoogen, 2001) and hMPV has been distinguished by RT-PCR in samples from 1976 (Williams, 2004). Phylogenetic examination of various hMPV quality successions recommends that hMPV veered from AMPV between 200–300 years back (de Graaf, 2008, Yang, 2009).

*Human metapneumonia virus* has an overall conveyance and has been recognized on each mainland (Ebihara *et al*., 2004; Al-Sonboli *et al*., 2005). In mild atmospheres, hMPV circles predominately in the late winter and spring, and the top of action at any given area regularly harmonizes with or takes after the crest of RSV action (Agapov *et al*., 2006; Bouscambert-Duchamp, 2005, Sarasini, 2006). In numerous groups, hMPV has been distinguished consistently, though at lower levels among the late spring, summer, and fall (Esper, 2004; Mullins, 2004). Attractive into account genomic sequencing and phylogenetic investigation, there are two notable genotypes of hMPV, assigned A and B (Williams, 2004; Chano, 2005).

These investigations depend on sequencing of the N, M, F, G, or L quality, whether these two genotypes express to obvious serogroups stays questionable. Every genotype seems to have no less than two distinctive subgroups (Bossert, 2002; Mackay, 2004).

A study reported that the highest point of the hMPV consistent cases is found middle March and April taking after the RSV and influenza malady seasons (Mizuta, 2013).

Earlier studies have been reported that the hMPV infection season covers with that of the RSV disease season (Chan, 2007). Being a respiratory infection, hMPV is transmitted by irresistible airborne droplets (Kahn, 2006). Seroprevalence studies have demonstrated that a high rate (90–100%) of children have been infected when they are 5–10 years of age, however re-infection can happen all through adulthood (van den Hoogen, 2001). It may occur because of lacking resistance obtained amid the initial infection and/or because of infection by diverse viral genotypes. The shading period differs from individual to individual, however is ordinarily somewhere around 3 and 5 days. Among animals experimentation, highest viral titers are seen between days 4 and 5 in BALB/c mice and cotton rat (Hamelin, 2005).

Phylogenetic examination of strains of hMPV discovers that the study of disease transmission of hMPV is perplexing and dynamic. Not at all like flu had infection, where a few strains spread over the globe every year, have flare-ups of hMPV had all the earmarks of being a neighborhood wonder. Strains of hMPV vary from group to group, and strains recognized in one area may be very like strains distinguished in different areas in diverse years. For instance, the model strain distinguished in the Netherlands is hereditarily like strains recognized in Australia; New Haven, Conn.; and Quebec, Canada in distinctive years (Pelletier *et al*.; 2002).Taking into account F quality successions, infections segregated in Australia (2001), France (2000 and 2002), Canada (1999, 2000, 2001, and 2002), and The Netherlands (2001) were firmly related, with couple of polymorphisms in the F quality (Boivin *et al*.; 2004).

In any given year, infections of both genotypes and subgroups in each genotype can circle (Peret *et al*.; 2002; Esper *et al*.; 2004, Carr *et al*.; 2005; Agapov *et al*.; 2006; Kaida *et al*.; 2006). In St. Louis, Missouri, the superior genotype of hMPV exchanged in back to back years from genotype A to genotype B (Agapov *et al*.; 2006). Comparable phenomena have been watched somewhere else (Gerna *et al*.; 2005).

In the study from St. Louis, the seriousness of illness connected with the two genotypes did not contrast. The observed epidemiological essentials of hMPV are like those of RSV, where infections of both A and B subgroups cocirculated every year and the transcendent strains change from area to area and from year to year (Peret *et al*.; 1998).

*Human metapneumovirus* is commonly found in the pediatric populations, with high susceptibility rates in children under 2 years of age. Infection with hMPV in grown-ups regularly indicates just gentle influenza like manifestations Nonetheless, in some grown-up cases, particularly elderly grown-ups, serious complication, for example, chronic obstructive pulmonary disease (COPD) can occur (Boivin, 2002). Dyspnea is more probable in grown-ups as thought about to children (Falsey *et al*.; 2003). The illness of hMPV has likewise been accounted for in a few immunocompromised patients, for example, lung transplant receivers, patients with hematological malignancies, and hematopoietic immature stem cell transplant recipients (Williams *et al*.; 2005; Dokos *et al*.; 2013). Two studies found that both genotypes of hMPV (A and B) glow among a regular respiratory infection season (Duchamp *et al*.; 2005), and incessant re-diseases with distinctive hMPV genotypes occur (Pelletier *et al*.; 2002).

A few lines of proof proposed that hMPV is a normal human respiratory pathogen; previous studies have established that hMPV causes mellow

respiratory tract diseases in sound grown-ups (Stockton *et al*., 2002). On the other hand, it has been revealed that children under 2 years of age, elderly individuals more than 50 years of age, and immunocompromised patients are at more grave danger of lower respiratory tract diseases, for example, bronchitis, pneumonia, and bronchiolitis (van den Hoogen *et al*., 2004). *Human metapneumovirus* has been revealed to be connected with intense wheezing in youngsters (Jartti. *et al*., 2002; Ebihara *et al*., 2004; Xepapadaki *et al*., 2004).

### 1.2.5. Seroepidemiology of *Human metapneumonia virus*

Infection with hMPV gives off an impression of being basic in childhood. A few techniques have been utilized to identify hMPV-particular antibodies in serum, including immunofluorescence utilizing hMPV-infected cells as the antigen (Ebihara, 2003), chemical connected immunosorbent methods utilizing recombinant hMPV proteins (Hamelin *et al*.; 2005), Chemical linked immunosorbent tests in light of recombinant hMPV F or recombinant hMPV N protein are both delicate and particular for the location of hMPV-particular antibodies (Ishiguro *et al*.; 2005).

A few studies have shown that hMPV disease happens right on time in adolescence (Leung *et al*.; 2005). By the age of 5 years, >90% of people screened have proof of hMPV infection; the seroprevalence of hMPV-particular immunizer in grown-ups is almost 100% (van den Hoogen, 2001). The seroprevalence of hMPV-particular counteracting agent in newborn children <3 months of age is >90%, showing that maternally incidental antibodies are available in young children (Leung *et al*.; 2005). Whether this hMPV-particular counteracting agent ensures against infection or reduces the seriousness of disease stays to be resolved. The risk factor connected with serious hMPV contamination incorporate untimely conception, young age, previous nosocomial

disease, and hidden continual aspiratory, heart, or neural disorders (Falsey *et al*.; 2003).

Studies researching the relationship in the middle of genotype and illness seriousness in youngsters have not discovered any huge connections. In some reports showed that genotype A may be more harmful that genotype B, while in other studies have been shown that it was genotype B that was connected with serious hMPV infection (Falsey *et al*.; 2003).

Compared with hMPV-negative adolescents, hMPV-infected children were observed to require supplemental oxygen, to have a more extended stay in the emergency unit, and more probable to have experienced mid-section radiography. Around 40% of children hospitalized with hMPV infections were found to have fundamental high hazard conditions, similar to asthma and chronic lung infection (Edwards *et al*.; 2013).

### 1.2.6. Clinical features of hMPV infection

*Human metapneumonia virus* has been associated with acute respiratory infection (ARI) in all age groups, with more extreme illnesses happening in young children, elderly people and immunocompromised hosts. The infection causes a range of clinical disorders in children that are run of the mill of the paramyxoviruses, including upper and lower respiratory tract diseases. The clinical attributes of hMPV infections are not particular, in this manner, separating it from other respiratory infections on clinical grounds is impractical (Stockton *et al*., 2002). A study ran at Vanderbilt University Medical Center on the relationship of the infection in a partner of 2000 subjects of age 0–5 years, took after within a 25-year period exposed that hMPV was associated with flu-like, bronchiolitis, pneumonia, croup and compounding of receptive respiratory airway illness (Williams *et al*., 2004) .The clinical profile of disease brought on by hMPV was like that brought on by RSV. Irregularity has additionally been

inspected all the more much of the time in hMPV infection when contrasted with RSV (Falsey *et al*. 2003). Further contrasted and RSV diseases, the children who create hMPV disease are to some degree more seasoned, and the seriousness of sickness is normally fairly not exactly RSV (Peiris *et al*., 2003; Viazov *et al*., 2003).

*Human Metapneumonia virus* disease is not limited to the extremely young children, additionally happens in grown-ups and elderly subjects. In grown-ups it more often than not causes flu-like sickness and colds (Boivin *et al.,* 2002). In frail elderly, hMPV causes more serious sickness than in complete elderly or young developed (Falsey *et al*., 2003).

In infants and children with lower respiratory tract disease brought by hMPV are like other viral related bronchiolitis, can't clinically recognized from the RSV, flu, and parainfluenza infections. Cough was accounted for in more than 90% of children; more than 75% had rhinorrhea and more than half had fever (Freymouth *et al*.; 2003; Nissen *et al*.; 2002) an increase of current asthma or wheezing was available in around one half. Hypoxia and atypical mid-section radiograph findings were normal in hospitalized patients; perihilar enters, peribronchial thickening, (Mullins *et al*.; 2004). Found less as often as possible were otitis media, the diarrhea, rash, conjunctivitis, and febrile seizures (Peiris *et al*.; 2003; Vicente *et al*.; 2003). Most infants and children got a discharge diagnosis of bronchiolitis, croup, pneumonia, bronchitis, or asthma overestimation (Viazov *et al*.; 2003, Esper *et al*.; 2003). Co-infection with other respiratory infections happens, and concurrent disease with hMPV and RSV has been hypothesized to bring about extreme sickness, frequently requiring concentrated consideration confirmation and ventilatory support (Greensill *et al*.; 2003). The illness is accepted to more serious in infants and in those with chronically illnesses (Maggi *et al*.; 2003).

### 1.2.7. Replication of *Human metapneumovirus*

*Human metapneumovirus* replication cycle initiates with linking of the infection to the host cell, which is thought to be coordinated by the G protein (Lamb and Parks, 2007). The G protein is the most variable protein among hMPV isolates (van Woensel *et al*.; 2006).

Schematic quick of the hMPV viral cycle; as in the figure (1-5), after linking of the virion to the plasma film, the viral and plasma layers join, bringing about uncoating of the virion and arrival of the RNP into the cytoplasm. After essential translation, the genome is repeated to create the antigenome. The antigenome is used to produce genomic RNA, which is joined into progeny virion or utilized as a template for supplementary translation. After translation, M proteins and RNPs are transported intracellularly to the plasma layer and the glycoproteins of virus F, G and SH are transported from the endoplasmic reticulum (ER) to the Golgi mechanical assembly and afterward to the plasma layer. At last, new virions are collected and are therefore discharged from the plasma layer by the emerging process as show in the accompanying (Mahalingam *et al*., 2006).

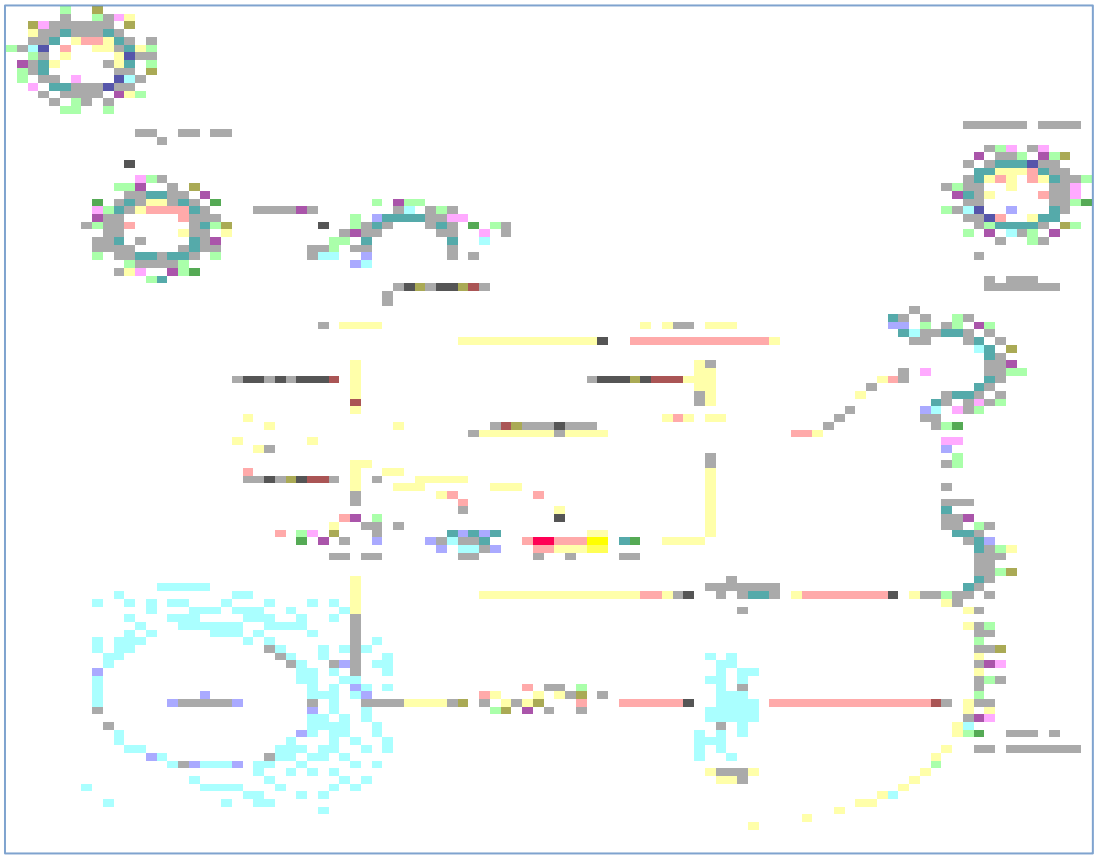


**Figure (1-3): Schematic representation of the hMPV life cycle (Lamb and Parks, 2007).**

### 1.2.8. Laboratory diagnosis of human metapneumovirus

#### 1.2.8.1. Isolation in cell culture

*Human metapneumovirus* isolation to cell culture such as HEp-2, LLC-MK-2 cells and Vero E6 cell lines, have been utilized for the growth and isolation of hMPV (Schildgen *et al*.; 2011).

In a late study utilizing 19 distinctive cell lines to cultivate hMPV, it was demonstrated that the most suitable cell lines for the development of hMPV were a human Chang conjunctiva cell line (clone 1-5C4) and a catlike kidney CRFK cell line.77 In cell culture, hMPV has a moderate development rate, with late cytopathic effects from the rounding of cells and their detachment from the culture matrix to small syncytium arrangement (Tollefson, 2010).

### 1.2.8.2. Serological tests

*Human metapneumovirus* antibodies were recognized in serum which picked up in 1958 that demonstrated that the virus has been circulated for at any rate for as long as 50 years in the Netherland (van den Hoogen *et al*., 2001).Recently, ELISA techniques utilizing viral N or F protein communicated in prokaryotic (Hamelin and Boivin, 2005) or renovated vesicular stomatitis virus (VSV) (Leung, *et al.*, 2005) and recombinant baculovirus framework (Liu *et al*., 2007) have been created to identify antibodies against hMPV utilization of these assays in sero-epidemiologic studies may be useful for recognition of immunized response against hMPV infection (Prins *et al*.; 2004). Serological tests are achieved for retrospective uncovering only, seropositive IgM anti-hMPV antibodies disclose within several days subsequently infection and frequently they late for 1 to 2 weeks can be predictable, in this way, IgM mean indicator for acute infection, whereas IgG upsurges later and stays longer and in this way they are great pointers of past hMPV infections (Hamelin *et al*., 2004).

### 2.1.8.3. Immunoflourescent tests

The cytospin-assisted direct immunofluorescence assay (DFA) is a substitute assay used uncommonly for hMPV detection as a part of Europe and has turned out to be entirely standard in the United States (Kawai and Akira, 2005; Landry *et al*., 2008). Direct immunofluorescence test (DFA) utilizing virus particular antibodies are a rapid performance to distinguish respiratory viruses and are usually utilized as a part of demonstrative research facilities. Commercially available hMPV specific antibodies have been produced for direct immunofluorescence tests; though this approach may not be as sensitive as RT-PCR for the recognition of hMPV (Ebihara *et al*., 2004; Percivalle *et al*., 2005).

Normally used antibodies are those from Chemicon/Millipore (Chemicon International, Temecula, CA) (Landry*et al*, 2005; Landry, 2008), these monoclonal antibodies are offered as complete units, e.g., the Light Diagnostics hMPV direct immunofluorescence test, or as the SimulFluor hMPV/RSV reagent. Likewise, Diagnostic Hybrids has fashioned and drove FDA-cleared measures, in particular, the D3 DFA metapneumovirus ID unit for the location of hMPV and the D3FastPoint L-DFA respiratory infection identifiable resistant pack, which permits the ID of 8 distinctive infections, including hMPV. The last test is declared to be as sensitive and exact as DFA, however a late study confirmed that it is less delicate than PCR and DFA; on the other hand, the ideal opportunity for multiplex discovery is shorter (Barger *et al*., 2010).

### 2.1.8.1. Molecular Diagnostic test of hMPV

Since of the inapproachability of rapid antigenic recognition technique in the previous time and difficulty and slow growth of hMPV in tissue cells culture, therefore the molecular techniques are considered a gold standard, and the excellent tool for diagnosis of hMPV is RT-PCR (Cote *et al*., 2003; Esper *et al*., 2003). The conserved region that may confer a target for detection of hMPV is within the F gene or N gene. As well as, the other target site is reported to bind with N and L gene, in addition, the P gene also reported to supply the fixed region for designing RT-PCR primers and satisfactory capriciousness to allow the precise genotyping of the virus into two major lineages and four sublineages (Mackay *et al*., 2004).

At least, there is two commercial protocols might be an available, firstly, single-plex assay, which are involved RT-PCR and NASBA assays. But, the facts on the usefulness of those tools are rare, in spite of the NASBA technique, the M gene is targeted for these assays, was appeared to be delicate as

information on the utility of those measures are uncommon, the way that NASBA was seemed to be as slight as a PCR upend diagnosis assay (Dare *et al*., 2007). Recently, the studies use multiplex methods to evaluate the patients who experienced with coinfection more than one pathogenic microorganism (Raymond, 2009).

### 1.3. *Respiratory syncytial virus* (RSV)

The novel of disclosure of RSV was shown individuals of chimpanzees in Washington, which had developed cold-like illness, and it was first recognized causes of bronchitis in children in 1957. The cytopathic effect (CPE) of RSV was noted by Morris and colleagues when the samples recovered from runny secretion of these illness chimpanzees to cell culture, As long as, they had noted like symptoms like rhinorrhea and malaise on human who was close contact with ill chimpanzees (Ogra, 2004). However, actual serious problem of the RSV effect in elderly persons was not predictable until the 1970s, when the care facilities were exposed to long term of an outbreak of RSV (Vikerfors *et al*.; 1987). Previous studies have utilized the imprecise tests to appraise of incidence and effect of RSV; therefore, there were variables assessment of illness, While the up-to-date studies have based on the new mathematical approach involving hospitalized children and death in elderly persons with the viral activity (Neuzi *et al*.; 2003; Thompson *et al*.; 2003).

#### 1.3.1. Biology of *Respiratory Syncytial Virus*

The virion of RSV is made up of lipid-enveloped with unequal spherical shaped, 150 to 300 nm in measurement, other morphology of virion may be present which are involved, 60-100 nm and up to 10 nm in length, filament-like and could be seen in both infected cultures and preparation of the virus (Brown *et al*.; 2004). The viral envelope is a lipid bilayer gained from the host plasmatic

layer. The viral transmembrane glycoproteins is the combination protein F, the association protein G, and the little hydrophobic protein SH sort out themselves to frame spikes, which are obvious under electron microscope and the figures (1-6, 7).


**Figure (1-6): Color enhanced transmission electron micrograph (TEM) of Respiratory Syncytial Virus (Phanie, 2007).**

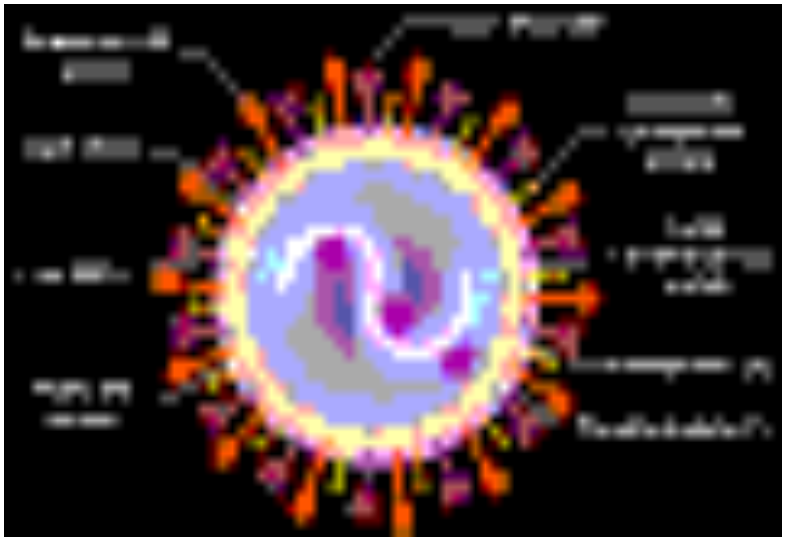


**Figure (1-7): Model structure of RSV**

(www.kuleuven.be/rega/mvr/images/RSV**)**

*Respiratory syncytial virus* is a ubiquitous infection that causes respiratory tract worsening, for example, laryngitis, croup and it likewise causes bronchitis (Brown *et al*.; 2002; Tripp *et al*.; 2005). The genetic materials of RSV encode eleven proteins (Hall, 2001; McNamara-Smyth, 2002).

In spite of the figure display ten proteins only; the M2 protein contains two overlapped proteins, which are M2-1 and M2-2. Proteins of RSV are divided into two groups, structural proteins (SP) and nonstructural proteins (NSP), functionally, the structural proteins can be divided into three groups, nucleocapsid (N) protein, phosphoprotein (P) and viral polymerase (L), all these proteins (N, P, and L) have been revealed as the replicase of RSV. The matrix (M) protein is lined externally by the outer envelope, the fusion (F) and attachment (G) glycoprotein are projected from envelope as spike-like projections, each F and G glycoproteins are assisting the virion to attach and inter into the target cells to initiate the infection and propagation of infection (Empey, 2010), another protein founds in viral envelope is M2 protein. The nucleocapsid proteins consist of a nucleoprotein, a phosphoprotein and a polymerase protein (C). The type 1 of transmembrane glycoprotein is the F protein, which is cleaved into two regions, N-terminal signal sequence on external surfaces, and another region is a transmembrane anchor adjacent the C-terminus. The F glycoprotein can be additional subdivided into two subunits, F1 and F2 subunits are interconnected by disulfide bonds, (Sullender, 2000).

The type II glycoprotein that held on the external envelope of the virion of RSV, is the G protein, which encoded by the G gene, the G protein have greatly glycosylated with N-linked and special region is O-linked sugar. As well as, comprise two regions which are characterized with hypervariabiltly, the hypervariable region ($1^{st}$ HVR and $2^{nd}$ HVR), adjoining a central conserved region. Furthermore, human RSV is divided into two subclass according to the

difference of their antigens, therefore, it is involved HRSV-A and HRSV-B (Collins and Karron, 2013), Recently, RSV subgroups are additional subdivided into genotypes depended on the variable genetic sequence of the 2nd HVR of the G gene. Also, RSV-A cab be other classified into eleven genotypes based on variability sequence, which are comprised GA1-GA7, SAA1, NA1-NA2 and the 72-nucleotide doubling genotype, ON1, as well as HRSV-B, likewise HRSV-A, can be divided into 9 genotypes: GB1-GB4, SAB1-SAB4 and the 60-nucleotide doubling genotype, BA, which has 12 minor groups (BA1-12) (Eshaghi *et al*.; 2012; Khor *et al*.; 2013).

There are two distinct strain of this virus, A and B, have been recognized, both are infectious, one strain has a tendency to overwhelm during an individual pandemic in an individual area (Hall, 2001). The predominance of each subtype changes over progressive seasons and is not connected with illness seriousness. It doesn't have neuraminidase and haemagglutinin surface glycoproteins that are existed in the flu virus (Black, 2003). Beside the 2 strains, a few subtypes of every strain have been recognized (Cane, 2001). Most of the variability among the RSV strains and subtypes can be followed to variability inside of the G protein, a glycoprotein situated on the surface of the viral coat, which is included in attachment of the virus to the host cell. Antibodies to RSV, which is responded to RSV infection, are particular to the G protein from the specific strain delivering the individual's infection. A few authors have predicted that the variability inside of this specific protein among different RSV strains and subtypes diminishes the viability of the body's immune response and permits continuous reinfections to occur (Wilson *et al*.; 2000).

### 1.3.3. Transmission of *Respiratory Syncytial Virus*

Clinical insights of the spread of RSV inside families, childcare, and different gatherings of children have noticed that the transmission of RSV requires close

contact with infected people or with their discharges (Hall, 2007; Lindsley *et al*., 2010). These perceptions and volunteer studies show that RSV is essentially spread by two instruments: (1) large size of aerosols products (10–100 μm) which are impelled short space about ≤ 0.9 m by coughing products, sneezing droplets and even still breathing; and (2) by contaminated environmental materials which followed by infectious inoculation (Lindsley *et al*., 2010). Low of humidity (≤30 %) increases survival of potential infectious of RSV, particularly it occurs during the winter season (Siegel *et al*., 2007).Transmission of RSV within a hospital is challenging in pediatric wards and immunocompromised patients (El Saleeby *et al*., 2008, Bont, 2009).

The essential mode of RSV transmission is close contact with the materials from an infected person and environments, blowout of RSV occurs when these resources come in close contact with the mucous membrane of nose, mouth, and eye, as long as, it can happen with inhaled substances, the source of infection such as fomites, unwashed hands and surface often persist infectious for several hours (Akhter and Al Johani, 2011). The major cause of bronchiolitis in worldwide is RSV, which form up to 70-80% of all other lower respiratory tract illness (Henrikson, 2004).

### 1.3.4. Clinical manifestations of *Respiratory syncytial virus* infection

The most practiced children with RSV are symptomatic, and have a prominent clinical signs, comparable with upper respiratory tract, the lower respiratory tract is more prevalent, and the predominant sign is wheezing, the infection of RSV is more harshly in the early of life, around the early months of life (Ogra, 2004; Hall, 2012). Often, the mild URT infection with RSV persists several days with low grade fever and cough. Worsening cough is typically indicate lower respiratory tract (LRT) involving ; therefore, the infants progress to grow tachypnea and may have ongoing more labored breathing, with difficulty

breathing with chest wall retraction. Frequently, crackles and wheezing are the auscultor signs, which are late from minutes to hours. Additional findings, which have been determined by radiologically, are hyperinfilteration and peribronchial thickening, A further radiological result which may be present is dispersed interstitial infiltrated (Wright and Piedimonte, 2011). Frequently reappearing wheezing and prolonged pulmonary disorder impediment has been recognized in up to 30-50% hospitalized infants who practice with RSV lower respiratory infection (Sigurs *et al*., 2010; Sly, *et al*., 2010; Stein and Martinez 2010).

The signs of URTI such as otitis media and sinusitis are not less than one fourth of adults who infected with RSV, develop have got signs of LRT like wheezing and cough (Walsh, 2011).

### 1.3.4. Laboratory diagnosis of *Respiratory syncytial virus*

Children who have lower respiratory infection have particularly bronchiolitis, the radiographical results show infiltrate, atelectasis, and hyperinflation, while the white blood cells count do not significant implement to determinate of rigorousness and detection of the virus, therefore have not been taken to guideline for treatment and follow up of response of therapy (Swingler and Zwarenstein, 2008; Baker *et al*., 2009), therefore, there are several techniques for diagnosis of RSV as the following shown.

#### 1.3.4.1. Protocol of clinical specimens

The suitable site and proper collection of clinical specimens has a substantial influence on the virus isolation and detection, the appropriate samples for diagnosis of RSV are nasal swab, nasopharyngeal aspirate have high sensitive and specific to determine RSV, it is not recommended to use the nasopharyngeal swab in hospitalized patients, it can be used to collect the sample in the outpatients, however, the flocked- nasopharyngeal swabs newly tool available for collection and extricate of virus infected –cells that covering the internal

surface of nasopharynx (Heikkinen *et al*., 2002; Abu-Diab *et al*., 2008, Lambert *et al*., 2008).

**1.3.4.2. Tissue culture methods for isolation RSV**

Even though the tissue cell culture of isolation of virus being more sensitive, but it takes long time for incubation, compared with other techniques, viral culture is considered a gold standard; the usefulness of viral culture is utilized for range of virus isolation (Langley and Anderson, 2011). As a consequence of time consuming and non-specificity test, it has not been adequate for initiating antiviral drugs and prevention and control assessment. Essential isolation of RSV in traditional culture takes three to seven days and probable ten days (Piedra *et al*., 2002). The tissues culture which have been utilized to RSV isolation are HEP-2, He La and human heteroploid, the cytopathic effect (CPE) features of RSV is comprising of syncytia formation (Hall & McCarthy, 2004; Machado *et al*., 2008).

**2.2.8.2. Antigen detection of RSV**

The direct immunoflourescent assay (DFA) uses fluorescein-labelled antibodies that distinguish RSV antigen in epithelial cells in respiratory secretions and has the advantage that the immunofluorescence form of the infected cells can be straight inspected by microscopy which provides additional confirmation of specificity, recognition of antigen techniques comprise enzyme immunosorbent assays (EIA), direct immunofluorescence assays, and chromatographic and optical immunoassays (Ribes *et al*., 2002; Ohm-Smith *et al*., 2004; Reina *et al*., 2004; Gregson *et al*., 2005; Borek *et al*., 2006). The immunosorbent assay (EIA) utilizes a specific RSV Antigen on a solid phase, the ELISA assays have several benefits include, swift, and ability to test a large numbers of samples in standard time, as a whole, the EIA has disadvantages comprise poorly sensitive and a vague results and an ambiguous results (hazy area) which need for confirmatory

via using other blocking ELISA technique. However, there are several kits of ELISA are commercially accessible for the valuation of specific Ag of RSV (Casiano-Colon *et al*.; 2003), while the DFA employs specific antibody which linked with fluorescein substances called fluorescein-labelled Ab, DFA detect the specific Ag of RSV on the surface infected epithelial cells in respiratory discharge that has been done by immunoflourescent microscope. Immunofluorescence assay and EIA which are used to RSV detection in respiratory discharges are largely employed in children and transfer the sufficiently amount of this material to identify infectious agent, but it requests a critical viral load in order to assure a positive outcome, these techniques, IFA and EIA, are not satisfactory to detect the RSV Ag in adults due to both methods exhibition poorly positive level, only IFA and 10% by EIA (Akhter and Al Johani, 2011), identification of RSV by IF methods show more sensitive detection than by tissue culture technique (85% to 99% versus 29% to 74%) respectively (Aldous *et al*., 2004).

**1.3.4.4. Polymerase Chain Reaction (PCR) for RSV**

The product of qPCR procedure, which often take <2 hours, can be directly measured without further beyond PCR steps. Real-time PCR Nucleotide sequences were considered using the primer express, and were directed also to a region located on the F gene of RSV, a highly preserved region in the genome and used successfully as target in laboratory in the classical PCR method (Mentel *et al.*, 2003).

Amplification and analysis in RT-PCR are occurring in concomitantly, amplification products have been notable via an increase in emission of fluorescence substance, which can be indicated by specific device provided in a PCR system. Real-Time PCR is adequate for quantitative approach, but recently there is commercially tool supplied to detect the nucleic acid in the respiratory

specimen only qualitative requests. The number of emission channels for multiplex methods are restricted in almost available RT-PCR system. Consequently, the multiplex RT-PCR is mostly limited for diagnosis of four or fewer genetic material particular. Recently, there is a new approach adapted of RT-PCR are provided for the recognition of RSV (involving A and B genotype), hMPV, parainfluenza viruses 1, 2, and 3, and influenza A (including subtypes) and influenza B viruses is called FDA-cleared real-time RT-PCR assays (Henrickson and Hall, 2007).

Such study indicated a method that recognized both types of RSV, A and B, and used disagreeing test to display that 63.9% specific and RT-PCR was 97.5% sensitive (Freymuth *et al*., 2006).

## 2.3. Expression of hMPVs' genes and Chemokines RSV- induced genes

### 2.3.1. Expression of G glycoprotein and M2-2 of hMPV

*Human metapneumovirus* encodes nine proteins, amongst them, phosphoprotein P, glycoprotein G, and small hydrophobic protein SH have been shown to modulate hMPV-induced innate immune response, the first mark of host protection against violent pathogens. Whether or not other hMPV proteins are involved in the regulation of host cellular responses is currently unknown, However the G protein is a transmembrane surface glycoprotein, which initiates the virus-host cell membrane attachment and so considered as a key player in viral replication (Bao *at al*., 2008; Kolli *et al*., 2011). The matrix protein of hMPV, M2, encodes two overlapping proteins: M2-1 and M2-2. The M2-1 open reading frame (ORF) of strain CAN 97-83 is assumed to initiate with the head AUG at nucleotide location 14 and encodes a protein of 187 amino acids. The M2-2 ORF possibly initiates with the AUGs at positions 525 and 537, overlapping the M2-1 ORF by 53 or 41 nucleotides, respectively (Bao *et al*., 2008).

The role of hMPV M2-2 protein in modifiable viral replication, both *in vitro* and *in vivo*, using a rodent and a primate model of infection, was recently investigated (Biacchesi *et al*., 2005; Schickli *et al*., 2008).

*Human metapneumovirus* infection of alveolar epithelial cells, the crucial goal of respiratory viruses, induces cytokine, chemokine, and type I IFN by RIG-I/MAVS-dependent signaling, but not through TLR-3- and MDA5-dependent, the M2-2 protein antagonizes MAVS-mediated innate antiviral response. This novel function of M2-2 in obstructive host innate immunity in addition to the M2-2 contributed to hMPV immune evasion as well. The M2-2 protein, but not other soluble hMPV proteins, inhibited the MAVS-activated IFN- promoter but

not the one definitely intermediated by downstream signaling particles, suggesting that MAVS is a target of M2-2 (Ren *et al*., 2012).

As of late, it have been recognized the M2-2 protein of hMPV is additionally a major suppresser protein in human airway epithelial cells. M2-2 straightforwardly targets characteristic antiviral signaling as well as by implication suppresses against hMPV responses by impeding the outflow of other virulence factors of hMPV, for example, G, Whether M2-2 controls host immunity in other cell types, including human dendritic cells (DC), a group of potent antigen presenting cells (APC), is not right now known (Ren *et al*., 2014). hMPV can infect human monocytes-derived DC (moDC) and plasmacytoid DC (pDC), and hMPV infection of these two cell-types can viably impede the formation of class I IFN because of TLR agonists, also taking after disease with hMPV, mice demonstrated a huge interference of IFN-b construction in the lung after intranasal vaccination with TLR9 agonist (Guerrero-Plata *et al*., 2005).

The replication of hMPV initiates with a fusion of the virus to the host cell directly by the G protein (Lamb& Parks, 2007). Suspected sequencing of amino acid of the glycoprotein G consists a one hydrophobic region, which is situated close the N terminus and is supposed aid for both an uncleaved signal peptide and a membrane anchor, anther region is C-terminal that form three-fourths of glycoprotein G and it thought to be extracellular. The glycoprotein G of hMPV contains of serine and threonine residues which serve as acceptor sites for O-glycosylation, in addition it has a high proline residue (van Woensel *et al*., 2006), and these features shared with heavily glycosylated mucin-like structures (Liu *et al*., 2007).

However the suggested role of glycoprotein G in attachment, the F protein of hMPV is adequate for fusion and attachment in the lack of glycoprotein G

(Biacchesi *et al*., 2004; Biacchesi *et al*., 2005), then the role of glycoprotein G is a minimal in attachment, a recombinant human metapneumonia virus which is lacking the G protein (rhMPV-ΔG) displays reduced replication in the upper and lower respiratory tract of African green monkey and Syrian hamster (Biacchesi *et al*., 2005).

At late time, it has been found the glycoprotein G is an essential virulence factor of the hMPV and its expression potently prevent type I interferon (IFN) production, furthermore secretion of cytokines and chemokine equally *in vivo and in vitro*. The G protein of hMPV inhibits interferon regulatory factor (IRF) and viral-induced nuclear factor- kB (NF- kB), proposing the G protein play a role in regulating early intracellular signaling events triggered by hMPV infection. Without a doubt, that G protein particularly, targets hMPV-affected cell reactions mediated by the cytoplasmic RNA helicase retinoic destructive inducible quality I (RIG-I) in respiratory route epithelial cells and by Toll-like receptor 4 (TLR4) 4 in primary immune cells (Kolli *et al*., 2011). Also the G protein of hMPV disturbs cellular signaling in respiratory route epithelial cells (Bao *et al*., 2008).

As of late, have been recognized the M2-2 protein of hMPV is additionally a major suppresser protein in human airway epithelial cells. M2-2 straightforwardly targets characteristic antiviral signaling as well as by implication suppresses against hMPV responses by impeding the outflow of other virulence factors of hMPV, for example, G, Whether M2-2 controls host immunity in other cell types, including human dendritic cells (DC), a group of potent antigen presenting cells (APC), is not right now known (Ren *et al*., 2014). hMPV can infect human monocytes-derived DC (moDC) and plasmacytoid DC (pDC), and hMPV infection of these two cell-types can viably impede the

formation of class I IFN because of TLR agonists (Guerrero-Plata *et al*., 2005).as well taking after disease with hMPV, mice demonstrated a huge interference of IFN-b construction in the lung after intranasal vaccination with TLR9 agonist (Guerrero-Plata *et al*., 2005).

### 1.4.2. RSV-induced Chemokines network in the lower airway epithelial cells

*Respiratory syncytial virus* infection in early life is associated with the succeeding initial allergic airway illness (Sigurs, 2000), in several previous studies had revealed no increased risk for rising asthma in children who earlier RSV infection (Carlsen *et al*., 1987). Newly, animal experimental practice have been used to additional inspect this relation, whether the infection with RSV, previously, during, or taking after allergen trial being displayed to modify allergic signs (Matsuse *et al*., 2000; Lukacs *et al*., 2001).

Chemokines are a superfamily of proteins can be organized into really diverse gatherings: three groups of small basic (heparin-binding) proteins, labeled the C, CC, and CXC chemokines (depend on the number and spacing of highly conserved $NH_2$-terminal cysteine residues), and a fourth, indirectly related group, the CX3C chemokines, made out of substantial, membrane-bound glycoproteins linked through a COOH-mucin-like area . Those receptors for the chemokines are express Edina cell sort limited design has permitted specificity in chemokine activity—for instance, individuals from the C group essentially stimulate lymphocyte chemotaxis, individuals from the CXC cluster affect neutrophil chemotaxis, and the CC group revitalizes monocytes, lymphocyte, and eosinophil chemotaxis **(**Baggiolini *et al*., 2007).

There are several CC chemokines network expression can be induced by RSV infection, for instance, thymus and activation regulated chemokine (TARC),

RANTES, MCP-1, macrophage-derived chemokine (MDC) and (MIP-1a and 1b), CXC, CX3C. However, TARC is a CC chemokine which is chemotactic for naïve CD4 T lymphocytes and immature dendritic cells **(**Lieberam *et al*.; 1999**).** The receptor CCR4 can be allowed to bind with CCL17/TARC and CCL22/ MDC. The CCR4 is expressed on several a subpopulation of peripheral blood such as mature dendritic cells, lymphocytes, thymocytes and on blood platelets (Lukacs, *et al*., 2001). The CCR4 expression associated with TH2 responses. Raised CCR4 levels can proceed up two days after induced by T-cell receptor (Vestergaard *et al*., 2003). Macrophage-derived chemokine (MDC/ CCL22) made up of 69 amino acid residues, synthesized by macrophage cells linage (Pinho *et al*., 2003), other cells are consider as a source of MDC production including monocyte-derived dendritic cells, natural killer (NK) cells and bronchial epithelial cells (Hirata *et al*., 2003), the MDC is clustered on chromosome 16q13, it contain of the 4 cysteine motif and highly conserved residues characteristic of CC chemokines, it is identity with other human chemokines such as TARC by less than 35%, and the later, TARC, is closed human comparative (Mantovani *et al*., 2000; Kakinuma *et al*., 2002).

Thymus-and activation-regulated chemokines (TARC) is a highly specific ligand of CCR4, and it is synthesized and produced by several cells including monocytes, dendritic cells and it as a serve assist recruitment, activation and development of Th2-polarized cells for CCR4 expression (Leung *et al*., 2002; Fujisawa *et al*., 2002).

### 1.5. Interleukin 8 (IL-8) and respiratory viral infections

Interleukin-8 (IL-8) is a fellow of CXC chemokine family and is categorized and denoted to as CXCL8 based on the new nomenclature system (Zlotnik, 2002). It has multifunctional chemokine that influence human neutrophil

functions, including chemotaxis, enzyme release, and expression of surface adhesion molecules. Recently, it was clarified that IL-8 and other ELR motif-containing CXC chemokines are mediators of angiogenesis (Belperio *et al*.; 2000).

Interleukin 8 has been alternatively named as T-cell chemotactic factor, neutrophil-activity peptide 1, Beta-thromboglobulin-like protein, and tumor necrosis factor-induced gene, amongst others. However, the name and character chemokine (C-X-C motif) ligand 8 and CXCL8, correspondingly, were adopted and have been officially endorsed by the Chemokine Nomenclature Subcommittee of the International Union of Immunological Societies (Bacon *et al*.; 2002).

Interleukin 8 gene (CXCL8) has been mapped to 4q12-q21 by using somatic cell hybridization and in situ hybridization. It is made up of 3211 bases in length and is encoded on 4 exons. Monocytes/macrophages, epithelial cells, smooth muscle cells and endothelial cells can all generate IL-8. It exists in two isoforms; a 72 amino acid form derived from endothelial cells and the more profuse 77 residue form secreted by monocytes and other cells (Zhang *et al*.; 2002).

A major property of IL-8 during the inflammatory process is chemotaxis of mark cells to the situation of inflammation, in particular neutrophils. IL-8 also has chemotactic activity against T cells and basophils. Neutrophil sticking and travelling across the endothelium are regulated by IL-8 and once neutrophils attain to the site of inflammation, IL-8 further stimulates those cells to carry out phagocytosis, thus increasing the competence of tissue restoration (Kobayashi, 2008).

*Human metapneumovirus* interferes with superantigen-induced T cell stimulation by infecting dendritic cells. Thus, the production of antigen-specific CD4+ T cells is restricted and the production of long-term immunity is impaired

(Cespedes *et al*.; 2013). Respiratory viruses are known to modulate cytokine responses. Compared to RSV and influenza, hMPV is a fewer effective stimulator of different cytokines like IL- 12, TNF, IL-6, IL-1b, IL-8, and IL-10 (Laham *et al*.; 2004).

The concentration of the proinflammatory IL-8 cytokine has also been described as elevated in nasal discharges attained from 10 hMPV-infected children who presented with the sudden onset of wheezing (Jartti, 2002).

### 1.5.1 Biological effect of IL-8 in asthma attack

Interleukin-8 was the first member recognized of a upward family ,currently more than 40 members, of proinflammatory chemokines belonging to the C-X-C subfamily that attract and activate immune and inflammatory cells (Hay and Sarau, 2001), It has been shown to be involved in the pathology of a wide range of disorders such as rheumatoid arthritis, gout, severe trauma, psoriasis, acute inflammation, infection, sepsis syndrome, cancer, systemic lupus erythromatosis, nephritis and number of pulmonary maladies, comprising chronic obstructive pulmonary disease (COPD), acute respiratory distress syndrome (ARDS), wheeze, chronic asthma, pulmonary fibrosis and bacterial pneumonia. Measurement of IL-8 in these conditions has been shown to be useful not only in understanding the basis of these disorders, but also as a prognostic and diagnostic marker of disease. Plentiful examinations are ongoing endeavoring to prevent the activity of IL-8 in these diseases as well as exploring the prospects of using IL-8 clinically to attract immune cells into tumors and to re-correct neutrophil function in myelodysplasia (Tsuji *et al*.; 2012)

IL-8 has also been suggested to play a role in asthma, where it is described to be included in lymphocyte, eosinophil and basophils activation and migration to the inflammatory site (Hollandera *et al*., 2007).

## 1.6. Bioinformatics application on particular viral proteins

### 1.6.1. Molecular phylogenetic

Molecular phylogenetic performs the structure and task of molecules and how they modification over time to infer these evolutionary relations. This division of study developed in the first 20th century but didn't initiate in intense until the 1960s, with the arrival of protein sequencing, PCR, electrophoresis, and other molecular biology techniques. Above the past 30 years, by way of computers have become further effective and more largely reachable, and computer algorithms more stylish, scientists have been able to challenge the enormously difficult stochastic and probabilistic difficulties that define evolution at the molecular level more effectively. Within past years, this field has been more revived and redefined as entire genome sequencing for complex organisms has become faster and less expensive. As banks of genomic data becomes widely obtainable, molecular phylogenetic is ongoing to raise and find novel applications (Hall, 2004).

Phylogenetic analysis is a universal technique in virology, founding an important section of inquiries describing viruses or viral epidemiology. However, several features of viruses' carriage specific tasks for phylogenetic: i) strong variances in development rates, ii) bulky latent for recombination and gene transfer equal between discrete viruses or their host type, iii) often strong evolutionary relations between viruses and their hosts and v) profusion of genomic 'fossil records' for example parts of antique viral genomes that happen within the genomes of present species, Phylogenetic trees are the most package presentation for virus phylogenies in the literature and numerous tree-building approaches and software exists like MrBayes (Ronquist & Huelsenbeck, 2003), BEAST (Drummond *et al*., 2012), PhyloBayes (Lartillot *et al*., 2009), RAxML (Stamatakis, 2014). Nevertheless, trees cannot denote complex evolutionary

relatives that are pertinent for viruses as horizontal gene transfer, interspecific recombination, or the evolutionary relationships between viruses and their hosts. Multiple distinctive categories of phylogenetic networks have been established in current years to designate such relations (Huson *et al*., 2011).

In the utmost common terms, an evolutionary tree, phylogeny, is an illustrative representation of biological objects that are related through shared descent, such as species or higher-level taxonomic alliances. A devastating body of indication supports the deduction that each organism active currently and altogether those who have always lived are members of a shared inheritance that spreads back to the beginning of life certain 3.8 billion years ago. One would consequently suppose it to be probable to rebuild the Tree of Life, division by division and branch by branch, from the recent variety exist at the furthest branches to a commonly shared origin (Gregory, 2008). Relationship among species is an idea that based on genetics besides history, and there is sufficient evidence that even remotely separated lineages need, at times, practiced noteworthy gene involvement, horizontal and vertical gene transfer (Doolittle *et al*.; 2008).

The ancient cliché struggles that an excessive effort on individual trees can inhibit one from attainment the grandeur of a forest. The opposite manage with respect to evolutionary trees, in that their cooperative significance is understandable, nonetheless a lot of people are unskilled with the basic topographies of distinct phylogenies. Whether they show associations among a small number of species or thousands (Bininda-Emonds *et al*., 2007) or of more groupings of species (genera, families, phyla), wholly evolutionary trees offer the same elementary facts: a historical model of ancestry, separation, and lineage. They do so by depicting a series of branches that unify at points expressive shared ancestors, which themselves are linked over more bulgy ancestors.

The overall anatomy of a phylogeny is concise in Fig (1-8) this tree displays the relations among three species.

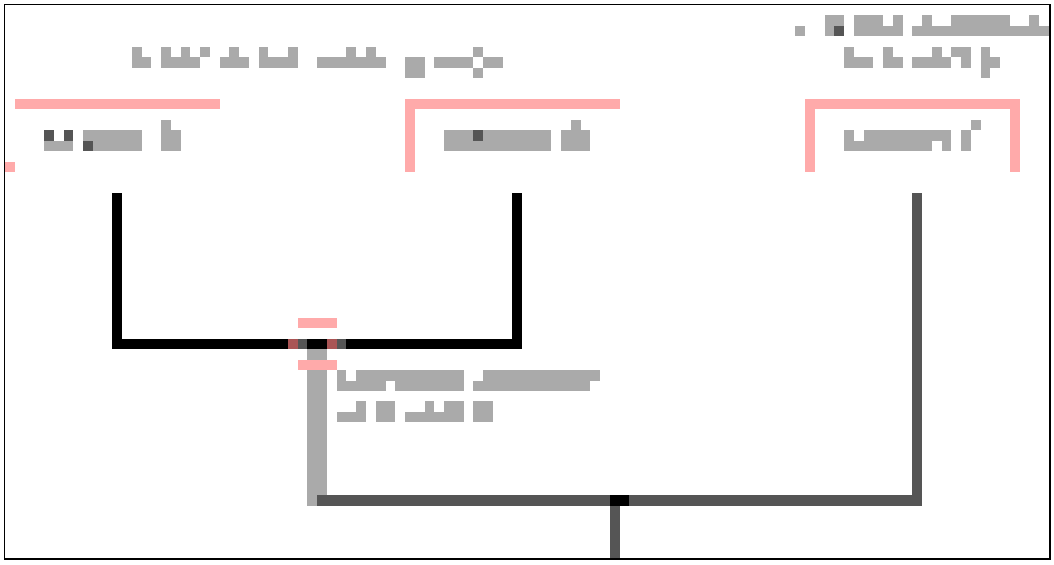


**Figure (1-8): Anatomical depiction of phylogenetic tree model, by Allen Collins in 1994. The current version (2006) is by Anna Thanukos.**

The phylogenetic tree is constructed by using a system of nodes and branches, the term node denotes to any ending end of a branch (a line). External nodes indicate the ending taxon, while internal nodes signify a shared ancestor that undertook some speciation happening (where organisms within that taxon stop reproducing due to causes like physical separation, such as the development of an island, or the favorite of a specific physical characteristic that a subsection of the population initiates to favor through the progression of sexual selection). Accordingly, speciation trials give rise to different lines of taxon and are denoted by horizontal twigs. These diverging line of taxa stem from a common ancestor, consequential in association named related taxa (such as taxon A and taxon B), denotation that they common the nearby evolutionary bond because they stem from the similar shared ancestor. Taxon external of that shared ancestor is designated to as outgroups as they are more evolutionarily detached in relation

than sister taxa are to one additional because of an extra expanse shared ancestor. Through every consecutive speciation incident, a new clade is made within the tree, permitting scientists to recognize shared ancestors between evolutionarily distant taxon (Xiong, 2006; Gregory, 2008; Dowell, 2008; Rastogi *et al*., 2009).

### 1.6.2. Sequence alignment protein

In bioinformatics a sequence alignment is an approach of ordering the sequences of DNA, RNA, or protein to find regions of similarity that may be a magnitude of functional, structural, or evolutionary relationships between the sequences. The first DNA sequences were obtained in the early 1970s by academic researchers using laborious methods based on two-dimensional chromatography (Mount, 2004). The multiple alignments of protein sequences have become a vital device in molecular biology. It has conventionally been used to discovery distinctive motifs and preserved regions in protein families, in the determination of evolutionary linkage and in the improved prediction of secondary and tertiary structure. With the swift upsurge in the number of protein sequences, particularly from the genome sequencing schemes, automatic means of probing protein databases for homologous sequences (Kemena and Notredame, 2009).

A multiple alignment is developed gradually by aligning the neighboring sequences first and consecutively adding in the more aloof ones. A number of alignment programs depend on this technique exist, for instance MULTALIGN, CLUSTALW, T-COFFEE, MUSCLE, CLUSTAL OMEGA and MSA prob. (Hang, 2008). Sequences can be aligned using their complete length (global alignment) or at particular regions (local alignment). Multiple sequence alignment for protein sequences is much more problematic than the DNA sequence corresponding (containing only 4 nucleotides) because of the point that

there are 20 diverse amino acids. Global optimization methods, established in practical mathematics and operations research, deliver a generic toolbox for resolving difficult optimization problems. Global optimization is currently used on a regular foundation, and its request to the MSA problematic has developed a repetitive (Do and Katoh, 2008).

Local alignments are desirable; nevertheless, they can be puzzling to estimate because of the struggle associated with the detection of sequence regions of resemblance. The two main characteristics of significance for MSA technique for the employer are biological truth and the computational complexity. Biological accuracy concerns how adjacent the multiple alignments are to the true alignment and are the sequences aligning acceptably, presentation insertions, deletions, or gaps in the right positions (Edgar, 2004). Motif detection algorithms are additional type of MSA algorithms that are used. These approaches are used to determine motifs in the long sequences; this procedure is observed as a "needle in a haystack" problem, because of the fact that the algorithm seeks a short stretch of amino acids (motif) in the long sequence. One of the most widely used tools for searching for motifs is Gapped Local Alignments of Motifs, GLAM2, (Frith *et al*.; 2008).

### 1.6.3. Protein prediction model

One of the first predictive algorithms GOR (Garnier, Osguthorpe & Robson,) for secondary structure was established through a co-operation between a laboratory attentive in developing the theory for protein secondary structure prediction methods and a laboratory concerned in using and matching such methods. The GOR algorithm definitely allocates each residue to one conformational state of a-helix, stretched chain, back turn or coil. In its early form GOR was approximately 50% accurate on a test sample of 26 proteins. .

The program gives two outputs, one eye-friendly giving the sequence and the predicted secondary structure in rows, H=helix, E=extended or beta strand and C=coil; the second gives the probability values for each secondary structure at each amino acid position. The predicted secondary structure is the one of highest probability compatible with a predicted helix segment of at least four residues and a predicted extended segment of at least two residues (Mount, 2004; Robson, 2005).

### 1.6.4. Prediction of Transmembrane Regions and Orientation

The TMpred program makes a prediction of membrane-spanning regions and their orientation. The algorithm is based on the statistical analysis of TMbase, a database of naturally occurring transmembrane proteins.

It is proposed to enable the subsequent jobs:

- discovery positional predilections of certain amino acids
- Originating an enhanced technique for the prediction of transmembrane domains.
- testing of such estimate outlines
- Statistical testing of general hypotheses regarding transmembrane proteins.

### 1.6.5. Predicted topology by OCTOPUS program

OCTOPUS presents a new technique of joining ANN-predicted residue scores with an HMM-based global prediction algorithm, where distinct paths are used for the prediction of inside/outside and membrane/non-membrane preference values. As alpha-helical transmembrane proteins institute approximately 25% of a typical genome and are vigorous parts of several crucial biological processes. Because structural knowledge of transmembrane proteins is problematic to

achieve experimentally, improved methods for prediction of structural features of these protein is important Functionality: OCTOPUS uses a combination of hidden Markov models and artificial neural networks. In particular, OCTOPUS is the first topology predictor to integrate modeling of reentrant-, membrane dip-, and TM hairpin regions in the topological grammar. OCTOPUS first does a homology search using BLAST to produce a sequence profile. This is used as the input to a set of neural networks that predict both the preference for each residue to be located in a transmembrane (M), interface (I), close loop (L) or globular loop (G) environment and the preference for each residue to be on the inside (i) or outside (o) if the membrane. In the third step, these predictions are used as input to a two-track hidden Markov model, which uses them to calculate the most likely topology (Feig and Jabri, 2002; Mayor *et al*.; 2003; Boyle , 2004; Bradley *et al*.; 2005).

## 2. Material and Methods

### 2.1. Materials

#### 2.1.1. Subjects

Two Hundred and thirty outpatients of children were suffering from respiratory tract illness (RTI), the database of them were registered in this study, which involved, name of the patient, age; gender and the major clinical symptoms of RTI, such as fever, cough, sneezing, nasal discharge (rhinorrhea),and asthma attack which evaluated, principally, by the pediatricians consultant via taking the major clinical features of asthma which encompassed (wheezing and dyspnea), the age of selected patients was from several days to fourteen years old of both genders, from Al- Sader Teaching Hospital at Al Amarah city during the period from December 2014 to April 2015. The information of each patient was taken based on the questionnaire format which has been constructed by the supervisors and pediatricians, as set out in the appendix.

#### 2.1.2. Instruments and Equipments

The Instruments and Equipments that used in this study with their companies and countries of origin are listed in the table (2-1)

**Table (2-1): The Instruments and Equipments used in this study**

| No. | Equipment & instrument | Company/ Origin |
|---|---|---|
| 1- | High Speed cold centrifuge | Eppendorf /Germany |
| 3- | Vortex | CYAN/ Belgium |
| 5- | Nano drop | THERMO/ USA |
| 7- | Real-Time PCR | BioRad /USA |

| | | |
|---|---|---|
| 9- | Exispin vortex centrifuge | Bioneer/S. Korea |
| 10- | ELISA System | Bioneer/S. Korea |
| 11- | Thermocycler apparatus | Bioneer/ S. Korea |
| 12- | Hot plat stirrer | Labtech /S. Korea |
| 13- | Sensitive balance | Sartorius /Germany |
| 14- | Digital camera | Sony /Japan |
| 15- | oven | Memmert |
| 16- | Electrophoresis apparatus | Bioneer/ Korea |

### 2.1.3. Chemicals

The chemicals with their companies and countries of origin used in this study are listed in the table (2-2)

**Table (2-2): Chemicals agents with their company/ state**

| No. | Chemical | Company/Origin |
|---|---|---|
| 1 | Absolute Ethanol | BDH /England |
| 2 | Isopropanol | BDH /England |
| 3 | DEPC water | Bioneer/ S.Korea |
| 4 | Free nuclease water | Bioneer/ S.Korea |
| 5 | RNase free water | Bioneer/ S. Korea |
| 6 | Agarose | Biobasic/ Canada |
| 7 | Ethidium bromide | Biobasic/ Canada |

| | | |
|---|---|---|
| 8 | TBE buffer | Biobasic/ Canada |
| 9 | Ladder 100bp | Promega / USA |
| 10 | PCR water | Bioneer/ S. Korea |
| 11 | Random Hexamer primer | Bioneer/ S. Korea |

### 2.1.4. Diagnostic Kits

#### 2.1.4.1. ELISA kit

The contents of ELISA kit, which is produced from Wuhan Elabscience Biotechnology Co/ Iwai North America Inc., are listed in the following table (2-3).

**Table (2-3): Human interleukin 8 (IL-8) ELISA kit components**

| Item | Specification |
|---|---|
| Micro ELIASA plate | 8 wells/12 strips |
| Reference standard | 2 vials |
| Reference standard & sample diluent | 1 vial /20ml |
| Concentrated Biotinylated Detection Ab | 1 vial /120ml |
| Concentrated Detection Ab | 1 vial /10ml |
| Concentrated HRP conjugate | 1 vial /120ml |
| HRP conjugate Diluent | 1 vial /10ml |
| Concentrated Wash Buffer (25*) | 1 vial /30ml |
| Substrate reagent | 1 vial /10ml |
| Stop solution | 1 vial /10ml |

### 2.1.4.2. Extraction total RNA and qRT-PCR components kits

The Molecular kits used in this study with their companies and countries of origin are listed in the table (2-4).

**Table (2-4): Molecular technique kit contents and their company and state.**

| No. | Kit | Company | Origin |
|---|---|---|---|
| **1-** | **AccuZol™ Total RNA extraction kit** | Bioneer | S. Korea |
| | Trizol 100ml | | |
| **2-** | **AccuPower RocketScript™ RT-qPCR PreMix** | Bioneer | S. Korea |
| | 8 wells strips containing RocketScriptTM reverse transcriptase and TaqMan probe premix | | |
| | DEPC water 4 tubes | | |
| | Adhesive film | | |
| **3-** | **AccuPower® PCR PreMix** | Bioneer | S. Korea |
| | Taq DNA polymerase | | |
| | dNTPs (dATP, dCTP, dGTP, dTTP) | | |
| | Tris-HCl pH 9.0, KCl, $MgCl_2$ | | |
| | Stabilizer and Tracking dye | | |
| **4-** | **AccuPower ® GreenStar ™ qPCR PreMix 96 plate** | Bioneer | S. Korea |
| | SYBER Green fluorescence | | |
| | Taq DNA polymerase | | |
| | dNTPs (dATP, dCTP, dGTP, dTTP) | | |
| | DEPC water | | |

### 2.1.4.3. Primers and Probes designing

The primers and probe were considered in this study by using the complete sequence of Nucleoprotein gene RSV-A (GenBank: KF973340.1), RSV-B (GenBank: KF893260.1), and hMPV (GenBank: KF891365.1) from NCBI-GenBank and Primer3 plus design. The primers were provided by (Bioneer. Company, Korea) as the following table (2-5):

**Table (2-5): primers and probes that used in this study**

| Primer | Sequence | | Amplicon |
|---|---|---|---|
| RSV-A primer | F | 5′-TGCAGGGCAAGTGATGTTAC-3′ | 86bp |
| | R | 5′-TTTCTGCTTGCACACTAGCG-3′ | |
| RSV-A probe | 5′-VIC-GGTGGGGAGTCTTAGCAAAATCAGTT-BHQ-1 | | |
| RSV-B primer | F | 5′-TGTGCACTTTGGCATTGCAC-3′ | 101bp |
| | R | 5′-TTACTTGCCCTGAACCATAGGC-3′ | |
| RSV-B probe | NED-TCCACAAGAGGGGGTAGTAGAGTTGA-BHQ-1 | | |
| hMPV primer | F | 5′-AGAAACTCAGGCAGTGAAGTCC-3′ | 130bp |
| | R | 5′-TCTCTTCCACCCAGCTTTTCTC-3′ | |
| hMPV probe | FAM-ACCAGAACGTACTCCTTGGGGAA-BHQ1 | | |

**F**: Foreword, **R**: Reverse

### 2.1.4.4. DNA sequencing primers of F gene of hMPV

These primers were considered by using the complete sequence of fusion protein (F) gene in hMPV (GenBank: DQ362940.1) from NCBI-GenBank and Primer3

plus design, 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 (2-5):

**Table (2-6): The F fusion gene primers of hMPV**

| **Primer** | **Sequence** | | **Amplicon** |
|---|---|---|---|
| F gene primer | F | 5′-TGATGTTGGAGAACCGTGCA-3′ | 428bp |
| | R | 5′-GTAGCAAGCAACCAAAGCCC-3′ | |

**F**: Foreword, **R**: Reverse

### 2.1.4.5. Gene expression study primers

The primers Chemokines (TARC and MDC) gene G glycoprotein and M2-2 of hMPV which were expressed from the airway epithelial cells that have been considered in this study by using NCBI-GenBank and Primer3 plus design GenBank codes: reference Actin gene (NM-001101.3), chemokine MDC gene (U83171.1), chemokine TARC gene (XM-011523256.1), M2-2 protein gene hMPV (AY530095.1), and G-protein gene hMPV (JQ309682.1), and these primers were provided by (Bioneer. Company, Korea), as following in the table (2-6).

**Table (2-7**): **The MDC, TARC G glycoprotein, M2-2, and Actin primers**

| **Primer** | **Sequence** | | **Amplicon** |
|---|---|---|---|
| Housekeeping gene (Actin) | F | 5′-TCGTGCGTGACATTAAGGAG-3′ | 133bp |
| | R | 5′-TTGCCAATGGTGATGACCTG-3′ | |
| MDC | F | 5′-TGTGAAGCCCCAAATTTGCC-3′ | 124bp |

| | | | |
|---|---|---|---|
| | R | 5′-AAGCCAAGACCACACCATTG-3′ | |
| TARC | F | 5′-TGGGGCAATGTCAATGTTGG-3′ | 125bp |
| | R | 5′-AGTTCTGTGTACCCAGCCAAG-3′ | |
| M2-2 gene hMPV | F | 5′-CAGTGAGCATGGTCCTGTTTTC-3′ | 95bp |
| | R | 5′-TCACTATCCCATCGGACAAAGC-3′ | |
| G-protein gene hMPV | F | 5′-ACAACCGCAGAGAAAAAGCC-3′ | 114bp |
| | R | 5′-TCCATTGCTGGTTTGGTTGG-3′ | |

**F:** Foreword**, R:** Reverse

## 2.2. Methods

### 2.2.1. Samples collections

The clinical specimens were nasopharyngeal aspirates, which are taken once from each patient by insert the sterile fine stick plastic, which is supplied with viral transport medium (VTM), into nasopharynx until the stick is challenging, then the stick was withdrawn gently, then put on the viral transport media (VTM) which prepared for this purpose, then the samples were transported by an ice bag from the hospital into the pharmacy department for storage in -70˚C.

The samples were divided into 2 tubes, first for the molecular tests, the second for the immunological test for measurement of IL-8.

### 2.2.2. Principle of ELISA test

This ELISA kit uses Sandwich-ELISA as the method. The micro ELISA plate delivered in this kit has been pre-covered with an antibody exact to IL-8. Standards or samples are additionally to the suitable micro ELISA plate wells and combined with the specific antibody. Then a biotinylated recognition antibody specific for IL-8 and Avidin-Horseradish peroxidase (HRP) conjugate is added to each microplate well consecutively and incubated. Free components are washed away. The substrate solution is added to each well. Only those wells that cover IL-8, biotinylated recognition antibody and Avidin-HRP conjugate will appear blue in color. The enzyme-substrate response is completed by the addition of a sulphuric acid solution and color turns yellow. The optical density (OD) is examined spectrophotometrically at wavelength 450 nm ±2 nm. The optical density value is proportional to the concentration of IL-8, and then the concentration of IL-8 in these samples was calculated by comparing the OD of the samples to the standard curve.

### 2.2.3. Sample preparation to measure IL-8 level

The nasopharyngeal (NP) aspirates of the particular patients are directly into VTM then they were well mixed, then kept at -70º C as long as, the ELISA kit were brought and prepared for this purpose, the NP aspirates were taken and thawed and then centrifuged for 20 minutes at 1000 rpm then the supernatant was collected to assay.

### 2.2.4. Procedure assay of ELISA method

It was carried out as described by the manufacturer's instructions

- One hundred microliter (100µl) Standard and sample was added to each well, and then incubated at 37˚C for 90 minutes.
- The liquid is removed and the 100 µl biotinylated detection Ab was added then incubated 1 hour at 37˚C.
- The well are aspirated and washed 3 times by ELISA washer.
- One hundred microliter (100 µl) HRP conjugated was added then incubated 30 minutes at 37˚C.
- The wells were aspirated and washed 5 times.
- Substrate reagent (90 µl) was added then incubated 15 minutes at 37˚C.
- Stop solution (50 µl) was added then the results were read immediately.

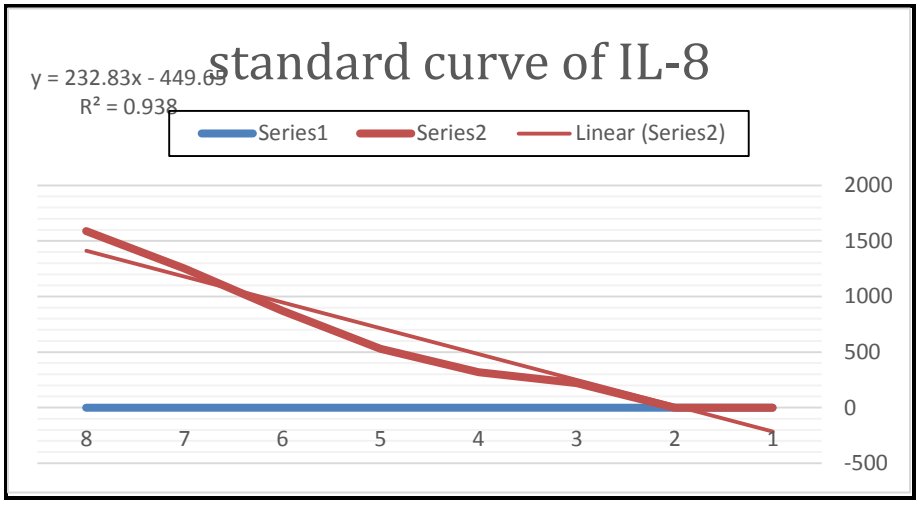



**Figure (2-1): show the standard curve to estimate the IL-8 in nasopharyngeal sample**

### 2.2.5. Viral RNA Extraction

- Viral RNA was extracted from frozen nasopharyngeal swab samples by using AccuZol$^{TM}$ Total RNA extraction kit (Bioneer, Korea) and done according to company instructions as in the following steps:

- The two hundred microliter (200µl) of nasopharyngeal fluid samples was transferred by sterile pipette into sterilized and fresh 1.5ml Eppendorf tube, then 1ml AccuZolTM reagent an mixed by vortex.
- The two hundred microliter (200µl) of chloroform was added to each Eppendorf tube and shaken vigorously for 30 seconds.
- The mixture was incubated in ice for 5 minutes.
- Afterward, the mixture was centrifuged at 12,000 rpm, 4°C, for 15 minutes.
- The supernatant was transported to a new Eppendorf tube, and 500µl isopropanol was added.
- The mixture was mixed by inverting the tube for 4-5 times and incubated at 4°C for 10 minutes.
- The mixture was centrifuged at 12,000 rpm, 4°C, for 10 minutes.
- The supernatant was thrown away.
- Ethanol alcohol (80%) was added into each tube and well mixed by vortex, then centrifuged at 12,000 rpm, 4°C for 5 minutes.
- The supernatant was discarded and the RNA pellet left to dry at room air for 5 minutes.
- After that, DEPC water (50µl) was added to RNA pellet tubes and well mixed by vortex to solute the RNA pellet.

- The extracted RNA sample was checked by Nanodrop spectrophotometer, and then kept at -20°C freezers.

### 2.2.6. Estimation of extracted total RNA yield

The total extracted RNA was assessed by a Nanodrop spectrophotometer (THERMO. USA), There are two quality controls were performed on extracted RNA. First one is to determine the quantity of RNA (ng/µL), and the second is the purity of RNA by reading the absorbance in a spectrophotometer at 260 nm and 280 nm in same Nanodrop device as follow:

- After opening up the Nanodrop software, the appropriate application was selected (Nucleic acid, RNA).
- The pedestal was cleansed by a dry kimwipe several times. Then carefully pipetted 2µl of free nuclease water and put on the surface of the lower pedestal to blanking of Nanodrop.
- After that, the pedestals ware cleansed and pipetted 1µl of RNA sample for measurement.
- Purity and concentration of obtained RNA was estimated according to the following equations:

  Purity of RNA= $A_{260nm}/A_{280nm}$ ratio

  Concentration of RNA= $40*A_{260}$*dilution factor

### 2.2.7. Reverse Transcription Real-Time PCR (RT-PCR)

One step RT-qPCR was performed for detection of RSV-A, B and hMPV based on nucleoprotein gene and this technique was carried out according to method pronounced in GoTaq® 1-Step RT-qPCR manual technique System.

### 2.2.7.1. Real-Time PCR master mix preparation

Real-Time PCR master mix was prepared by using one step Reverse Transcription and Real-Time PCR detection and it was done according to the company instructions as in the following table (2-10):

**Table (2-8): The RT-PCR mastermix constituents**:

| **RT-PCR mastermix** | **Volume** |
|---|---|
| Total RNA template | 5µL |
| RSV-A, RSV-B, hMPV<br>Forward primer (20pmol) | 2.5µL |
| RSV-A, RSV-B, hMPV<br>Reverse primer (20pmol) | 2.5µL |
| RSV-A, RSV-B, hMPVprobe (25pmol) | 2.5µL |
| DEPC water | 37.5 µL |
| Total | 50µL |

The RT-PCR master mix reaction components that mentioned in table (2-8) above were added into RT-PCR tube containing (8 wells strips tubes which containing RocketScriptTM reverse transcriptase and TaqMan probe premix). Then all strips tubes vortexing for mixing the components and centrifuged for 3000rpm for 3 minutes in Exispin centrifuge, after that transferred into Real-Time PCR thermocycler.

### 2.2.7.2. Real-Time PCR Thermocycler conditions

A real-time PCR thermocycler condition was set based on the primer temperature and RT-PCR TaqMan kit instructions as in the following table (2-11):

**Table (2-9): The RT-PCR thermocycler conditions**

| Step | Condition | Cycle |
|---|---|---|
| Reverse transcription | 50°C, 15 min | 1 |
| Pre-Denaturation | 95°C, 5 min | 1 |
| Denaturation | 95°C, 20 sec | 50 |
| Annealing/Extension | 60 °C, 30 sec | |
| Detection (Scan) | | |

### 2.2.7.3. Real-Time PCR Data analysis:

The quantitative PCR (qPCR) data analysis was performed by calculating the threshold cycle number (CT value) of the positive amplification in Real-Time PCR cycle number.

### 2.2.7.4. The DNA sequencing study

The DNA sequencing was performed for genotyping of positive Human metapneumovirus by qPCR technique. The DNA sequencing method was based on F- fusion gene primers that done in PCR technique as following steps:

**1- Preparation of the PCR mastermix**

The PCR mastermix was prepared by using (AccuPower PCR PreMix Kit) and this master mix done according to company instructions (2-12)

**Table (2-10): The PCR master mix of gene expression contents**

| **PCR Master mix** | **Volume** |
|---|---|
| DNA template | 5 µL |
| F gene Forward primer,(10pmol) | 1.5µL |
| F gene Reveres primer,(10pmol) | 1.5 µL |
| PCR water | 12 µL |
| Total volume | 20 µL |

After that, these PCR master mix component that mentioned in table (2-13) placed in standard AccuPower PCR PreMix Kit that encompassing all other components that needed to PCR reaction which are involved Taq DNA polymerase, dNTPs, Tris-HCl pH: 9.0, KCl, $MgCl_2$, stabilizer, and tracking dye. Then, all the PCR tubes transferred into Exispin vortex centrifuge at 3000rpm for 3 minutes, and then placed in PCR Thermocycler (MyGene. Australia).

## 2- The conditions of the PCR Thermocycler

The PCR thermocycler conditions were done by using conventional PCR thermocycler system as following table (2-13):

**Table (2-11): The PCR thermocycler condition of gene expression**

| PCR step | Temp. | Time | repeat |
|---|---|---|---|
| Initial Denaturation | 95°C | 5min | 1 |
| Denaturation | 95°C | 30sec. | 30 cycle |
| Annealing | 58°C | 30sec | |
| Extension | 72°C | 1min | |
| Final extension | 72°C | 5min | 1 |
| Hold | 4°C | Repeatedly | - |

## 3- The product analysis of PCR

The PCR products of hMPV F gene were analyzed by agarose gel electrophoresis following steps:

- The 1% Agarose gel was prepared in using 1X TBE and dissolving in water bath at 100 °C for 15 minutes, after that, left to cool 50°C.
- Then, 3µl of ethidium bromide stain were added into agarose gel solution.
- The agarose gel solution was poured in tray after fixed the comb in proper position after that, left to solidified for 15 minutes at room temperature, then the comb was removed gently from the tray and 10µl of PCR product were added in to each comb well and 5ul of (100bp Ladder) in one well.

- The gel tray was fixed in electrophoresis chamber and fill by 1X TBE buffer, and then electric current was performed at 100 volt and 80 Amp for 1hour.
- The products of PCR (428bp) for F gene were visualized by using UV transilluminator.

**4- DNA sequencing method**

The PCR product of hMPV F gene (428bp) was purified from agarose gel by using (EZ-10 Spin Column DNA Gel Extraction Kit, Biobasic. Canada). As the following steps:

- The specific PCR product was removed from the gel by clean, sharp scalpel. And then, transferred into a 1.5mL microcentrifuge tube.
- Four hundred microliter (400µl) of binding buffer II was added to gel fragment, then, incubated at 60ºC for 10 minutes and mixed until the agarose gel is completely dissolved.
- Add the above mixture to the EZ-10 column and let stand for 2 minutes. Centrifuge at 10,000 rpm for 2 minutes and discard the flow-through in the tube.
- Seven hundred and fifty microliter (750µl) of washing solution was added to each tube and centrifuged at 10000 rpm for one minute. Then, the supernatant was discarded.
- After that, the step 4 was repeated and centrifuged at 10000rpm for an additional minute to remove any residual wash Buffer.
- The column was placed in a clean 1.5ml microcentrifuge tube and added 30µl of Elution Buffer to the center of the column and incubated at room

temperature for 2 minutes, then, the tube was centrifuged at 10000 rpm for 2 minutes to elute PCR product and store at -20ºC.

After that, the purified F gene PCR products were directed to Bioneer Company in Korea for achieved the DNA sequencing by AB DNA sequencing system.

Phylogenetic study was done based on NCBI-Blast alignment documentation and Neighbor Distance Phylogenetic tree analysis (Mega ver. 6).

### 2.2.8. Gene expression study

The gene expression study was performed for the estimation of relative gene expression of some chemokine genes, MDC and TARC, which induced by hRSV as well as the G-protein of and M2-2 protein gene of hMPV and in only cDNA template of positive samples by using Real-Time PCR based Syber Green dye amplification. This method was carried out according to method described by Zhang *et al*., (2001) as following steps:

**1- Real-Time PCR (qPCR) mastermix preparation**

Quantitative PCR mastermix was prepared by using AccuPower$^{TM}$ Green Star Real-Time PCR kit based SYBER Green dye detection of gene amplification in Real-Time PCR system and include in the following (2-14):

**Table (2-12): The Real-Time PCR (qPCR) mastermix preparation of gene expression**

| The qPCR mastermix | Volume |
|---|---|
| cDNA template (100ng) | 3µL |
| Target genes or reference gene Forward primer(10 pmol) | 1 µL |
| Target genes or reference gene Reverse primer (10 pmol) | 1 µL |
| DEPC water | 15 µL |
| Total | 20 µL |

After that, these qPCR mastermix component that mentioned above AccuPower Green star qPCR premix standard plate tubes that have the Syber green dye and other PCR extension constituents, then the plate mixed by Exispin vortex centrifuge for 3 minutes, then placed in Miniopticon Real-Time PCR system.

After that, the qPCR plate was loaded and the following thermocycler condition in the following table (2-13).

**Table (2-13): Thermocycler protocol of qPCR of gene expression**

| The qPCR steps | Temperature | Time | Repeated cycle |
|---|---|---|---|
| Initial Denaturation | 95 °C | 5min | 1 |
| Denaturation | 95 °C | 15 sec | 45 |
| Annealing\Extension Detection(scan) | 60 °C | 30 sec | |
| Melting | 60-95°C | 0.5 sec | 1 |

### 2.2.9. Data analysis of qRT-PCR

The data results of qRT-PCR for target and housekeeping genes were analyzed by the relative quantification gene expression levels (fold change) ΔCT Reference technique that designated by Livak and Schmittgen, (2001). The relative quantification method, quantities obtained from q RT-PCR experiment must be normalized in such a way that the data become biologically meaningful. In this process, one of the investigational samples is the calibrator such as (Control samples) each of the normalized target values (CT values) is separated by the calibrator normalized target value to generate the relative expression levels. After that, the ΔCT Way with a Reference Gene was used as following equations:

**Relative expression formula:**

$Ratio_{(reference/target)} = 2(CT_{(reference)} - CT_{(target)})$

**Table (2-14): The ΔCT Method for estimation the relative gene expression.**

| **Gene** | **Test (treatment, group)** | **Cal. (control, group)** |
|---|---|---|
| Target gene | $CT_{(target, test)}$ | $CT_{(target, cal.)}$ |
| Reference | $CT_{(ref, test)}$ | $CT_{(ref., cal.)}$ |

First, normalize the CT of the reference (ref) gene to that of the target gene, for calibrator sample:

$$\Delta CT_{(calibrator)} = CT_{(ref., calibrator)} - CT_{(target, calibrator)}$$

Second, normalize the CT of the reference (ref) gene to that of the target gene, for test sample:

$$\Delta CT\ (test) = CT_{(ref., test)} - CT_{(target, test)}$$

$$\Delta\Delta CT = \Delta CT_{(test)} - \Delta CT_{(calibrator)}$$

$$Fold\ change = 2^{-\Delta\Delta CT}$$

$$Ratio_{(reference/target)} = 2^{CT_{(reference)} - CT_{(target)}}$$

So, the relative expression was separated by the expression value of chosen calibrator for each expression ratio of the test sample.

### 2.2.10. The Real-Time PCR Data analysis

RT-PCR data analysis was performed by calculation the threshold cycle number (CT value) that presented the positive amplification in Real-Time PCR cycle number.

## 2.3. Bioinformatics application methods

There are several programs have been performance in the current study which pointed in the following

- EMBL-EBI (protein sequence analysis and classification)
- Protein Data Bank in Europe
- SWISS-MODEL (Model Building Report)
- Structure protein and classification
- ExPASY (transmembrane helices): *web.**expasy**.org/protparam/*

In this division, some of these will be outlined.

### 2.3.1. Clustal omega for multiple sequence alignment (MSA)

Clustal omega is a general purpose multiple sequence alignment program for DNA or proteins. It tries to estimate the finest match for the chosen sequences, and align them so as to the characteristics; similarities and differences can be seen. Successively a tool from the web procedure is a simple multiple steps method,

**Step 1 - Sequence**

**Sequence Input Window**

Note:

- Sequences can be in GCG, FASTA, EMBL, PIR.
- Partly formatted sequences are not accepted.

**Step 2- Sequence File Upload**

FASTA uploaded and used as input for the multiple sequence alignment

**Sequence Type: DNA and proteins**

**Step 3 - Multiple Sequence Alignment Options**

Multiple alignment protein sequence comparison matrix series used to score the alignment.

**Step 4 – Submission:** Job title

(It's possible to recognize the tool outcome by giving it a name).

**Results:** Output of multiple sequence alignment of Clustal omega.

### 2.3.2. Prediction of Transmembrane Regions and Orientation

The TMpred program makes a prediction of membrane-spanning regions and their orientation. The algorithm is based on the statistical analysis of TMbase, a database of naturally occurring transmembrane proteins, which has designated by Hofmann & Stoffel (1993).

**Procedure**

- Paste your sequence in one of the supported formats into the sequence field below
- Press the "Run TMpred" button.
- Make sure that the format button (next to the sequence field) shows the correct format.
- Choose the minimal and maximal length of the hydrophic part of the transmembrane helix.

### 2.3.3. The Protein Model Portal

The Protein Model Portal (PMP) is part of the Protein Structure Creativity Knowledgebase (PSI KB). The aim of the portal is to give cohesive access to the

different models that can be leveraged from PSI targets and other experimental protein structures.

### 2.3.3.1. Predicted topology by OCTOPUS program

**Method**

- Enter one amino acid sequence in FASTA format**.**
- Submit OCTOPUS.

### 2.3.2.2. GOR IV secondary structure prediction method

1- Paste a protein sequence in box tool

2- Submitted to GOR IV.

## 2.4. Statistical analysis:

Data of the current study were analyzed by SPSS ver.16 and Microsoft office excels 2010.

## 3. Results and Discussions

### 3.1. Quantitative RT-PCR of hMPV and RSV

In the current study, two hundred and thirty outpatients of children had been suffered from respiratory illness, out of 230, one hundred of them, were selected, and the nasopharyngeal aspirate of them were undergone to laboratory examination, only 7 (7%) were encountered with the hMPV, 8 (8%) and 14 (14%) were detected with RSV-A and B respectively, as in the following figures (3-1, 2, and 3). There are several studies revealed different results of detected of hMPV, the lowest prevalence has been reported in Cambodia, 1.7%, and 2.6%% in the USA, and the highest prevalence rate reported was 54.4% in Al-Ahwaz province (Maggi *et al*., 2003; Kuypers *et al*.; 2005; Sarasini *et al*.; 2006) while the prevalence rate of RSV was 29.3% in infants with median age 13.5 months as in the study of João *et al*., (2011).

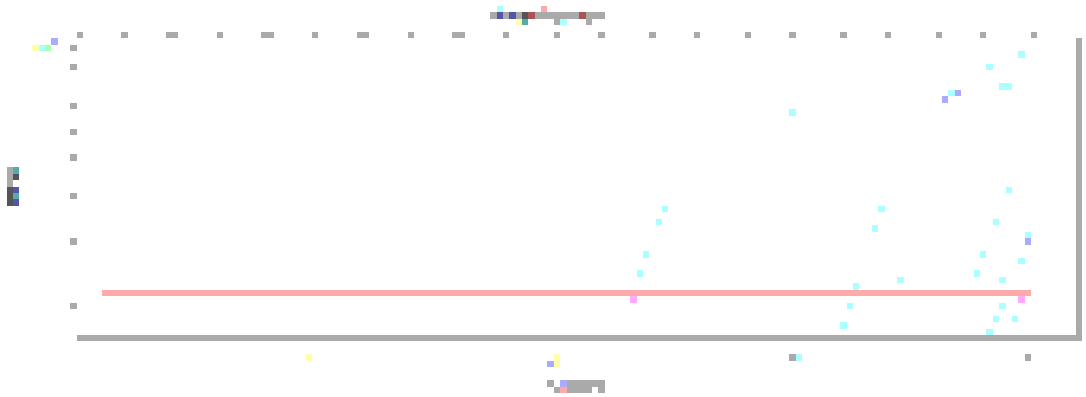


**Figure (3-1):Real-Time PCR amplification plot of positive samples for Human metapneumovirus based nucleoprotein gene primers and probe (FAM) dye of nasopharyngeal swabs, where the positive samples were shown positive amplification ranging from 23.24 into 39.56 threshold cycle (CT).**

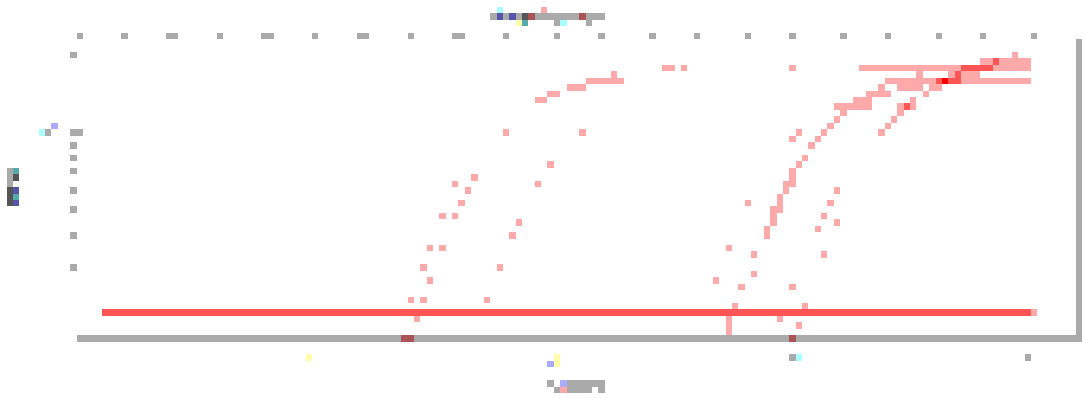


**Figure (3-2):Real-Time PCR amplification plot of positive samples for Human respiratory syncytial virus type A based nucleoprotein gene primers and probe (VIC) dye in nasopharyngeal swabs samples, where the positive samples were shown positive amplification ranging from 13.79 into 30.28 threshold cycle (CT).**

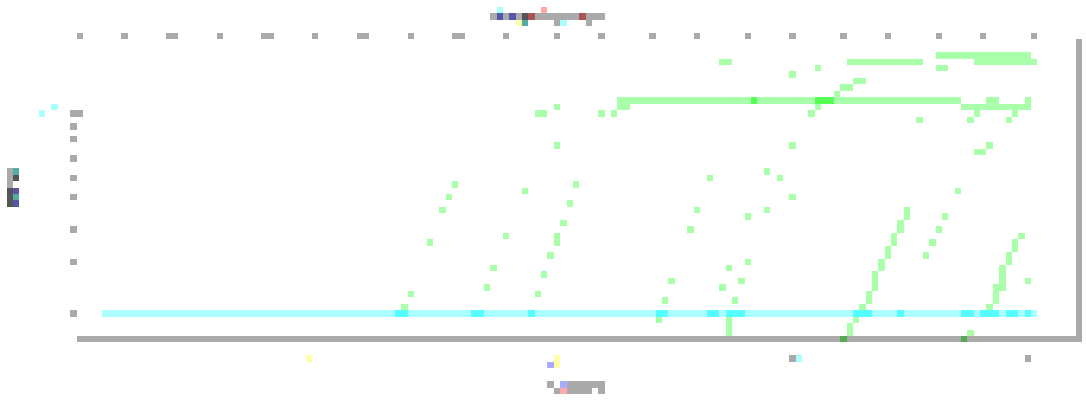


**Figure (3-3):Real-time PCR amplification plot of positive samples for Human respiratory syncytial virus type B based nucleoprotein gene primers and probe (NED) dye in the nasopharyngeal swab samples where the positive samples were shown positive amplification ranging from 13.72 into 39.94 threshold cycle (CT).**

### 3.2. Relationship between *Human metapneumovirus* and *Respiratory syncytial viruses* with the age group.

In the table (3-1) represents the distribution of *Human metapneumovirus* (hMPV) and *Respiratory syncytial virus*-A & B (RSV-A, RSV-B) based on the age groups. The highest percentage was 4(57.1 %) of children (4-7) years old in whom have hMPV infection, and highest percentage, 6(75 %) in age group (<1) and 5(35.7 %) for (<1) in whom have encountered RSV-A and B respectively. Several studies reported roughly percentage for hMPV with the same age groups like Nandhini *et al*., (2016) showed 23 (5%) of 447, out of all, 8 (34.7%) in children with (1-5 yrs.), other studies such as that achieved by Sahar *et al*., (2015) presented 21 (18.7%) during 2011-2012, the RSV-A & B was identified in 23 (20.5%) with high percentage 63.6% in 2-5 years of both years (2011, 2012). Other study which worked by Broor *et al*., (2008), they displayed in 2000 outpatients with age (1-5) years old, only 12% of cases have shown hMPV. 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. However, infection of hMPV and RSV-A and B are detected in all age groups of children from several days to 12 years old, and the results which observed by several studies such as Fabbiani *et al*., (2009) and Xiao *et al*., (2010), whose referred to similar results concerning hMPV and they concluded that these viruses could infect all age groups of children, the age groups in these study were 1-15 years and ≤ 14 years old, respectively.

**Table (3-1): Distribution of hMPV and RSV-A& B based on age group**

| Age group (years) | hMPV | RSV-A | RSV-B |
|---|---|---|---|
| <1 | 2(28.5 %) | 6(75%) | 5(7.14 %) |
| 1-3 years | 1(14.2%) | 1(12.5 %) | 4(28.5 %) |
| 4-7 years. | 4(57.1 %) | | 4(28.5 %) |
| 8-12 years. | | 1(12.5 %) | 1(7.1%) |

### 3.3. The clinical features of hMPV, RSV-A and RSV-B infection

The table (3-2) displays the main clinical spectrum of upper respiratory (sneezing, rhinorrhea, croup) and lower respiratory (cough, wheezing, dyspnea, and crepitation) infection that caused by hMPV, for comparison with other studies in different places, it would to display the results clearly; it's summarized in table (3-3) and their corresponding authors.

**Table (3-2): the clinical features of human metapneumovirus infection**

| Clinical sings &symptoms | Frequency | Percentage |
|---|---|---|
| Fever | 5 | 71.4% |
| Cough | 5 | 71.4% |
| Wheezing | 4 | 57.1% |
| Sneezing | 3 | 42.8% |
| Rhinorrhea | 2 | 28.5% |
| Dyspnea | 4 | 57.1% |
| Crepitation | 2 | 28.5% |
| Croup | 1 | 14.2% |

**Table (3-3): the clinical features of hMPV infection of the some other studies**

| | No. of references | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| % of patients with symptoms and signs | | | | | | | |
| Fever | 96.5 | 67 | 13 | 52 | 81 | * | 85 |
| Cough | 95.5 | 100 | 16 | 90 | 56 | 40 | 85 |
| Wheezing | 47.4 | 83 | 6 | 52 | 72 | 40 | 32.1 |
| Sneezing | * | * | * | * | * | * | 25 |
| Rhinorrhea | 91.2 | 92 | 2 | 88 | 59 | * | 14.2 |
| Dyspnea | 26.3 | * | * | 52 | 86 | 13 | 71.4 |
| Crepitation (pneumonia) | 64.4 | 67 | * | 59 | * | 40 | 46.4 |
| Croup | * | * | * | 18 | * | 28 | * |

*= not reported

1- Takashi *et al*., (2004) 2- Boivin *et al.* (2003) 3- Beneri *et al*., (2009)

4- Viazov *et al*., (2003) 5-Aziz TAG, (2015) 6-Al-Mola, (2013) 7-Marie *et al*., (2004)

The clinical manifestations of upper respiratory (rhinorrhea, sneezing) and lower respiratory tract infection (cough, dyspnea, crepitation) which have been referred in the tables (3-2) and (3-3) may be concomitant with hMPV infection.

They are not pathognomonic signs of hMPV infection, but are rather overlapping with other common viral respiratory infection; therefore it cannot be differentiate among them clinically. Comparably with other studies, fever and cough were occurred in high frequent vast majority of studies cited in this study, as in the table (3-3), also the studies in the same table, recorded rhinorrhea in considerable frequent than other signs, Furthermore, it can be back to the tables (3-2) and (3-3), croup (Laryngotracheobronchitis) was recorded is least frequent, (16%) in present study and (18%- 28%) in table (3-3).

In the table (3-4) which displays the main clinical features that related to RSV-A, B infection, cough is the highest frequent, 7 (87%); 12 (85.7%) respectively, for both serotypes, after that, fever, wheezing and dyspnea are less, 6 (75%) of the serotype A, while, of serotype B are 10 (71.4%) for fever and 11 (78.5%) for wheezing and dyspnea, it is worth noting the signs of the upper respiratory illness, sneezing and rhinorrhea are the least one of that, they were 2 (25%); 3 (37.5%) for serotype A, and 4 (28.5%) for both signs of serotype B. The others signs such as crepitation and croup are also reported for both serotypes of RSV, 3 (37.5%) for both signs of serotype A and 7 (50%), 5 (35.7%) for crepitation and croup of serotype B respectively.

There are several studies have revealed similar results such as the study that done by Xixiang *et al*., (2013) who noted that the common clinical feature of positive cases of RSV (70.1%) were suffered from cough and dyspnea which was reported in 41.1% of positive cases, while the least frequent sign was rhinorrhea. Moreover, other signs which had been recorded in these studies were sore throat (18.4%) and cough with sputum production (11.5%), these signs were not noticed in the present study, other study which has been accomplished by Hemalatha *et al*., (2010) recorded 4(19.0%) of upper respiratory tract illness,

23(60%) of bronchiolitis and 29(43.3%) of pneumonia, in certain study like that achieved by Sahar *et al*., (2015) by using RT-PCR as a diagnostic tool, they have stayed the clinical symptoms associated RSV-positive results related with age groups which separated into classes <2 years and 2-5 years, with clinical symptoms such as bronchiolitis 12(27.3%); pneumonia 19(43.2%); asthma 3(6.8%) and allergy 1(2.3%) for <2 years old children whereas, for 2-5 years were 11(25%) bronchiolitis; 2(4.5%) pneumonia; 9(20.5%) asthma; and 8(18.2%) allergy.

It has be noted some studies focused only the relationship between RSV and acute lower respiratory infection in hospitalized-children such as the study which done by João *et al*., (2011), they recorded cough 88.2%; 50(64.0%) bronchiolitis; 31(25.8%) pneumonia with pleural effusion; 68(16.2) acute wheezing.

The respected notes through these studies with the recent study were these two viruses, hMPV and RSV prone to cause lower respiratory illness than upper respiratory infection, and the variation of these studies may reflect various epidemiological approaches of these viruses recognition in different places which produce different factors that linked with the environment, geographical distribution, as well as factors associated with the host such as genetic susceptibility, immune response status, exposed to anther pathogen or the children may have experiencing defect immune system such as hypersensitivity type 1 when the IgE is raising level (atopic), these state lead to severity of the bronchopneumonia and exaggerating of asthma, whenever the children are exposing to respiratory viral infection.

**Table (3-4): the correlation between RSV-A and RSV-B with the major clinical features**

| Clinical picture | RSV-A | RSV-B |
|---|---|---|
| | Frequency & % | Frequency & % |
| Fever | 6 (75%) | 10 (71.4%) |
| Cough | 7 (87%) | 12 (85.7%) |
| Wheezing | 6 (75%) | 11 (78.5%) |
| Sneezing | 2 (25%) | 4 (28.5%) |
| Rhinorrhea | 3 (37.5%) | 4 (28.5%) |
| Dyspnea | 6 (75%) | 11(78.5%) |
| Crepitation | 3 (37.5%) | 7 (50%) |
| Croup | 3 (37.5% | 5 (35.7%) |

## 3.4. Bioinformatics application of F fusion protein and local isolated of hMPV

### 3.4.1. Genetic variability of *Human metapneumovirus*

In the subsequent study, it will have been considered the genetic variation (genotyping) of hMPV to divulge the local isolates and align with the reference isolates that have been brought from NCBI-GenBank hMPV and reveal distinctiveness of particular isolates, beyond that, this section will focus on the comparison with other studies in different countries and different places, and then will have an idea about the predomenant strain (genotype) of human metapneumovirus, finally, will try to shed light on the possible causes of the predomanent local isolates.

The figure (3-4) expresses the sequence alignment of the partial F gene, fusion protein (F), sequence of local isolates of hMPV, F gene has several characteristics that made it the best gene to analyze the genotyping of hMPV, first of all, the F gene is more conserved than other genes, rather than it encodes a 539-amino acids, protein, this length of putative fusion protein ORF is neither so short such as M2-2 protein, 71, nor so long such as L protein which is 2005 in length, therefore the analysis comparison are adequate to determine the genetic diversity of hMPV, however, the analysis sequence of hMPV relied on sequencing of the otherwise genes such as N, M, G, and L in the genotype grouping is concordant anyhow of which is studied ( Bastein *et al* 2003; Biacchesi *et al* 2004; Boivin *et al* 2004; Peret *et al* 2002). Besides these essential features of F-protein, there is another feature is not less important than others, the F-protein is capable to provoke the immune response to produce potent immunoglobulin that neutralizes the virus that regardless of majorly homologous, the cross-productivity can occur (Ulbrandt *et al*., 2008; Ryder *et*

*al*., 2010). However, depending on genetic sequencing of hMPV and construction of phylogenetic tree, there are two major genotypes of hMPV which designated hMPV-A and hMPV-B, likewise, these genotypes also separated into 2 sublineages as A1and A2 so B1 and B2. In the current study, nucleotides sequencing were gained from 5 (5/100, 5%) hMPV positive samples. Amplified products are elected to concede to the partial genome sequencing of the F gene for determination of the local isolates of hMPV subtypes, the income of these sequencing is two genotypes A and B, hMPV, genotype A1 is found in (1/5, 20%), A2 is not existing in the current study, and B1 is present in (3/5, 60%) and B2 (1/5, 20%), besides that, the homology of local current isolates were 100% of hMPV-S1,hMPV-S3, at the nucleotides sequencing level with the KM408076.1, KJI96323.1respectively, and 99% of hMPV-S4 with JQ041689.1 strain, three of five hMPV-S2,S3, and hMPV-S5 were identical (100%) so, the genotype B is predominant, (80%).

The predominance rate of hMPV is problematic and dynamic, hMPV's strains, unlike the influenza viruses which responsible for a global pandemic due to exhibits swift genetic evolution through antigenic shift and antigenic drift, they reveal two or three spread across the globe each year, the outbreak of hMPV has shown to be of local occurrence (Esper *et al*., 2004), however, RNA viruses reveal a genetic mutation frequently because these RNA viruses absence of proofreading capability of RNA-dependent RNA polymerase **(**Drake, 1993**)**. In comparison with other studies from every continent, which showed distinctive proportional than current study, strains of hMPV display fluctuation from community to another, and pick strains of hMPV in one location may be likely to strains that observed in somewhere else in various years. In Malaysia at University Malaya Medical Center, a tertiary hospital placed in Kuala Lumpur,

Nor'e *et al*., (2014), they have shown that the Malaysian isolates of hMPV grouped predominantly into two sublineages, A2b (54%) and B1 (42%), moreover in the same study, B1 is the predominant in 2012, make up 9/11 (81.8%) of the strains. Another study of Loo *et al*., (2007), it displayed only one subgenotype A2 was obtainable. In the study of Barbara *et al*., (2006) in Germany, they have stated that all genotypes and sublineages (A1, A2; B1, and B2) are cocirculated during 2 seasons with subtype A predominating than subtype B, moreover, subgroup A2 made for most cases. In the study of Qaisy *et al*., (2012), in Jordan, the linage A was detected in 93%, while 28.6% with the lineage B. Although these previous studies differ from the current study in recognition and analyzing of the predominant genotype, other studies have revealed the similar genotype, which has recognized in the recent study, an example is the work conducted in Upper Egypt by Mona *et al*., (2014) in which the subgroup B2 was the predominant. Similarly, in Cambodia between 2007 - 2008, Alicia, *et al*., (2014), have observed the predominant of sublineage B2 strain is predominant. However, reemerged and recirculation of genotype and sublineages have been observed to differ every year, which are substituted every 1-3 years within a particular community, which is believed to happen rely on upon development of acquired immunity to infection with specific strain of the predominant coursing genotype (Chung *et al*., 2008; Carneiro *et al*., 2009; Aberle *et al*., 2010; Williams *et al*., 2010).

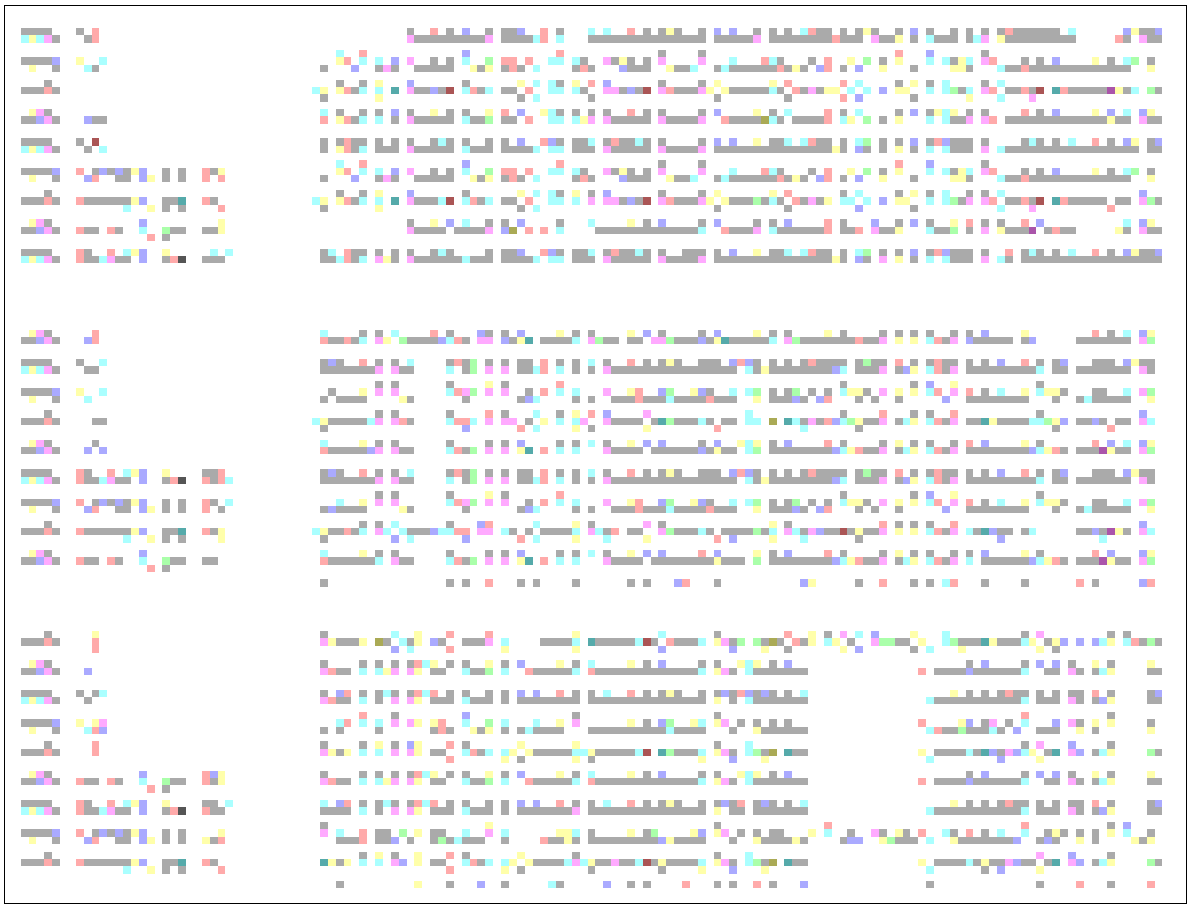


**Figure (3-4): Multiple sequence alignment analysis of the partial fusion protein (F) gene sequence in local Human metapneumovirus isolates with for NCBI-GenBank *Human metapneumovirus* based ClustalW alignment analysis by using (MEGA 6.0, multiple alignment analysis tool).**

### 3.4.2. Homology of F fusion protein of local isolates of hMPV

**Table (3-5): Similarities and identities sequencing of local isolates by using NCBI- BLAST alignment tool.**

| Local isolate of hMPV | Homology sequence identity (%) | | | |
|---|---|---|---|---|
| | Genotype A1 (KM408076.1) | Genotype A2 (KJ196309.1) | Genotype B1 (KJ196323.1) | Genotype B2 (JQ041689.1) |
| S1 KY441445 | 100% | 91% | 93% | 89% |
| S2 KY441446 | 86% | 86% | 100% | 94% |
| S3 KY441447 | 86% | 86% | 100% | 94% |
| S4 KY441448 | 86% | 85% | 93% | 99% |
| S5 KY441449 | 86% | 86% | 100% | 94% |

The local HMPV isolates (S1) were shown to be closely related to NCBI-Blast hMPV genotype A1 (KM408076.1), The local HMV isolates (S2, S3, and S5) were show closed related to NCBI-Blast Human metapneumovirus genotype B1 (KJ196323.1), The local HMV isolates (S4) were show closed related to NCBI-Blast Human metapneumovirus genotype B2 (JQ041689.1).

### 3.4.3. Sequence alignment of Fusion F-protein of local isolates

**Figure (3-5): similarity and difference among hMPV locals isolates: HMV-S1:0.19500, HMV-S4:0.09500):0.10500, HMV-S2:0.00000):0.00000, HMV-S3:0.00000, HMV-S5:0.00000);>HMV-S4 > HMV-S1 _P_ 4dagA> HMV-S3 > HMV-S2 > HMV-S5. Clustal Omega**

The colors of the residues above figure based on their physicochemical properties:

| Residue | Color | Property |
|---|---|---|
| AVFPMILW | | Small (small+ hydrophobic (incl. aromatic -Y)) |
| DE | | Acidic |
| RK | | Basic |
| STYHCNGQ | | Hydroxyl+sulfhydryl+amine+G |
| others | | Unusual amino/amino acids etc. |

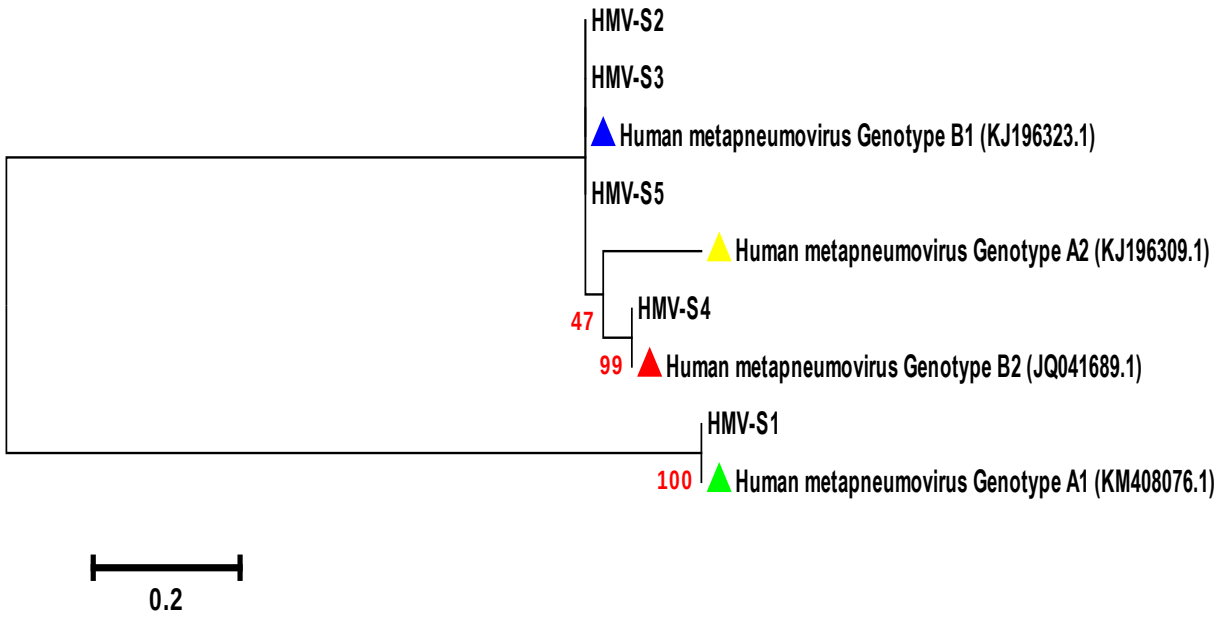



**Figure (3-6): Phylogenetic tree analysis based on the fusion protein (F) gene partial sequence that used for Human metapneumovirus genotyping detection. The phylogenetic tree was built using Maximum Likelihood tree method in (MEGA 6.0 version).**

### 3.5.1. The Model Building (Doman and loop) of F Fusion protein prediction

The following tables and figures, which are numbered (3-7a, 3-7b, 3-7c, 3-7d, and 3-7e)**,** show predication of the secondary structure of F protein.

| | |
|---|---|
| QMEAN | -3.73 |
| Cβ | -4.03 |
| All Atom | -1.56 |
| Solvation | -1.26 |
| Torsion | -2.61 |

Figure (**3-7a): Oligo-state**: monomer (matching prediction); ligands: sugar (n-acetyl-d-glucosamine)

| | |
|---|---|
| QMEAN | 0.05 |
| Cβ | -0.57 |
| All Atom | 4.82 |
| Solvation | 2.40 |
| Torsion | -0.79 |

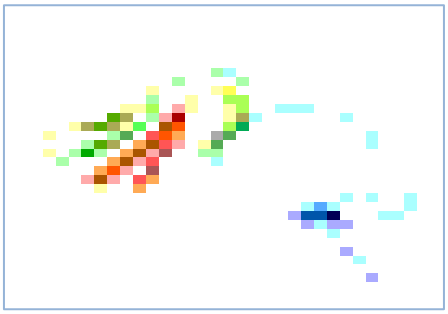
**Figure (3-7b): Oligo-State**: MONOMER (matching prediction); ligands: ALPHA-L-FUCOSE; gmqe: 0.12.

| | |
|---|---|
| QMEAN | -5.05 |
| Cβ | -3.97 |
| All Atom | -1.79 |
| Solvation | -0.42 |
| Torsion | -4.05 |

**Figure (3-7c): Oligo-State**: monomer; ligands: sugar (n-acetyl-d-glucosamine); gmqe: 0.07

| | |
|---|---|
| QMEAN | -0.06 |
| Cβ | -0.59 |
| All Atom | 5.44 |
| Solvation | 2.24 |
| Torsion | -1.21 |

**Figure (3-7d): Oligo-State**: monomer; ligands:  chloride ion.



| | |
|---|---|
| QMEAN | -5.23 |
| Cβ | -1.14 |
| All Atom | -2.43 |
| Solvation | -2.71 |
| Torsion | -4.35 |

**Figure (3-6e): Oligo-State**: monomer; ligands:  2-[n-cyclohexylaminoethane sulfuric acid].

### 3.5.2. Predicted topology of F fusion protein of hMPV

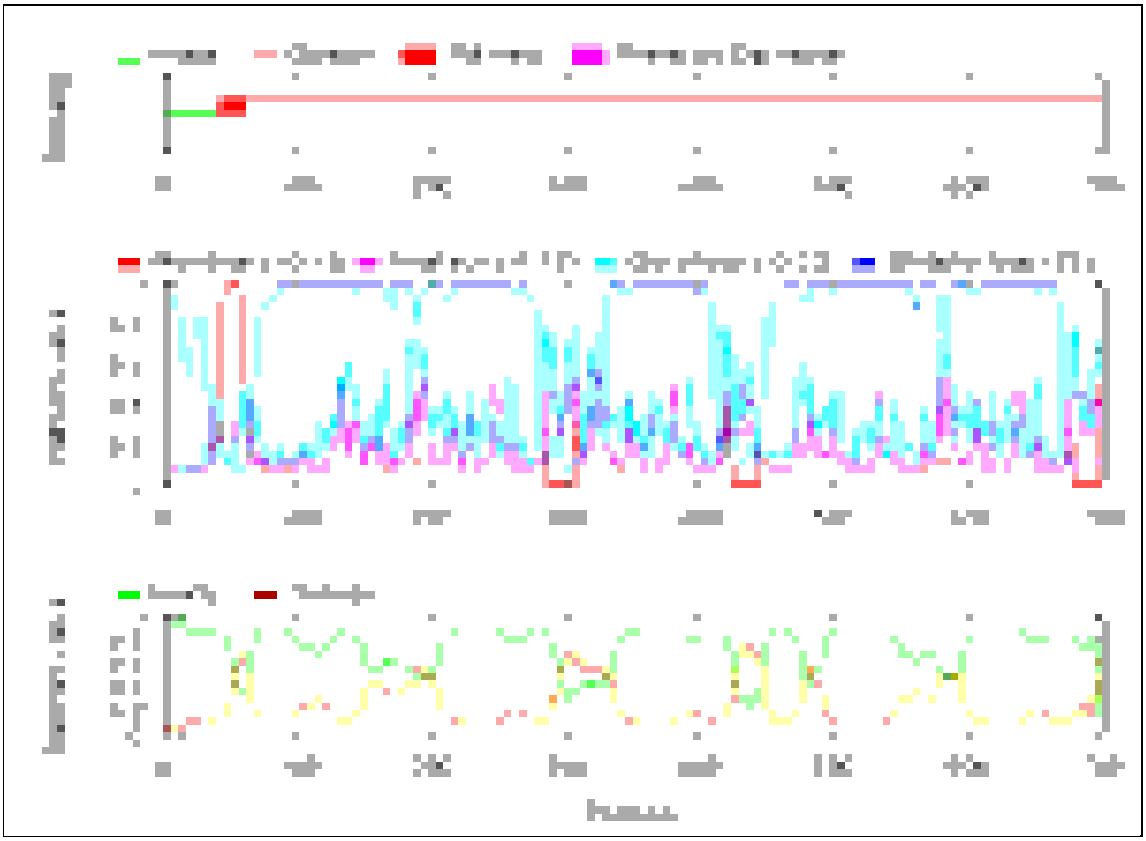


**Figure (3-8): Display the topology prediction of F fusion protein of hMPV.**

Every residue is expected to be either inside or outside the virion particle and situated in a transmembrane (M), interface (I), close-loop (L) or globular loop (G), it is done by OCTOPUS program.

### 3.5.3. TMpred output for F fusion protein of hMPV

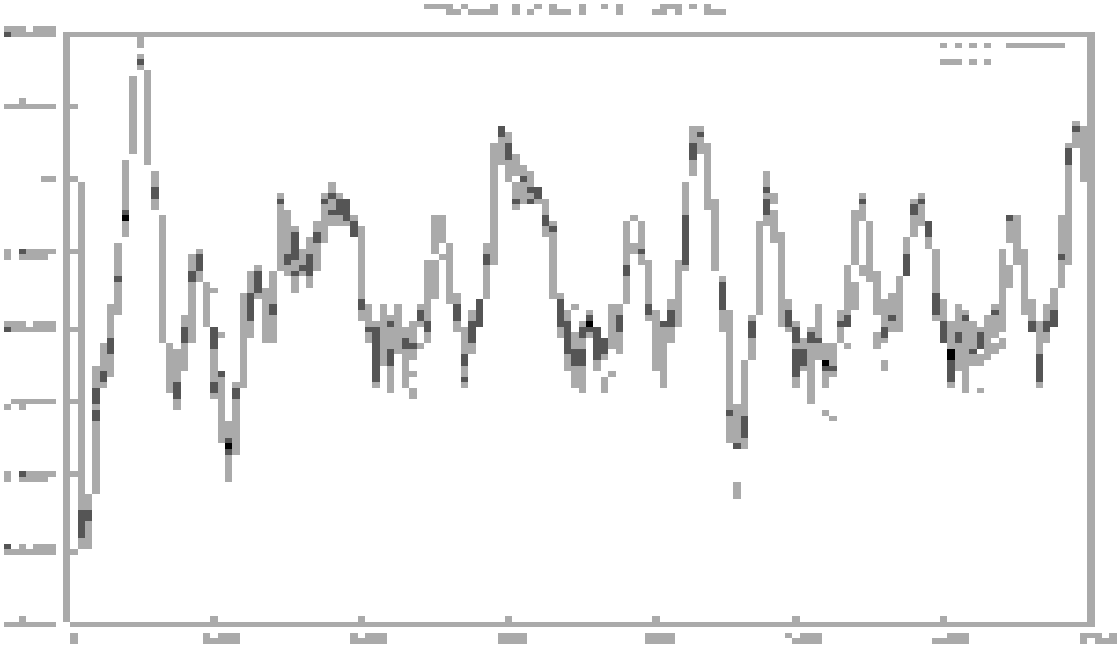


**Figure (3-8): Displays the properties of F-fusion protein of hMPV.**

The above figure indicates discrimination district of hydrophobicity of particular protein and potential spanning fields, and distinguish collected alpha helices and beta sheets transmembrane domain, the numbers more than 0 denote increased hydrophobicity, while less than 0 represent increased hydrophobicity of amino acids

## 3.6. Characteristics of F fusion protein of hMPV

### 3.6.1. Physical characteristics of F fusion protein of hMPV

**Table (3-7): physical features and figures which follow this table of F protein of hMPV depend on the GOR method**

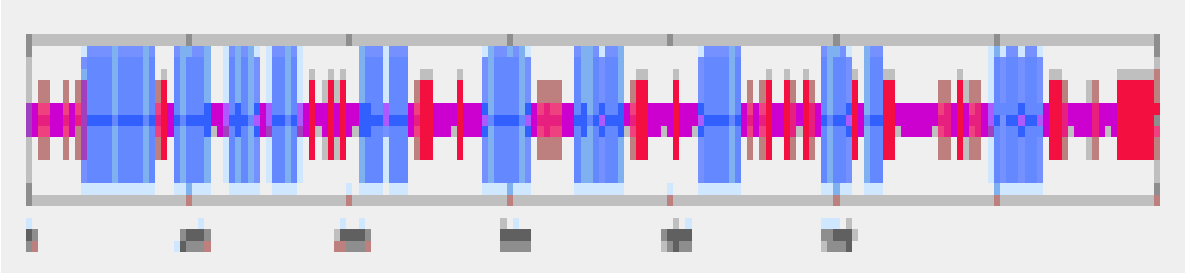
| **Physical features of F protein** | No. | % |
|---|---|---|
| Alpha helix(**Hh**): | 260 | 37.9% |
| $3_{10}$ helix (**Gg**) : | 0 | 0% |
| Pi helix (**Ii**) | 0 | 0% |
| Beta bridge (**Bb**) | 0 | 0% |
| Extended strand (**Ee**) | 132 | 18.83% |
| Beta turn (**Tt**) | 0 | 0% |
| Bend region (**Ss**) | 0 | 0% |
| Random coil (**Cc**) | 309 | 44% |
| Ambiguous states | 0 | 0% |
| Other states | 0 | 0% |

### 3.6.2. Chemical properties of F fusion protein of local isolates of hMPV

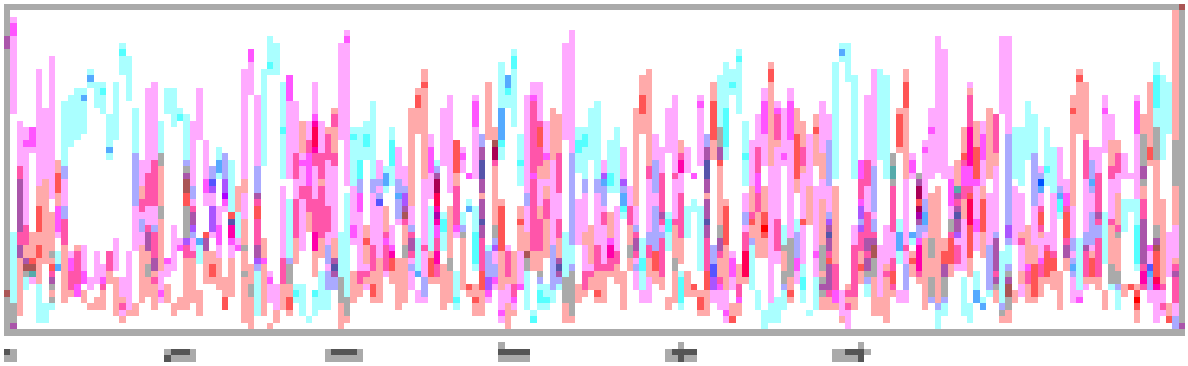
**Table (3-8): Chemical properties of F-fusion protein of hMPV local isolates**

| Chemical properties | hMPV-S1 | hMPV-S2 | hMPV-S3 | hMPV-S4 | hMPV-S5 |
|---|---|---|---|---|---|
| Aliphatic G,A,V,L,I | 47.01 | 35.29 | 35.29 | 27.07 | 35.29 |
| Aromatic F,W,A | 3.73 | 8.09 | 8.09 | 5.26 | 8.09 |
| Sulphuric C,M | 2.99 | 3.68 | 3.68 | 6.77 | 3.68 |
| Acidic B,D,E,N,Q,Z | 17.91 | 13.97 | 13.97 | 19.55 | 13.97 |
| Basic K,R,H | 11.94 | 13.24 | 13.24 | 12.78 | 13.24 |
| Aliphatic hydroxyl S,T | 14.93 | 16.91 | 16.91 | 18.05 | 16.91 |
| tRNA synthetase class I Z,E,Q,R,C,M,V,I,L,Y,W | 39.55 | 42.65 | 42.65 | 45.86 | 42.65 |
| tRNA synthetase class B,G,A,P,S,T,H,D,N,K,F | 60 | 52.21 | 52.21 | 46.62 | 52.21 |

### 3.7. Secondary structures prediction of F fusion protein of hMPV



**a-**



**b-**

**Figure (3-10 a&b): GOR4 result for: human metapneumovirus F fusion protein**

The GOR method (Garnier-Osguthorpe-Robson) is an information theory-depend on technique for the estimate of secondary structures in particular proteins as well as the state of amino acid which composed the particular protein to arrangement a secondary structure (Garnier *et al*., 1996).

### 3.8. Membrane Protein Secondary Structure Prediction

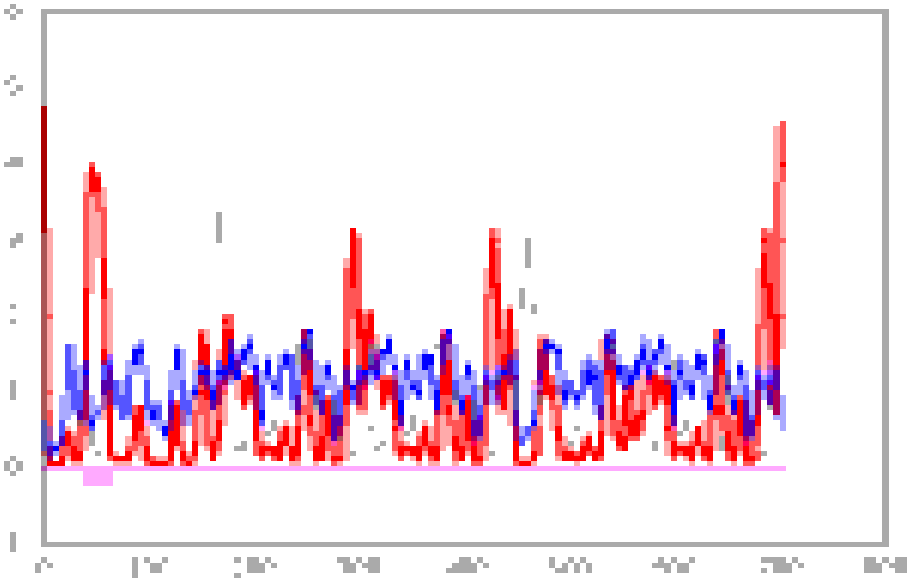


**Figure (3-11): predicted F fusion secondary structure**

Red line: Transmembrane helix preference.
Blue line: Beta preference.
Gray line: Modified hydrophobic moment index.

Violet boxes (below abscissa): Predicted transmembrane helix position.

## 3.9. Graphic model prediction of F fusion protein of hMPV

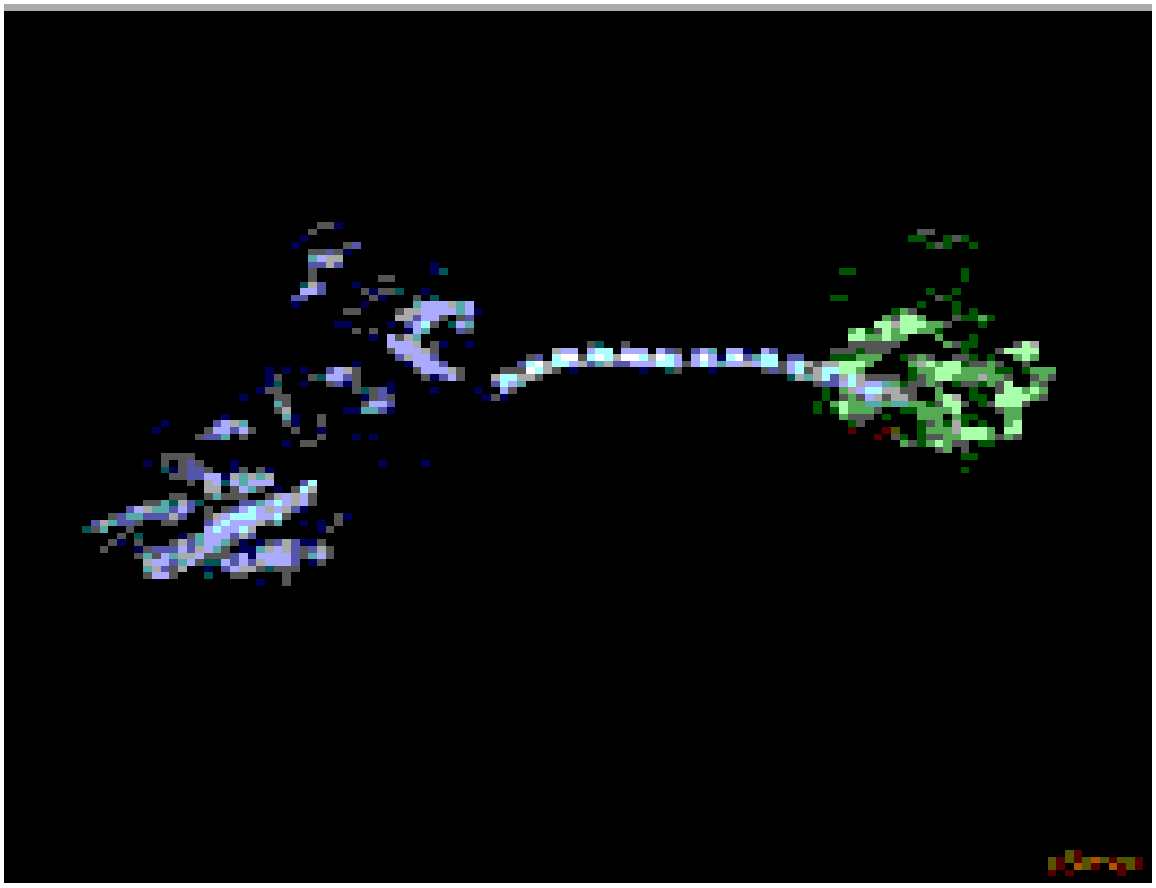


**Figure (3-12) show assembly model of F fusion of hMPV performance by FFAS-3D and AIDA).**

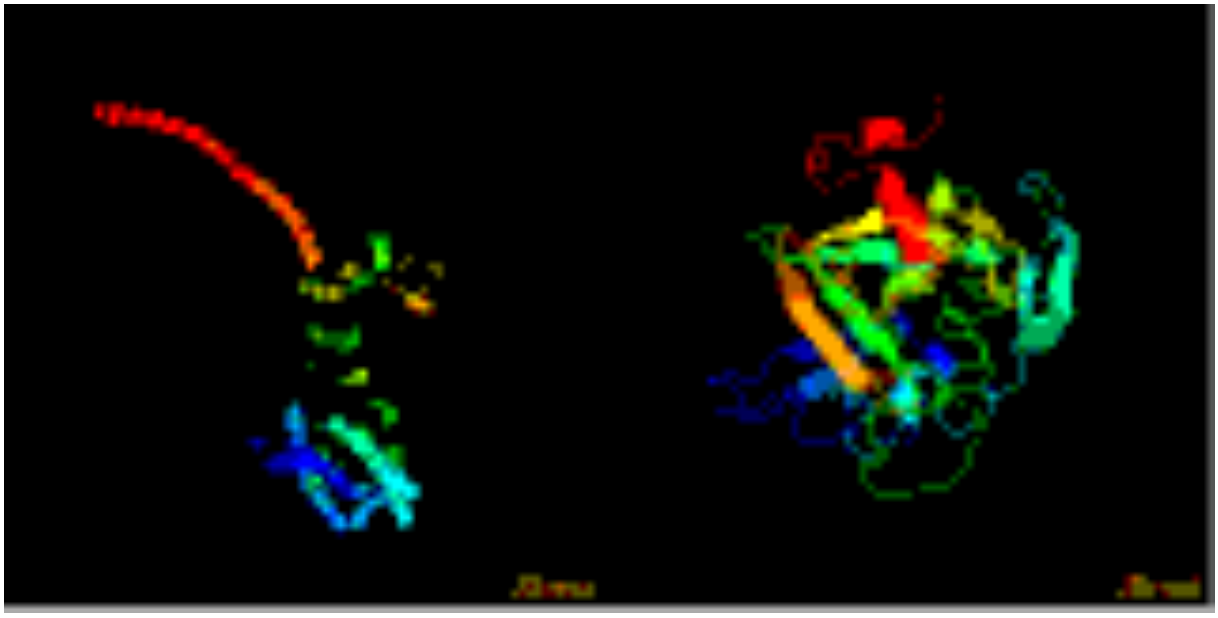


**Figure (3- 12a)**

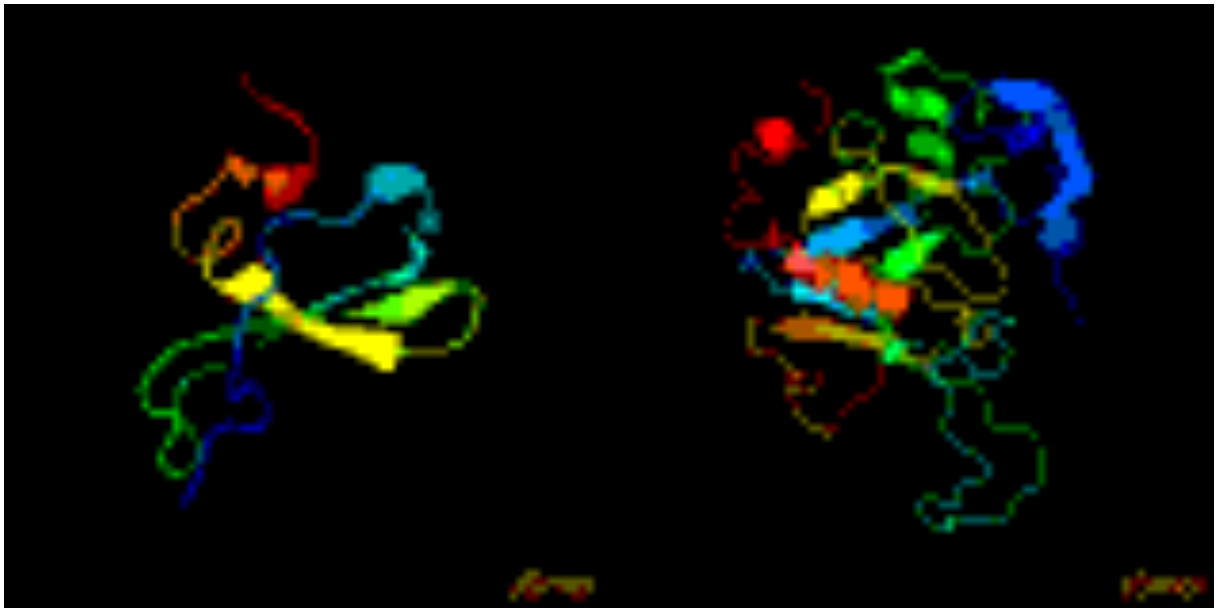


**Figure (3-13b):**

**Figure (3-12a &b) show assembled model of F fusion protein level 1 (a) and level (b)**

### 3.10. Gene expression of G-glycoprotein and M2-2, of hMPV and RSV- host cells induced cytokines, TARC and MDC.

The following results that are represented by below tables and graphs display the gene expression of viruses and respiratory epithelial cell. In figures (3-11) and (3-12) show of all positive results of hMPV are folding change expression of G-protein gene, with a higher level range from 13.642 into 50.213 folds, however, these results which indicate this virus does with reproductive hyperactivity in the airway epithelial cells, however, the G protein has a crucial role in the pathogenesis of hMPV, also the G-protein is a major surface fusion glycoprotein attaches the virion to the receptor on the host cell membrane by cooperating glycosaminoglycan, announcing the infection, as well as the essential role of G-protein in the attachment of virion, it has play interface role with host cell, it impedes synthesis and production type I interferon via RIG-I **(**Bao *et al*., 2013 **).** The surface glycoprotein G slices no noteworthy sequence similarity with other paramyxoviruses attachment glycoprotein; nevertheless it has a high amount of serine, threonine and proline residues (van den Hoogen, 2002). Afterward this nippy review of biological features of G-glycoprotein, then introduce the focus point of this the current study, in the table (3-2) which is involved the correlation between the major clinical pictures and hMPV infection, it seems 66% is dyspnea and also the same percentage of wheezing of all other signs, they have made the vital signs of lower respiratory illness such as asthma, pneumonia and allergy, but these clinical signs, dyspnea and wheezing, are more established with asthma than others lower respiratory diseases such as bronchiolitis and pneumonia, however, there are several study have founded relationship between hMPV and asthma exacerbate, such as the study that was achieved by Williams *et al*., (2004) they found 14% have exacerbated asthma,

by way of the study that worked by Jartti *et al*., (2002) who have studied human metapneumovirus and acute wheezing in children, their results suggested that hMPV is a causative agent of acute wheezing in young children, moreover the other study which worked by Williams *et al*., (2010), their study, which did attain the role of viral respiratory infections in asthma and asthma exacerbation, their conclusion was there is strongly linked between respiratory virus infection such as RSV, hMPV, coronavirus, and other viruses and development and exaggeration of asthma.

At least, that it might be hMPV cause as a risk factor to asthma exaggeration in children which are sustained by limited studies in guinea pig, showing a long-term pulmonary inflammation parallel with hyperresponsiveness and obstruction of airway induced by hMPV infection **(**Hamelin *et al*., 2016**).** In further study has proven that G-protein of hMPV does as a virulence factor which upsets signaling pathway of mitochondria in airway epithelial cells (Bao *et al*., 2013).

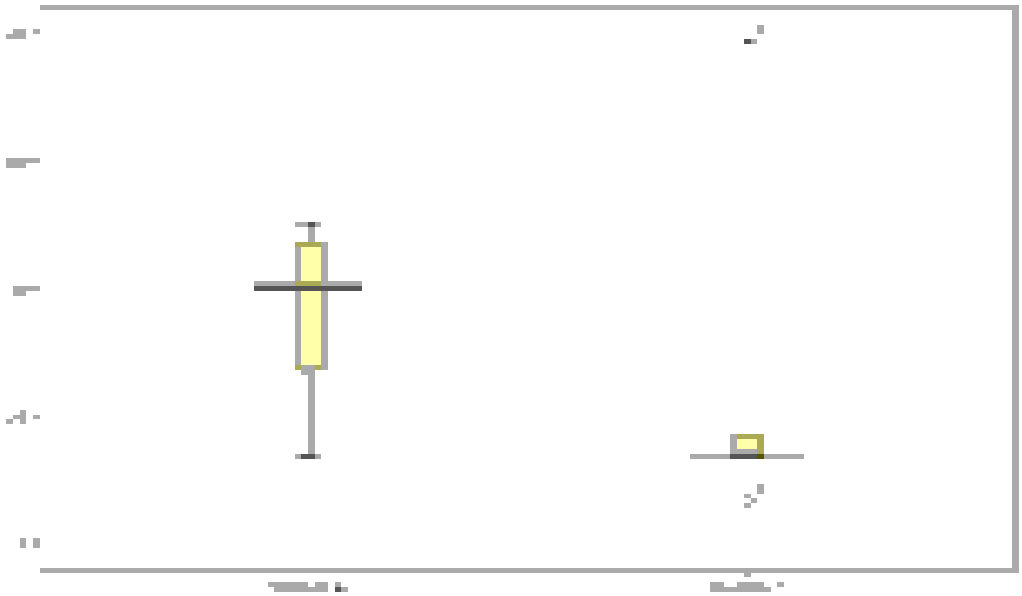


**Figure (3-14): Boxplot descriptive of G-protein and M2-2 gene expression of human metapneumovirus.**

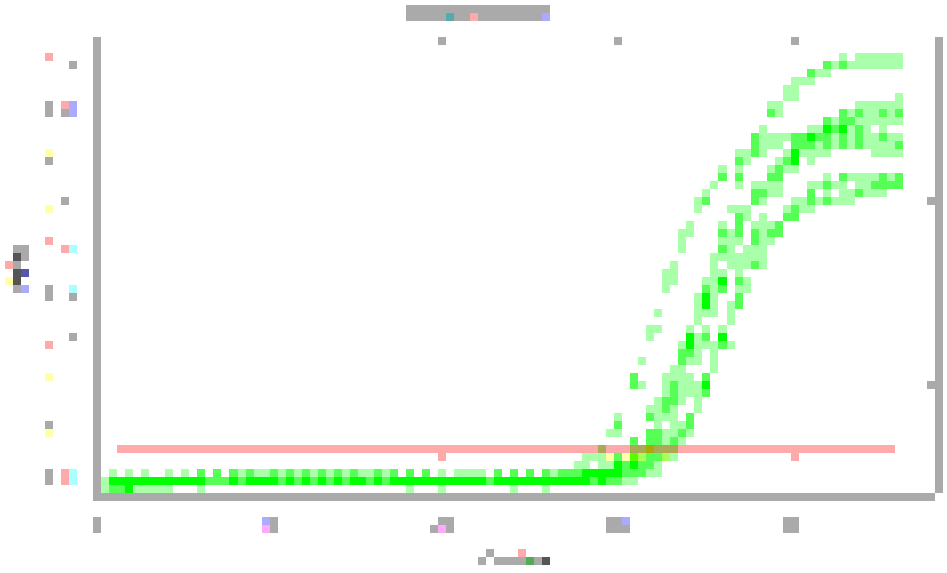


**Figure (3-15): Real-Time PCR amplification plot for G-protein gene expression in hMPV patient samples**

Other critical factor of hMPV in the pathogenesis mechanism is M2-2, in the figure (3-13) that display the results of M2-2 gene expression of hMPV which

reveal higher level of M2-2 ranging from 6.543 to 78.793 folds that represent the hyperactivity of hMPV in the airway epithelial cells. There are two overlapping proteins encoded by hMPV M2, M2-1 and M2-2. The essential role of M2-1 is for full replication of the virus, while the critical task of M2-2 is in blocking the innate immunity of the host, these fact is proven by the work which done by Ren *et al*., (2014), they have demonstrated that M2-2 inhibits signaling pathway which mediated to induce antivirus response, furthermore they showed that hMPV G protein blocks type I INF synthesis by targeting RIG-I. Other studies showed the essential role of M2-2 of hMPV as the study which is done by Ren *et al*., (2015) they did reveal that M2-2 is a substantial protein for RNA synthesis of hMPV. Additionally, it has been presented to modify and control immune response of the host.

In order to explain these effects of G-protein and M2-2 protein, we have some studies for this fact, one of them is the work that has achieved by Kolli *et al*., (2011) they demonstrated several evidences that showed the dynamic role of these proteins, G-protein and M2-2 protein, through the experiment that is proved the recognition of hMPV's proteins are doing as an opponent of the signaling pathway of the host innate immunity, they revealed that M2-2 of hMPV is an essential antagonist of antiviral signaling path, in fact, the delta M2-2 infected epithelial cell of the airway system formed a higher level of INF-β and other mediator immunity, for that, they concluded the mechanism of M2-2 may interrupt of the airway epithelial cells. As already mentioned, they founded the G-protein of hMPV interfaces with RIG-I, it happens initially through the CARD domains of its N-terminus which lead to block RIG-I linked with the adaptor protein MAVS, in addition to that, the G-protein expression precludes the interplay between mitochondria and mitochondria-associated membrane

(MAM) constituent of endoplasmic reticulum (ER), which involves stimulator of IFN genes (STINGS) that is essential of the viral-induced RIG-I/ MAVS signaling pathway.

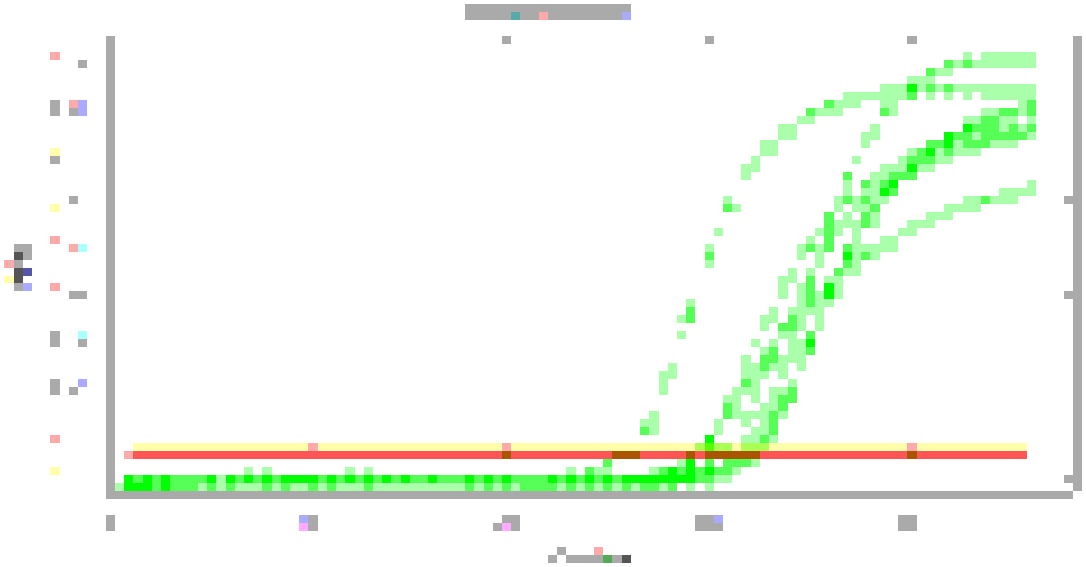


**Figure (3-16): Real-Time PCR amplification plot for M2-2 protein gene expression in hMPV positive patient samples.**

The following data involved the display certain chemokines (CC) which induced by RSV and their role in pulmonary diseases such as allergy and asthma exaggeration, these CC are represented with thymus-and activation-regulated chemokine (TARC) and macrophage-derived chemokine (MDC), each those CC is expressed on bronchial epithelial cells and alveolar macrophage, they have common receptor which is chemokines receptors-4 (CCR4) that selectively expressed on Th2 cells which play a crucial role in asthma development. Whatever, the TARC and MDC are also elevated in other chronic diseases such as atherosclerosis, Crohn's disease, and cigarette smoke-induced acute and chronic airway inflammation (Jugde *et al*., 2001), but these diseases are beyond the scope of the current study.

**Interpretation results of RSV-A&B TARC and MDC gene expression**

In the subsequent figures show the expression level of TARC and MDC gene, which induced by both genotype RSV (A and B), all these tables of positive RSV (A and B) have shown a high level fold change expression, and compared to the negative-RSV-A patients which represented in the subsequent table and their figures, there is a significantly different, the p-value is (0.0001), it is detonating RSV-A & B have induced the infected bronchial epithelial cells and alveolar macrophage to produce TARC and MDC that ligand the specific receptor on the Th2, CCR4,. Th2 cells have an essential role in allergic diseases like asthma and allergic rhinitis (Panina-Bordignon *et al*., 2001; Berin, 2002; Leung *et al*., 2004). Several studies have convinced the association between RSV and asthma development, as the study that worked by Zhang *et al*., (2001), they have concluded through their experiment, that RSV has a potent inducer to produce several chemokines such as TARC, RANTES, MCP-1, and MDC, and then they explained their result which encompassed the importance of TARC and MDC in asthma development and exaggeration, TARC an effective chemotactic for $CD_4^+$ T lymphocytes and immature dendritic cells, RSV-infected A549 cells can be induced for TARC production which has implicated in atopic asthma in human. In addition, other studies such as that achieved by Monick *et al*., (2007), they proved RSV induce Th2 cytokines such as IL-4 and IL-13 that improve production of TRAC; also they observed the IL-4 alone did produce very small amount of TRAC mRNA. In one study that done by Takeuchi, *et al*., (2005) they have determined that TARC is a considering target for immunotherapy (IT) which has been observed the reduction amount of TRAC in patients who have suffered from allergic rhinitis after IT demonstration. MDC is another potent chemotactic, it has the

capability to recruit Th2 lymphocytes, monocytes, immature DC, and IL-2-activated NK cells (Chantry *et al*., 1999). MDC is, as well, recognized in normal bronchial epithelial cells in human atopic asthma (Sekiya *et al*., 2000). The study like that done by Gonzalo *et al*., (1999) they have reported role of MDC which is essential chemokine in allergic ovalbumin-stimulated pulmonary inflammation in mice, MDC is neutralized by antibodies that lead to block eosinophil recruitment. Furthermore, in original article that published by Egypt Journal Pediatric Allergy in 2005 which have been done by Mohammad and his collogue, Karim, they have measured MDC (CCL22) and its receptor CCR4 on peripheral blood T lymphocytes of asthmatic children, they founded the level of MDC was significantly higher in asthmatic children (Mohamed and Karim, 2005). However, the MDC might recruit Th2 lymphocytes into airway of asthmatic case, Th2 lymphocytes intensify the allergic inflammatory reaction through IL-4, IL-5 and IL-13 production which provoke the B lymphocytes into plasma cells which responsible for IgE production, moreover the IL-5 stimulate eosinophil for activation and differentiation (Brombacher, 2000).

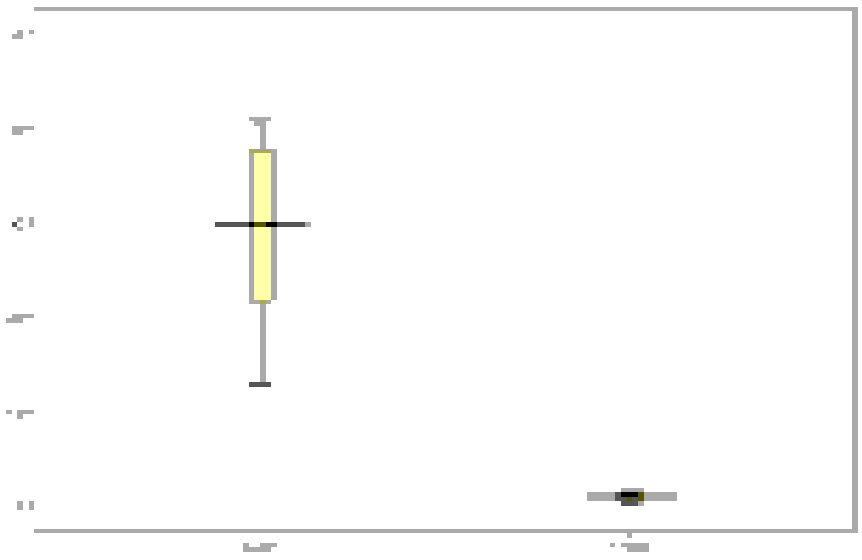


**Figure (3-17): Boxplot shows comparison between RSV-A-induced TARC with control (RSV-A negative).**

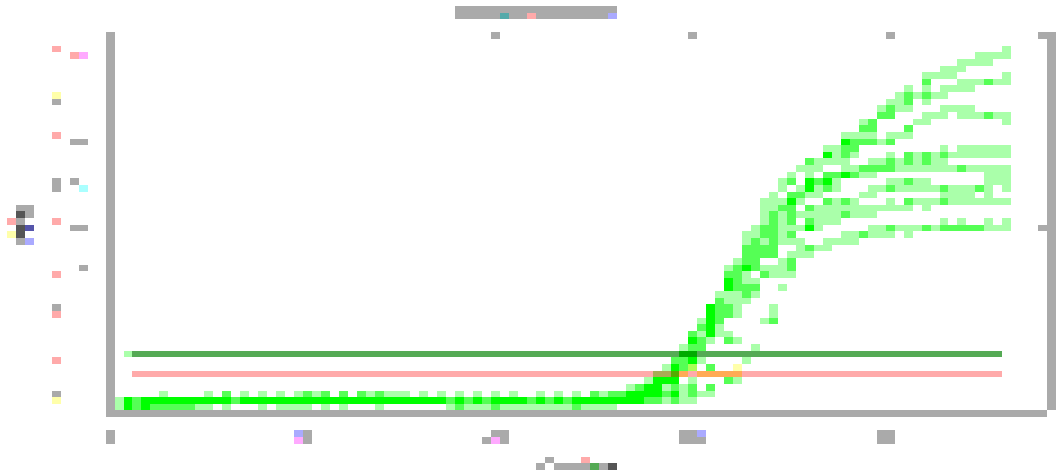


**Figure (3-18): Real-Time PCR amplification plot for TRAC gene in Respiratory Syncytial Virus type A (RSV-A) positive patient samples.**

In the above figure shows the expression level of TRAC gene, which induced by RSV-A that ranging from the higher level 40.786 to lower level 19.160 folds.

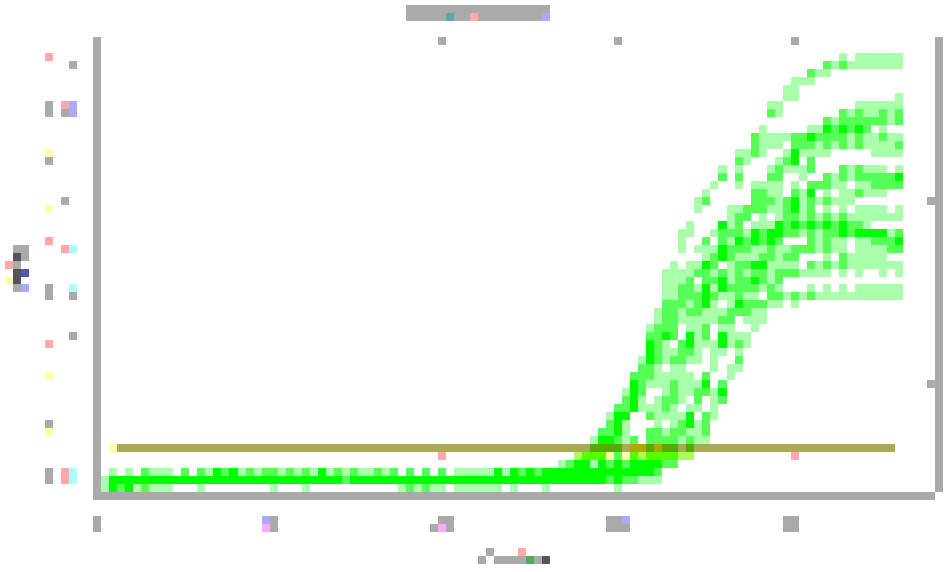


**Figure (4-19): Real-Time PCR amplification plot for TRAC gene in Respiratory Syncytial Virus type B (RSV-B) positive patient sample**

In the above figure shows the expression level of TRAC gene, which induced by RSV-B that ranging from the higher level 64.000 to lower level 9.646 folds.

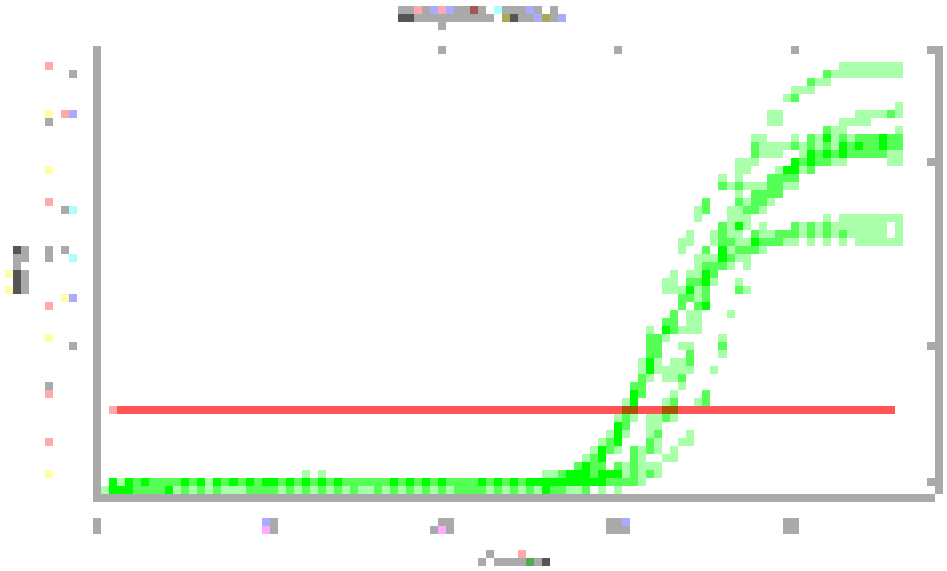


**Figure (4-20): Real-Time PCR amplification plot for MDC gene in Respiratory Syncytial Virus type A (RSV-A) positive patient samples.**

In the above figure shows the expression level of MDC gene, which induced by RSV-A that ranging from the high level 37.792 to lower level 7.727folds.

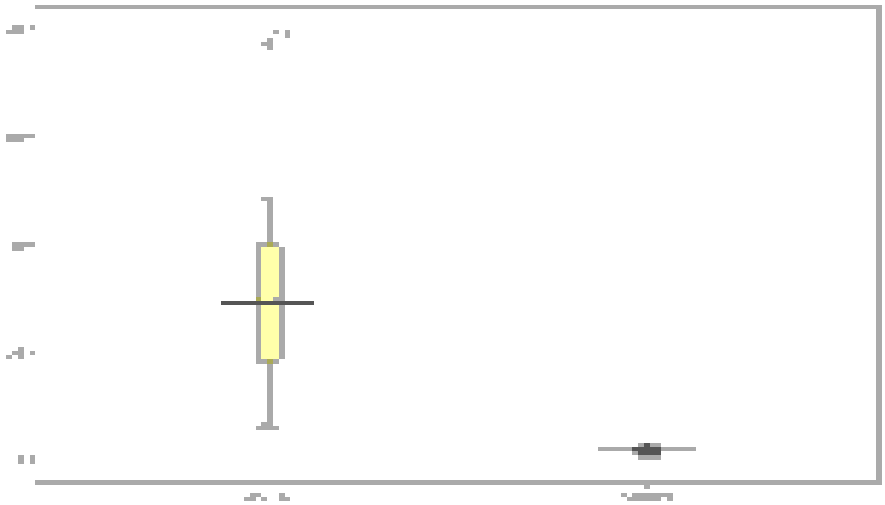


**Figure (4-21): Bar box of RSV-B induced MDC and control (RSV-B negative)**

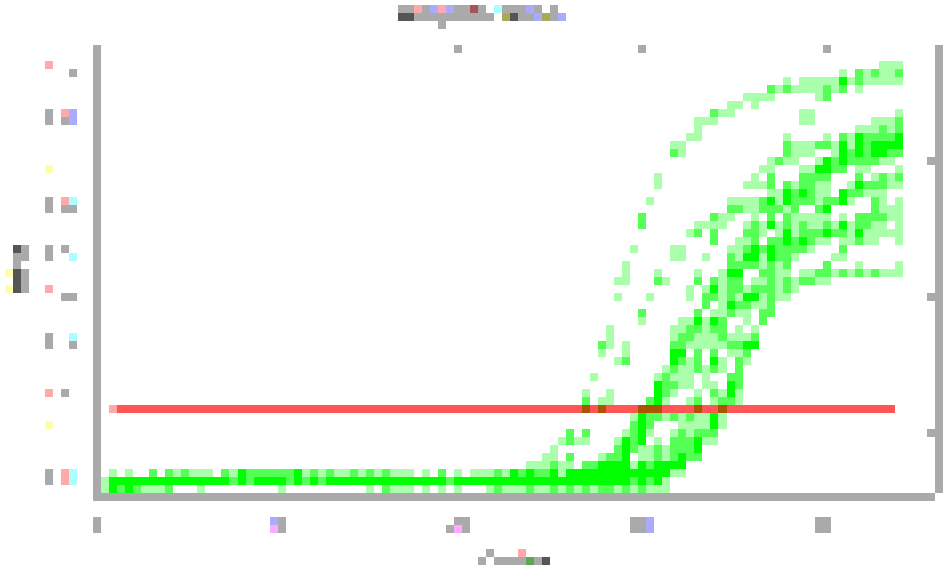


**Figure (4-22): Real-Time PCR amplification plot for MDC gene in Respiratory Syncytial Virus type B (RSV-B) positive patient samples**

In the above figure show the expression level of MDC gene, which induced by RSV-B that ranging from the high level 48.840 to lower level 6.063folds.

### 3.14. Interleukin8 (IL-8) results interpretation

In the current study, there are 4(4%) are positive result to interleukin 8 (IL-8), out of 7 of hMPV, 3(42.8%) are IL-8 positive, while there's only 1(7.14%) is IL-8 positive for RSV-A, statistically, the aforementioned results are significant, since the p-value is (0.001), as clarify in below table (3-9).

**Table (3-8): Shows percentage of interleukin8 (IL-8) positive results in patients infected with hMPV and RSV.**

| hMPV | RSV-A | RSV-B |
|---|---|---|
| 3(42.8%) | 1(7.14%) | 0(0%) |
| p-value<0.001 | p-value >0.001 | |

this indicate that raising of IL-8 is usually affected by this virus under consideration, hMPV and RSV-A & B, or others factors likely effect the increased the IL-8 in the nasopharyngeal such as duration of illness, since the IL-8 appears and detected at the first few hours of inflammation because IL-8 is one of the most important proinflammatory cytokines such as IL-1, TNF, IL-6 and others, all these cytokines are appearing through the onset of illness. Several studies showed variant results, such as the study that done by de Graaf M *et al*., (2008), showed that hMPV induces the tissue cells to release IL-8 through 3to 6 hours and vanished at 15 hrs. While in other study that have been done by Laham *et al*., (2004), displayed different results, they observed that the samples of patient who had infected by hMPV were poor elicit to release cytokines which involved IL-8 comparably with infected by RSV and influenza virus, they proposed that hMPV may has different pathway than other respiratory virus which based on it to produce symptoms, as well as, the study that done by Shum, (2007), through his study, he observed that tissue cell line, A549, which infected

by hMPV and RSV-infected epithelial cells displayed decreased production of antiviral cytokines such as IFN-α and IFN-β, while the hMPV-infected epithelial cells abortive to elicit proinflammatory cytokine like IL-6 and TNF- α, RSV is powerful to stimulate IL-8 and monocyte chemoattractant protein 1(MCP-1) at the first three hours of infection, However, hMPV did not provoke IL-8 and RANTES mRNA and seemed to be a less effective stimulator of MCP-1 than RSV. There is noteworthy, it found several other researches showed the relationship between respiratory viral infection and cytokines involved IL-8 that paly an essential role for asthma exaggeration, their results which have been obtained from their studies may be different than the current study, there are many factors cause these difference, some of them, type and quantity of clinical specimen which may contain a few amount of cytokines that make the diagnostic tool, ELISA, unable to get this protein, while, they used molecular techniques.

# 4. Conclusions and Recommendations

## 4.1. Conclusions

**The conclusions of the current study are in the following points:**

1- Human metapneumovirus have recently recognized as the etiologic agent of respiratory tract infection predominantly in children, the distinguishing proof of new viruses will keep on occurring as molecular methodology tests turn out to be more contemporary.

2- The prevalence rate of hMPV is less than respiratory syncytial virus, and both subtypes of hMPV, A and B may existence and circulate in one season, and the predominant sublineage of hMPV shifts in progressive season.

3- The clinical manifestations of hMPV as like as the clinical features of RSV, and both of them prone to cause bronchiolitis and they contribute to allergy, such as asthma exaggeration.

4- The genotypes of hMPV, A1; A2; B1; and B2 are prevalent varied, and they do reveal different to other isolates of other places in the world, these genetic diversity of hMPV strains produces recurrent infection and subterfuge a challenge for future vaccine development.

5- The genetic diversity of both groups of RSV is as similar as hMPV, which can co-circulate with various level of prevalent rate.

6- Consideration of the physical and chemical properties of F fusion protein of hMPV by using certain bioinformatics tools and revealed graphical models of this protein.

7- Identification of viral gene expression level, particularly those are responsible for virulent, such as G-protein of hMPV, and that does as immunomodulation factor as M2-2, is a principal to have knowledge the patient indeed illness from the particular identified virus.

8- Measurement of host gene expression such as TARC and MDC, which increased level in allergy such as asthma, particularly in childhood, that induced by RSV are essential role to have known severity and hyperresponsiveness of allergy.

## 4.2. Recommendations

1- The studies of the simplest techniques such as serological tests and advanced molecular tests of the identification of viruses mainly in children are scanty, then there are no databases available that are related with the pathogenic viruses particularly with the childhood, therefore, it is so necessary to assign the appreciative work to study the virological and molecular characteristics of these viruses and do comprise among them.

2- Augment to orient researchers and students to study the genes expression of the factors that associated with the pathogeneses of virus such as the factors that overcome of the immune system (innate arm and acquired arm).

3- In spite of the fact that hMPV has been all around described as a pathogenic virus of respiratory tract infection mainly in children, the advancement of prevention strategies and remedial are challenging, along these lines, this point is creative study.

4- Extended the study that reveals the coinfections between hMPV and other respiratory tract viruses such as flu virus, adenovirus, rhinovirus, and parainfluenza virus, by using molecular techniques.

5- Further studies that associated with the pathogenicity of hMPV on animals' model have to be conducted in future.

6- Studying the immunohistochemistry of hMPV and RSV-inducing receptors and their CC ligands such as IL-4, IL-5, IL-8, IL-13 and other

which allow using as a tool for knowing the severity and monitoring to therapeutic response from the secretion specimens.

7- Evaluate the potential mechanism of how hMPV and RSV are inducing asthma exacerbation, through exposed the animal experiment (mouse and rats) to allergen substances with RSV/ hMPV or both viruses.

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# الخلاصة

استهدفت هذه الدراسة تشخيص اثنين من اهم الفايروسات الممرضة والتي تكون مسؤولة عن اعتلال الجهاز التنفسي في الاطفال واللذان يشملا الفايرس التالي لذات الرئة الانساني (ت, ذ, ن)و كذا الفايرس المخلوي التنفسي (م, ت), وكان الغرض من أنتقاء هذين الفايروسين كعنوان الى البحث هو على نحو تأسيس الخطوة الأولى لوضع قاعدة بيانات وأرشفة للدراسة الجزيئية لهذين الفايروسين, , تم اختيار مسحات البلعوم الأنفي كعينة سريرية, حيث أخذ مأتان وثلاثون طفلا قد تعرضوا لأعتلال الجهاز التنفسي والذين أوتي بهم الى مستشفى الصدر التعليمي في مدينة العمارة خلال الفترة بين تشرين الثاني لعام 2014 الى شهر نيسان لعام 2015,وبعد جمع العينة فقد وضعت في أنابيب خاصة لجمع العينات الفايروسية والتي تحوي على وسط للمحافظة على حيوية الفايرس تم اعدادها لهذا الغرض , بعدها نقلت بواسطة حقيبة الثلج من مكان جمع العينة (مستشفى الصدر التعليمي), الى قسم الصيدلة (المذخر العام) لحفظها تحت درجة حرارة -70 °م , وقد انجزت كل ذلك بأستخدام الطرق الجزئية, التفاعل المتسلسل المكوثر ضمن الوقت المناسب والحقيقي (و. م. ت. م. م) لغرض الكشف والتحري عن الفايروسين قيد الدراسة بينما نتائج التعبير المورثي تم قياسها بأستخدام معادلة ليفاك و شمتجن (ضعف التغيير = $2^{-\Delta\Delta CT}$ )

**أما النتائج المحصلة من هذه الرسالة فقد أوجزت في النقاط الاتية :**

1. من بين 230 طفلا يعانون من امراض الجهاز التنفسي تم أختيار 100 عينة عشوائية وكانت 7% فقط قد اصيبوا بفايرس (ت, ذ, ن), و 8% و 14% بفايرس (م, ت) بكلا نوعيه أ& ب على التوالي.
2. الأعمار الأكثر أنتشارا للأصابة بكلا الفايروسين (ت, ذ, ن), (م, ت) بنوعيه أ& ب كانت ضمن (4-7) سنين بواقع 4(56.1%) للذين اصيبوا بفايرس (ت, ذ, ن), ولأقل من سنة ( 1> ), 6(75%) و 5(35.7%) للذين اصيبوا بفايرس (م, ت) بكلا نوعيه أ& ب, على التوالي.
3. طيف الاعراض السريرية لأعتلال الجهاز التنفسي العلوي ( العطاس, رشح الأنف و الخانوق) وكذا السفلي ( السعال, الازيز, صعوبة التنفس و الصرير) المتسببة بواسطة فايرس ( ت, ذ, ن) لمجموعة الدراسة كانت بواقع (5, 83%) للحمى ; ( 5, 83%) للسعال ; (4, 66%) للأزيز; (3, 50%) للعطاس ; (2, 33%) لرشح الأنف ; (4, 66%) لصعوبة التنفس ; (2, 33%) للصرير ; (1, 16%) للخانوق.

4. الأعراض السريرة الرئيسة المتسببة بواسطة فايرس (م, ت) بنوعيه أ& ب , كان السعال الأكثر ترددا عن باقي الأعراض حيث شكل 7(87%) و 12( 85.7%) على التوالي, أما الأعراض الأخرى مثل الحمى و العطاس و وصعوبة التنفس كانت اقل, حيث شكلت 6(75%) لنوع أ, أما النوع ب فكانت كالتالي 10( 71.4%) للحمى و 11(78.5%) للسعال ولصعوبة التنفس ايضا, ومن الجدير بالذكر ان اعراض الجهاز التنفسي العلوي كانت الأقل حظوة عن غيرها من الاعراض , فقد كانت 2(25%) ; 3(37.5%) للنوع-أ و 4(28.5%) لنفس لفايرس (نوع- أ) و 4(28.5%) لكلا العرضين لفايرس (نوع- ب). والاعراض الأخرى كالصرير التنفسي و الخانوق الفايروسي لكلا نوعي الفايرس (م. ت) فهي 3( 37,5%) لكلا العرضين لنفس الفايرس (م. ت) نوع أ و 7(50%) , 5( 35.7% ) للصرير التنفسي و الخانوق الفايروسي لفايرس (م. ت) نوع ب.
5. التنوع الوراثي للعزلات المحلية لفايرس التالي لذات الرئة الأنساني (ت. ذ. ن) قد أخذ بعين الأعتبار وتم دراسة توالي الاسس النتروجينية لوصف الذرية أو السلالة الأكثر انتشارا والتي كانت النوع-ب1 (5/3 ,60%) اما السلالات الاخرى فكانت (5/1, 20%) لكلا النوعين-أ1 و النوع-ب2 , أما النوع –أ2 فلم يعزل.
6. دراسة التشابه والمقارنة بين العزلات المحلية و العزلات الاخرى من خلال رصف المتواليات الاسس النتروجينية و توالي الاحماض الأمينية, حيث كانت العزلتين 1و 3 متشابهتين 100% مع العزلة KM408076.1 و KJI96323.1 على التوالي و 99% للعزلة -4 مع السلالة JQ041689.1 و العزلات الثلاثة الاخرى ( 2و 3و 5) كانت متشابهة 100% و النوع –ب الاكثر أنشارا.
7. تم تطبيق ادوات علم المعلوماتية الحيوية على نتائج توالي الأسس النتروجينية والأحماض الأمينية , حيت دراسة تخمين الشكل الثانوي لبروتين الالتصاق من نوع –أ من خلال معرفة الطبقة المحبة للماء والكارهة له, او اشكال التراكيب التي تكونها توالي الأحماض الأمينية مثل انواع الأشرطة (ألفا و بيتا) و العروات , كل ذلك امجز من خلال حزم من البرامج المعدة لذلك الغرض.
8. كل العينات الموحبة لفايرس التالي لذات الرئة الأنساني قد عبرت وراثيا للبروتين السكري نوع-س وبمدى مستوياته 13.642 الى 50.213 ضعفا.
9. و النوع البروتيني القالبي ق2-2 ايضا عبر وراثيا وبمدى مستوياته 78.793 الى 6.543 ضعفا, وهذا يمثل الفاعلية النشطة لفايرس (ت.ذ,ن) على خلايا المسلك التنفسي.

10.أما مستوى التعبير المورثي للبروتين الحركيات الكيمائية , الجاذبات الكيموية المنشطة التفعيل (ج. ك. م . ت) والمستحثة بواسطة الفايرس المخلوي التنفسي نوع –أ بمدى19.160الى 40.786 ضعفا.

11.و مستوى التعبير المورثي للبروتين الحركيات الكيمائية , الجاذبات الكيموية المنشطة التفعيل (ج. ك. م . ت) والمستحثة بواسطة الفايرس المخلوي التنفسي نوع - ب بمدى 9.1646الى 64.000 ضعفا.

12. والكيمويات المشتقة من الوحيدات الخلوية (ك . م . و) المستحثة بواسطة الفايرس المخلوي التنفسي نوع –أ كانت بمستوى من 7.727 الى 37.792 ضعفا.

13.والكيمويات المشتقة من الوحيدات الخلوية (ك . م . و) المستحثة بواسطة الفايرس المخلوي التنفسي نوع –ب كانت بمستوى من 6.063 الى 48.840 ضعفا.

14.واخيرا, تم دراسة وتقييم الأنترلوكين -8 حيث كانت النتائج 4(4%) موجبة لأنترلوكين 8 ومن بين ال 6 من المصابين بفايرس التالي لذات الرئة , 3(60%) موجبة لأنترلوكين 8 وهناك فقط 1(7.14% ) لفايرس المخملي التنفسي.

الأستنتاجات الرئيسية في هذه الدراسة هي ان كلا الفايروسين يميلان لتسبيب وزيادة شدة الاعراض التنفسية السفلى وأزمات الربو, اضافة لذلك أن التنوع الوراثي للعزلات المحلية لفايرس التالي لذات الرئة الأنساني (ت. ذ. ن) وكذا التنوع الوراثي للعزلات المحلية لفايرس المخلوي التنفسي تختلف من حيث معدل انتشارهم.

وزارة التعليم العالي والبحث العلمي
جامعة القادسية
كلية الطب
فرع الأحياء المجهرية الطبية

# التحري الجزيئي لفايروسي التالي لذات الرئة البشري وفايرس المخلوي التنفسي عند الاطفال في مدينة العمارة

**اطروحة مقدمة**

**الى مجلس كلية الطب/ جامعة القادسية**

**وهي جزء من متطلبات نيل شهادة دكتوراه فلسفة في علم الأحياء المجهرية الطبية**

**من قبل**

**مطلك مهدي خلاوي الموسوي**

**ماجستير تقنيات التحليلات المرضية/ 2010-2011**

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