INTRODUCTION

Influenza, commonly known as " flu", is an infectious disease of birds and mammals caused by RNA viruses of the family Orthomyxoviridae ***(Eccles, 2005).***

There are three types of influenza viruses: A, B and C. Human influenza A and B viruses cause seasonal epidemics almost every winter. The emergence of a new and very different influenza virus can lead to an influenza pandemic ***(CDC, 2009).***

Influenza A viruses are divided into subtypes based on two proteins on the surface of the virus: the hemagglutinin (H) and the neuraminidase (N). Influenza A viruses can be further broken down into different strains. Current subtypes of influenza A viruses found in human are influenza A (H1N1) and influenza A (H3N2) viruses. In the spring of 2009, a new influenza A (pdm H1N1 09) virus emerged to cause illness in people. This virus was very different from regular human influenza A (H1N1) viruses. That virus also called “2009 H1N1" has now mostly replaced the H1N1 virus that was previously circulating in humans ***(CDC, 2009)***.

Highly pathogenic avian influenza virus H5N1 originated in poultry and has been occasionally transmitted to humans resulting in high mortality. There have been no reports thus far to indicate that H5N1 is readily transmissible from human to human ***(Kuiken et al., 2011).***

Influenza B viruses are not divided into subtypes, but can be further broken down into different strains, but Influenza type C infections cause a mild respiratory illness and are not thought to cause epidemics **(*CDC, 2009).***

Typically, influenza is transmitted through the air by coughs or sneezes. Influenza can also be transmitted by direct contact with nasal secretions, or through contact with contaminated surfaces, avian influenza "H5N1" transmitted by direct contact with bird droppings. Airborne aerosols have been thought to cause most infections ***(Brankston et al., 2007).***

The most common symptoms are chills, fever, runny nose, sore throat, muscle pains, headache (often severe), coughing, weakness/fatigue and general discomfort. Although it is often confused with other influenza-like illnesses, especially the common cold, influenza is a more severe disease caused by a different type of virus ***(Eccles, 2005)*** Flu can occasionally lead to pneumonia, either direct viral pneumonia or complicated by secondary bacterial infection , even for persons who are usually very healthy ***(Ballinger et al., 2010).***

Molecular diagnostic techniques as the real-time reverse transcription polymerase chain reaction (rRT-PCR) are rapid and sensitive methods for the detection and identification of influenza viruses, from clinical samples ***(WHO, 2012).***

AIM OF THE WORK

The aim of this study is to determine the prevalence of common influenza viruses. Flu A (pdm H1N1 09, H3N2, H5N1) and Flu B in different governorates in Egypt.

Chapter (1)
INFLUENZA VIRUS

Influenza viruses circulate and cause disease in humans every year. Disease tends to occur seasonally in the winter months, spreading from person-to-person through sneezing, coughing, or touching contaminated surfaces. Seasonal influenza viruses can cause mild to severe illness and even death, particularly in some high-risk individuals. Persons at increased risk for severe disease include pregnant women, the very young and very old, immune-compromised people, and people with chronic underlying medical conditions ***(WHO, 2014a).***

There are three large groupings or types of seasonal influenza viruses, labeled A, B, and C. Type A influenza viruses are further divided into subtypes according to the specific variety and combinations of two proteins that occur on the surface of the virus, the hemagglutinin or "H" protein and the neuraminidase or "N" protein ***(WHO, 2014a).***

Structure of Influenza Viruses

All influenza viruses contain a segmented, linear, negative sense, single stranded RNA genome.. Influenza type A and B viruses have 8 genes. The number of segments differs with influenza A and B viruses which contain 8 segments, And influenza C virus has 7 segments ***(WHO, 2011a).***

The single stranded RNA genome of Influenza A and B viruses occur at eight separate segments. Most of the segments code for a single protein. The complete nucleotide sequence is known for many influenza viruses. The first 12-13 nucleotides at each end of each genomic segment are conserved among all eight RNA segments, these sequences are important in viral transcription ***(Lund et al., 2004).***

Because of the segmented nature of the genome, when a cell is co-infected by two different viruses of a given type, mixtures of parental gene segments may be assembled into progeny virions. This phenomenon, called genetic reassortment, may result in sudden changes in viral surface antigens, a property that explains the epidemiologic features of influenza viruses and posses significant problem for vaccine development ***(Trampuz et al., 2004).***

Influenza viruses are relatively stored at 0-4°C for weeks without loss of viability. Lipid solvents, protein denaturants, formaldehyde and irradiation destroy infectivity. Both infectivity and haemagglutination are more resistant for inactivation at alkaline pH than at acid pH ***(Brooks et al., 2004a).***

Influenza viruses are roughly spherical although somewhat pleomorphic. They are enveloped viruses with a segmented genome. Influenza A and B made of eight single-stranded negative RNA segments of 890 to 2,341 nucleotides,

ranging from 80 to 120 nm in diameter ***(Noda et al., 2006).*** While Influenza C viruses contain seven segments of RNA lacking a neuraminidase gene ***(Capua et al., 2003).***

The Influenza virus is covered with a lipid envelope (figure:1) which is derived from the cell surrounding the virus particle ***(Trampuz et al., 2004).*** The lipid envelope is covered with about 500 projecting spikes, 10 nm long on the surface of the particle, 'which can be seen easily under the electron microscope (Figure 1) these spikes represent the envelope glycoprotein HA and NA ***(Cox and Zeigler, 2003).***

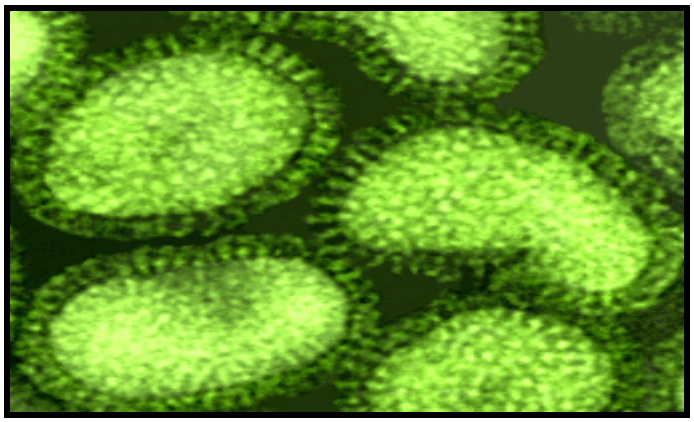

Figure (1): Electron micrograph of Influenza virus particles ***(WHO, 2011a).***

Influenza virus particles contain different structural proteins and non-structural proteins (figure:2). The structural proteins include in addition to the HA and NA proteins,M1 protein, M2protein, viral polymerase and the nucleoprotein (NP) which associates with the viral RNA to form a

ribonucleoprotien (RNP) structure. The RNP is 9 nm in diameter, assumes helical configuration and forms the viral nucleocapsid ***(Voyles, 2002).***

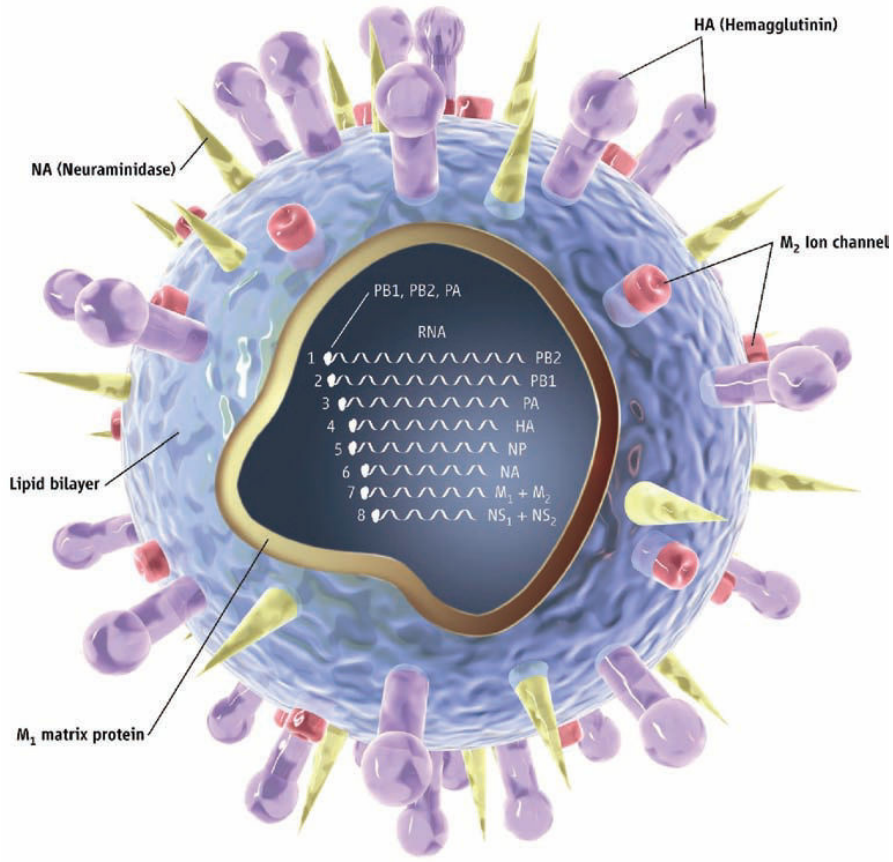

Figure (2): Structure of the influenza virus ***(WHO, 2011a).***

A) Structural proteins:

1. Haemagglutinin Protein:

The HA protein resembles a spike shaped trimer extending outward from the lipid layer and is the most abundant viral surface protein. HA molecules attach the virus to sialic acid receptors on the cell surface and mediate the release of viral ribonucleoprotein particles (vRNPs) into the cytoplasm. HA is cleaved into HA1 and HA2. HA1 contains the sialic acid binding site. After binding, the virus is internalized in endosomes. Acidification of the endosomes triggers a marked, irreversible conformational change in HA. This change includes dissociation of HA1 from the endosomal membrane and its movement away from HA2 ***(Harrison, 2008)*** and promote the fusion of the viral and endosomal membranes, resulting in the release of the vRNPs into the cytoplasm ***(Das et al., 2010).***

2. Neuraminidase protein:

The NA protein is a spike-shaped tetramer extending from the lipid layer, and is integral in both viral attachment and viral release from the host cell. The complete virion is released from the cell membrane by the enzymatic activity of NA which cleaves the α-ketosidic linkage between the terminal sialic acid and its adjacent sugar residue to which the HA is bound. The cleavage of sialic acid leads NA to play a role in both viral attachment, viral release, and viral spread by removing nearby

sialic acid receptors from carbohydrates on the viral glycoprotein, thereby preventing aggregation of viral progeny ***(Matrosovich et al., 2004)***.

Neuraminidase, like HA also plays a major role as an antigenic determinant that undergoes antigenic variation. This activity requires a delicate balance between HA and NA so that viral particles do not aggregate at the cell membrane and so released progeny can continue the cycle and infect other cells ***(Wang et al., 2002).***

3. Matrix 2 Protein:

The third integral membrane protein is the matrix 2 (M2) protein, is the target for the adamantane drugs amantadine and rimantadine (Figure 2). The structure and function of M2 protein have been characterized by selective proton transport at low pH and drug binding ***(Cady et al., 2009).*** M2 protein has a transmembrane (TM) domain and a C-terminal cytoplasmic amphiphilic helix. The proton channel features a tetrameric arrangement of TM helices ***(Pielak et al., 2009)***.

4. Matrix 1 Protein:

The matrix 1 (M1) protein lies beneath the lipid envelope in a layer extending the circumference of the virion and interacts with RNPs, forming a bridge between inner core components and membrane proteins, and allowing assembly of viral products and budding of the virion from the host cell, M1

not only promotes binding to RNA but it also acts as a nuclear localization signal (NLS) based on a specific signal sequence at amino acids 101-105 to promote transport from the cytoplasm to the nucleus ***(Latham & Galarza, 2001).***

5. Nucleocapsid protein (NP)

The nucleocapsid protein (NP) which coats the RNA, and the complex of three proteins which constitute the RNA dependent RNA polymerase ***(Cros et al., 2005).***

It is a major structural protein that encapsidates viral RNA. NP protein is involved in RNA synthesis and RNA nuclear export, and is required for the import of viral RNA ***(Cros et al., 2005).***

6. Viral Polymerase:-

The viral polymerase (P complex) is a heterotrimer of subunits polymerase acidic (PA), polymerase basic 1(PB1) and polymerase basic 2(PB2). P complex is bound to viral RNA and it is responsible for transcription and replication. Transcription involves (i) binding of the 5′ cap of a host mRNA to the PB2 subunit (ii) cleaving of a phosphodiester bond 10–13 nucleotides downstream of the cap (iii) initiating transcription of viral mRNAs at the cleaved 3′ end of the capped segment. The P complex also replicates the viral RNAs in a distinctly different process of unprimed initiation that requires nucleoprotein molecules ***(Coloma et al., 2009)***.

Polymerase basic1 (PB1) gene also encodes a second protein (PB1-F2) which regulates influenza A virus-mediated apoptosis by targeting the mitochondria, causing destabilization of the mitochondrial membrane with some H1N1 strains, but not in H5N1 strains possibly due to its cellular localization; it contributes to viral RNP activity and aids in viral RNA replication ***(Chen et al., 2010).***

Polymerase basic 2 (PB2) binds to the 5' cap of host messenger RNA molecules, after which PB1 cleaves the cap for incorporation into viral RNAs (so called "cap snatching"). While most PB2 protein localizes in the nucleus, PB2 also localizes to the mitochondria and interacts with mitochondrial antiviral signaling protein, it also shows differences in strain specificity like PB1, with seasonal strains targeting the mitochondria but nonmitochondrial targets in H5N1 viral strains ***(Graef et al., 2010)***.

Polymerase acidic (PA) is the third component of RNA polymerase that interacts with PB1 and has protease activity. Its role not only with protease degredation of both viral and host proteins but endonucleotic cleavage of capped RNA primers and transcript elongation. Like PB1-F2 and PB2, PA also has some involvement with mitochondrial proteins and regulation of apoptosis ***(Bradel-Tretheway et al., 2011).***

B) Non-structural proteins:

The final set of proteins encoded by the genome of influenza virus are the non-structural proteins 1&2: The nonstructural protein 1 (NS1) is considered multi-functional and works as a viral interferon antagonist by suppressing the host's immune response induced by the viral infection ***(Kochs et al., 2007)***. NS1 can be divided in two parts, the RNA-binding domain and the C-terminal effector domain, which mediates both the interactions with host cell proteins and functionally stabilizes the RNA-binding domain. ***(Wang et al., 2002)***

Nonstructural protein 2 (NS2) which is also called the nuclear export protein is involved in nuclear export of viral RNPs. NS2 binds to M1 through ionic interactions in the C-terminal domain and is responsible for both the nuclear export of viral RNPs and for blocking re-entry of vRNPs into the nucleus by blocking the action of nuclear localization signal (NLS) of the M1 protein. ***(Shimizu et al., 2011)***

Classification of Influenza Viruses

Influenza viruses belong to the *Orthomyxoviridae* family. The antigenic differenses exhibited by structural protein NP and M protein are used to divide influenza virus into types A,B and C. Antigenic variation in the surface glycoprotein HA and NA are used to subtype the virus ***(WHO, 2011a).***

Influenza types A and B are responsible for epidemics of respiratory illness that are often associated with increased rates of hospitalization and death. Influenza type C is a milder infection that does not cause epidemics, and does not have the severe public health impact of influenza types A and B ***(WHO, 2011a).***

In the case of influenza type A viruses, further classified into subtypes according to the properties of their major membrane glycoproteins, the haemagglutinin (HA) and the neuraminidase (NA). 18 HA subtypes and 11 NA subtypes ***(WHO, 2011a).***

The serotypes that have been confirmed in humans are: H1N1 which caused Spanish Flu in 1918, H2N2 which caused Asian Flu in 1957, H3N2 which caused Hong Kong Flu in 1968, H5N1 which caused Bird Flu in 2004, H7N7 which has unusual zoonotic potential, H1N2 endemic in humans, pigs and birds, H9N2,H7N2,H7N3,H10N7,H1N1 cause Swine Flu in 2009) ***(Wang and Palese, 2009)*** and H7N9 cause flu in china 2013 ***(Wiwanitkit, 2013)***.

Antigenic shift and Antigenic drift:

In order to evade the immune response from natural infections or vaccinations, influenza viruses evolve by two types of antigenic variation: antigenic drift and antigenic shift. Antigenic variation renders the population susceptible to new

strains despite previous experience with other influenza viruses ***(WHO, 2004a).***

A) Antigenic drift:

There are small changes in the genes of influenza viruses that happen continually overtimes as the virus replicate. It is the process by which the HA and NA gene segments change by mutations in the genetic code, through nucleotide substitutions, insertions and deletions. It is the gradual alteration by point mutation of the HA and NA within a given subtype of influenza A viruses. The lack of mechanisms for repair of errors during replication, result in uncorrected errors. As a result of accumulation of these constant, permanent, and usually small errors, result in viruses what are antigenically different, Antigenic drift is usually a random and slow process ***(Brooks et al., 2004a).***

Antigenic drift is thought to be a result of immunological pressure on the virus, so it is more in human viruses than in avian ones due to lower immunological pressure in short lived birds. Antigenic drift variants are responsible for periodic epidemics that occur between pandemics ***(Alexander, 2000).*** The tendency of influenza viruses to undergo antigenic drifts necessitates constant monitoring of the global influenza situation and composition of influenza vaccines ***(Hien et al., 2004).***

B) Antigenic shift

It is an abrupt major change in the influenza A viruses. In contrast, antigenic shift denotes a sudden and profound change in antigenic determinants, i.e. a switch of H and/or N subtypes, within a single replication cycle, due to reassortment ***(Belshe, 2005).***

Genetic reassortment can occur following co-infection of a single cell with different strains of influenza A virus, Conditions favorable for the emergence of reassortant strains have long been involved humans living in close proximity to domestic poultry and pigs, because pigs are susceptible to infection with both avian and mammalian viruses, including human strains. They can serve as a mixing vessel for the scrambling of genetic material from avian viruses resulting in the emergence of novel subtypes ***(WHO, 2004a).***

Replication of influenza viruses

Influenza viruses of humans and other mammals are spread by aerosols, The virus replicates in the cells of the upper and lower respiratory tract,, Avian influenza viruses replicate in both the respiratory tract and the lower intestinal tract, hence the shedding of high concentrations of virus into feces ***(Lamb 2010)***.