# Virology

Lec (6)

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#### **Teaching Objectives:**

- 1. To list general characteristic of Orthomyxoviruses.
- 2. To distinguish different strains.
- 3. To recognize the mechanism of entry and replication.
- 4. To analyze result of diagnosis.
- 5. To the mechanisms of treatment and prevention.



*Influenza:* True influenza is an acute infectious disease caused by a member of the orthomyxovirus family (influenza virus A, B or to a much lesser extent influenza virus C). However, note that the term 'flu' is often loosely used for any febrile respiratory illness with systemic symptoms that may be caused by many different bacterial or viral agents.

Important properties of Orthomyxoviruses.

- Orthomyxoviruses from Greek worth-straight, myxo-mucus.
- Spherical pleomorphic in shape, measuring about 80-120 nm in diameter.
- Single strand RNA, segmented (8 molecular in the influenza virus type A, B while C contain seven segment of RNA), negative sense total 13.6 Kb.
- Helical nucleocapsid.
- -Matrix protein which give rigidity to the lipid bilayer and connected the envelope,
- -Envelop contain hemagglutinin (HA) and neuraminidase (NA).

-Viruses replicate in both cytoplasm and nucleus.



## Figure (1): Morphology of Influenza virus

## Proteins classification according to location:

-The internal antigens (M1 and NP proteins) are the type specific antigens used to determine if a particular virus is type A, B or C. The M1 and NP proteins of all members of each type show no cross reactivity.

-The external antigens (HA and NA) show more variation and are the subtype and strain specific antigens. These are used to determine the particular strain of e.g. influenza A responsible for an outbreak.

## - Proteins classification According to functions:

**A- Hemagglutinin(HA):** Homotrimer glycoprotein composed of 5 specific antigenic site in HA1 and HA2 that undergo change during shift and drift the HA spike important in attachment activity, fusion activity and neutralizing antibody.

**B-Neuraminidase(NA):** It's composed of four monomer glycoprotein give a final shape tetramer, each NA has 4 active site (catalytic site) can remove sialic acid from the glycoconjucate and facilitate the release of virus from infected cells during budding process and prevent the aggregation of virus inside the cell.

## Other protein

- Nucleoprotein associates with the viral RNA to form a ribnucleoprotein (RNP)
- Three large protein (PA, PB1, PB2) are bound to the viral RNP and are responsible for RNA transcription and replication
- M2-protein (M2) matrix protein, which form a shall underneath the viral lipid envelope, M2-protein ion channel protein.
- Nine structural protein and non-structural protein.



Figure (2): Types and sub types of Influenza virus

## Influenza types

- Influenza virus type A: Usually infected human and animal, antigenic changes continually occur within this type.
- Influenza virus type B: Usually infected human only, antigenic changes lesser degree in this type.
- Influenza virus type C: Usually infected human and swine, antigenically stable.

## Spread:

- 1- Person to person spread is primarily via small particle of aerosols that can get into the respiratory tract.
- 2- The virus can also survive for a short time on surfaces and can be spread by this (Contact with contaminates surface and hand).
- 3- Avian influenza A viruses may be transmitted from animals to human in two main ways (directly from birds or in directed from avian virus-contaminated environments to people).
- 4- Through an intermediate host, such as a pig.

# Summary of replication cycle:

- 1- Influenza virus infects the epithelial cells of the respiratory tract which are rich in viral receptors (neuraminic acid, sialic acid).
- 2- The next steps after virus attachment to its cellular receptor(s) and endocytosis, the envelope fuses with vesicular membrane
- **3-** Uncoating is facilitated by the low ph within the endosome.
- 4- The released ribonucleoprotein (RNP) capsid segments, each containing a specific negativesense genomic segment, migrate to the nucleus where transcription of positive-sense RNA takes place using virion-associated transcriptase, in the nucleus because a methylated guanosine 'cap' is required, the cap is obtained from cellular nuclear RNAs in process called Cap snatching.
- **5-** Most of the mRNA moves to the cytoplasm, where they are translated into viral protein, some of the viral mRNA remain in the nucleus where they serve as the template for the synthesis of the negative strand RNA genomes for the progeny virions.

**6-** The helical ribonucleoprotein assembles in the cytoplasm, matrix protein mediates the interaction of the nucleocapsid with the envelope and the virion is released from the cell by budding from the outer cell membranes.

The incubation period is short 18-72 hours. Within short time many cells die due to the direct effects of the virus on the cell, also possibly due to the effects of interferon, and also due to cytotoxic T-cells at later times. The efficiency of ciliary clearance is thus reduced, this leads to impaired function of the mucus elevator, and thus there is reduced clearance of infectious agents from the respiratory tract.



Figure (3): Replication cycle of Influenza Virus.

## Clinical manifestation.

**A- Uncomplicated infection:** Following a typical incubation period of 48 hours, the typical symptoms of influenza appears, Fever (38-40°C), headache, shivering, dry cough, nasal discharge, ocular symptoms - photophobia, tears, retro-orbital ache, myalgias.

**B- Complicated infection:** Are commonly associated with patients with preexisting cardiovascular disease, rheumatic fever, elderly patients, pregnant women and babies.

**1.** *Pneumonia (Pulmonary complications):* Serious complications usually occur only in the elderly and debilitated especially those with underlying chronic disease and young children, pregnancy appear to be a risk factor for lethal pulmonary complications in some epidemics. Croup (acute laryngotracheobronchitis) in young children - symptoms include cough (like a barking seal), difficulty breathing, stridor (crowing sound on inspiration). Primary influenza virus pneumonia, secondary bacterial cause by (Streptococcus pneumoniae, Staphylococcus aureus, Hemophilus influenzae) or a combination of the two. The build up of fluids, increased mucus production helps carry agents into the lower respiratory tract as well as lack of mucociliary clearance in the respiratory tract and dysfunction of phagocytic cell, these entire factor provide a good environment for bacterial growth.

2. Reye's syndrome (Non-pulmonary complications): Is acute encephalopathy (edema) and liver degeneration (fatty deposits) of children and adolescent, usually between 2 and 12 years of age, the mortality rate are high 10-40% of cases. The origin of the syndrome is unclear but it seems to follow

certain viral infections such as influenza or chicken pox (varicella zoster/herpes zoster), especially in the young and especially if they have been treated with aspirin. Aspirin is contraindicated for childhood or adolescent fevers because it is a risk factor in the development of Reye's syndrome.

#### <u>Note</u>

Influenza A virus infection can lead to liver problem but the mechanism remains unclear. So some studies investigated the correlation between virus and liver injury, it is postulated that massive expansion of virus specific CD8 T cells and activation of kupffer cells in foci of apoptosis lead to release of inflammatory cytokines, hepatic oxidative stress and hepatocyte injury.

Recently, it has been demonstrated that infection with some influenza strain results in kupffer cells mediated reduced hepatic fatty acid oxidation. Other study reported that three different subtypes (H1N1, H5N1, and H7N2) after histological analysis, the results showed increase serum aminotransferase (ALT and AST) levels and evident liver injury on days 3 and 7, especially on day 5 post infection. These data are interesting because liver damage occurs after infection, but no viral pathogen is detected.

#### **Diagnosis.**

*-Clinical diagnosis:* Rapid test which can be used in the physician's office have been approved depend on the clinical symptoms (fever, cough, runny nose, malaise), but considered non-specific

#### -Laboratory diagnosis

**1- Virus isolation**- throat swab, nasal-washing and nasopharyngeal aspirate may be used for virus isolation, inoculate in embryonic egg or primary monkey kidney cell for 3 to 10 days at 33-37°C then recognized the virus inside the culture by two ways.

A- Hemagglutination inhibition test (HIT). Hemagglutination with chicken RBCs.

**B-** Hemadsorbtion test. hemadsorbtion with genia pig RBCs.

**2- Detection of viral antigen** HA, NA, NP and M protein using **Rapid diagnosis** such as immunofluorescence (Exfoliate cell in nasal aspirated using specific fluorescent antibody then measured by IFA or ELISA, CFT used in acute and convalescent phase.

**3- Molecular test** like polymerase chain reaction (PCR) and RT-PCR tests are being developed to detect viral RNA.

**Recovery:** Interferon may play a role by decreasing virus production. Many of the symptoms of uncomplicated influenza (muscle aches, fatigue, and fever) are associated with the efficient induction of interferon. The cell-mediated immune response is important in viral clearance. The antibody response is usually not significant until after virus has been cleared. Repair of the respiratory epithelium begins rapidly, but may take some time to complete.

#### **Prevention and treatment**

## Influenza vaccines

-Inactivated vaccine is given by injection. It has a short lived protective effect.

-Live attenuated influenza virus (LAIV) vaccine is also available, it is only approved for healthy individuals (those not at risk for complications from influenza infection) 5-49 years old. It is given nasally and should provide mucosal, humoral and cell-mediated immunity. It needs to be given every year.

#### Treatment

**1-Amantadine and Rimantadine** block virus entry across the endosome, and also interfere with virus release. They were good prophylactic agents for influenza A.

**2-Zanamivir [Relenza] and oseltamivir [Tamil].** this drug inhibit neuraminidase They are active against influenza A and influenza B. Both drugs are approved for prevention (about 70-90% effective in healthy adults). These drugs can reduce the duration of uncomplicated influenza (by ~ 1day) and decrease symptoms if taken within 2 days of the onset of illness.

#### How influenza viruses changes.

Influenza viruses can changes in the antigenicity hemagglutinin and neuraminidase protein, this property contributes to their capacity to cause devastating worldwide epidemic, there are two different types of antigenic changes: Antigenic shift and antigenic drift, influenza type A viruses under both kinds of changes: influenza type B, C viruses changes antigenic drift only because are restricted to human.

### 1-Antigenic shift

Which are major changes based on the reassortment of segment of the genome RNA, major change to produce a novel influenza A subtype in humans that was occur every(10-20)years, lead to pandemic result.

- A- Genetic reassortmente. Antigenic shift can occur either through direct animal (poultry) to human transmission or through mixing of human influenza A and animal influenza A virus gene to create a new human influenza A subtype virus ex. In 1968 human H2N2, H3N8 type A in duck and equine, H3N2 Hong Kong subtype flue.
- **B-** Reemergence of strain. Influenza virus has stay dormant for a long time then remerges infected new population lack immunity of this strain ex. In 1918 appear H1N1 and remerge in 1968(Spanish type).



Figure (4): Summary of the events leading up to antigenic shift of human



Figure (5): Recombination (between an existing human Influenza virus A and chicken influenza virus

2-Antigenic drift (due to mutation): Occurs all the time, small, gradual changes that occur through point mutations in the two genes that contain the genetic material to produce the main surface protein, hemagglutinin and neuraminidase. Antigenic drift result in minor changes to these surface proteins newer virus strains appear, the antibodies against the older strains might not recognize the newer virus and infection with a new strain can occur. This is one of the main reasons why people can become infected with influenza viruses more than one time.

Flu pandemic	Years	Death	Subtype
Asiantic (Russian flu)	1889-1890	1 million	H2N2
Spanish flu	1918-1920	40 million	H1N1
Asian flu	1957-1958	1-1.5 million	H2N2
Hong Kong flu	1968-1969	0.75 million	H3N2
Swine flu	2009-2010	-	H1N1