Sheet number: 7

Refer to slide number: 2 and 3

Written by: Leen Musharbash.

Corrected by: Tala Ghishan.

Culture and diagnosis of viral infections

* In general in our country as well as most other countries, most common viral infections such as (rubella, measles, mumps and influenza etc) are diagnosed based on clinical features and rarely we do lab tests to confirm the presence of a virus. However relating to research we might collect samples and look for a type of organisms or to see epidemiology of infection in relation to serotype and genotype.
* In research they use certain type of tests e.g.:

1-living animals but in the last 30-40 years they have rarely been used. They still use chick embryos to study influenza viruses and produce vaccine for influenza.

2- Tissue culture, we have two types the first **primary cell line** which can be subcultured up to 50 times and this type usually carry normal chromosomal number which mean it can be used to produce vaccines where as the second type **continuous cell line** can be subcultured up to 100 -1000 times, this type is usually used to study cytopathic effect of viruses (how viruses change morphological structure of certain cells like elongation or produce inclusion bodies...). Continuous cell line can be used in lab diagnosis of a virus, it originated from tumors or from normal cells which have become transformed during subculture.. have abnormal chromosomal count. And so they cannot be used to produce vaccines.



* In the figure above we have special petri dishes or what we call cell culture bottle, having all necessary nutrients (certain amino acids and vitamins) and the PH is usually controlled. We inoculate this culture with few cells and allow them to grow on the surface of the medium and at the end they produce a sheet of cells which are similar to sheets of cells in our skin and if you want to study any virus in the Respiratory tract or in any other part of the body we inoculate cells of URT for example on the surface of these bottle and we incubate it under CO2 usually 10% and we observe a change in morphology of the cells over a period of one to six weeks to recognize if there is inclusion bodies or elongation in cells...etc and we might take some of these cells and stain them in order to look exactly for a morphological change. And as we said this process requires 1 to four weeks to recognise causative agent and morphological changes.
* We might study the quantity of the virus, by counting something known as **plagues** which are holes present at the surface of the sheets of a tissue and they are counted to estimate the titer of the virus in clinical specimens and this is mainly in research and not in routine culture for diagnostic cases.
* Serological tests are important in certain cases for example rubella virus titer must be known in a young lady before pregnancy in order to know if these ladies must be vaccinated or not,to prevent any complication in pregnancy like abortion or damage to fetus.

* Normally our body produces 3 important types of immunoglobulin's
1. IgA: mainly related to mucosal cells and protect us against attachment of virus.
2. IgM
3. IgG

In a serological test IgG usually develops first then IgM, IgM is more stable and can be recognized for a longer time therefore we rely mainly on IgM to study the status of immunity in an individual to any viral infection.

* Molecular technology represented by polymerase chain reaction(PCR) can be used to detect specific RNA or DNA of a virus (usually we have a primary sequence; a known number of genes only found in a specific type of virus) and now used to study new types of viruses causing upper respiratory tract infection like CORONA virus and also used in research to study genotype where a certain genotype is more common in our country as compared to others which might help in relation to prepare vaccines specific to that genotype circulating in our community.
* The doctor will send us a paper about human papilloma virus.
* Vaccines, as you know already, are important to prevent us from lots of viral infections, more than bacteria. In relation to bacteria we have very limited amount of vaccines like influenza typeB, Streptococcus pneumonia, Neisseria meningitis etc while viruses we have more than 20 vaccines and the first vaccine to eradicate a disease was a viral vaccine which is small pox.
* Viral vaccines are composed of two types:

1) Live attenuated vaccines **(Majority)**: we reduce the pathogenecity of vaccine by treating virus with formalin or certain reagent keeping the RNA or DNA intact as well as envelope, the virus might multiply in our body but slowly and without any clinical manifestations. It allows our body to respond and produce antibodies against virus without getting infected.

2) Killed vaccines.

* Viruses might mutate changing their genes (DNA or RNA) and this means we always have to follow these changes and change the vaccine!

**Viral Respiratory Tract Infection**

* Generally as you know humans are exposed to viral as well as bacterial and fungal agents but most commonly viral agents. Once an infection is established in the upper respiratory tract it might directly reach to the lung or throat or it might disseminate to larynx and pharynx or even might start at nose sinuses but rarely a virus starts at oral cavity(Usually in mucosal part of upper respiratory tract (pharynx and throat))
* Viruses might only produce localized infections/mild infections for example sore throat only mild inflammatory reaction, erythema, slight burning sensation and normally this is not a serious condition but severity might increase and later might spread to the lung and produce pneumonia.
* To understand the pathogenicity and the epidemiology of viruses, we have to divide the common clinical viruses into upper respiratory tract infections and lower respiratory tract infections. This is because some viruses only produce infections in the upper respiratory tract (they cannot easily reach the lung) while others start in the upper respiratory tract but can spread to the lower respiratory tract and produce more serious infections.
* A proper number of viral particles (1,000 to 10,000 cells) should reach the respiratory tract for a virus to establish an infection. The number of viral particles reaching the respiratory tract differs from one human being to another depending on their susceptibility.
* Transmission of a virus occurs through a cough, sneeze, and saliva.
* A human cough usually is associated with aerosol droplets which contain a few numbers of epithelial cells and water; these might carry viruses.
* Sneezing is always associated with a greater number of virus particles than a cough does.
* All viruses are excreted in ones saliva in the oral cavity, hence highly contagious with close contact (even with a hand shake). Wearing a mask is advised.
* Lower respiratory tract infections remain localised in mucosal cells of the respiratory tract. Viruses rarely reach other parts of the body especially in healthy adults. Under certain conditions (measles, mumps..etc) however viruses might produce blood sepsis and meningitis in children. Similarly upper tract respiratory infections might be associated with pneumonia.
* The term “cold” involves a watery component being discharged from the nose (runny nose), this might be an indication of a viral infection without the presence of fever or sore throat, usually caused by Rhinoviruses.
* In immunocompromised patients, infections (especially upper respiratory tract infections) can be associated with secondary bacterial infections especially in children and elderly. Infections in this case might reach the lung and cause pneumonia as a complication.
* Pharyngitis/sore throat is not associated with every upper respiratory tract virus but is often recognized in influenza and adenovirus..etc. Pharyngitis is characterised by erythema, edema, and inflammation. Viral inflammation (being mild) is easier to recognise than bacterial inflammation (more severe).
* 90% of all upper respiratory tract infection is caused by viral infections. Only 10% are caused by bacterial infections. 1-2% is a mix of both.
* Tonsilitis is often a bacterial infection more than a viral infection.
* Sinusitis and Otitis media might be caused by a viral infection or a bacterial infection. The inflammation might spread in children and end up affecting the lung, this is because at this age the pathological barrier cannot prevent the spread.
* In our community any type of upper respiratory tract infection involving runny nose and cough is considered influenza, however most cases are not caused by influenza but instead other types of viruses.
* Influenza involves other pathological changes in the body including high fever, malaise, cough, severe weakness..etc
* Influenza like viruses produces mild infections and do not interfere with your level of activity.

**Lower repiratory tract infections**

* A virus is more dangerous when it reaches the lower respiratory tract.
* Laryngo-Tracheo Bronchitis occurs mostly in children as a complication, while laryngitis occurs in children and adults equally. Severe inflammation and accumulation of fluid in the lung causing pneumonia is observed. Patients who are immuno-compromised, children and adults might require hospitalization.
* Acute bronchitis involves the inflammation of the bronchi. There is always fever and cough and wheezing. Easily recognized infection.
* Acute bronchiolitis is more common in children. Associated with more severe coughing and cyanosis compared to acute bronchitis. Patients are hospitalized.
* Pneumonia & Bronchopneumonia could be observed in any individual following complication of an influenza virus which could be associated with (more dangerous) or without bacteria. Easily recognized during clinical examination. Xray of the lung will show patches due to the accumulation of fluid. Symptoms include inflammation, general weakness in the body, and cyanosis. Patient is hospitalised to support respiration..etc
* Treatment of viral infections: Administration of antibiotics might complicate the case especially if the patient has an underlying disease; hence careful evaluation of the case by physician is necessary.

**Viral Respiratory Pathogens**

**Orthomyxovirus: Influenza virus**

* Ortho = orthodox =classical form
* Myxo = mucosa
* This is a typical type of virus producing inflammation in the respiratory mucosa.
* This virus is related to the mucus.
* There are 3 groups of influenza viruses: A, B and C
* Group A is found in humans, animals especially aquatic birds like ducks and swine (pigs). This is the first group which has resulted in a pandemic outbreak of influenza disease. Group A was first isolated in 1933 by using the electron microscope.
* Group A and B usually spread together and produce clinical infection of influenza. We rarely recognize one of them as the causative agent.
* Group B can produce infections alone but is less virulent/pathogenic in comparison to group A. Both however are highly contagious between humans.

Note: highly contagious means that only a few numbers of the virus A or B are needed to establish an infection in the respiratory tract of humans and animals.

* The clinical features are related to the mucosa of the upper respiratory tract causing sore throat, fever which might be high within a short recognition period of 48 hours, fatigue, headache, chills, runny nose and under certain conditions might spread to the lower respiratory tract and cause a more dangerous complication in the form of Hemorrhagic & necrotizing tracheo-bronchitis. Might be fatal especially if the viral infection is associated with bacterial infection.
* Morphology of Orthomyxovirus:



* Two types of spikes are present in this virus; Neuramindase and Hemagglutinin. These spikes are composed of a special type of glycoproteins. They project from the envelope of the virus (which is composed of a lipid bilayer).
* Hemagglutinin produces agglutination to the erythrocytes.
* Neuraminidase is an enzyme; which acts on the cell membrane of the respiratory tract.
* Two important proteins are also present; M1 and M2. M1 is a membrane protein while M2 is a proton ion channel allowing the inflow of the specific enzymes from the outside during infection of the mucosa.
* Looking at the genome of the virus, we see 8 strips of RNA. Hence, this is a RNA virus of single stranded –ve cells. This means that before it can produce the necessary components of the virus it first has to produce a messenger RNA.
* The RNA segments of the virus are never stable (more than 2 yrs) and are constantly mutated, this is important in developing new genotypes which involve producing new glycoproteins in the envelope. This virus is now perceived as a foreign virus in our bodies.
* There is no solid immunity for viruses.