University of Jordan
Faculty of Medicine

Medical Committee
The University of Jordan

Introduction to

Microbiology

Title :

Herpes (DNA Viruses)

Professor:

Dr. Ashraf Al-Khasawneh -11

: 31

- ○ Slides
- ○ Handout
- ● Sheet

Done by: **Baraa' Al-Subaih**

Price: .....

Designed by:
Waseem Kamal

M.D University of Jordan Class of 2018

groups/Doctor2012
http://medstudygroup.weebly.com

In the name of Allah the most gracious, the most merciful

Sheet contents: 1- Continuation of antiviral drugs**. 2 –**Herpes viruses in details

***…** Antiretroviral drugs:*

****Fusion inhibitors**:

- We have 2 drugs work by inhibiting fusion: 1- ***Fuzeon*** or the (infuvirtide)
  (2- The prof didn't mention it.)
- This class of inhibitors inhibits viral fusion by inhibiting viral replication.
- Fusion inhibitors are new class of antiviral drugs.
- They're used in combination with other drugs against HIV as all other HIV drugs.

****Entry Inhibitors**

- Inhibit viral entry into macrophages and T-cells.
- It blocks the co- receptor (CCR5) and we have a drug used as entry inhibitor which is ***Maraviroc***.
- It is used in combination with other drugs active against HIV as well.

****Antiretrovial Drugs (Slide 48) General Notes:**

It is common for HIV drugs to be used in combination with multiple antiretroviral medications. Every drug used in the combination should work on a different stage of the virus replication.

Side effects vary with each drug and may be severe so monitoring for dose-limiting toxicities is required. We mentioned that fusion inhibitors cause side effects such as neuro/nephro-toxicities and so monitoring is necessary. We need to monitor for the signs of opportunistic infections since that when HIV patients develop the full blown picture of Aids the Immune system is suppressed and they are susceptible to opportunistic infection by a wide range of microorganisms.

** Interferons (IFNs):

- They are natural proteins produced by the cells of the immune systems in response to challenges by foreign agents such as viruses, bacteria, parasites and against some cancer cells. There are 3 classes of interferons (α, β, γ):
- **(α, β):** Most important, produced by all the cells and are more associated with viral infection
- **(γ):** Produced only by T lymphocyte and NK cells in response to cytokines – immune regulating effects. Less anti-viral activity compare to (α, β)
- Mechanism of action: mentioned when we discussed viral immunology. Refer to slide 51.

<u>Antiviral spectrum:</u>

Interferon α

- The interferon is used for: Hepatitis B virus (HBV), Hepatitis C virus (HCV) and Human Papilloma virus (HPV)
- **Pegylated interferon:** Slow release form of interferon, it is used in addition to ***ribavirin*** in hepatitis C patients.
- Anti-proliferative actions may inhibit the growth of certain cancers (like Kaposi sarcoma)

<u>Pharmacokinetics of Interferons:</u>

- Oral bioavailability: < 1%. Therefore it can't be given orally, it can be administrated: Intralesionally, S.C (subcutaneously) and I.V.
- They can reach all body systems except eye and C.N.S
- Half life: 1-4 hours.

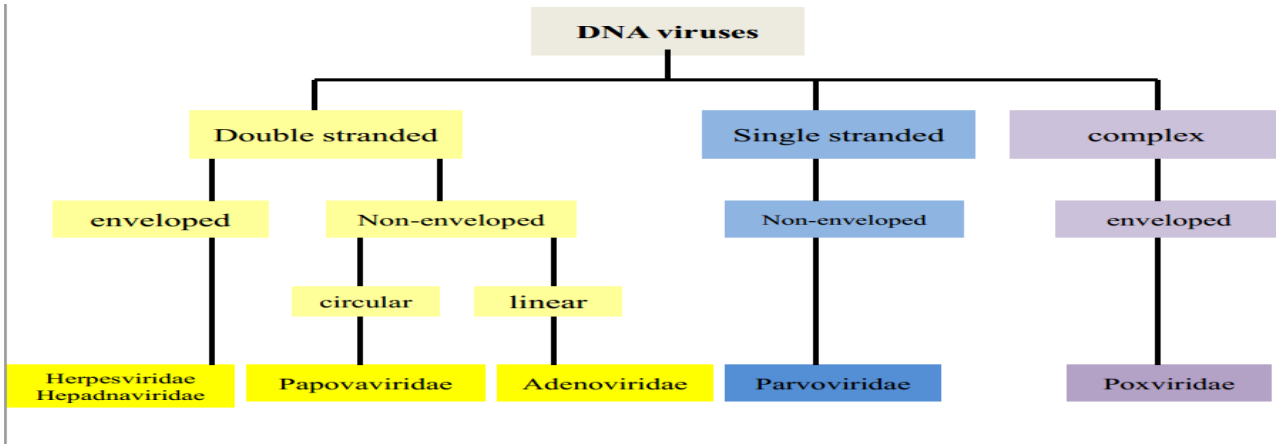
<u>Adverse effect of interferons:</u>

1- Bone marrow suppression
2- Neurotoxicity (confusion, seizures)
3- Cardiotoxicity (arrhythmia)
4- Acute flu-like symptoms (fever, headache)

<u>Therapeutic uses of interferons:</u> HBV, HCV and HPV. Please refer to slides 55-57.

NEW TOPIC: DNA VIRUSES - HERPES

We have 6 families of DNA viruses and 13 families or RNA viruses. (He read this figure)



Notes on this diagram: (all are info you should already know)

- 1- Hepadna viruses (hepatitis viruses). it is a partial double stranded DNA virus

- 2- Paroviruses are the smallest viruses (non-envelope ss-DNA). Poxviruses are one of the largest viruses and have a complex capsid, they are enveloped viruses.

Herpesviridae

- They are double stranded DNA viruses, have a linear genome, enveloped viruses.
- Their genome's size varies from 125 kilo base pair - 240 Kbp: Large genome, which in turn encode for 75 viral proteins (large number)
- The large number of proteins is required for the early stages of viral entry and because herpesviredae can infect non-dividing cell especially when during the latency stage it can infect neurons (non-dividing cells).
- Some viruses may have up to 9 glycoproteins on their surface and there is 50-70% similarity between different herpesviredae viruses.
- There are 8 types of HHV (Human Herpes Virus):

- **HHV 1**: Human Simplex Virus 1 (HSV1)
- **HHV 2**: Herpes Simplex Virus 2 (HSV2)
- **HHV 3**: Varicella Zoster Virus (VZV).
- **HHV 4**: Epstein–Barr Virus (EBV)
- **HHV 5**: Cytomegalovirus (CMV)
- **HHV 6**: Human Simplex Virus 6
- **HSV 7:** Human Simplex Virus 7
- **HHV 8:** Human Simplex Virus 8, also known as: Kaposi's sarcoma-associated herpes virus because it is associated 100 % of the cases with Kaposi sarcoma.

Reason behind the name of the Cytomegalovirus?

Have intracytoplasmic and intranuclear inclusions. The intracytoplasmic inclusions lead to the enlargement of the affected cell and the name of the virus was acquired from this cytopathic effect.

** What is the target cell of each type, what is the target of acute infection and where does each virus go and acquire latency?

- Herpesviridae as a group are known for **latent infection**, they go and stay dormant in certain cells.

- Here is some target cells for some herpes viruses according type of infection:

A) Acute infection sites:

- *Mucoepethilial cells:* **HSV1**, **HSV2**, **VZV**
- *B-lymphocytes*: **HHV4** (EBV), **HHV8**
- *B and T-Lymphocytes*: **HHV5** (CMV)
- *T-lymphocytes*: **HHV 6, 7**

B) Latent infection sites: (rotation of the latent viruses)

- *Dorsal Ganglia*: **HSV1**, **HSV2**, **VZV**
- *B-lympocytes*: **HHV 4** (EBV)
- *Neutrophils and monocytes*: **HHV 5** (CMV)
- *T-lymphocytes*: **HHV6, HHV 7**
- Kaposi sarcoma lesions: **HHV 8**

**** There is cross reactivation between HSV and VZF**

- This means that there is a similarity between the antigens and as a result the antibodies produced might be a bit similar.

**** How can we differentiate between HSV-1 and HSV 2?**

- Nearly all glycoprotein on their surfaces are similar but they differ in one glycoprotein through which we can differentiate between them, this is called **glycoprotein B 1 (gp-B1)** in HSV-1, and **gp-B2** in the case of HSV-2.

**** Herpesviridae Morphology:**

All herpes viruses have similar morphologies and cannot be distinguished from each other under electron microscopy.

Structure:

- Capsidated with an icosahedral capsid, enveloped and there is a space between capsid and envelope, we call this space the **"Tegument"** (you can look at p 109 Sherries Fig D)**.**

- In the Tegument, you can find the proteins and enzymes required for the early stages of the viral infection.

**** Herpesviredae classified into 3 subclasses (subfamilies):**

1- *Alpha*: HSV-1, HSV-2, VZV.
2- *Beta*: CMV, HHV-6, HHV-7.
3- *Gamma:* EBV, HHV-8.

**** Replication cycle of Herepes Viruses:**

First: adsorption to the cell in which the viral glycoprotein attaches to cellular receptors, one of them is *heparin sulfate*. **Second:** virus enters the cell and uncoats. **Third:** the linear genome travels to the nucleus (since it's a DNA virus) where it becomes circular. **Fourth:** Replication using the viral *DNA –Polymerase.* Since it is a circular genome we will get **DNA Concatemers**: a high molecular weight, long stranded DNA molecule that contains multiple genomes attached

to each other and are cleaved later on as a result of this complex pattern of DNA replication.
Finally: Virus assembly; each genome is cleaved and enters an individual capsid.

** Protein synthesis:

In protein synthesis we have 3 stages: The immediate early, the early, and the late.

- **First process, the Immediate Early process:**

- The proteins and enzymes required for transcription are synthesized.
- This occurs as an individual step (there is only this step going on inside the cell at this time, the immediate early is shutdown once the early proteins synthesized)

- **Second: Early protein synthesis stage:**

Once this stage starts, the first stage shuts down and replication also starts to take place.

- Non-structural proteins are synthesized in this process: DNA polymerase and Thymidine Kinase (TK) enzymes which are unique for Herpresviridae viruses (بالأحرىdistinct from the host cell enzymes), those enzymes are targeted by antiviral drugs.

- **Third: Late protein synthesis stage: (structural proteins)**

Some of late proteins might be synthesized at the same time as when replication occurs but other late proteins might be produced only after replication has finished.

- Structural proteins are synthesized: Glycoproteins and Capsid

After these 3 processes the genome has replicated, structural and non-structural proteins synthesized, now we move on to assembly.

** Herpes viruses assembly:

- The proteins travel from the cytoplasm to the nucleus because the assembly takes place in the nucleus.
- Concatemers are cleaved, consequently, one genome will enter the capsid
- The structural and non structural proteins are arranged, and then the viruses bud from the nucleus acquiring the nuclear membrane as their envelope (Inner lamella of the nuclear membrane is acquired as envelope).
- Then viruses travel through the endoplasmic reticulum and Golgi acquiring another membrane which surrounds the whole virus and then exits the cell via exocytosis, this envelope is temporary and is lost upon exiting the cell, it is only needed for exocytosis.

** Latency (Latent infection):

The virus is not cleared; it lies dormant in cells (each type of virus lays in a specific cell).

- The viral linear genome goes to the nucleus of the cell where it lies dormant there as a circular genome we call it an **Episome**. It is not integrated with the cellular genome.

Note: Latency in certain DNA viruses is associated with integration of the viral genome (either a part or whole) into the cellular DNA and it is associated with oncogenic viruses, but it is **not** the case with herpesviredae.

Factors that reactivate the virus

Include: Immune-suppression, emotional/physical stress, sunlight/UV exposure, trauma, radiation.

How does reactivation occur?

There are 2 theories about reactivation:

- **First:** metabolic changes occur in the cells mainly as a result of immune suppression or other reactivation factors and this leads to replication of the virus travels down along the nerves axons into the skin making lesions and illness. Here we are targeting specifically simplex and VZV viruses

Note: when the virus goes to the cell where and lies dormant there as a circular episome, it only produces the immediate early proteins and doesn't produce early and late proteins. So, there is **NO** production of thymidine kinase and DNA polymerase. As a result, **it** cannot be treated with antiviral drugs since they are prodrugs and need activation by thymidine kinase

- **Second:** during latency, there is slow chronic replication of the virus (in the ganglion). Every now and then when the reactivation conditions (especially the immune suppression) occurs, the virus is shed and is released through the nerve axons to the skin, making lesions.

The probability of the first theory is more.

- Note:
  - **Latency in HSV-1** occurs in trigeminal, superior cervical and vagal nerve ganglia.
  - **Latent HSV-2** has been demonstrated in the sacral **(S2-S3)** region**.** Remember the virus is transmitted through the nerve axons**,** most commonly in VZV, they are transmitted through the superficial innervations of the body: dermatomes)

Only 25% of DNA/protein produced are incorporated into virions. What does this mean?

Replication of herpesviredae is one of the most wasteful replication cycles of the viruses because only 25 % of newly produced genomes and proteins are incorporated into new virions. The rest aggregate and accumulate inside the cell and are lost once the cell lyses.

And even of this 25 % when we are looking at purely produced viruses, the ratio of healthy (infectious) viruses to unhealthy viruses (non-infectious) is 1 – 1000. (In other words. the ratio of complete virus particles to incomplete virus particles is 1-1000).

****HSV-1:** mainly causes "above the waist" infection. Most commonly in the circumoral and buccal mucosa, can also cause infection in genitalia.

****HSV-2:** causes infection in the genitalia only.

- **Mans is the only natural host for herpes simplex viruses:**

  Herpes simplex viruses can infect animals but they don't cause diseases .They can only replicate in humans and if you need to grow herpes viruses in the lab you should use human cell line as they are unable to replicate except in it.

- **Seroconversion:** Development of detectable, specific antibodies to microorganisms.

We detect production of specific antibodies toward specific virus.

- Seroconversion in HSV-1 seen in 90% of those who are aged 30 and above in the population.
- Seroconversion in HSV-2 is associated with sexual activity in women. It is seen in 15-30% of women are aged 15-20 and are sexually active.

**** Primary infection:**

- As a general rule in herpesviredae, the primary infection goes unnoticed, maybe there is a lesion but the patient doesn't recognize it, or is too small or is not problematic to him.
- Following primary infection in HSV-1, the chance of recurrence of the oral infection is 45 % while in genital HSV-2, 60% of patients with genital herpes will experience recurrences.
- During the primary infection, HSV spread locally.

**** Recurrent infection**

- Lesions which are painful and are recognized are recurrent most of the time (due to reactivation).

**** Clinical Manifestations of herpes simplex viruses:**

- Acute Gingivostomatitis
- Herpes Labialis (cold sore)
- Ocular Herpes

- Herpes Genitalis (caused by HSV-2 in 70 % of cases, HSV-1 is also involved)
- Meningitis
- Encephalitis
- Neonatal herpes

Acute Gingivostomatitis:

- It is the commonest of primary herpes infection.
- There is pain and bleeding from the ulcers in the mouth.
- It could be as small as 1-8 millimeter ulcers with necrotic bases are present
- There is alignment of lymph nodes.
- It associated with fever.
- It resolves completely in 5-12 days.

Herpes Labialis (cold sore, fever blisters):

- Most of the time it is a recurrent infection.
- When there is a recurrence of the virus, the patient will feel tingling sensation (prodrome tingling) at the site where the viral infection is going appear.
- Tingling sensation, warmth 1-2 days. After that there is a macular rash followed by formation of a vesicle, pustule and crust of the lesion.
- It last for 1-week and can benefit from topical acyclovir treatment.

Herpetic Whitlow:

- Infection of the finger or nail area through small cuts
- The virus might enter through these cuts and cause a pustule there, this pustule mimics bacterial paronychia (bacterial infection)
- Most of the time it is treated as a bacterial infection

Ocular Herpes

- The virus can infect different parts of the eye **causing** keratitis, keratoconjunctivitis, irdocyclitis, and chorioretinitis.
- Patient can benefit from oral and topical acyclovir.

Herpes Simplex encephalitis

- **Focal form:**

- It is seen in children and adults
- Most commonly the temporal lobe is involved. Symptoms depend on the area involved.
- The mortality rate is (70%) without treatment

- **Neonatal form:**

- It is seen in newborn infants.
- Infants get infection when passing through the birth canal during delivery by coming in contact with HSV -2.
- Neonatal encephalitis is associated with 100% mortality: lead to liquefaction of brain matter.

For both, you MUST investigate presence of HSE early by taking blood /CSF sample or blood smear. IV acyclovir is given in all cases of suspected HSE before laboratory results are available because it might be lifesaving.

Genital herpes:

- It is caused most of the time by HSV-2; HSV-1 might also cause it.
- Many sites include in genital herpes including penis, vagina, cervix, vulva and bladder.
- Incubation period: 5 days
- Lesions begin as small erythematous papules, and then become vesicles which postulate and are cleared without leaving any scars.
- It is associated with Dysuria (burning sensation on urination)
- Asymptomatic shedding may occur. Most of the time primary infections go unnoticed especially in females, so the female could be still shedding the virus (producing it, the replication virus still taking place and it is present in vaginal secretion) and so her partner might be infected although he doesn't know that his female partner was infected.

***

** **Laboratory Diagnosis:**

- **Tznack test:** A smear from the base of the lesion. Stained with Giesma stain to detect inter-nuclear inclusions**.**
- **Direct detection**
- **Virus isolation**
- **Serology**

**** Management**

- **Famciclovir** and **Valacyclovir** (oral only), and **Acyclovir** (oral or IV according to the case), are all beneficial antiviral drugs.
- In the case of **acyclovir resistant herpes infection** we use **Foscarnet**
- The treatment previously mentioned in points **:**

1-*Acyclovir* is used against Herpes Labialis as a topical treatment.

2-Both cases of herpes simplex encephalitis (HSE) either focal or neonatal we use IV Acyclovir as a lifesaving drug**.**

- **For prevention** of **HSV-2 infection:**

- Safe sexual practices.
- Screening for pregnant mothers. **If** she has (HSV-2) infection she should deliver her baby by a caesarian section (C-section) to avoid neonatal infection.

Varicella - Zoster Virus

Infections:

- In ***Acute*** form it causes chickenpox
- In ***Latent*** form it causes Shingles (Herpes Zoster)

- There is only one antigenic serotype only. So, infection with VZV (chickenpox, commonly) gives **life-long immunity** because of the production if antibodies
- Most seen in **winter** and early **spring** months.
- **Incubation period**: 2-3 weeks (14-21) days.
- Transmitted through:
- **Inhalation** by aerosols and droplets
- **Direct contact** with vesicular or pustular lesions which have fluids inside

- Chickenpox is a **high communicable** illness so if you get in direct contact with the patient the probability for getting the disease is 90% especially in children.
- **Communicability** of chickenpox:
- Most contagious 2 days before appearance of the rash and 3-4 days after appearance of the rash.
- Chickenpox is an illness of childhood, 90% of the cases are seen during the childhood, but, there is still 10% of chickenpox can be seen in adults.
- In the adults the symptoms of Chickenpox are more sever in the amount of the rash, the pain associated with the lesions and more probable to bacterial contamination because of the increased amount of rash, the individual lesions might form large lesions and this is good entry point for bacteria.

- The immunity caused by infection of VZV causing Chickenpox doesn't protect the body against shingles since the antibodies are formed toward the VZV. That doesn't mean the virus will not be reactivated.
- Chickenpox is seen mostly kids 4-10 years.
- Herpes zoster (shingles) cases can be seen all year round.

Pathogenesis:

- Pathogenesis of the virus: starts in the upper respiratory tract follo→ primary viremia → lymphatic organs → secondary viremia generated → goes to skin →you can see the lesions there
- Following the primary infection, the virus remains latent in the Cerebral or posterior root ganglia.
- In 10 - 20% of individuals, a single recurrent infection occurs after several decades. Most of the time after 50 or 60 years of infection or might be developed in immune-suppression cases or previously mentioned reactivation factors.
- The virus is reactivated in the ganglion and tracks down the sensory nerve to the area of the skin innervated by the nerve, producing a varicella form of rash in the distribution of a dermatome, most of the time it is unilateral distribution.
- Since the nerve replicates in the nerve, it might leads to nerve damage and this will result in what's called: **Post-herpetic neuralgia**.
- Sever pain is accompanied with shingles lesions

Treatment:

- Chickenpox (Varicella) is self-limited, doesn't need any treatment and is resolved without it.
- Shingles: Most patients might benefit from antiviral treatment with acyclovir especially immune-compromised patients. Sometimes you should give him pain killer**s** as wel**l,** if the pain is too severe you might use the strongest pain killers.

Manifestations:

- Presentation of chickenpox starts as fever with enlarged lymph nodes followed by the rash.
- The rashes in chickenpox you can see it different stages of development (you will see some lesions that have just erupted and some lesions in the healing stage)
- The rashes in Smallpox caused by Poxvirus are all in the same level of development
- Rash stages of chickenpox (refer to slide # 20 note):

1- Red spot 2- Blisters. 3- Hard crust

- A question asked during the lecture, doctor said it is not included.

Could a mother infected with varicella transmit it to the fetus?

Yes, if the fetus infected in less than 7 days before delivery or 2 weeks after delivery he needs to be given immunoglobulins. But if the infection occurs more than 7 day before delivery he will be protected.

Prevention:

- Do we have immunoglobulins and vaccine for varicella?

Yes. Immunoglobulins are not given routinely and are expensive. Vaccines are not part of national vaccination program; you can find it as MMRV (measles, mumps, rubella and varicella). The first shot of vaccination is given at 1-1.5 years of age, and the second booster dose is given at (4-6) years of age.

HHV-4 Epstein Bass virus:

- It infects B- lymphocytes and causes their immortalization (Increase in life span).
- There are two strains of the virus: **A** and **B**. Cellular receptor **CR-2** and **CD-21**.

Infectious Mononucleosis (IM):

- Caused by EBV, referred to as the "kissing disease". Most seen in college aged persons
- It is contracted through contact and kissing
- It results in *fever*, *enlargement of the lymph nodes* and *splenomegaly*. The patient is also susceptible for *splenic rupture* and internal bleeding with strong physical activity therefore young patients must be prevented from doing any form of sports or strenuous activity.
- It is usually a self-limited disease, there is no specific treatment.
- Diagnosis of IM is usually made by the heterophil antibody test and/or detection of EBV IgM.

Diseases associated with (EBV):

- Infectious Mononucleosis
- Burkett's lymphoma
- Nasopharyngeal carcinoma
- Lymphoproliferative disease and lymphoma in the immunosuppressed.
- X-linked lymphproliferative syndrome
- Chronic infectious mononucleosis
- Oral leukoplakia in AIDS patients
- Chronic interstitial pneumonitis in AIDS patients.

As u see in previously mentioned diseases, EBV is associated with multiple cancers. The exact mechanism of how (EBV) produces cancer is unknown.

How to differentiate between EBV (HHV-4) and CMV (HHV-5):

- In (CMV) the heterophil antibody is negative and in (**EBV)** the heterophil antibody is **positive**.

EBV Diagnosis:

As all others and it includes**:** Serology, PCR isolation

Cytomegalovirus HHV-5 (CMV):

- If it infects new competent humans it is a marked illness.
- ***Vertical transmission*****:** might occur at 3 different stages; *during pregnancy*, *during delivery*, *after delivery*.
- During delivery and after delivery: the infection is mostly a mild, asymptomatic and doesn't need a treatment.
- During the pregnancy: infection is associated with multiple congenital anomalies and we call it **Cytomegalic Inclusion Disease**.
- Immunocompromised patients such as transplant recipients and AIDS patients are prone to severe CMV disease such as pneumonitis, retinitis, colitis, and encephalopathy.
- Reactivation or reinfection with CMV is usually (asymptomatic) except in immunocompromised patients.
- This the range of congenital anomalies if the infection is transmitted during pregnancy:
    - Eye: choroidoretinitis and optic atrophy.
    - CNS: microcephaly, mental retardation, spasticity, epilepsy, periventricular calcification.
    - Ear: sensorineural deafness.
    - Liver: hepatosplenomegaly and jaundice which is due to hepatitis.
    - Lung: pneumonitis.
    - Heart: myocarditis.

Diagnosis:

- There are 2 distinct tests that we can do to detect CMV infections:
    1- pp65 CMV antigenaemia test
    2- DEAFF test

Both tests are serological in which they detect a viral protein using an antibody.

Treatment:

- In immunocompetent there is no need for treatment.
- In immunocompromised: **ganciclovir**, **forscarnet**, and **cidofovir**.
- In congenital cases (during delivery): nothing can be done but if it is diagnosed you can give the mother the option of abortion.
- In Perinatal and postnatal infection: no need for treatment.

HSV-6 and HSV-7

- **Cause: Rosella Infantum** (Exanthem Subitum) disease, has the following features:
- It is seen in those who are aged 6 months-4 years.
- Associated with a sudden rash. This starts centrally in the trunk then goes to extremities.
- The rash resembles the rash caused by other viruses and it is associated with fever and convulsions (sometimes). The rash is a faint macular rash so it doesn't have vesicles.

Diagnosis: Seroconversion, culture and PCR.

Treatment: The patient can benefit from ganciclovir and foscarnet.

HHV-8

- Originally isolated from 100% of the cases with Kaposi's sarcoma (KS) patients
- Now appears to be firmly associated with Kaposi's sarcoma as well as some lesser known malignancies such as Castleman's disease and primary effusion lymphomas.
- Most patients with KS have antibodies against HHV-8.
- The high risk groups are the homosexuals (same as AIDS target group) especially those who have AIDS.

Forgive me for any mistake. This is a very tough lecture and the Dr used to say lot of things that contradict with the book.

Test yourself (past paper questions)

1) In CMV infection, one statement is wrong:

A) Teratogenic. B) Primary infectio0n is usually symptomatic. C) May cause infectious mononucleosis like illness. D) May cause severe pneumonia in immunocompromised patients.

-- The answer is (B). CMV is usually (asymptomatic) except in immunocompromised patients.

2) In chickenpox one of the following statement is wrong:

A) IP is 3-4 week. B) Vesicular rash is a characteristic C) the disease can be prevented by vaccination. –The answer is (A)

3) The following viruses are associated with congenital infections of newborn except: A) Varicella Zoster. B) CMV. – The answer is (A) '' the baby can be infected in special condition mentioned but with no congenital anomalies mentioned.

4) One of the following can cause Gingivostomatitis:

A)HSV-1. B) CMV. C) HSV-2. D) Hepadna viruses. - The answer is (A).

5) Which of the following is not a diagnostic approach in virology:

A) Isolation of virus in cell culture. B) Detection of antibodies against viral proteins. C) Interferon estimation in proteins. (n.b. Serroconversion id detection of antibody against the viral protein. - Here the right answer is (C).

***** Good Luck *****