HIV & AIDs

Objectives:

- By the end of the lecture student should know:
  - Have an overview of the epidemiology of HIV worldwide and in Saudi Arabia
  - Understand the risk factors for HIV
  - Know the life cycle of HIV and have a brief overview of antiretroviral therapy.
  - Describe the infections and opportunistic diseases expected to occur in AIDS

Team Members: Mohammad almutlaq - Dina aldossari - Amal AlQarni - Badriah alsabbagh

Team Leader: Fahad Alzahrani

Revised By: Basel almeflh and Yara aldigi

Resources: 435 team + Davidson + Kumar + Recall questions step up to medicine.

- Editing file
- Feedback

HIV and AIDS

★ **Definition of HIV :** Infection with Human immunodeficiency Virus which typically begins with a brief acute retroviral syndrome. then transitions to a multi-year chronic illness that progressively depletes CD4 T- lymphocytes critical for maintenance of effective immune function and ends up with life-threatening immunodeficiency.

- Characterized by :
  A. Long latency period
  B. Progressive immunodeficiency
  C. Opportunistic infection when CD4 count reaches low levels any kind of bacteria, viruses, or parasites will attack "عدوى انتهازية" .
- HIV causes diseases by disrupting the immune system function as measured by CD4 cell depletion called: AIDS (Acquired Immune Deficiency Syndrome). we don't call someone AIDS patient unless he reached this stage of immune deficiency .
- HIV is an RNA Lentivirus virus
- It belongs to retrovirus family.
- Retrovirus: Information in the form of RNA is transcribed into DNA in the host cell.

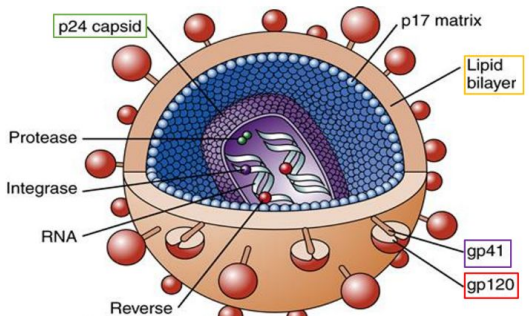
★ Types of HIV:

- **HIV1 :** Predominate world wide,↑virulent &↑susceptible to mutation.
- **HIV2* :** Predominate(in specific regions)in western Africa. closely resemble HIV-1 BUT is a much slower progression to AIDS, ↓virulent & ↓ susceptible to mutation.
  - It causes diseases by disrupting the immune system function as measured by CD4 cell depletion.
    * Type 2 is slower (less aggressive) but the end result is the same as type1 .
    *You can find the two types in one patient.

★ The hallmark of HIV Disease:

- Infection and viral replication within T-lymphocyte expressing the CD4 antigen resulting in **Progressive depletion in CD4 cell counts** مهم جدًا⟵ This effect on helper-inducer lymphocyte will increase the risk of:
- Opportunistic infections such as Pneumocystis Jiroveci عدوى انتهازية
- Neoplasms such as lymphoma especially NON Hodgkin lymphoma & Kaposi sarcoma

- **It is an icosahedral[1] structure of :**
  - **Lipid Envelope** (env) derived from infected cell, containing numerous external spikes formed by two major envelope proteins:
    - The external **gp 120** it's the most important one
    - The transmembrane **gp 41**



- **Nucleocapsid (gag)** with **P24** major core protein, the core contains two single strands of RNA.
- **Polymerase (pol)**

History:

First recognised in USA 1981, CDC reported the occurrence of :

1. Unexplained occurrence of pneumocystis pneumonia in 5 healthy homosexual in Los angeles so they called it "men syndrome"
2. later on in the same year they found Kaposi sarcoma in 25 healthy homosexual men in NY and LA
3. Later on the disease became recognised in both male and female with IV drug users 'IVDU'.
4. As well as recipients of blood transfusion and haemophiliacs (factor A)

- Around 4 millions of children with HIV are considered victims, they either get it from their mothers during pregnancy and delivery or due to sexual abuse.

Transmission:

- The mode of transmission does not affect HIV disease .
- HIV is a fragile virus (It cannot live for very long outside the body)
- HIV is primarily found in the blood, semen or vaginal fluid of an infected person
- **it is transmitted through:** (arranged by most common to least common)
    1) Sexual (heterosexual ,men who sex with men ,others): Heterosexual is the most common mode of transmission worldwide.
    2) Vertical transmission: from pregnant woman to the newborn (MTCT)is the main mode of infection in children. during pregnancy, after delivery or from breastfeeding
    3) Blood and body fluid not very common
    4) IV Drug Users

No evidence of spread by casual contact

-لا تخافوا من الفايروس لما تتعاملوا مع المريض، الفايروس ضعيف ومايقدر يعيش على الأسطح خارج الجسم .

HIV life cycle & replication: انتبهوا للانزايمز مهمة

HIV needs CD4 receptors to enter the cell, it will leave the envelope outside and enter as ssRNA, after entering the cell, reverse transcriptase will convert the viral RNA into DNA. So, if we do anti-reverse transcriptase in the treatment we will prevent this step. Then the pro DNA (viral DNA) enter the nucleus to integrate with the host DNA by the enzyme which will make them dsDna, after that it will multiply inside the cell making the cell produce large amount of the provirus (millions) which will use the protease enzyme to convert it back to RNA then it will release and infect other cells * the basic pathophysiology of HIV is **depletion of CD4 !!** IF YOU NEED TO REMEMBER ONE THING FROM THE LECTURE IS CD4 لما تجيك بالاختبار غمضي عينك وحطيها :))

① **Binding:** the virus binds to host CD4 receptor containing cells (T cell, macrophages, and microglial cells) via the envelope glycoprotein **gp120** pls remember this one is very important !! then gp120 and gp41 bind to the chemokines CCR5 and CXCR4.

⏬

② **Fusion** (between cell membrane and the virion) → **Penetration** → **Upcoating**.

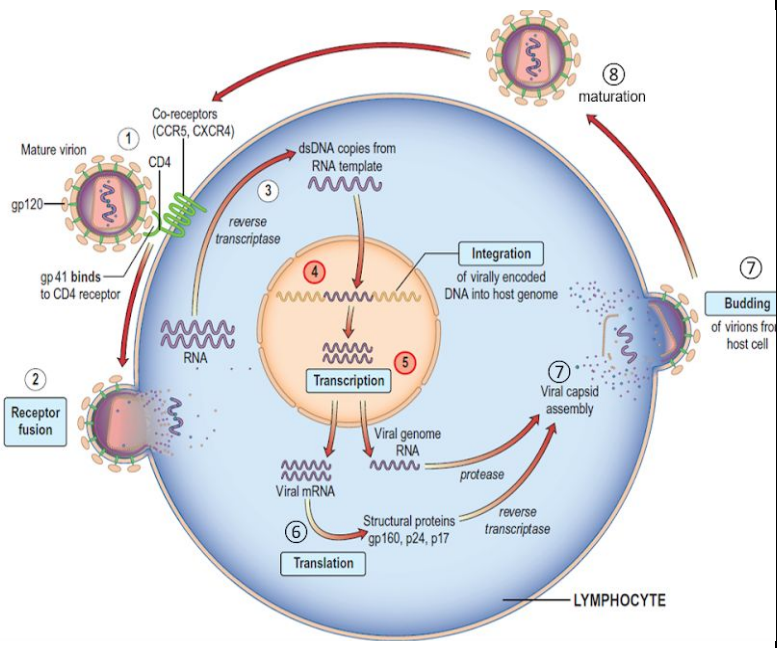
⏬

③ **Reverse transcription**, Formation of cDNA

⏬

④ **Integration**: it goes inside the nucleus & Integrate with the host DNA by the enzyme integrase (in the nucleus, virally encoded DNA is inserted into the host genome)

⏬



⑤ **Transcription** of proviral DNA
- formation of genomic RNA
- formation of structural mRNA

(an RNA molecule is now synthesized from the DNA template!!!). now it'll replicate using host's DNA, once it replicate into large number it'll go back to RNA by an enzyme called protease

⑥ **Translation** of structural m RNA:
-Formation of viral structural protein
- Packaging of genomic RNA of structural protein

⑦ **Final assembly:**
a) Insertion of viral specific glycoprotein into plasma membrane
b) Budding
c) Release of mature virions

⑧ **Final maturation :** by cleavage of gag and pol by polymerase enzyme.

- ليش ما نقدر نقضي على الفايروس نهائياً؟ لأنه يدخل في تركيبة الجينات بحد ذاتها ولازم نقتل كل الجينات وهذا الشيء مستحيل حالياً، المشكلة الثانية انه حيكون موجود ومنتشر في كل الجهاز اللمفاوي مو بس الخلايا اللي تمشي في الدم.
- you know from biochemistry that DNA transcript into mRNA and then mRNA translates into protein, so: DNA to RNA to Protein, but what happens when the virus has RNA ? it goes back to DNA ! that is what retrovirus means ! it doesn't end up with protein.
- Mr X acquired HIV through drug abuse, WHAT WILL HAPPEN? the virus will replicate in huge amount and stays as a reservoir in the body.

- an example of infectious diseases is brucella, the pathway would end with spontaneous recovery in 2-3 weeks and get cured. unlike HIV pts they recover from symptoms but never cured.

Pathophysiology and clinical presentation

★ Pathophysiology:

- Massive replication of the virus in the lymphatic tissues .subsequently
- Permanent viral reservoirs containing proviral DNA are established in the latent T cell or macrophages.

★ Clinical presentation:

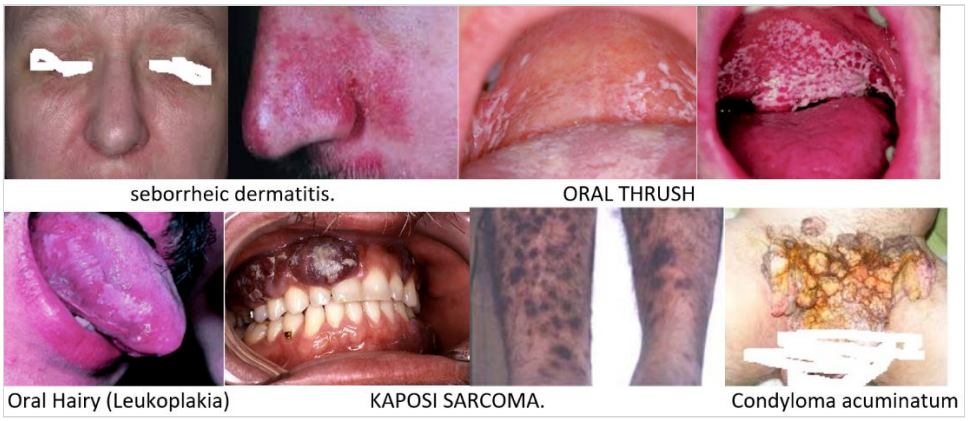
1- Acute HIV infection (primary infection) nothing special	- Acute HIV occurs 1-4 wks after transmission . - 50%-90% of persons develop symptoms within the first few weeks after they become infected with HIV. it resembles **infectious mononucleosis** with: ● Fever, sweats, Pharyngitis, lymphadenopathy. ● truncal maculopapular rash, malaise, lethargy, headache, arthralgias/myalgia, fatigue, oral ulcer. ● Diarrhea, anorexia. - duration of illness is brief 3 days to 2 weeks. →**THEN:** ● HIV RNA level falls and the symptoms resolve. ● CD4 cell count rebounds but remains below the baseline
2- Asymptomatic chronic phase here we discover them accidentally when we screen them for another reason.	- Asymptomatic chronic phase - Active viral replication is ongoing and progressive. - Patient with high HIV RNA may progress to symptomatic disease than those with low HIV RNA level. - Chronic immune activation lead to increase in various inflammatory markers. - This increase the risk of Non-AIDS related comorbidities: CVD, Renal dysfunction and cancer. 🌟Patient with high HIV RNA may progress to symptomatic disease THAN those with low HIV RNA level.
3- Symptomatic HIV infection (pre-AIDS)	- First evidence of immune system dysfunction. - without treatment, this phase lasts about 1-3 years. - **the following frequently appear:** 1. **Skin:** condition associated with HIV, **seborrheic dermatitis** إلتهاب الجلد الدهني skin disease happens in normal people but much more common in HIV patients you see it close to hair follicles like back of head and eyebrows or nose.

2. **Oropharynx:** oral thrush: it's oral candida & when it's happen it usually means the patient is immunocompromised, you can see it in pregnant women, newborn, diabetic patients but if you see it in young healthy patient it's AIDS, hairy leukoplakia: it always indicate AIDS, mucosal kaposi sarcoma.

> ⚠ **Note:**
> Careful examination of the mouth is important, as oral candidiasis and oral hairy leucoplakia are common and important conditions that require the initiation of antiretroviral therapy and prophylaxis against opportunistic infections, irrespective of the CD4 count.

3. **Lymph node:** Generalized lymphadenopathy (TB , **Lymphoma**).very common! non Hodgkin lymphoma .
4. **Eyes: Fundoscopy:** CMV retinitis. (CD4 less than 50).
5. **Genital exam:** ulcers, condylomatous lesions (genital warts)
    a. Condyloma acuminatum: A pointed papilloma typically found on the skin or mucous membranes of the anus and external genitalia. if you see it in pts you MUST screen for HIV, syphilis and other STDs
        i. **caused by:** human papillomavirus (HPV) type 6 or 11
        ii. **Transmitted through:** sexual contact.
    - Diagnosis by visual inspection and confirmed by biopsy

6. **Constitutional symptoms:** night sweats, weight loss and diarrhea.



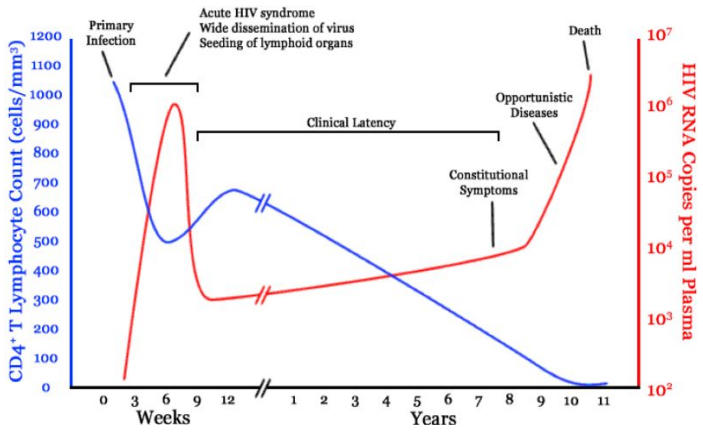
4- AIDS

1. Marked immune suppression leads to disseminated opportunistic infections and malignancies.
2. CD4 count is < 200 cells/mm^3
3. pulmonary, GI, neurologic, cutaneous and systemic systems are common.

> ▶ **Notes in The Natural History:**
> The average time from HIV to an AIDS is about 10 yearsthen survival averages 1-2 years.
>
> BUT!!!
>
> There is tremendous individual variability in these time intervals:Patients progress from acute HIV infection to death within 1-2 years and others not manifesting HIV- related immunosuppression for 20 years
>
> ※The mode of transmission does not affect the natural history of HIV disease

CD4 Cell Count	Viral Load (HIV-1 RNA Levels)
✓ CD4 positive T lymphocytes level is the main method of assessing the immune status of the HIV positive patient. ✓ It is used to determine when to initiate antiretroviral therapy and PCP prophylaxis. It is also useful in assessing the response to antiretroviral therapy. **Immunological staging:** 1. >500 cells/mm³ normal immunity. 2. 350-500 cells/mm³ mild deficiency. 3. 200-350 cells/mm³ moderate immune deficiency. 4. <200 cells/mm³ severe immune deficiency.	- Used to assess response to and adequacy of antiretroviral therapy: - If the viral load is still >50 after about 4 months of treatment, modification in the regimen may be needed. - Do not stop antiretroviral therapy even if viral loads are undetectable for years;Latently infected cells can lead to reappearance of viral RNA once therapy is stopped.
Measure the plasma HIV RNA levels and the CD4-cell count at the time of diagnosis and every 3 to 4 months thereafter.	

HIV progression:



blue line: CD4 will drop rapidly due to cell destruction after multiplication of the virus ' the virus is قليل خاتمة enters the cell, multiply, and then destroys it and attack other cells'. then the immune system try to overcome this and increase the CD4 and here we see a decrease of -viral load-red line. then الجسم يبدا يتعافى ينزل (viral load)والخط الاحمر CD4 شوي فترتفع the patient remain asymptomatic or ' clinical latency' for 5-11 years. then CD4 drop and viral load increases and the immune system will be completely damaged and pts are really sick.

★ HIV serology :

To sum up stages of HIV infection: just go through it

Viral Transmission→Acute HIV infection (occurs 1-4 wks after transmission) →Seroconversion[2]: development of a positive HIV antibody test within 4 weeks and always by 6 months (occurs 2-12 weeks after the development of symptoms) →Asymptomatic HIV infection (last 8-10 yrs "variable amount of time") → symptomatic HIV infection (last 1-3 yrs) →AIDS (Average survival 1-2 yrs)

[2] **Seroconversion** is the period of time during which HIV antibodies develop and become detectable.

★ Diagnostic investigations :

Screening Tests (initial tests):
1- **Combo test:** combination detection of antibodies+antigens ● will detect HIV1 and HIV2 and **P24** antigen:Sensitivity of more than 99.5%. for BOTH Antigens and Antibodies, help in EARLY detection.
2- **ELISA:** ● Screening test for detecting antibody ONLY to HIV; becomes positive 1 to 12 weeks after infection Could give us false negative
Confirmatory Tests:
1- The INNO-LIA™ HIV I/II Score is a Line Immuno Assay (LIA®) *to confirm :* ■ The presence of antibodies against the human immunodeficiency virus type(HIV-1) and (HIV-2) in human serum or plasma. ■ Also differentiates between HIV-1 and HIV-2 —infections ■ Sensitivity: 100%, specificity : 96%
2- Western blot assays: ● can also be used to confirm infection, but they are expensive and sometimes yield indeterminate results.
3- PCR (polymerase chain reaction): *for quantitative **RNA assay*** NOT routine testing BC expensive and time consuming used as : ● Confirmatory test for undetermined cases. ● To assess the viral load. to monitor response to medication ● Babies born to HIV-positive mothers, because their blood contains their mother's HIV antibodies for several months. ● **Blood supplies** we use it for blood donors to protect others فيها خطورة انتبهوا Why it's Not for routine testing: ● Decreased sensitivity at lower viral load ● Significant cost

Home-testing kits only detect HIV antibodies and therefore will not. detect acute HIV infection.

HIV Screening and Diagnosis:

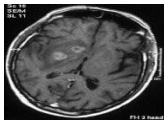
Indication and benefit:
HIV-infected persons aware of their status.
Benefit: Early HIV care and adherence to antiretroviral therapy (ART) prolong life, Decrease the chances of HIV transmission.
16% of the estimated 1.2 million persons with HIV infection in the United States are unaware of their infection. huge problem thinking this is in the educated USA then what about Africa?!

Screening:

- DC recommends HIV screening for patients aged 13–64 years in all health-care settings.
- Persons should be notified that testing will be performed, but retain the option to decline or defer testing.

- A separate consent form for HIV testing is not recommended.
- HIV screening is recommended for all persons with STD. syphilis, gonorrhea, and chlamydia.

Complications of HIV/AIDS :

Tuberculosis	Candidiasis	Toxoplasmosis
TB is the most common opportunistic infection and a leading cause of death TB depends on cell mediated immunity (T cells) that's why it's almost always with HIV. we won't say pneumonia is more common BC it depends on humoral immunity(antibodies-immunoglobulins)	It causes inflammation and a thick, white coating on the mucous membranes of the mouth, tongue, esophagus or vagina.	This potentially deadly infection is caused by Toxoplasma gondii, a parasite spread primarily by cats. It causes meningoencephalitis. ○ **DX:** Serology and MRI. ○ **Treatment:** Combination of (pyrimethamine+sulfadiazine) Respond very well. it doesn't mean anything when you are + and you have good immunity, but it's a big problem for HIV patients, it may affect their BRAINS. 

Cancers common to HIV/AIDS:	
Kaposi's sarcoma: A tumor of the blood vessel walls, common in HIV-positive patients. Rare in none. i. Kaposi's sarcoma usually appears as pink, red or purple lesions on the skin sole of foot and shaft of legs and mouth gums and soft palate and can also affect the internal organs, including the digestive tract and lungs.	**non-hodgkin lymphoma.** مهمة!!

Early management:

- Reduces risk for HIV transmission,
- Decreases individual morbidity and mortality risk, Provides the opportunity to modify risk behaviors.

Special Considerations:

- All pregnant women should be tested for HIV infection during the first prenatal visit TO:
- maintain the health of the woman, enables receipt of interventions that can substantially reduce the risk for perinatal transmission of HIV.

Antiretroviral therapy (ART) : Just Read it مو مطلوب منكم تحفظونها

Goals of Antiretroviral Therapy (ART):

> NOTE: Eradication of HIV? Not possible with currently available antiretroviral medications. We are able to treat them but not able to cure them!

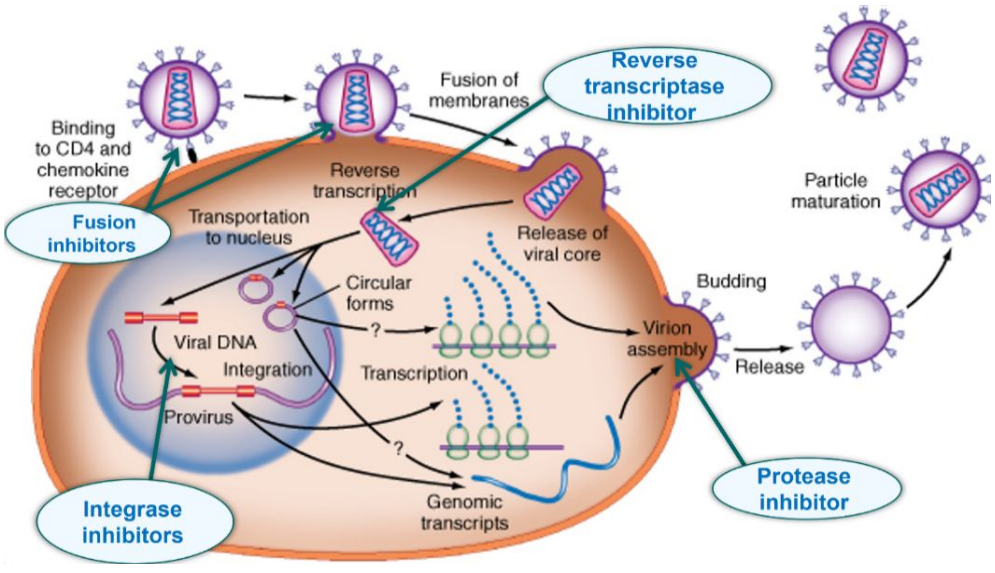
- Improvement of quality of life
- Reduction of HIV-related morbidity and mortality
- Restoration and/or preservation of immunologic function
- Maximal and durable suppression of viral load.

Indications of initiation of antiretroviral drugs:

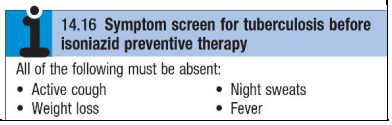
- Symptomatic HIV patients regardless of CD4 count. - Asymptomatic HIV patients with: ● CD4 count less than 350. ● Pregnancy	-Post exposure prophylaxis:

Antiretroviral drugs:

first line medications:			second-line agents:	
RTI's **prevent replication**	non-nucleoside RTI's	protease inhibitors	Entry (fusion) inhibitors	Integrase inhibitors



Chemoprophylaxis : This is Important!

Chemoprophylaxis:	
What is it: Chemoprophylaxis is the use of antimicrobial agents to prevent infections. **Types of prophylaxis:** • Primary prophylaxis is used to prevent opportunistic infections that have not yet occurred. • Secondary prophylaxis is used to prevent recurrence of opportunistic infections because many may recur after an initial response to therapy. Secondary prophylaxis can be discontinued when ART results in immune reconstitution, with CD4 counts increasing to over 200 cells/mm3, but for CMV and MAC, prophylaxis can be stopped if CD4 counts increase to more than 100 cells/mm3	
Indications of Opportunistic infection chemoprophylaxis: مهم تعرفون هالدوائين ويستخدمون للوقاية من أي انفكشن	
In HIV patients It's recommended to prevent Pneumocystis jirovecii when their CD4 count below 200 cells/mm3 according to current guidelines you treat any infection immediately and you don't wait until the count drops to 200. • **Prophylaxis: co-trimoxazole** 1 ds OD • Pneumocystis jirovecii: Can Cause Pneumonia نستخدم هالدوئيين عشان نمنع الانفكشن الين تبدأ ال CD4 ترتفع الى مستوى اعلى من 200 ، تقريبا مدة 6 شهور .	In HIV patients It's recommended to prevent Mycobacterium Avium-Intracellulare when their CD4 count below 50 cells/mm3 ○ **Prophylaxis: clarithromycin** 500 mg orally twice a day.
Read it just in case(Isoniazid preventive therapy (IPT): • Isoniazid preventive therapy (IPT) has been shown to reduce the risk of tuberculosis **ONLY** in HIV-infected patients with a positive tuberculin skin test(induration of 5 mm or more) • Screen all patients with a yearly tuberculin skin test is require. • There is no CD4 count or clinical threshold for starting or stopping IPT. • It is important to rule out active tuberculosis before starting IPT -->	

Prevention:

1. The only absolute way to Prevent sexual transmission of HIV infection is:
   اتباع قول الله تعالى: { وَلَا تَقْرَبُوا الزِّنَى إِنَّهُ كَانَ فَاحِشَةً وَسَاءَ سَبِيلًا}
2. **Abstinence** from sexual relation completely.
3. Safer sexual contact: Use of condom, 10% failure rate.
4. Circumcision: results in 50% reduction of HIV acquisition.
5. Stop using IDUs (intravenous drug users) .
6. Screen all blood and blood products.
7. The **cornerstone** of an HIV prevention strategy is:
   - Education
   - Counseling
   - Behaviour modification
8. If more than 25% of infected patient does not know, What to do? Routine testing between 13 and 64 years. (CDC recommendations without written consent)

Counseling for Persons with HIV Infection:

Health-care providers should :

- assess the need for immediate medical care and psychosocial support.
- substance abuse counseling and treatment
- treatment for mental health disorders emotional distress, reproductive counseling, risk-reduction counseling, and case management
- determine whether any partners should be notified concerning possible exposure to HIV.

Mother to child transmission:

<table>
<tr><th colspan="3">Pregnancy and HIV infection:</th></tr>
<tr><td colspan="3">Pregnant women infected with HIV infection caries risk to infect her baby by:</td></tr>
<tr><td>In-utero,
25-40%</td><td>Intrapartum, 60-75%</td><td>Breast feeding:</td></tr>
<tr><td></td><td>Current evidence suggests <u>most transmission</u> occur during the intrapartum period.</td><td>• Established infection 14%
• Primary infection 29%</td></tr>
<tr><td colspan="3">• Overall risk for mother to child transmission (MTCT) is 16%-25% (Without antiretroviral Rx) MTCT:Mother To Child Transmission</td></tr>
<tr><th colspan="3">Perinatal HIV transmission</th></tr>
<tr><td colspan="3">Today the risk of perinatal transmission is: Less than 2% now 1% with:
1. Effective antiretroviral therapy (ART)
2. Elective caesarean section when appropriate
3. Formula feeding</td></tr>
</table>

Summary

- **Definition:** Infection with Human immunodeficiency Virus which typically begins with a brief acute retroviral syndrome.
- **Characterized by**: Long latency period, Progressive immunodeficiency, Opportunistic infection. that will end up with AIDS.
- **Types**: HIV I (predominant worldwide), HIV II (slower progression).
- **Hallmark**: Progressive depletion in CD4 cell counts which predispose to opportunistic infections such as Pneumocystis Jiroveci and neoplasms such as NON Hodgkin lymphoma & Kaposi sarcoma
- **Structure**: RNA Lentivirus belongs to Retrovirus family with a lipid envelope with the envelope proteins external gp120, transmembrane gp41, nucleocapsid (gag) with P24 major core protein, and polymerases.
- **Transmission**: Sexual Heterosexual is the most common mode of transmission worldwide, Vertical transmission Blood and body fluid, IV Drug Users.
- **Life cycle**: Binding, Fusion, Penetration, Upcoating, Reverse transcription, Integration, Transcription, Translation, Assembly, Maturation.
- **Disease Course:** Viral Transmission → Acute HIV infection (occurs 2-4 weeks after transmission) **infectious mononucleosis** →Seroconversion (occurs 2-12 weeks after the development of symptoms) → Asymptomatic HIV infection (last 8-10 yrs) → symptomatic HIV infection (last 1-3 years) →AIDS **cell count < 200** (Average survival 1-2 years).
- **Diagnostic Tests**:Screening tests; (combo for P24 detection + Elisa for antibodies). Confirmatory tests; (the INNO-LIA™ HIV I/II Score is a Line Immuno Assay (LIA®) antibodies against HIV I,HIV II + western blot assays + PCR to confirm the undetermined and asses viral load).
- **Complications**: TB most common, Candidiasis, Toxoplasmosis, Kaposi Sarcoma, Lymphoma non Hodgkin's.
- **Mother to child transmission**: In-utero, 25-40%. Intrapartum, 60-75% most common. Breast feeding, Established infection 14%, Primary infection 29%. now with available treatment the risk has dropped to 1%.
- **Prevention and Counseling**.
- **Treatment**: according to current guidelines you treat any infection immediately and you don't wait until the count drops to 200.

Questions

1-Which one of the following cells are the target for HIV virus?

A. CD8 receptor containing cells.
B. CD3 receptor containing cells.
C. CD4 receptor containing cells.
D. CD6 receptor containing cells.

2-The most common opportunistic infection in HIV positive patients is:

A. Pneumonia
B. Cryptococcal meningitis
C. Toxoplasmosis
D. TB

3-The lipid envelope derived from HIV infected cell contains major external envelope proteins which are:

A. gp41
B. gp120
C. P24
D. gp20

4- A 42-year-old man presents to his GP complaining of deterioration in his vision in the right eye and the presence of floaters. The change in his vision has been causing him to suffer from headaches. He has been HIV positive for ten years. Fundoscopy reveals haemorrhages and exudates on the retina. What is the most likely diagnosis?

A. Retinal detachment
B. CMV retinitis
C. Kaposi's sarcoma
D. Optic atrophy

5- One of the following drugs reduces the risk of tuberculosis **ONLY** in HIV-infected patients with a positive tuberculin skin test(induration of 5 mm or more):

A. Co-trimoxazole.
B. Isoniazid preventive therapy (IPT).
C. Clarithromycin.
D. Protease inhibitors.

6- A 42-year-old man presents to his GP with ‘blotches’ over his legs. He has been HIV positive for ten years. On examination, there are multiple purple and brown papules over his legs and his gums. What is the most likely diagnosis?

A. Malignant melanoma
B. Squamous cell carcinoma
C. Basal cell carcinoma
D. Kaposi’s sarcoma
E. Toxoplasmosis

1- ANS: C, 2- ANS: D, 3- ANS: B, 4- ANS: B, 5- ANS: B, 6- ANS: D.