- READ ME: I wrote everything important + what the dovtor mentioned in the lecture. But you have to see the figures only because not everything is written here so please forgive me it was a heavy lecture + the record full of noises and ma saddaget a5alle9 .. Forgive me for any mistakes and GOOD LUCK ☺

Slide 2

- **Disease:** acquired immune deficiency syndrome (AIDS), the virus is HIV but the end stage of the disease is AIDS :P !
- Two attributes make AIDS unique among infectious diseases: it is uniformly fatal if untreated, and most of its devastating symptoms are **not due to the causative agent**

- **Transmission**: Sexual contact, Blood (who takes drugs like *Heroin),* Vertical

- **Sexual Contact:** Male to Male sexual partners are the highest risk group in developed countries. Also, male to female transmission is higher than female to male. Because for males, their skin barrier is only a single epithelial layer (simple epithelial in the Rectum) so it's much more likely to transmit the virus through it! In comparison with the Vagina which is stratified epithelial cells (more than one layer).
- **(Male to male > male to female > female to male),** why male - female carries a higher risk? Because the female is exposed to higher amount of secretions than the male (remember the virus is transmitted by FLUIDs)
- Injured skin (e.g. STD lesions) increases risk of transmission, especially un-protected sex with a partner having STD lesions in the genitalia.

- **Contaminated blood products** have mostly been eradicated as a cause of transmission. Don't forget to mention health care worker to/from transmission, you as a doctor may be exposed to contaminated needle or blood through surgery or sth but it is not the end of the world because there is PROPHILACTIC therapy, if exposed combined **Anti-retroviral** therapy has shown efficacy.

- **Vertical transmission:** may occur transplacentally, perinatally during the birth process, or postnatally through breast milk with varying percentages depending on the location of the study and if anti-viral therapy is given.
- Recommendation in developed world: if the child has not been infected during delivery or pregnancy, then the breast feeding is *contraindicated*, as long as there is an alternative food source (difficult in developing countries). The anti-retoviral therapy given to pregnant and breastfeeding mothers has reduced the probability for the infection to reach the baby (before this, 40% of children who were born to mothers with HIV were also infected; this has now been reduced to 5%).

Slide 3

- 1984-1985, virus identified as the causative agent and ELISA test produced which showed the extent of the epidemic
- It is of the **Lentiviral** family (means **slow** and **unremitting** disease), and a Retrovirus

- Highly cytopathic in human peripheral blood mononuclear cells (target cells), specifically killing CD4+ T Lymphocyte in cell Culture (that means the most affected T Helper but not the one's only affected). And you know that CD4+ T cells are required for both Humeral and Cellular Immunity. CD8+ T are also affected since they require CD4+ T cells to develop an immune reaction!

Subtypes:

1) HIV 1 ; more common / US, Europe , Central Africa
2) HIV 2 ; some western Africa

- HIV 1 and HIV2 share about 40% of their genome sequence, which indicates that there are two separate sources for these two types!
- Before HAART (highly active antiretroviral therapy): HIV-1 infection increases mortality 40x and HIV-2 infection 2-5x, also indicates two distinct origins.

- **HIV** is remarkably similar to the simian immunodeficiency virus **SIV;** Because of the close contact between humans and monkeys, which are hunted for food (raw) or kept as pets in West Africa, it is currently thought that HIV represents a zoonotic transmission of SIV from monkeys to humans

Slide 4

The dr read the slide but added these points:

1- It's an epidemic disease (worldwide)
2- Increases very quickly, in 2009, the estimates were about 33 million! It doubled in the last five years.
3- Most infected people in low and middle income countries, where most AIDS-related death occur (lack of medication and access to health care
4- Death among ages 15-59 years, due to sexual activity

We are in the midst of a Global epidemic (actually multiple local epidemics), and we may still be early in the epidemic to really see how many are infected (long term disease)

Molecular Biology

Slide 6

- Has a membrane, matrix, membrane proteins, a capsid, cone- shaped cylindrical core, two ss RNA genome
  - Because it is **ssRNA** (degraded when found in cells) it needs a **capsid**, for protection.
  - Also carry within it (capsid) its own **Reverse Transcriptase** to produce DNA.
  - Envelope Glycoprotein: **gp160** is processed into: gp120 (surface) and gp41 (transmembrane)

- **gp120** Highly variable envelope protein, multiple versions of this protein can be found, and even in the same infected person, multiple mutations may occur. The body will produce Ab against the virus, but the effect of the specific CD8 cells won't last long since the mutated viruses will have different surface proteins that won't be recognized or affected by the immune response and will begin to divide again (**natural selection**) and new recognition and Ab production will be required. This will be repeated several times, and this exhausts the immune system.

- **gp41** highly conserved induces a **non-neutralizing Ab response** why? Because it's inside the envelope, but we can use it for detection

- The virion buds from the surface of the infected cell and incorporates a variety of host proteins, including major histocompatibility complex class I and II antigens, into its lipid bilayer.
- Although of positive polarity, HIV genome is not infectious

Slide 7

*Typical retrovirus genomes contain three major genes; **gag-pol-env**. These are synthesized as polyproteins which produce proteins for: virion interior (called **Gag**, group specific antigen); the viral enzymes (**Pol,** polymerase) or the glycoproteins of the virion envelope (**Env**).* HIV was found to have multiple open reading frames in addition to those usual ones.

- Genes and gene products:

- **GAG**: The genomic region encoding the capsid proteins (group specific antigens). The precursor is the **p55** protein, which is processed to p17 (MAtrix), p24 (CApsid), p7 (NucleoCapsid), and p6 proteins (important for viral budding).
- **POL**: The genomic region encoding the viral enzymes protease, reverse transcriptase, and integrase. These enzymes are produced as a Gag-Pol precursor polyprotein **P160** (it's not like gp160, it is not glycosylated).
- **ENV** Viral glycoproteins produced as a precursor **gp160**, which is processed to give the external glycoprotein gp120 and the transmembrane glycoprotein gp41

LTR = Long terminal repeats, in part, are used to integrate viral genetic material into the host cell "عالاطراف"

Slide 8

Replication:

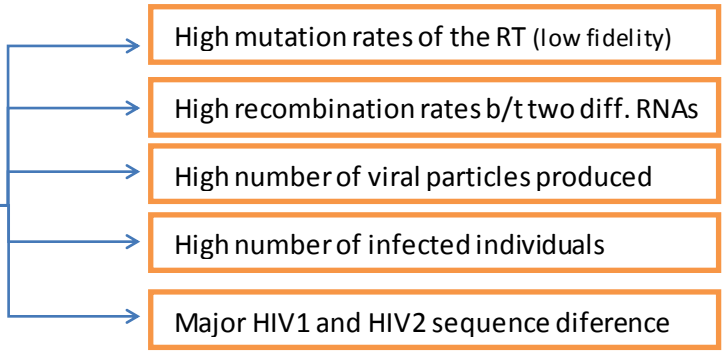
- gp120 contains the binding site for the CD4 receptor and binds to it
- Co-receptors CCR5 and CXCR4 (Chemokine Receptors on the WBC, belong to the family of seven-transmembrane-domain G protein–coupled cellular receptors) → along with gp41 induce fusion of the plasma membrane and the viral membrane
- The genomic material and the viral proteins get inside the cell, after that the Reverse transcriptase produces linear DNA which will be integrated into the cellular genome by integrase to be transcribed.
- mRNA produces large proteins which are modified post-tranlationally, this doesn't happen normally (normally, it produces mRNA and then the introns are spliced). Here, the virus produces splicing inhibitors which allow for the whole sequence which is formed to be exported out of the nucleus and gets translated into PolyProtein which then will be cut into viral proteins.
- Exit by budding

- HIV directly infects and kills cells that are critical for effective immune responses
- The CD4 molecule is found **predominantly on T helper cells**. It is **also expressed on the surface** of monocytes/macrophages and dendritic/Langerhans cells.
- **Major targets of anti-HIV treatments are**: Reverse transcriptase, Integrase, and p10 Protease

Slide 9

- HIV and treatment escape:

- **Extensive Glycosylation (gp120)**
- **Extensive genetic heterogeneity**



- Recombination; due to the presence of two ssRNA.
- The same target cell may be infected from more than one virus.
- **Extensive glycosylation** means the gp120 will be covered by the sugar so can't be recognized!
- AIterations of antibody production & viral adaptation. While some of the antibodies produced may be able to neutralize the virus (if they can see the protein from all the glycosylation) this induces evolution of the virus.

Slide 10

- **Activated infected CD4 cells** are the main producers of the viruses in your blood, but typically we don't have many activated CD4 cells in the blood, although we have many latently infected CD4 cells. The active cells die in 1-2 days which is the generation time for a new viral particle. The resting cells have a $t_{1/2}$ of 5-6 months; especially the latently infected cells where the virus is integrated into the genome but doesn't produce proteins or progeny (transcriptionally silent) and is therefore undetectable and un-affected by treatments (escape treatment and immune recognition). This occurs whether or not persons are started on treatment in the initial symptomatic stage of infection.

Slide 11

- **Mucosal transmission**

- Exposure to the virus → If there is a damaged mucosal barrier: **direct access**. If there is no damage, the virus can also infect **dendritic cells** and gain access to the inside through them.
- Once the virus is inside, it will infect both activated and resting cells. Typically, there are not many activated T CD4 cells, so not a lot of the viral progeny are produced initially, but then ***the virus activates the immune response***, which activates the resting CD4 cells so they start producing more viruses as well. ***The virus also affects the mucosal barrier and damages it***, so more microbial organisms enter and induce the recruitment of more CD4 cells, and hence there are more reservoirs for the virus to replicate.

- Eventually, Abs and CD8 cells will be produced during the acute stage, but since there is excessive viremia now and abnormal regulation of CD8 cells, the CD8 cells can't keep up with the infected cells. The CD8 cells are not allowed to become mature memory cells (no proper CD4 cells stimulation) so they constantly replicate and this exhausts them, so they start to produce a death protein called **programmed death (PD) 1 molecule**, and you end up with a sustained HIV infection.
- CD8 T cells recognize viral infected cells through the MHC I proteins, ***the virus actually down-regulates MHC I production***, so they are not able to recognize infected cells as well.

Slide 12

- The virus starts its viremia by establishing the infection in the ***gut-associated lymphoid tissue*** (GALT), since it is rich in CD4 cells, once they are killed by both direct viral effects and by activation-associated apoptosis, the mucosal membranes are no longer protected, the immune system is activated, more microbes enter the immune system and activate it further (microbial translocation) → massive viremia + dissemination, establishes a state of chronic immune activation. The loss of CD4 cells depletes many of the CD4 memory cells.

- Eventually the immune system will not be able to keep up any longer with the massive amounts of viremia, eventually CD8 cells will drop and opportunistic infections begin to develop.

Slide 13,14

Other genes:

Essential Genes: no viral progeny produced without them

- **TAT**: Transcription activator, bind to transactive region in the LDR, so LDRs are not only important for integration but also for the initiation of transcription.

- **REV**: Regulator of viral gene expression, inhibits splicing, allows long mRNA strands formation which will be translated to large proteins. HIV as we have seen produces several intronic containing proteins to be processed post translation. Rev binds and allows nuclear export of these sequences despite the usual cellular machinery requirements

Accessory Genes: the virus can live without them, may increase virulence

- **VPR** – Viral Protein R: inhibits replication, we want the cell to be completely devoted to viral proteins production. Also associated with HIV infection in macrophages

- **NEF** – Negative Effecter: down regulates MHC I expression. resulting in the lack of ability of the CD8+ CTL to recognize and kill the infected target cell.

- **VPU** – Viral Protein U: CD4 degradation. *Specific to HIV1*; this is the major difference between them. (HIV 2 has a VPX gene not present in HIV1, VPX and VPR in HIV-2 share the function of vpr here.)

- **VIF** – Viral infectivity factor: overcomes inhibitory effect of APOBEC (a cytidine deaminase that results in G to A mutations in the viral geome). VIF prevents APOBEC from being incorporated into the virion particle normally to prevent this hypermutation. It does this by inducing it's destruction by the ubiquitin proteasomal pathway

Clinical Syndrome

Slide 16

- The course of the HIV infection can be divided into 3 stages:
    - **Acute/ Primary** - resembles MN, very not specific
    - **Chronic** – asymptomatic; clinical latency (Clinical latency not to be confused with viral latency, here there is continuing viral replication and clearance until replication outpaces the immune system)
    - **Advanced disease** – AIDS

- AIDS constellation of clinical illnesses, because proper cellular immunity is lost! Primarily **Opportunistic Infections** and **Malignancies**.
- This is all caused by the progressive loss of the CD4 T cells.

Slide 17

This is the typical course of an **UNTRAETED** HIV patient:

Initially: **Acute,** mononucleosis-like symptoms:

- Lasts for a few weeks
- Massive Viremia
- Massive drop in CD4 cells
- Abs produced and Cd4 Cd8 specific cells produced.
- A lot of the virus-producing cells destroyed, but this doesn't last and the infection is not controlled.
- Best indicator of future disease progression is viral load after acute symptoms abate, but viral load at any time point is an important determinant of disease state.
- Mutations occur, new viruses, immune system can't keep up and is exhausted, CD4 cells drop.
- When CD4 cell numbers drop below 200 mark → opportunistic infections start to develop, eventually death occurs.

- This period including the **latency** can last anywhere between **6 months – 25 years**, depends on the type of virus, the effectiveness of the immune response, and the level of viremia at the acute infection: it is a prognostic factor of how long the latency will be.
- **Viral load** throughout the lifetime is a major prognostic factor for any point of the disease. The higher, the worse the prognosis.
- The duration of each stage is highly variable and can be altered by antiretroviral therapy.

Slide 18

Acute HIV syndrome:

- IP: few days – 3 months
- Non-specific symptoms: fever, pharyngitis, headache, arthralgia, myalgia, malaise
- Non puritic maculopapular rash
- Generalized Lymphadenopathy
- Musculocutaneous ulceration and weight loss help distinguish primary HIV from other viruses, this only raises a red flag if the patient is in a high risk group (IV drug users, homosexuals, promiscuous) and must be screened for HIV.
- This lasts for 2-3 weeks and results in clinical recovery.
- Seroconversion occurs in 1-10 weeks, even up to 3 months. The person is super infective in the period between the point of infection and massive viremia (occurrence of symptoms), and if you are still using the 3rd generation methods of screening which only detect HIV Abs, you will get a false negative result, because the patient hasn't produced Abs, this is called the Window Period.
- There is a new method of screening which is called the 4th generation where the viral particle itself is detected.

From the slides**:**

- **Aseptic meningoencephalitis** is the most common neurologic manifestation of primary HIV-1 infection
- Persistence of symptoms beyond 8 to 12 weeks, along with a severely depressed CD4+ T-lymphocyte count and high plasma HIV-1 RNA levels may predict more rapid progression of disease
- Laboratory characteristics of primary HIV-1 infection include **lymphopenia** and a decrease in the absolute CD4+ T-lymphocyte count, usually accompanied by an increase in circulating activated CD8+ T cells

Slide 19

  - If this patient progresses to the **symptomatic HIV infection** (clinical latency period is over, CD4 count decreased), there is a whole constellation of reasons why the patient gets the diseases:
- HIV replication, lots of regulatory cells, toxicity from AntiViral treatment: dystrophy/dylipidemia/hyper coagulation, opportunistic infection, microbial translocation, further increase in inflammation, activation of monocytes and t cells.
  - Affect all the organ systems!

- From the slides: *Markers of Progression to AIDS*

1) Increased viral load 2) P24 antigenemia 3) Decline of anti P24 antibodies 4) CD4+ T cell count below 200 cells/ul

Slide 20

Originally, they were called AIDS defining conditions. Those are no longer the main killers of HIV infected individuals in developed countries. Fewer than 50% Death among AIDS patients receiving HAART are a direct result of an AIDS-defining illness.

A growing number of end-organ complications not traditionally considered "AIDS-defining" events have been recognized to occur more frequently in HIV-1–infected patients. These include an increased risk of cardiovascular disease, non-AIDS defining malignancies, renal and hepatic disease, and other changes typically associated with increasing age, suggesting that HIV-1 infection may **accelerate the aging process**.

Slide 21+ 22 – very important

Diagnosis:

- **ELISA** enzyme-linked immunosorbent assay: You can either have a capture an Ab or a viral Ag on the solid support, and then you can detect the Ab if you have the Ag (and the opposite). These are combined in one small well, where HIV1 and 2 protein antigens as well as p24 capture Ab are stuck on the bottom of the well. Add the patient serum (contain Ab and viral proteins) add detection: either a viral protein with a detection method (fluorescent…etc) or an Ab against p24 that has a detection method (something that changes in color or light).

- This is the 4th generation method previously mentioned and reduces the window period to a week because of the added p24.
- Some centers still use the 3rd generation, if you do you must be careful and not forget the window period. NAT could be useful at these cases:
  - **NAT** Nucleic acid testing: There are very few circumstances when this is justified e.g. diagnosis of HIV infection **in babies** born to HIV-infected mothers (babies have maternal antibodies but not necessarily virus)

- Sensitivity: used for screening, you want to catch as many cases as possible.
- Specificity: catching the disease and only the diseases.

Example: in a population of a million and 10 people are HIV infected, if a test catches all those cases, it is a highly positive cases, if you found another 5 people with those test that are false-positives, it is still a highly sensitive test but it is not a very specific test

- **ELISA**: highly sensitive, some false-positives could be found (not very specific), therefore we confirm by using **Western Blotting** which is not very sensitive but it is highly specific (ready-made strips with all the viral proteins on a gel and transferred onto a nitrocellulose membrane. Pt serum added, if they stick → +ve, you can only say the test is –ve if you get nothing.)

From the slide:

- Tests are divided into screening and confirmatory assays, Screening assays should be as sensitive as possible whereas confirmatory assays should be as specific as possible, Virus is readily detectable in peripheral blood and lymph nodes throughout the course of infection
- There are different criteria for the interpretation of HIV Western blot results e.g. CDC, WHO, American Red Cross.
- The most important antibodies are those against the envelope glycoproteins gp120, gp160, and gp41
- p24 antibody is usually present but may be absent in the later stages of HIV infection

Slide 23

Treatment:

- 6 categories of drugs:
    - CCR5 antagonist
    - Fusion inhibitor
    - Nucleoside RT inhibitor
    - Nonnucleoside RTi's
    - Integrase Inhibitor
    - Protease Inhibitor

- The drugs mentioned are only examples (i.e. non-exhaustive list). More than 30 approved drugs are now available.
- Commonly used in combination. The introduction of combination therapy in the mid-1990s in the developed world resulted in the decrease of the AIDS-related mortality by more than 80%.
- Remember that Most people with HIV/AIDS (95%) reside in low and middle income countries, and these are the countries least equipped to respond.

Slide 24

Prevention:

- Education - the most important one
- Lifestyle changes (applicable to western societies, use of condoms decrease infection rate massively)
- Prophylactic treatment (vertical transmission, exposed health care workers)
- Blood product screening – no longer a problem
- Vaccine – under trials