Human Immunodeficiency Virus (HIV): A comprehensive review

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Introduction

With the invention of new anti-retroviral medications, regular screening tests and public awareness, new cases of HIV infections are declining. This gives a hope to HIV positive patients to experience a normal and healthy lifespan regardless of their HIV status.

There are two type of HIV virus, HIV-1 which is high virulence and HIV-2 which is less virulent compared to HIV-1. HIV-1 is spread throughout the world, while HIV-2 is mainly limited to West Africa.

Both, like all other Lentiviruses, cause chronic illnesses. The incubation period therefore, is between 6 months to 10 years. During the incubation period, the individual may look healthy despite being capable of passing virus to others. After the incubation period, the virus takes over the immune system and leads to the final stage of the disease which is called AIDS (Acquired Immunodeficiency Syndrome). In this stage, since there is no effective immune system, the body is susceptible to infections, which in most cases can be limited by the immune system in a healthy person. One of the most common opportunistic infections in AIDS is oral candidiasis. Weight loss ubiquitous with candidiasis highly suggests a decreased number of cells that express CD4, the primary cellular receptor for HIV entry. This phenomenon can be due to AIDS. Tuberculosis and cryptosporidium-associated illnesses are also important opportunistic infections in AIDS (13). Meningitis, encephalitis, chronic parasitical diarrhea, and cancers such as Kaposi's sarcoma and non-Hodgkin lymphoma are also some other AIDS related opportunistic illnesses (14).

Transmission

HIV virus can be transmitted through exchange of bodily fluids (blood, semen, vaginal discharge, milk and pre-ejaculate fluid). Due to the long incubation period of HIV virus, individuals may be HIV positive without knowing. According to the CDC, 12.8 % of individuals are HIV positive and do not know about their infection (9). Unprotected sex with an HIV positive individual is the most common cause of HIV transmission in the United States. In 2009, 57% of the people who were tested HIV positive were men who had sex with men (MSM). The term MSM refers to all men, regardless of sexual orientation, who have sex with men (10).

Transmission of the virus through an oral-genital route is extremely low, with only one confirmed case of oral-genital transmission reported (7). There are reports of possible HIV transmission through the oral route when the oral mucosa was compromised due to chemotherapy, dental procedures, allergy and pharyngitis (8).

Mother-to-child transmission of HIV during pregnancy, delivery, labor or breastfeeding without medical or supportive intervention varies between 15% to 45%. This rate may be as low as 5% with proper medical intervention. Antiretroviral therapy (ART) for both mother and child, and medical support during pregnancy to prevent the baby from acquiring HIV are possible interventions (11).

Risk groups

MSM still have the highest rate of infection among all groups in 2010. It also shows a 12% increase compared to MSM incidence rate (new infections) in 2008. Although MSM only make up 4% of the male population in the US, 78% of new HIV infection was reported among them (9). Since the anal mucosal surface can easily be damaged during anal intercourse, HIV-infected semen may get in contact with the

injured tissue and virus can easily enter the receptive partner's body. With regards to this fact, the receptive partner in anal intercourse has 4 to 14 times higher chance of getting infected, although the insertive partner is also at risk (10).

Vaginal intercourse is the second most common transmission mode of HIV in the United States, although, it is the most common route of infection worldwide. Male-to-female transmission rate is significantly higher than female-to-male transmission during vaginal intercourse (10).

Incidence rate of about 50,000 of cases per year has remained stable during the past few years in the US (9). In spite of this constant rate, some groups are showing higher incidence rate compared to others. For example, African American males show excessively higher incidence rate. African Americans constitute only 12% of the US population, but include 44% of the new cases of HIV infection (Figure 1) (9).

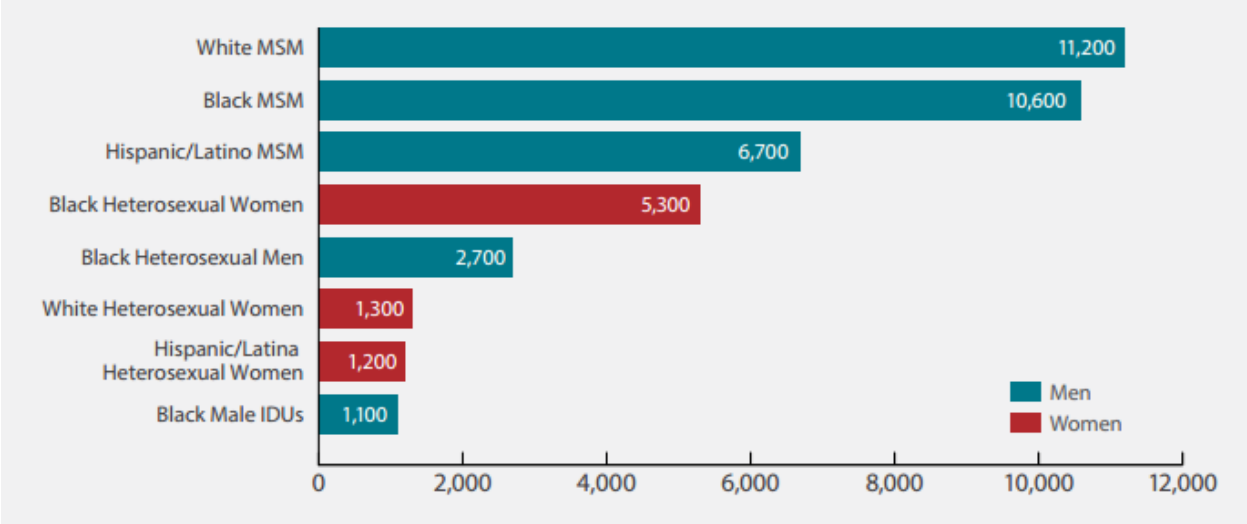

Figure 1: New HIV cases based on the race/ethnicity in the US in 2010

In Figure 2, Miami has the highest rate of new HIV infections, followed by New Orleans. Also Washington DC is the 5th, Atlanta 8th, New York 9th, Orlando 11th, Los Angeles 19th and San Francisco 21th cities in the US with the highest rate of new HIV cases in 2013 (12).

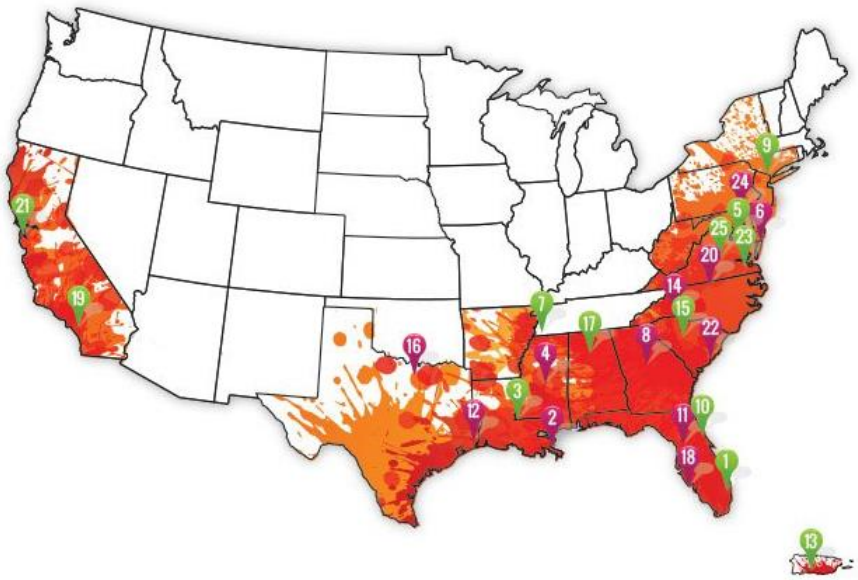

Figure 2: Map of new HIV cases in 2013, CDC.

HIV tropism

HIV tropism is the tendency of the virus to infect a certain type of cell which is determined in patients by a blood test called the Trofile Assay. HIV needs two main tools to enter the cell and consequently infect it. These tools include the CD4 receptor and chemokine co-receptors. Chemokine co-receptors that HIV uses to enter the cell are mainly either CCR5 or CXCR4. CCR5 is a surface protein receptor on macrophages and some types of T cells (15). CXCR4 is an alpha chemokine receptor which is expressed on the surface of T cells (16).

Based on the viral utilization of CCR5 or CXCR4 co-receptors, HIV tropism is categorized. Some HIV strains use the CCR5 co-receptor to attach to the host cell. These are called R5 strains (18). Some others that use CXCR4 are called X4 (16), and the ones that use both are X4R5 or dual/mixed (D/M) (17).

There are other co-receptors that some strains of HIV recruit to enter the host cell such as CCR3, which is expressed on the eosinophil and microglial cells (19). It's important to note that, although HIV tropism is categorized as above, not all R5 strains can cause infection by using CCR5 co-receptors on macrophages (17).

HIV structure

HIV structure is relatively simple, being composed of two identical copies of positive single stranded RNA, composed of 9749 nucleotides, a capsid and a lipid bilayer envelope. Inside the core along with the single stranded RNA, there are three enzymes, which include Integrase(IN), Reverse Transcriptase(RT), and Protease (PR). There are several proteins that are important in HIV virion maturation, including p7, p17, and p24. Nucleocapsid protein p7 binds to the single stranded RNA, while matrix protein p17 is responsible for the virion particle integrity. Between 1500 and 3000 copies of p24 capsid protein compose the viral capsid (1). However, most of the viral envelope proteins are derived from the host cell. The virus itself encodes one envelope protein, gp160, which after translation will be cleaved to a

transmembrane glycoprotein gp41, and an extracellular glycoprotein gp120. gp120 and gp41 are crucial for attachment and fusion of the virus to the host cell (Figure 3).

Virus infects three types of immune system cells: $CD4^+$ T helper cell, macrophages and microglial cells. gp120, along with a CD4 molecule and co-receptors are the initial entry complex into the host cell. Co-receptors used in this complex vary in different host cells. CCR5 co-receptor exists on the surface of macrophages, while CXCR4 co-receptor exists on the surface of T lymphocytes. Co-receptors are absolutely necessary for virus entry to the host cell (2).

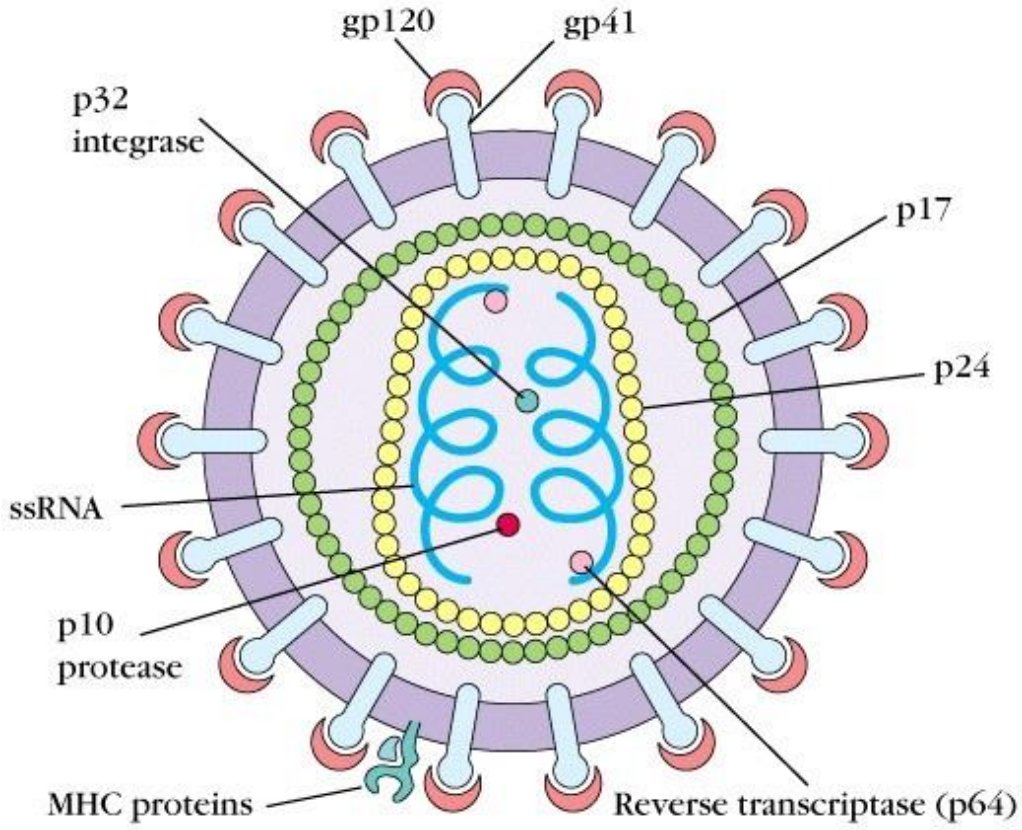

Figure 3: General structure of HIV particle (20)

HIV pathophysiology

In order to better understand how HIV infects patients, knowing the mechanisms of HIV replication is necessary.

HIV replication

The replication of HIV consists of few different steps, including attachment, fusion, viral genome replication, and assembly.

Attachment

To establish the infection, HIV needs to attach to a host cell, which can include $CD4^+$ T helper cells, macrophages or microglial cells. In order to attach, HIV needs to recruit certain receptors on the cell surface and viral envelope glycoproteins which make up the envelope glycoprotein (Env) complex. The

Env complex is composed of three gp120 subunits, three gp41 subunits, a CD4 receptor and co-receptors CCR5 and/or CXCR4 (24).

Initial formation of the complex occurs by interaction between CD4 antigen receptor on the surface of the host cell and gp120. Binding gp120 and $CD4^+$ leads to the conformational change in gp120. To finish the process of attachment, co-receptors (CCR-5 or CXCR-4) should be bound to the complex as well. Attachment of co-receptor makes further conformational change in gp120. This conformational change will lead to exposure of gp41 to interaction with the host cell surface. (23)

Fusion

This step is mediated by gp41, a glycoprotein which is composed of two domains, HR2 and HR1. HR2, the hydrophobic domain of gp41, inserts itself into the cell membrane. This causes gp41 to fold back on itself and consequently, the whole complex is pulled toward the host cell membrane. Then HR2 facilitates merging of the bilayer phospholipid, which is called gp41 zipping. (35)

Cell entry

After integration of the virus envelope and host cell membrane, the viral nucleocapsid is released to the host cell cytoplasm along with protease, reverse transcriptase and integrase.

Viral genome replication

In viral genome replication, Reverse Transcriptase(RT), Integrase (IN) and Protease (PR) are the main contributors. RT promotes the reverse transcription of viral RNA. It is composed of 2 domains, which are ribonuclease H active site and polymerase active site. First, polymerase active site transcribes a single stranded viral RNA into an RNA-DNA double helix. Then, ribonuclease H breaks the RNA-DNA double helix to allow the polymerase active site to make the complementary DNA strand by the use of the viral ssDNA. This results in a double stranded DNA helix. The newly synthesized viral dsDNA is now ready to be integrated into the host cell genome (20).

Integrase first removes a dinucleotide from each end of the produced DNA double helix and makes two sticky ends. Then both integrase and the sticky DNA are transported to the nucleus where the viral DNA is now ready to be integrated to the host (21).

Upon activation and transcription of the host cell genome, viral mRNA and viral genomic RNA are transcribed from viral DNA. After completion of this process, all transcribed genetic material is transferred to the cytoplasm. Once in the cytoplasm, viral proteins are made and some of them are post-translationally modified via viral protease. Protease activity is essential for viral maturation. Proteases cleave more than 12 viral proteins. Several of the viral protease products become viral core proteins that make the viral capsid (22).

Assembly

Two viral RNA strands, replication enzymes and core proteins make an immature viral particle that buds out of the host cell. Viral envelope around the virion is formed from the host cell membrane when it buds out of the host cell. Viral Gag polyprotein is essential for viral assembly since the expression of Gag begins the formation of viral envelope (Figure 4) (24).

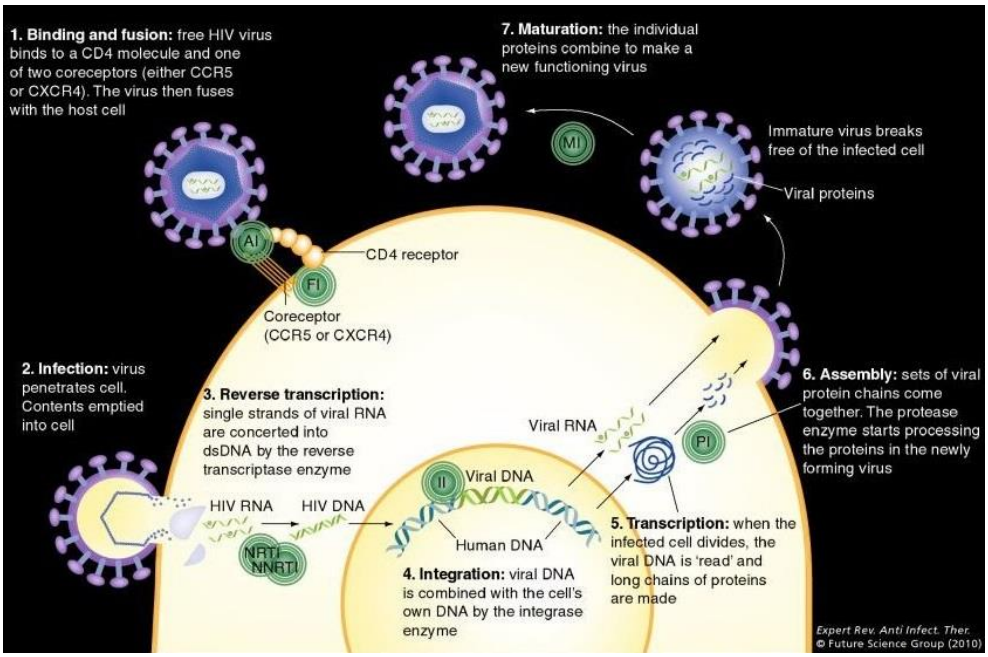

Figure 4: General pathogenesis of HIV (3)

HIV Diagnostics

HIV can be diagnosed by HIV antibody, antigen or nucleic acid tests. HIV antibody test was the first way of diagnosing HIV-1 and HIV-2. Several testing protocols were released by the CDC between 1989 to 2004. In 2013, a more recent guideline of HIV diagnostic tests was released by the CDC (5).

In the new guideline, the presence of HIV-1 and HIV-2 antibodies along with HIV-1 p24 antigen is evaluated in the specimen. If any sample is reactive in the initial test, a supplemental test will be performed. Supplemental testing is composed of an immunoassay that can distinguish HIV-1 antibodies from HIV-2 antibodies. If the result comes back indeterminate or nonreactive, the nucleic acid test is performed to determine whether or not the individual is actually HIV positive (5).

If the nucleic acid test is positive, the patient should seek antiretroviral therapy (ART) as soon as possible to help the $CD4^+$ T lymphocyte cell count remain and/or return to an acceptable level. Once the patient starts ART, the CD count test should be performed in order to measure and monitor the number of CD4 T lymphocytes (CD4 cells) in the blood. If the ART is successful, the viral load will be dropped to undetectable levels, which is defined as 40 to 75 viral particles per 1 cubic millimeter of blood (6).

HIV treatment

Currently there are no cures available for HIV, however there is one documented cure from HIV. Timothy Brown, cured from HIV when he was injected the stem cell from a donor who lacked CCR5 co-receptor. This treatment was done to save his life from Leukemia.

In less than 1% of human population, a mutation can cause complete deletion of CCR5 gene. This mutation is called CCR5-Δ32 and removes the 32 base pairs that encode CCR5 protein from the p arm (short arm) of position 21 on human chromosome 3 (42) (43).

Treatment with anti-retroviral medications can greatly affect the viral load, and maintain an acceptable $CD4^+$ count in HIV positive patients. Anti-retroviral medications mainly target HIV replication. Each step of HIV pathogenesis can be blocked or interfered causing failure of the virus to replicate. For instance, fusion of HIV to the host cell can be blocked by gp120 antagonists. Therefore, the Env complex cannot continue with its conformational change and gp41 will not be able to unfold, inhibiting it from being exposed to the host cell. Enfuvirtide is a fusion inhibitor that blocks gp41 (25, 26). Enfuvirtide can only limit the fusion of HIV-1 and restriction of HIV-2 by Enfuvirtide is not significant (27).

Action of protease is needed for HIV particle maturation. If processing of viral proteins is blocked, viral particles cannot mature and therefore the action of HIV will be compromised. Indinavir, Nelfinavir, Ritonavir and Saquinavir are four protease inhibitors (PIs) that block the cleavage of gag and gag-pol proteins. Consequently, this also inhibits maturation of the virion. Immature virion has no infectivity characteristic (28).

Furthermore, primary and/or secondary mutation can happen in the gene that encodes viral protease. Primary mutation usually happens in the substrate-binding cleft of viral protease. Due to the structural similarity of PIs, resistance against one PI can affect the function of other PIs as well. Neither primary, nor secondary mutations can seriously affect the function of PIs by themselves. However, if both mutations happen, significant resistance against PIs is seen (32).

Nucleoside reverse transcriptase inhibitors (NRTIs) or non-nucleoside reverse transcriptase inhibitors (NNRTIs) are being used along with other anti-retroviral medication to decrease the activity of HIV, and are a standard part of ART regimen.

NRTIs are modified nucleotides that are integrated by reverse transcriptase to make the DNA double helix based on the viral RNA genome. Due to the faulty nature of these nucleotides, the resulting DNA product is also faulty and therefore cannot be integrated into the host DNA. Emtricitabine (Emtriva ®), Lamivudine (Epivir®), and Zidovudine (Retrovi®) are examples of NRTIs (29).

NNRTIs directly block the action of reverse transcriptase, so RT will not be able to make any dsDNA from viral RNA. A few examples of NNRTIs are Rilpivirine (Edurant®), Etravirine (Intelence®), and Doravirine (30). Viral mutation is common against NNRTIs and it mostly occurs in amino acid sequences forming the NNRTI binding site. A single mutation of reverse transcriptase gene can cause a high level of resistance to multiple NNRTIs. In the case of known mutation, this group of anti-retroviral medications should be cautiously administered (31).

Raltegravir (Isentress®), Dolutegravir (Tivicav®), and Elitegravir (Vitekta®) prevent the action of HIV by blocking the viral enzyme integrase. These medications hold great hope for the patients whose HIV strain has developed resistance against reverse transcriptase inhibitors and protease inhibitors (33). Due to the low genetic barrier of integrase inhibitors against viral resistance, primary and secondary HIV mutation is inevitable and can greatly decrease the effectiveness of Elvitegravir and Raltegravir (34).

HIV triple therapy

Due to the susceptibility of HIV to mutation and developing resistance against anti-retroviral medications, successful treatment is characterized by minimization of the possibility of mutation. Newer anti-retroviral medications are designed to have combinations of at least three different medications with different mechanisms of action so that it lowers the possibility of emergence of resistance. For

instance, Triumeq, a new highly recommended anti-retroviral medication, is composed of three medications, Dolutegravir (integrase inhibitor), Abacavir (NRTI) and Lamivudine (NNRTI) (36) (37)(38).

Pre-Exposure Prophylaxis (PrEP) and Post-Exposure Prophylaxis (PEP) medication

In order to decrease the chance of getting HIV, Pre-Exposure Prophylactic medication is now available for individuals who have high risk behavior that may lead to HIV infection. The current medication is Tenofovir/Emtricitabine, which is available under the name of Truvada® and being manufactured by Gilead pharmaceutical company. Despite being pricey, most insurance companies cover a considerable price of the medication. According to the CDC, Truvada, if taken consistently, can lower the risk of HIV infection by up to 92% in high risk HIV negative individuals. These individuals should perform full blood tests, including renal function tests, to ensure side effects of the medication are not too extreme (39).

Unfortunately, there is one case of HIV contraction that is documented in a 43-year old Canadian MSM who was taking Truvada on a regular daily basis. Although this is a very rare case, multi drug resistance can cause failure of PrEP regimen. In this case, the HIV strain was resistant to both Tenofovir and Emtricitabine (40).

PEP regimen, which is composed of two different medications, Truvada and Isentress, is prescribed for people who have been exposed to HIV due to their job or sexual contact for 28 days. In the case of known exposure to HIV, the individual should seek immediate medical assistance. The sooner PEP regimen is taken, the more effective it can be and therefore the chances of HIV contraction will be lower. PEP should be taken no longer than 72 hours post exposure (41).

HIV vaccination

According to the National Institute of Allergy and Infectious Disease, NIAID, there is no HIV vaccine available at this point. Since HIV pathogenesis is complicated for the immune system, and also due to capability of the virus for mutation, developing a safe and effective vaccine for HIV hasn't yet been successful (44).

HIV and other STDs

Infection with Gonorrhea, Chlamydia and certain strains of Human Papilloma Virus (HPV) can increase the risk of HIV infection (45). It was shown in a cohort study on women in Africa that gonorrhea can cause a 7-fold increase in HIV contraction. Also, there was 7.9% increase in the HIV incidence rate in individuals with Herpes Simplex Virus 2 (HSV-2) infections (46). Furthermore, in another study on MSM in the US in 2010, high chances of HIV incidence with anal re-infection with chlamydia or gonorrhea was documented. This study showed 8-fold increase in HIV contraction in men with anal re-infection due to chlamydia or gonorrhea (47). Moreover, there was 4-fold increase of HIV incidence in men who were infected with high-risk strains of HPV (48).

HIV host cells are $CD4^+$ cells. Therefore, if an HIV positive individual gets infected with another STD such as gonorrhea, chlamydia or HSV, due to the recall for more CD4 cells to the damaged genital tissue, there will be more contracted $CD4^+$ cells which can be passed to the HIV negative partner and consequently, higher chance of HIV contraction (49). The higher chance of HIV contraction can be because of tissue damage associated with chlamydia, gonorrhea, and HSV. It also can be due to the

recruitment of CD4 cells as part of the inflammatory response to the damaged tissue in the genitals or the rectum (50).

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