T.R.N.C.

NEAR EAST UNIVERSITY INSTITUTE OF HEALTH SCIENCES

CORRELATION BETWEEN CD4 C868T POLYMORPHISM AND THE SUSCEPTIBILITY TO HIV IN DRC POPULATION

DORCY MANYINGU MUSANGILAYI

MEDICAL MICROBIOLOGY AND CLINICAL MICROBIOLOGY PROGRAMME MASTER OF SCIENCE THESIS

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SUPERVISOR; Assist Prof.DR. UMUT GAZI

NICOSIA

2018

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ABSTRACT

DORCY, M. Correlation between CD4 C868T polymorphism and the susceptibility to HIV in Democratic Republic of Congo Population. Near East University, Graduate School of Health Sciences, M. sc. Thesis in Medical Microbiology and Clinical Microbiology Programme, Nicosia, 2018.

CD4 gene which expresses the main receptor for the initiation of HIV infection, is associated with a number of synonymous polymorphisms. Among the identified polymorphic molecules, CD4 C868T possesses the same sequence as the normal CD4 gene except for a cytosine-to-thymidine substitution at nucleotide position 868. Up to today, the possible correlation between *CD4 C868T* polymorphism and HIV-1 susceptibility has only been investigated in Kenyan and Chinese populations, and these studies have yielded conflicting results. Our study is the first study to investigate the correlation in the Congo population and aims to contribute to the clarification of conflicting findings.

Forty-five HIV patients and 29 HIV-patient control groups from the Bandundu Hospital of the Democratic Republic of the Congo were included in this study. The collected blood samples were dried on a whattman-labeled filter paper and sent to BM Laboratories in Ankara for molecular analysis (since the HIV + samples were prohibited from entering the TRNC). By DNA sequencing, samples expressing the *CD4 C868T* gene were identified, which is then followed by statistical analysis to assess the effect of the polymorphism on HIV susceptibility.

Our study did not reveal any significant difference in CT and TT genotype as well as T allele distributions of *CD4 C868T* polymorphism between patient and control groups. In addition, no correlation was observed in the recessive and dominant genetic model analyzes. Our study found no association between *CD4 C868T* polymorphism and HIV-1 susceptibility in the Congo population. Further work is needed to confirm these results.

ÖZET

DORCY, M. CD4 C868T polimorfizmi ile Demokratik Kongo Cumhuriyeti'nde HIV duyarlılığı arasındaki korelasyon. Yakın Doğu Üniversitesi, Sağlık Bilimleri Enstitüsü, M.sc. Tıbbi Mikrobiyoloji ve Klinik Mikrobiyoloji Programında Tez, Lefkoşa, 2018.

HIV

virüsütarafındankonakhücreyepenetrasyoniçinkullanılanreseptörlerarasındaenönemli CD4'ü ekspreseeden polimorfikbirgendir. sisayılan gen, TanımlananpolimorfikmoleküllerdenCD4 C868T normal CD4 molekülündensadece 868 nükleotidpozisyonundafarklılıkgösterirvesitosininyerinetimidiniekspreseeder. BugünekadarCD4 *C868T*polimorfizmiile HIV-1 duyarlılığıarasındakiolasıkorelasyon, sadece Kenya veCinpopulasyonlarındaaraştırılmışvebuçalışmalarbirbiriileçelişkilisonuçlarvermişler dir. ÇalışmamızKongopopulasyonundasözkonusukorelasyonuaraştıran ilk çalışmaolup, çelişkilibulgularınaydınlatılmasınakatkıdabulunmayıhedeflemektedir.

KongoDemokratikCumhuriyeti Bandundu Hastanesi'nden 45 HIV+ hastasıve 29 HIV- hasta kontrolgrubuçalışmamızadahiledilmiştir. Toplanılankannumuneleri, whattman etiketlifiltrekağıdındakurutulmuşvemoleküleranaliziçin (sözkonusu HIV+ numunelerinKKTC'yegirişiyasakolduğundan) Ankara'dabulunan BM Laboratuvarları'nagönderilmiştir. DNA dizianaliziile*CD4 C868T*geniniekspresedennumunelertespitedilmişvebunutakibenyapılanistatikselanali zilesözkonusupolimorfizmin HIV duyarlılığınaolanetkisideğerlendirilmiştir.

Yaptığımızçalışmada, CD4*C868T*polimorfizminin CT TT ve Т genotipfrekansınınyanında alleldağılımlarında, hasta vekontrolgruplarıarasındaanlamlıfarktespitedilememiştir. Ekolarakyapılanresesifve dominant genetik model analizlerinde de herhangibirkorelasyongözlemlenememiştir. *C868T*polimorfizmiile HIV-1 Calışmamız, Kongonüfusunda*CD*4 duyarlılığıarasındabirilişkininolmadığınıtespitetmiştir. Bu sonuçlarıteyitetmekiçindahaileriçalışmalaragerekduyulmaktadır.

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1. INTRODUCTION

The human immunodeficiency virus (HIV) and acquired immunodeficiency syndrome (AIDS) stays one of the world's most critical challenge for public health especially in low- and middle-income countries. Fortunately, due to late advances in access to antiretroviral therapy (ART), infected people can now live healthier and longer. It has been recently stated that, antiretroviral therapy stops forward transmission of HIV (WHO, 2017). The World Health Organization (WHO) has reported an overall figure of 36.7 million people living with HIV and a scope of 1.8 million individuals became recently infected in 2016 (WHO,2017). Universally, 54% of adults and 43% of youngsters living with HIV are currently under the treatment procedure which is a lifelong process. The African region has the most elevated number of infected people in the world which is estimated to be 25.6 million people (WHO, 2017). Presently, just 70% of people living with HIV are aware of their status, while the rest or 7.5 million individuals need to undergo HIV testing (WHO, 2017).

The Democratic Republic of Congo (DRC), through its Ministry of Public Health and the AIDS program, has established a strategy to reach more than 200,000 people to initiate antiretroviral treatment by June 2018. An intensive HIV testing campaign has reached the coverage of 2 million individuals who were informed on their serological status which also allowed people to initiate HIV treatment. Scope of antiretroviral treatment remains at only 33% of individuals living with HIV since 2015, yet much lower than the scope in eastern and southern Africa which is 54%. As indicated by government estimations, by April 2017, around 34.000 more individuals were on treatment. The expansion puts the nation on track to achieve the June 2018 target, which would see 73% of individuals living with HIV on treatment (UNAIDS, 2017)

However, single nucleotide polymorphisms (SNPs) are recognized as genetic variety among individuals. Every SNP brings about a differentiation of a nucleotide base along the DNA chain. It occurs typically once in average of 300 nucleotides, which implies around 10 million SNPs throughout the genome. These varieties can be found located within the genes and used as markers for analysing genes that are related to diseases (USNIH gov, 2017).

The effect of C868T single nucleotide polymorphism due to cytosine to thymine replacement on the third domain of CD4 receptor at nucleotide position 868 has been shown to bring about an increased vulnerability of HIV acquisition and the progression of the disease (Lederman et al., 1991). Other studies have also demonstrated the same correlation in infants, and in female commercial sex workers in Kenya (Choi et al., 2010; Oyugi et al., 2009). However, another published study did not report any effect of CD4 C868T polymorphism on HIV susceptibility in the Chinese population (Y. lu et al., 2015).

Our purposed aim is to find out whether this polymorphism could be found responsible for the susceptibility to the HIV infection in Democratic Republic of Congo (DRC). DRC is located in Sub-Saharan African region which is known to be the highest by its incidence in coverage of HIV infections over the world (WHO, 2017).

2. GENERAL INFORMATION

2.1 Origin of the HIV and AIDS

HIV is the origin of AIDS in human. Most African primates have been naturally infected by simian immunodeficiency virus (SIV) whose multiple interspecies transmissions have given rise to HIV. Viruses resulting from the majority of these transfers have spread to humans through body fluids and blood infected by the consumption of bush meat while hunting (Hahn B et al., 2011). However, the main cause of the AIDS pandemic which is caused by HIV-1, results from transmission that involves SIV isolated from chimpanzee in southeastern Cameroon (Keele B et al., 2006).

According to Takehisa J *et al*, different viruses such as HIV-1 group N and O and group P as well as species non P and non O have been transmitted from chimpanzees. It is evident that these transmissions are from the main chimp which is named as Pan Troglodytes (Figure 2.1). Eastern chimpanzees can also be infected with SIV which does not have any descendant that is capable of causing infection in human (Takehisa J et al., 2009).

In the face of this genetic resemblance between human and chimpanzee (Figure 2.2), it is clear that SIV has undergone an adaptation to its new human host. (Paul M et al., 2010).



Figure 2.1 Location of different gorilla species distributed in West Africa and the main P troglodytes shown in middle Africa region close to DRC (Paul S et al., 2010).



Figure 2.2 Genetic origin of HIV in red and SIV in blue, rooted from P troglodytes (Paul sharp et al., 2010).

2.2 Epidemiology

2.2.1. Global Statistic on HIV/AIDS

According to WHO estimations (Figure 2.3), approximately 36.7 million people are infected with HIV worldwide (WHO, 2017). Other estimates found that 0.8% of adults in the 15 to 49 age group were also infected with HIV (Figure 2.4). The prevalence of the disease varies from one country to another accordingly to different regions (WHO, 2017). Sub-Saharan Africa has the highest proportion of HIV in the world with nearly one in 25 adults (4.2%) living with HIV (WHO, 2017). Other estimates have shown that 20.9 million people were treated with antiretroviral therapy in June 2017 (WHO, 2017). Globally, of the 36.7 million people living with HIV, only 53% have received antiretroviral therapy (WHO, 2017).

Recent estimates have also shown globally, the number of new cases of HIV stands at 1.8 million people which means a total of 5,000 new cases per day (Figure 2.5). These are children under the age of 15 whose number rises to 160,000 (Figure 2.6) (WHO, 2017). The largest number of these children are from Sub-Saharan Africa, whose contamination is caused by seropositive mothers at the time of breastfeeding and at the time of delivery (WHO, 2017).



Figure 2.3. Estimated numbers regarding HIV infected individuals in the world (WHO, 2017).







Number of people newly infected with HIV

Figure 2.5. Estimated numbers regarding new HIV cases (WHO, 2017).



Number of children (<15 years) receiving ART globally, and by WHO region, 2000-2015

Figure 2.6. Estimated numbers of children on ART per WHO regions (WHO, 2017).

2.2.2Current Epidemiological Status on HIV/AIDS in DRC

According to UNAIDS, recent estimations report for DRC in adults, women and children were 33,000, 200,000, 42,000 respectively. Furthermore, the number of deaths is 22. 000 and orphans were 330, 000. Also 121,762 individuals were on antiretroviral treatment including 66% of pregnant women in DRC were receiving the therapy as a prevention of the birth-transmission. In overall, the number reported of people living with HIV is approximately 370,000 (UNAIDS, 2016).

2.3 Biology of Human Immunodeficiency Virus

2.3.1 Taxonomy

HIV (Figure 2.7) belongs to the family of retrovirus and it is a member in the genus of lentivirus. Once enter into the human cell, RNA is transcribed into DNA by reverse transcriptase. The formed DNA is integrated into the DNA of the infected cell. The process is the opposite of what happens in human cells in which DNA is transcribed into RNA. Because of this process of reverse transcription the name of "retrovirus" was used for these viruses. DNA copy of HIV is latent when the virus is present and does not cause any damage. It becomes active when the virus takes control of the functions of the infected cell which results in the production and release of many new copies of HIV which then invade other cells (Jonathan D et al., 2013).

HIV has two mains strains; HIV-1 and HIV-2. HIV-1 is widespread in the world while HIV-2 is mainly found in West Africa. Clinically, HIV-1 disease progresses more rapidly than HIV-2 (Nyamweya S et al., 2013). Based on the genetic distances found in virological studies, HIV-1 categorized into three groups subdivided into subtypes:

- The major group M comprises different subtypes (A,B,C,D,F,G,H,J,K)
- The group O discovered in Cameroon and Gabon is much rarer
- The group N discovered in Cameroon is also rare (Paul S et al., 2010).



Figure 2.7. Different sections of Human Immunodeficiency virus (Jonathan D et al., 2013).

2.3.2 Structure of the HIV Particle by Electron Microscopy

HIV structures (Figure 2.7) consists of; the envelope where the glycoproteins (gp120 and gp 41) are fixed, the matrix which consists of the viral protein (p17), and a nucleus protected by a capsid made by the protein (p24). There are also, two identical RNA strands and viral enzymes such as integrase, protease and reverse transcriptase (Alan E et al., 2012). All retroviruses express three major genes (Figure 2.8) Gag which expresses a polyprotein for viral matrix, capsid, nucleoproteins and protease (Alan E et al., 2012). Pol gene that encodes reverse transcriptase and integrase involved in provirus integration, and Env gene involved in the expression of the surface glycoprotein (Alan E et al., 2012).

Other important genes and their functions are;

- Transcriptional activator [**Tat**]; increases the cellular transcription of the viral proteins (Atze T et al, 2011).
- Regulator of viral expression [**rev**]; permits export of unspliced RNA that is incompletely spliced transcripts from nucleus (Maik B et al., 2010).
- Viral infectivity [**vif**]; influences infectivity of the viral particle in the replication (Andrew A et al., 2014).
- Viral protein R [vpr]; transport of DNA to nucleus. Enlarges virion generation and stops cell cycle (Zhao R et al, 2014).
- Viral protein U [vpu]; promotes intracellular degradation of CD4 and the release of the genetic material in the host cell (Andrew A et al, 2014).
- Negative regulation factor [**nef**]; increases viral replication *in vivo* and *in vitro*, diminishes CD4, MHC class 1 and 2 expression (Alan E et al., 2012).



Figure 2.8. Difference regarding the genome layouts of HIV-1 and HIV-2 (Delia M et al., 2017).

2.3.3 Transmission

Blood or body liquid that is obviously defiled with blood might be viewed as fit as for transmitting HIV infection. Semen and vaginal discharges are also considered potentially ready to mediate the transmission of HIV (Lohiya GS et al,. 2013). Furthermore, cerebrospinal liquid, amniotic liquid, pleural liquid, synovial liquid, and peritoneal and pericardial liquids pose a significant risk of transmitting the HIV virus (Lohiya GS et al., 2013).

Medical staff whose exercises include contact with patients and their practices including blood or other body liquids can transmit the virus. Nurse, trainees and students are at particularly high risk for remarkable morbidity from these exposures (West CP et al, 2012). However, non-medical staff may face exposure by sexual activity (including rape) with infected individual, traumatic situation with contaminated materials, drug abuse, inoculation of non-attenuated vaccine that contains virulent strains (Chaiwarith R et al., 2013).

Exposure to body fluid may have a significant risk of transmitting HIV. The probability of developing HIV after trauma or needle-stick injury in a contaminated HIV patient is around 0.3% (West CP et al,. 2012). Factors that increase the chance of HIV transmission after percutaneous exposure include profound cut or sore, blood present on the instrument, trauma from a needle-stick that has been placed in a vein or course of the source patient. Wearing gloves may lessen around 50% of the volume of blood introduced by an injury (Farsi et al., 2010; Chaiwarith R et al., 2013).

HIV does not survive long outside the human body and it cannot even drive replication process outside a human host. It does not spread by water, air, mosquitoes, ticks, diverse bugs, saliva, tears and also sweat only if they are not mixed with the blood of a HIV contaminated person. Moreover, by shaking hands, grasping, sharing toilets, sharing dishes/drinking glasses or close mouth or "social" kissing with some individual who is HIV-positive, drinking water fountains and others sexual activities that do exclude the contribution of body fluids such as touching are not involved in the spreading of the disease (CDC gov, 2017).

2.3.4 Life Cycle



Figure 2.9. Cycle of viral replication (Roger J et al., 2003).

When HIV approaches to host cell, it binds to the CD4 receptor expressed on the host cell via gp120, which is followed by the engagement with chemokines receptors CXCR4 or CCR5 (Clapham et al., 2002; Freed et al., 2001). Further conformational changes in the viral envelope protein complex occur as a result of a second interaction between gp 120 and the co-receptor (CCR5 / CXCR4). These changes are involved in the dissociation of the gp 120 and in the exposition of the gp41 which causes a fusion of the viral membrane to the host cell membrane (Clapham et al., 2002; Freed et al., 2001). After fusion, the virus is progressively disassembled and then, subjected to reverse transcriptase (RT) whose role is the formation of a new DNA from the viral genomic RNA (Smith J et al., 2006; Zheng Y et al., 2005). After moving to the nucleus of the host cell, the recently produced DNA will integrate into the host DNA following the action of the integrase which results in the formation of the provirus. Thus, the cell continues to produce new viral proteins that constitute building block to produce more virus (N. Klima et al., 2008). In addition, there is an assembly of viral proteins into immature viruses. Once outside of the cell, these virus esbecomes capable of infecting other cells (US gov.2017).

2.3.5 Immunopathogenesis of the HIV Infection

HIV harms our immunity by approaching, penetrating and producing cellular death of CD4 T-cells that eventually results in immunodeficiency when the disease progresses significantly. HIV can bind to proteins surface other than CD4 on the surface of T-cells and diverse range cells including macrophages and dendritic cells (Naif, 2013). Interestingly, CD4 binding alone remains insufficient to allow viral penetration into the cell. This disclosure has led to the discovering of chemokine receptors as essential co-receptors for HIV-1. Among these, two which are perceived as to play a significant part in HIV entrance are CXCR-4 and CCR-5 (Naif, 2013).

HIV tropism depends on the difference in binding to CCR5 and CXCR4 expressed on immune cell surfaces (Figure 2.10). CCR5 is essential for M-tropic strains (R5) of HIV during early contamination. CXCR4 generally is correlated with strains (X4) that are more pathogenic and they show up in individuals with disease progression (Naif, 2013).

Once HIV has bound to CD4 and one of the chemokines receptors, the virus is exposed, and can fuse with the human cell and permit entry of viral genetic material into the cell (Marmor et al., 2006). The knowledge of this mechanism of HIV entry into the cell has resulted in the development of a new class of HIV therapy called entry inhibitors, which blocks the CCR5 or CXCR4 co-receptors and prevent the HIV penetration into the susceptible CD4 T-cells (Marmor M et al, 2006).



Figure 2.10. Dual tropism and disease progression (Naif, 2013).

The immune damage caused by HIV infection is characterized by;

- 1. Sequestration of Lymphoid Organ
- 2. Heightened Destruction
- 3. Diminished Production

2.3.5.1 Sequestration of Lymphoid Organ

A significant part of the harm associated with HIV infections presumably comes about because of viral replication in lymphoid tissues. In early phases of contamination, generalized lymphadenopathy can be detected. In untreated HIV disease, lymph nodes demonstrate inflammation with elevated levels of cytokines, such as; interferon-gamma (IFN- γ), interleukins (IL-1, IL-2 and IL12) (Lederman et al., 2006). The inflammatory state of the lymphoid tissues likely is a result of abnormal replication of the virus at these sites. These inflamed lymph nodes are likewise described by increased expression of intercellular attachment particles such as tight and gap junctions, and also vascular cell binding molecules such as integrin and junctional adhesion molecules (JAM). This "sticky" and inflamed state results in sequestration of lymphocytes that are in circulation in these sites. As the infection progresses, the destruction of lymphoid architecture and eventually the depletion of lymphocytes in circulation occurs (Lederman et al., 2006).

2.3.5.2 Heightened Destruction

The high level of lymphocytes in the circulation is the result of the hyperactivity of the immune system caused by HIV acting as an antigen that over time produces immunodeficiency. Strangely, this increased passage to the cell cycle usually fails most of the time after *in vitro* cultivation as the activated cells tend to die by apoptosis and furthermore, by necrosis. This might be true particularly for CD4 T-cells, where telomere length investigation does not show successful division in spite of increase cell multiplication and turnover *in vivo or ex vitro*, CD8 T-cells were shown to be are actively depleted with a shortening of telomere length that reflects several cellular replication cycles (Lederman et al., 2006).

2.3.5.3 Diminished Production

HIV disease is described by intense cellular destruction with evidence that cell production could be damaged at specific phases of contamination. With advanced phases of HIV infection, there is confirmation of cell hypo-productivity in bone marrow. Pancytopenia is common in advanced stage of disease and hypoplasia is evident in bone marrow biopsies. Also, CD34hematopoietic progenitor cells in bone marrow seem to be vulnerable to HIV contamination. The process by which bone marrow efficiency is weakened in HIV infection is not fully understood and it is likely that simultaneous disease with opportunistic pathogens such as; cytomegalovirus and *Mycobacterium aviumcomplex* may participate in this effect. (Lederman et al., 2006).

2.3.6 Immune Mechanisms against HIV

2.3.6.1 Innate Immune Responses

In the blood, as long as viremia increases, there is an enhanced level of cytokine production such as; interleukin (IL10 and IL-15) (Figure 2.11). Furthermore, tumor necrosis factor (TNF) as well as interferon gamma (IFN- $_{\rm Y}$) which possess antiviral activity to inhibit HIV replication (Stacey AR et al., 2009; McMichael et al., 2010).

Dendritic cells(DCs) are particularly diminished in number within primary phase of HIV-1 contamination. Exposed plasmacytoid dendritic cells release IFN- α which improves adaptive immune reactions. HIV-exposed plasmacytoid dendritic cells produces indole amine 2, 3-dioxygenase that induce CD4T-cell differentiation into Regulatory T-cells involved in suppression of HIV-specific responses. Conventional DCs initiate a particular reaction through CD4 and CD8 lymphocytes following exposure to HIV (Mc Michael et al., 2010).

Similarly like in most viral diseases, natural killer T-cells (NKT) and natural killer cells (NK) get activated during the primary phase that represents acute HIV infection (Borrow et al., 2008; Clay CC et al., 2007; Ward J et al., 2007). The antiviral role played by NK cells may have more significant impact further in the course of the disease. Preceding the peak levels of viremia, NK cells were shown to multiply and possess increase cell activity during *ex vivo* infections models (Goonetileke N et al., 2009).



Nature Reviews | Immunology

Figure 2.11. Profile of cytokines during acute HIV infection (Mc Michael et al., 2010).

2.3.6.2 Adaptive Immune Responses

In those individuals that are not vulnerable to the infection, multipara metric cytometry examinations have shown CD8T cells are more polyfunctional because of their ability of secreting various chemokines and cytokines such as; IL-2, IFN- γ , TNF- α , MIP-1 β in addition to degranulation through CD107 upon HIV specific stimulation (Migueles SA et al., 2008; Ferre A et al, 2009).Polyfunctionality is related with a higher levels of cytokines production and therefore to a more proficient generation of effector CD8 T-cells. It is not yet known how cytokine/chemokine provide protection to viral control. The current distinguishing proof is that when a particular subsets of polyfunctional CD8T-cells is supplied either with IL-2 secretion or with the ability to upregulate perforin, they were shown to be more effective in controlling disease (Migueles et al., 2008; Makedonas G et al., 2010).

Generally in those infected individuals, immune reaction occurs through CD8 T-cells. These have considerably higher reactivity compared to CD4 T-cells that are depleted during the progression of the disease. Memory CD4 T-cells are greatly reduced from the lymphoid organ especially in the gut as a result of activation-induced apoptosis (Walker B et al., 2012). However, the levels of HIV-specific CD4T-cells that are contaminated even during abnormal state viremia is usually lower. These cells somehow avoid infection in spite of being activated during a period of high viremia level (Walker B et al., 2012).

Humoral reactions to HIV develop after about1 week of significant replication. However, antibody binding were reported not to have a significant impact on the viremia (Tomaras GD et al., 2008) and not to provide any specific resistance against the envelope of the virus (Keele BF et al., 2008).

2.4 Clinical Forms

2.4.1 Stages of Infection

- 1. **Primary Stage ;** This first phase of infection lasts for two to four weeks (figure 2.12). During this phase, influenza-like symptoms can be observed (for example; high body temperature and headache). The virus produces more copies and spreads inside the body. Infection causes attenuation of immune defense by destroying or killing CD4 T-cells (US gov, 2016).
- **2. Secondary Stage** ; In this stage, the disease is named as asymptomatic HIV disease or latency phase (Figure 2.12). During this phase, HIV keeps on multiplying in the body at low levels. Individuals could not show the presence of symptoms but they are capable of transmitting the disease to others people. The disease advances to AIDS in couple of years if the treatment is not initiated as soon as possible (US gov, 2016).
- **3. AIDS** ; It is the last stage of the disease progression (Figure 2.12). The virus has already damaged the immunity and it cannot initiate the battle against opportunistic infections (opportunistic infections occurs in people that are infected and have a

weakened immunity.). CD4 count less than 200 cells/mm³ indicate a confirmation of acquired immunodeficiency syndrome (AIDS). Life expectancy is around 4 years in absence of the treatment (HIV gov, 2016).



HIV Progression

Figure 2.12. Progression of the disease according to different phase of contamination (US gov, 2016).

2.4.2 **Opportunistic Infections**

1. Toxoplasma gondii Encephalitis

The most well-known clinical consequence of *Toxoplasma gondii* infection is encephalitis with cerebral pain, confusion, fever and weakness. Patients may have specific manifestations, including specific cerebral pain and psychiatric side effects. Central neurological abnormalities might be available on physical examination. In the absence of initiation of proper treatment, seizures can occur and the infection can lead to coma (Joanne P et al., 2013).

The illness is rare when patients have CD4 number higher than 200 cells/µl. Patients with CD4 count lower than 50 cells/µl are at most serious risk. The disease is transmitted via ingestion of undercooked meat containing cyst or after ingestion of oocysts that have been excreted by feces from a cat, and that had been sporulated in the environment (Joanne P et al., 2013).

2. Pneumocystis Pneumonia

Pneumocystis pneumonia (PP) is the most common opportunistic infection in people living with HIV / AIDS (Nicholas B et al., 2017). Africa is experiencing an increased incidence of *Pneumocystis* in up to 80% of infants with HIV infection. In infected patients, symptoms include; dry cough, chest discomfort and fever (Murray JF et al., 2005). In HIV-infected people with severe PP, corticosteroids are prescribed as first-line therapy. More effective, trimethoprim-sulfamethoxazole is chosen as the treatment of choice (Nicholas B et al., 2017).

3. Coccidioidomycosis

The risk of developing symptomatic coccidioidomycosis is higher in patients whose CD4 count is under 250 cells/mm³ and when AIDS is confirmed. The lower CD4 number and the lack of viral suppression are also linked with the intensity of coccidioidomycosis infection. Symptoms include; meningitis, diffuse pneumonia and localized pneumonia (Jennifer B et al., 2013). The rate of HIV-related coccidioidomycosis have declined since introduction of antiretroviral treatment (Jennifer B et al., 2013).

4. Tuberculosis

Rather than a long inactivity stage between contamination and proper disease, individuals with HIV can turn out to suffer from tuberculosis with longer active duration that can last for weeks to months with greater intensity (WHO, 2008).

Infected people with pulmonary tuberculosis may have typical symptoms of tuberculosis such as; pleural effusion, blood sputum, cough of more than 3 weeks, chest pain, pericardic disease. People who are co-infected with tuberculosis and HIV could have symptoms that are often possible not to be regarded as tuberculosisrelated, such as deterioration of the general wellbeing. In that case, this causes a delay in initiating a proper treatment and diagnosing tuberculosis (Sterling, 2010).

5. Cryptosporidiosis

Human cryptosporidiosis is caused by apicomplexan protozoans named as Cryptosporidiumspp. It was once thought to be caused by a one specie, however molecular investigations have shown that it is caused by no less than 15 distinct species. Among the more typical species are *Cryptosporidium hominis*, for which human are the most common host and *Cryptosporidium parvum*, which can infects bovines (White AC, 2015; Checkley W et al., 2015). When the immune system is weaken, cryptosporidium causes delayed and persistent diarrhea in people with AIDS (Bouzid M et al., 2013).

6. Mycobacterium Avium Complex

Mycobacterium avium complex (MAC) comprises two species: *Mycobacteriumavium* and *Mycobacteriumintracellular*. Since it is are hard to identify them, they are also referred as *Mycobacterium avium-intracellular* (MAI). MAC is the atypical *Mycobacterium* most ordinarily related to human disease (Nishiuchi et al., 2007).

Mycobacteria are basically pneumonic pathogens that affect people who are immunocompromised. In clinical perspective, mycobacteria has been associated with osteomyelitis, tenosynovitis, synovitis and has spread to the bone marrow, liver, spleen and ultimately to the central nervous system (CNS). MAC is well-known causative agent for nontuberculous mycobacterial infection among patients with AIDS. *Mycobacterium* strains are often isolated in around 95% of patients at the AIDS phase (Nishiuchi et al., 2007).

7. Cytomegalovirus

HIV-related cytomegalovirus (CMV) encephalitis is a disease that affects the peripheral and the central nervous system (CNS). Neurological symptoms of CMV include encephalitis, ventriculitis, myelitis, retinitis, radiculoganglionitis and neuropathies. They occur in patients with severe immunodeficiency conditions in which the number of CD4 lymphocytes is generally lower than 50 cells/µl (Gulshan et al., 2015).

HIV patients with a low CD4 count under 50 cells/µl are prone to be affected by CMV neurologic infection. The rate has reduced since highly antiretroviral treatment (HAART) became accessible. CMV infection of the CNS can be seen in 18-28% of patients with AIDS. Histologic investigations reveal ventriculoencephalitis, microglial nodule, central parenchymal necrosis (Gulshan*et al.*, 2015). A fast initiation of antiviral therapy is crucial. If left untreated, HIV-related CMV encephalitis typically advances to death in days to weeks. Patients may die of various complications other than neurologic conditions in last phase of AIDS (Gulshan et al., 2015).

8. Genital Candidiasis

Genital candidiasis may occur in vagina and under the prepuce in men. It causes burning sensation, itching and pain. Women are more susceptible to candidiasis when they are pregnant. Candidiasis can be passed on from mother to child at the moment of delivery. Among individuals with HIV, candidiasis is more typical when CD4 cell number is low, and repeated infections can be an indication of the progression of the disease (Goncalves B et al., 2016).

9. Genital Herpes

HIV and the genital herpes are working in collaboration. One can worsen the impacts of the other. Studies have confirmed that when the herpes infection is active, the more HIV reproduces itself by replication and more cells killed and this leads to AIDS. Individuals with both HIV and the herpes infection may have longerenduring, more regular and more extreme episodes of herpes symptoms because of the attenuated immune system (Nat India, 2010; Reynold SJ *et al*, 2012).

10. Viral Hepatitis

a. Viral Hepatitis type B

Viruses such as Hepatitis Bvirus (HBV) and HIV produces blood borne infections transmitted principally through sexual contact and injection of drug. On these methods of transmission, a high probability for HIV contamination poses additionally a further risk for HBV disease. Furthermore, people who are co-infected with HIV and HBV can present serious complications including high risk liver-related morbidity and mortality. To combat against HBV in HIV-contaminated patients, immunization against HBV is recommended in all defenseless patients with HIV/AIDS (CDC gov, 2017).

b. Viral Hepatitis type C

Around one fourth of HIV patients are evenly contaminated with Hepatitis C virus (HCV). HCV is a blood borne infection transmitted through direct contact with the blood of a contaminated individual. Accordingly, co-infection with HCV and HIV is more frequent around 50%–90% in HIV-contaminated drug users. HCV is amongst the most frequent causes of chronic liver disease, HCV progresses faster to produce liver damage in HIV-infected people (CDC gov, 2017).

2.4.3 AIDS Related Cancers

Patients with HIV / AIDS are more prone to develop the following cancers: Kaposi's sarcoma (cancer of the cutaneous, blood vessels and mucous membranes of the mouth, nose and anus), Lymphoma (cancer of the lymphatic system) cervical cancer, lung cancer, liver cancer (FCC, 2017).

HIV is not a direct cause of cancer. But it favors disease. As a result of the

attenuation of immune system, the body can less effectively destroy cancer cells and less effectively fight infections that can cause cancer. Statistics show that infections with viruses likely to cause cancer occur more often in people with HIV (FCC, 2017). HIV-positive people generally smoke and drink more than average and this behavior weighs heavily because of their weakened immune system (FCC, 2017). Infected people have a chance to develop lung and liver cancers. They are highly recommended to avoid smoking and drinking (FCC, 2017). The cancer cases in patients suffering from AIDS result from other viruses other than HIV such as Human herpes virus 8 (HHV-8) which is one of the causes of Kaposi sarcoma, Epstein-Barr virus that induces cancers such as of Hodgkin and non-Hodgkin lymphoma, and human papillomavirus can cause cervical cancer (FCC, 2017). Highly Active Antiretroviral Therapy (HAART), a treatment that reduces viral load but cannot destroy the virus and, also can reduce the risk of cancer. If they already have cancer, HAART improves their overall survival (FCC, 2017).

2.5 Laboratory Diagnosis

The Centers for Disease Control and prevention (CDC) recommends diagnosis by using 4th generation ELISA test that detects HIV infection earlier than antibody detection tests. It detects the protein p24 in the blood (CDC, 2014). Antibodies from both HIV-1 and HIV-2 should be distinguished. The advantage of this test is the fact that results can be obtained faster than western blot (US FDA, 2013). Polymerase chain reaction (PCR) needs to be performed in order to confirm the presence of infection (US FDA, 2013; CDC, 2014).

Distinguishing feature of HIV-1 antigen test allowing the detection of HIV-1 earlier than HIV-1 antibody test. This test can be utilized as a parameter to recognize contaminated people who may not be tested by traditional settings (Qaseem et al., 2009; Lowes R., 2013). It is recommended that all patients be routinely screened for the benefits of prior treatment (Brooks M, 2014).

2.5.1 Screening for HIV

ELISA is the most sensitive test that should be used for screening. Most ELISA tests can be used to recognize different strains of HIV-1 such as M, N and O and HIV-2. For a negative ELISA result there is no need to confirm by western blot. However, a false positive result needs twice western blot testing for confirmation (Torian L et al., 2010).

Serology is not successful test when this is used as early detection test. In addition, a viral RNA detection test may help to recognize past infections before the onset of seroconversion. This would likely decrease transmission and general costs (Claassen et al., 2012).

2.5.2 CD4 Count

The reference range for CD4 number is around 500-2000 cells/ μ l. After seroconversion, CD4 numbers have a tendency to fall by 700 cells/ μ l approximately and continues to decline with the time. For this reasons, a CD4 number below 200 cells/ μ l, that increase susceptibility to opportunistic diseases, should be regarded as a sign for AIDS infection (Hull M et al., 2012).

2.5.3 Viral Load

Viral load must not be considered as a tool to make a decision because of false-positive results. A progression of the infection from AIDS to death is indicated by the viral load. Patients with high loads (30,000 copies/ml) are more subject to die, and are 18 times more vulnerable to AIDS (Nicholas B et al., 2017; Raper JL et al., 2014).

With treatment, the burden can be decreased to an imperceptible level under 20-75 copies/ml. This is viewed as ideal viral suppression. Meanwhile, the probability to die is lessened. Commonly, patients that are successfully treated will display viral suppression less than 200 copies/ml. Failure of treatment is

characterized by viral burden to stay at high level more than 500 copies/ml. The importance of this point of view might be helpful in clinical practice (Nicholas B et al., 2017; Raper JL et al., 2014).

2.5.4 Secondary HIV Testing

Viral culture technique costs high and has less sensitivity compared to others test such as ELISA and PCR. It can be used as phenotypic perspective in drug-resistance testing. Molecular techniques may identify proteins and viral DNA. PCR is often performed in infants as serologic test is not useful due to maternal antibodies usually that usually last for 6 months or more. At least 2 negatives results over a period of one month is required to be considered as negative. The detection of viral DNA or RNA is very important as it enables sequencing to detect the mutations that may confer resistance to different antiretroviral drugs. It is also an indication in the choice of drugs that is more effective in the therapy (Nicholas B et al., 2017).

2.6 Treatment

Treatment with medication against HIV is called antiretroviral treatment (ART). It cannot result in cure, however can control the infection with the purpose of extending life expectancy, leading to a more healthy being and decreases the risk of transmission. This includes taking a mix of HIV drugs (called a HIV regimen) precisely as prescribed. These HIV prescriptions effects HIV replication (making duplicates of itself) which diminishes the measure of HIV in the body. Having less HIV in the body allows immune system to recover from the diseases to fight against invading agent as well as cancers. Despite the fact there may still be some HIV in the body, the immunity is sufficiently solid to battle off contamination and tumors. The treatment is prescribed for all individuals with HIV regardless of the duration they have been infected or in good health. In the untreated case, the virus weakens the immune system by destroying CD4 T-cells (HIV gov, 2017; Willem D et al., 2017).

2.6.1 Mechanisms of Actions of Different Classes of Drugs

HIV approved medicine (FDA, 2014)

Mechanisms	Generic	Brand	FDA
		names	Approval
			Date
	Abacavir (ABC)	Ziagen	December
			1998
Inhibition of the action	Didanosine (DDL)	Videx	October
of reverse transcriptase			1991
that is used to make	Emtricitabine (FTC)	Emtriva	July 2003
several copies of HIV			
-	Lamivudine(3TC)	Epivir	November
			1995
	Stavudine(d4T)	Zerit	June 1994
	Tenofovir(tenofovir,TDF)	Viread	October
			2001
	Zidovudine	Retrovir	March
	(azidothymine,AZT,ZDV)		1987

1. Nucleoside Reverse Transcriptase Inhibitors (NRTIs) (Table 2.1).

2.Non-nucleoside Reverse Transcriptase Inhibitors (NNRTIs) (table 2.2).

Mechanisms	Generic	Brand names	FDA date Approval
Binding to and	Efavirenz (EFV)	Sustiva	September 1998
transcriptase enzyme.	Etravirine (ETR)	intelence	January 2008
	Nevirapine (NVP)	Viramune XR	March 2011
	Rilpivirine (RPV)	Edurant	May 2011

3. Protease Inhibitors (PI) (Table 2.3).

Mechanisms	Generic	Brand names	FDA date approval
	Darunavir	Prezista	June 2006
Inhibition of the action of	Atazanavir	Reyataz	June 2003
protease enzyme	Fosamprenavir	Lexiva	October 2003
	Indinavir	Crixivan	March 1996
	Nelfinavir	Viracept	March 1997
	Ritonavir (RTV) Used in Adults and pediatric HIV infection	Norvir	March 1996
	Saquinavir(SVQ)	Invirase	Decembre 1995
	Tipranavir (TPV)	Aptivus	June 2005

4. Mechanisms of other Antiretroviral Drugs(Table 2.4).

Mechanisms	Generic	Brand Name	FDA date approval
Fusion inhibitors stops the penetration of the virus into the CD ₄ lymphocyte	Enfurvirtide (T20)	Fuzeon	April 20013
Entry inhibitors inhibits a protein on the CD_4 lymphocyte which allow the penetration of the virus into the cell	Maraviroc (MVC)	Selzentry	September 2007
Integrase inhibitors stops	Dolutegravir (DTG)	Tivicay	August 2013
the action of the viral integrase	Eltegravir (EVG)	Vitekta	September 2014

enzyme	Raltegravir (RAL)	Isentress	October 2007

2.7 Prophylaxis

2.7.1Pre-Exposure Prophylaxis

Pre-exposure prophylaxis is a strategy in which antiretroviral drugs are initiated in non-HIV infected people before being exposed to the infection. Medicines such as Tenofovir disoproxil fumarate (TDF) combined with Emtricitabine (FTC) has been found to be efficient in protection against HIV-1 contamination and decreasing the susceptibility to be contaminated by HIV in homosexual men, in people who use intravenous drug and have HIV-1–negative partner, and in serodiscordant couples (Grant et al., 2010; Thigpen et al., 2012).

2.7.2 Post exposure Prophylaxis

This kind of prophylaxis consists of the utilization of antiviral therapy for a short period of time to diminish the risk of getting infected by HIV virus after exposure. Current rules suggest to take medications over a period of 28 days just after 36 to 72 hours following exposure. Usually, it take up to 3 days for the virus to be detected in lymph nodes at least 5 days in the blood, and 8 days in the cerebrospinal fluid. This window period gives opportunity to stop the infection to proceed after exposure by initiating antiretroviral therapy that can inhibit the replication of the virus, and also the expansion of the viral infection (Sultan et al., 2014).

2.8 Prevention

2.8.1. Sexual Transmission

Condom decreases the chance of transmitting HIV in heterosexual contact by around 80% over the long-term (Bounse et al., 2012; Galo et al, 2012). Where one partner of a couple is contaminated, reliable condom utilization brings about rates of HIV disease for the uninfected individual below 1% every year. The utilization of the spermicide nonoxynol-9 may expand the risk of transmission since it causes vaginal and rectal irritation (Baptista et al., 2009). A vaginal gel containing Tenofovir, a reverse transcriptase inhibitor when utilized promptly before sex was reported to diminish disease rates by approximately 40% among African women (Celum et al., 2012).

Circumcision in sub-Saharan Africa lessens the risk of HIV transmission in heterosexual men around 38 and 66% in every two years (Siegfried et al, 2009). Based on these investigations, WHO and United-Nations program for HIV/AIDS (UNAIDS) have both suggested male circumcision as a strategy for counteracting female-to-male HIV transmission (WHO and UNAIDS, 2007). However, its effect on the prevention during male-to-female transmission is disputed (Larke, 2010). Furthermore, preventing sexual transmission by condoms remains an advantage among men who intercourse with men, and in infected hemophiliac individuals in which the blood clot is replaced by recombinant factor 8 as this induces excessive bleeding (Kim et al., 2010; Templeton et al, 2010; Wiysonge et al., 2011). On the other hand, women who have experienced female genital cutting have are at great risk of HIV (Utz B et al., 2008).

Projects encouraging sexual abstinence do not seem to influence HIV risk (Underhill et al, 2008). Evidence for an advantage from peer instruction is similarly poor (Tolli, 2012).Comprehensive sexual education given at school may diminish the risk of HIV infection (Ljubojevic et al., 2010). It is currently not known whether treating other sexually transmitted diseases is also a better way in the prevention of HIV transmission (Butler, 2011).

2.8.2 Blood Transfusion

- Screening ; It is important to ensure the safety of blood supply. It is recommended that a donor must be free of any transmissible infection by blood transfusion. There is a list of deferred blood which in order to differentiate blood samples from donors such as those with a past infectious disease (e.g. Hepatitis C, syphilis etc...) must undergo additional test. Also blood samples taken from subjects with past intravenous drug abuse are often delayed for additional investigations (OL1, 2014).
- **Quarantine :** Blood must be isolated until proven to be safe for transfusion (OL1, 2014).
- Blood testing ; After donation each unit of blood is required to undergo several tests that can detect antigens from any type of infectious disease including; HIV (1 and 2), *Trypanozoma cruzi* for Chagas disease, West Nile virus and also *Treponema pallidum* for syphilis. Additionally, testing for all strains of hepatitis is recommended (OL1, 2014).

2.8.3 Mother to Infant Transmission

Reducing the transmission of HIV by ART from mother to infants can lessen rates of transmission by 92-99% (Kurt et al., 2011; Coutsoudis et al., 2010). This fundamentally includes the utilization of antivirals at the moment of pregnancy and after birth in the newborn child. Additionally, bottle feeding instead of breastfeeding can also be practiced for prevention since HIV is still able to pass through the breast milk to contaminate the newborn who is not able to initiate a strong immune response (Siegfried et al., 2011; WHO, 2008). Furthermore, antiretroviral prophylaxis to the baby may diminish the risk to acquire infection from the mother (Horvath et al., 2009; Singh K et al., 2009).

3. METHODOLOGY

3.1. Study Zone

The city of Bandundu has only one health zone in which there are one General Reference Hospital (GHR) where this study was carried out, four Secondary Reference Hospitals (RSH) and ten Health Centers (HC). The General Reference Hospital offers emergency, maternity, internal medicine, surgery, pediatrics, medical imaging (with radiography and ultrasound) and dentistry services (CAID, 2016).

In the city of Bandundu, HIV / AIDS prevention and care are integrated in 40 of 50 health zones in the province's coverage for the three axes of 77%. The province has several branches of HIV / AIDS awareness (Churches, Schools, Community Based Associations, etc.). Fight against HIV / AIDS is coordinated by the Provincial Multisectoral HIV Committee, chaired by the provincial governor. The technical aspects of the fight are managed by the provincial coordination of the PNLS. (PNLS, 2014).

3.2 Sampling

Both patient and control samples were collected at the Bandundu General Hospital in the National HIV/AIDS program laboratory (PNLS/Bandundu). For the patient group, 45 blood-tested samples were collected. Positive HIV samples were confirmed by western blot testing which has detected different viral antigens such as p17, p24, gp41 and gp 120. For the control group consisting of non-HIV infected individuals, 29 blood samples were collected, and were later confirmed by western blot testing. Written consent was provided and blood sampling was carried out from September 2016 to January 2017. Our project has received support from the Near-East Hospital Ethics committee in Nicosia, North-Cyprus. The code of ethical approval is project N^o YDU/2017/43-347.

The biological material consists of 5 ml of blood collected with all biosecurity measures and was dried on whattman-labeled filter paper and carefully packaged in an aluminum envelope. The filter papers were then sent from DRC to the BM laboratory (Ankara/Turkey) for molecular analysis.

3.3. Molecular Methods

3.3.1. DNA Extraction

The procedure was performed following the DNA extraction protocol from the manufacturer (Eurx, Poland). Various chemicals such as proteinase K(10 μ l), Sol QB buffer (400 μ l), PBS (100 μ l), and 96% ethanol (200 μ l) were used to dissolve blood stains contained on the filter paper which was placed in 1.5 ml eppendorf tubes in order to extract the DNA from the blood. All chemicals used were from Eurx Quick Blood DNA purification kit (Poland). The same method of extraction was applied for patients and controls samples.

3.3.2. Nested Polymerase Chain Reaction (PCR)

Nested PCR procedure to amplify the target DNA containing nucleotide at the position 868 was performed as previously described by Choi *et al.* Two sets of primers were used; outer amplifying primers 5-GTCCAGGAATCCTAAGGACAGC-3and 5-CCACCAGGTTCACTTCCTGATG-3 and inner amplifying primers 5-GTGGCCTGCTGTAGGAAAATGC-3and 5-CACCAGGTTCACTTCCTGATGC-3 (Choi *et al.*, 2009). Furthermore, chemicals used in the procedure were; DNA polymerase, 10X reaction Buffer BD, MgCl₂, dNTPs contained in the kit (Solis Biodyne company, Estonia). However, 11.66 µl is the concentration of all the chemicals used during the nested PCR procedure.

After amplification, PCR products were ran in electrophoresis for 70 minutes at 100 volt in 1.2% agarose gel (Bioshop, Canada) supplemented with 1x TBE buffer and visualized by ultra-violet light using ethidium bromide (Solis Biodyne, Estonia).

3.3.3 Detection of the C868T Polymorphism by DNA Sequencing

The PCR products were sent to Macrogen Laboratory Company (Netherlands). With ABI 3730XL Genetic Analyzer, nucleotides were sequenced by Sanger's method via Finch TV software. At the 868 position, homozygous sequences were indicated by same type of nucleotides (CC or TT) while heterozygous sequence was indicated by 2 different nucleotides (CT).

3.4 Statistical Analysis

All analyses were carried out by SPSS version 18.0. Any significant association was supposed as p value<0.05 at 2-sided values. Genotyping and allele frequencies from the comparison between controls and patients samples were performed by using Binary logistic regression. Odds ratios with 95% confidence intervals were calculated. With respect to demographic characteristics, the chi-square and student t test were applied.

4.1 Demographic Characteristics of Subjects

This study included samples from 74 subjects (45 HIV+ and 29 HIV-) obtained from the Bandundu Reference General Hospital. The mean age of the patient group was 40.62 ± 11.9 years while for the control group it was 37.38 ± 15.6 years. There was not any significant difference detected between the two groups regarding the age of subjects included (p=0.318). Furthermore, the HIV+ group included 17 male (37.8%) and 28 female subjects (62.2%) while the HIV- group included 9 male (31.0%) and 20 female (69.0%) subjects. The comparison of the gender ratios between both groups revealed no statistically significant difference (p=0.623) (Table 4.1).

Characteristics	Patients n=45	Controls n=29	P value
Age (mean±Std)	40.62±11.9	37.38±15.6	0.318
	Years	years	
Gender			0.623
Male	17 (37.8%)	9 (31.0%)	
Female	28 (62.2%)	20 (69.0%)	

Table 4.1 Characteristics of the study populations.

4.2 Effect of CD4 C868T Polymorphism on HIV Susceptibility.

The frequency of individuals with wild type genotype C/C was 64.4% (n=29) and 72.4% (n=21) in the HIV+ group and HIV- group, respectively. The frequency of individuals with heterozygous genotype C/T in the patient and the control group were, 26.7% (n=12) and 24.1% (n=7) respectively, with no statistically difference detected between the groups (OR= 0.34, 95% CI= 0.03-3.31, p= 0.357). The same statistical indifference was true from individuals with TT genotype (OR= 0.42, 95 % CI= 0.04 - 4.6, p value= 0.486), in which the

frequency in the patient and in the control groups accounted for 8.9% (n=4), and 3.4% (n=1) respectively. Both groups conformed to the Hardy-Weinberg equilibrium test (p= 0.518 for patient group, and p= 0.183 for control group). Furthermore, no difference was found for both dominant (OR= 0.69, 95% CI= 0.2 – 1.91; p value= 0.476) and recessive model (OR= 2.7, 95% CI= 0.29 – 25.7, p value= 0.380), in addition to allelic model of overall analysis (OR= 1.5, 95% CI= 0.65 - 3.70, p value= 0.318) (Table 4.2).

Genotypes	Patients n=45	Controls n=29	Odd ratio(95%	Р
	(%)	(%)	CI)	value
CC	29 (64.4)	21 (72.4)	1.00 ^{ref}	-
СТ	12 (26.7)	7 (24.1)	0.34 (0.03-3.31)	0.357
TT	4 (8.9)	1 (3.4)	0.42 (0.04 - 4.6)	0.486
Dominant model	16 (35.6)	8 (27.5)	0.69 (0.25-1.91)	0.476
Recessive model	4 (8.9)	1 (3.4)	2.7 (0.29-25.7)	0.380
C allele	70 (77.8)	49(84.5)	1.00 ^{ref}	-
T allele	20 (22.2)	9(15.5)	1.5 (0.65-3.70)	0.318

Table 4.2 Genotypes and Alleles Results.

*Dominant model is used to compare TT+CT versus CC Recessive model is used to compare TT versus CT +CC

The frequencies for control group genotyping from this study (CC; 72.4%, CT; 24.1%, TT; 3.4%) were found to be similar to the Hap-Map genotypes frequencies (Y Lu et al., 2015), in which the comparison included people from different countries such as African ancestry from Southwest United-States (CC; 65.3%, CT; 28.6%, TT; 6.1%), Yoruba from Nigeria (CC; 59.3%, CT; 33.6%, TT; 7.1%) and female commercial sex workers from Kenya (CC; 75.9%, CT; 13.8%, TT; 10.3%) (Oyugi et al., 2009) (Table 4.3).

Table 4.3 Comparison of CD4 C868T frequencies in healthy subjects from the present study and that from the Hap-Map Project. Table adapted from Y Lu *et al*, 2015.

Genotypes	The present	ASW n=98	YRI	CSW
	study n=29(%)	(%)	n=226 (%)	n=87
				(%)
CC	21(72.4)	64(65.3)	134(59.3)	66(75.9)
СТ	7(24.1)	28(28.6)	76(33.6)	12(13.8)
TT	1(3.4)	6(6.1)	16(7.1)	9(10.3)

*ASW; African ancestry in Southwest United-States (USA)

YRI; Yoruba in Ibadan (Nigeria)

CSW; Commercial Sex Workers (Kenya)

5. DISCUSSION

This study is the first to investigate any effect of on HIV susceptibility in Congolese population recruited in Bandundu General Hospital. Our results showed that there is not any correlation, and that therefore *CD4 C868T* expression could not be a factor contributing to susceptibility to HIV at least in Congolese population studies. This correlates with data previously published by Y Lu *et al*, which did not show any effect of the *CD4 C868T* SNP on HIV susceptibility in Chinese population (Y. Lu et al., 2015). In contrast, two previously published studies in infants and female commercial sex workers in Kenya have shown an increased susceptibility to the disease (Choi et al., 2010; Oyugi et al., 2009).

In this study, sequencing was performed in order to detect the mutations over *CD4* gene (rs28919570). Regarding the demographic characteristics, no significant difference has been demonstrated. The frequency of genotyping among patients accounted for 64.4 % of wild type (CC), 26.7% of heterozygous (CT) and 8.9% of homozygous (TT) variants. Among control subjects 72.4% were wild type (CC), 24.1% were heterozygous (CT) and 3.4% were homozygous variants (TT). No significant difference was detected. Recessive and dominant model analyses did not reveal any association with susceptibility to HIV. Furthermore, analyzes of alleles frequencies were performed and no significant difference was obtained.

Our results could be explained by the fact that, a low sample size of 74 individuals was collected. Our goal was to obtain more samples, but few HIV infected patients and healthy controls from Bandundu General Hospital agreed to participate in our study. Additionally, the study was therefore insufficient to apply the data for the whole DRC population, and for more precise conclusion.

Our finding on the current study (Bandundu/DRC), people who carried the wild-type genotype in patients and control groups were 64.4%, and 72.4% respectively. These were also found comparable to the Oyugi*et al* finding, in which the frequencies in patients and controls were 69.0%, and 75.9% respectively (Y. Lu et al., 2015).

Compared with the data the published by Y Lu *et al*, the percentage of heterozygous people was found to be higher in the present study (Bandundu/DRC). This consists of 26.7%, and 24.1%, in patients and controls subjects, respectively. However, in the Chinese population these consisted of 5.88%, and 8.91%, patients and controls subjects, respectively (Y Lu et al., 2015). As it is reported that, *CD4* polymorphism was not associated with the susceptibility to HIV, it has been demonstrated that, the stronger effect of some other genes such as those encoding HLA molecules influenced the protection against HIV and delayed the disease (Saksena N et al., 2007; Y Lu et al., 2015). The effects of *HLA B-27* and *HLA B-57/58* polymorphisms on HIV infection are recommended for future work. Also in the current investigation, dominant and recessive models did not report any effect of the CD4 SNP on the risk of HIV infection.

According to Marmor M *et al*, interleukin 4 (IL-4) was shown to provide protection against HIV by a downregulation of the CCR5 level on the CD4T-cell surface, which eventually results in the inhibition of the penetration of the virus into the host cell. Furthermore a polymorphism such as *IL-4-589T* SNP was found to protect against heterosexual transmission of HIV (R5) in Japanese population (Marmor M et al, 2006). Thus, the impact of *IL-4-589T* SNP on HIV infection can also be investigated in DRC as well as other countries in Sub-Saharan Africa countries in since the major transmission of HIV remains by sexual contact.

The other candidate genes that can be used for future studies on defining genetic pool responsible for the HIV susceptibility include; *TRIM5α*, *APOBEC3G*, *CCL5*, *CCL3*, *CXCL12*, *HBD 2 and 3*, *RIG-1 and CCR5 delta 32*.

TRIM5 α plays a role in the inhibition of HIV-1 replication once inside the host cell. However, its polymorphism *TRIM5* α *136Q* exhibit a stronger inhibitory activity against HIV infection (Javanbakht H et al., 2006; Speelmon E et al., 2006). Further investigations on the effect of the *TRIM5* α *136Q* polymorphism with the transmission of HIV are recommended.

Another factor named as human apoliprotein B (*APOBEC3G*) is an intracellular inhibitor of HIV-1 replication(Mangeat et al, 2003; Harris et al, 2003).

APOBEC3G has low molecular mass and can be found in the cell in enzymatically active form and can restrict HIV-1 replication (Esnault C et al, 2005). A Total of 29 polymorphisms of *APOBEC3G* were found not to be associated with the disease progression in a French cohort (Do H et al, 2005). Projects on the *APOBEC3G* polymorphisms with the susceptibility to HIV infection are encouraged.

The chemokine CCL5 (*RANTES*) (Figure 5.1) inhibits the HIV (R5) strains by preventing the attachment of the virus to the chemokine receptor CCR5 on CD4 T-cell surface. Also, the high level of this chemokine has been reported to inhibit HIV in some of European cohorts (Koning F et al, 2004). Previous investigations demonstrated that, *CCL5-403A* SNP and *CCL5-28G* SNP delayed the progression of the HIV infection in Japanese and Thai cohort, and also decrease the susceptibility of the disease in a Chinese cohort (Wichukchinda N et al, 2006; Zhao X et al, 2004). *CCL5-403A* SNP and *CCL5-28G* SNP effects on HIV infection has not been yet investigated in African population. For this reason, it is encouraged to be investigated for future studies.

According to Lama *et al*, the protection against HIV infection is also mediated through chemokine ligand CCL3 which seems to inhibit strongly the infection by R5 (HIV) strains. The ligand CCL3 known as *MIP-1a* (Figure 5.1) was shown to exhibit a great affinity for the receptor CCR5 (Lama et al., 2007). In fact, in African population, the copy number of the *CCL3L1* gene was the highest, and it was shown to restrict HIV infection (Lama et al., 2007). To date, no research has yet investigated the number of copies of the gene *CCL3L1* in Congolese population. Thus, we hope that, next research will shed more light in this area among other populations different than Africans.

Another ligand CXCL12 (*SDF-1*) blocks the penetration of HIV (X4) strains by internalization of the chemokine receptor CXCR4 into the CD4 T-cells (Figure 5.1). However, at the position 801 of the DNA, the polymorphism *SDF1-3'A* was also associated with the delay of the disease in homozygous people (Lama et al., 2007). The efficiency of the *SDF-1* gene on HIV infection can be investigated by future studies.



Figure 5.1. The black arrows shows especially the protection mechanisms against HIV (R5 and X4) strains entry mediated by different ligands over chemokines receptors. (O'Brien S et al., 2004).

According to Weinberg *et al*, human beta defensin 2 and 3 (HBD2 and HBD3) have greater activity against HIV(X4) strains with a disruption of the viral particles through a direct interaction with the viral cell surface and decrease the level of CXCR4 in order to block the viral entry into CD4 T-cells (Weinberg et al., 2006). Investigations on the impact of Human Beta Defensins with the susceptibility to HIV infection are recommended.

A previous published data by Yizhong W *et al*, indicated that *RIG-1* gene play antiviral defense against HIV by secreting type 1 interferons (IFN α and IFN β) in macrophages, and not in CD4 T-cells (Yizhong W et al, 2013). It would be exciting to investigate *RIG-1* and its susceptibility to HIV in CD4 T-cells for further purposes.

The protection against the HIV disease is also mediated throughout the chemokine receptor CCR5 by which the virus penetrate into CD4 T-cells for its replication (Fatima B et al., 2013). However, the truncation of CCR5 is involved in the absence of the receptor at the cell surface, so the entry of the virus into the cell is inhibited (Fatima B et al., 2013). Since the virus does not enter the cell, the susceptibility to HIV infection cannot exist and the disease lasts for many years to be developed, it is also one of many reasons why many peoples of different countries, cultures and ethnicities are not vulnerable to the HIV infection (Fatima B et al., 2007). Accordingly, a previous study demonstrated that among heterozygous people, the mutant CCR5 delta 32 delayed the disease, and showed lower level of expression on the cell surface than in homozygous individuals in which CCR5 receptor was totally absent on the cell surface (Lama et al., 2007). Therefore prevalence studies on any other gene polymorphisms such as CCR5 delta 32 in DRC population can provide a further data on the genetic pool in Congo involved in HIV susceptibility. This type of data, in future, can be used for genetic-based therapy approaches to stop HIV spread.

Our results for control group genotyping frequencies from this study (CC; 72.4%, CT; 24.1%, TT; 3.4%) were found to be similar to the Hap-Map genotypes frequencies (Table 4.3) (Y Lu et al., 2015), in which the comparison included people from different countries such as African ancestry from Southwest United-States (CC; 65.3%, CT; 28.6%, TT; 6.1%), Yoruba from Nigeria (CC; 59.3%, CT; 33.6%, TT; 7.1%) and female commercial sex workers from Kenya (CC; 75.9%, CT; 13.8%, TT; 10.3%) (Oyugi et al., 2009). The proximity of these results might be explained by the fact that African people could have the same ancestor

6. CONCLUSION

This is the first study studying the possible effect of CD4 C868T SNP on HIV susceptibility in DRC population using patients and control samples from Bandundu General Hospital (DR Congo). The total of 74 individuals subjects were divided into 2 groups; control group and patient group consisting of 29 and 45 subjects, respectively. The presence of the polymorphism was investigated by PCR using blood samples collected.

Our results showed that, C868T SNP has not any effect on HIV susceptibility to in DRC population. Further studies on CD4 C868T SNP are encouraged to be conducted including high number of patients in order to have a clear picture for the whole DRC population. Furthermore, researches on the effect of other genetic factors on the susceptibility to HIV infection will implement the development of new and more efficient drugs.

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