TURKISH REPUBLIC OF NORTH CYPRUS

NEAR EAST UNIVERSITY

HEALTH SCIENCES INSTITUTE

EVALUTAION OF *CCR5* GENE VARIATION FREQUENCIES IN NIGERIA AND ZIMBABWEAN POPULATIONS LIVING IN NORTH CYPRUS

BASIL CHUKWUEBUKANDIKOM

MASTER THESIS

MOLECULAR MEDICINE DEPARTMENT

Thesıs Supervısors:

Assoc. Prof. UMUT FAHRIOGLU

Assoc. Prof. MAHMUT CERKEZ ERGOREN

NICOSIA-2020

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THESIS APPROVAL

Directorate of Institute of Health Sciences

This work has been adopted as a master thesis in the program of molecular medicine by the jury.

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This thesis has been approved by the above jury members in accordance with the relevant articles of the NEU postgraduate education, training and examination regulations and has been accepted by the decision of the board of the Institute.

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COMPLIANCE AND APPROVAL

His master thesis “Evaluation of *ccr5* gene variation frequencies in Nigeria and Zimbabwean populations living in north Cyprus” was written in accordance with the NEU Postgraduate thesis proposal and thesis writing directive.

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DECLERATION

I hereby declare that I have no unethical behavior at all stages from the planning of the thesis to the writing, I have obtained all the information in this thesis within the academic and ethical rules, I cited all information and interpretations in the text and added these citations to the references part. I hereby, I did not violate patents and copyrights during the study and writing of this thesis.

Basil Ndikom

Signature:

Date:

DEDICATION

This thesis is dedicated to my loving family. I love you all.

ACKNOWLEDGEMENTS

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LIST OF ABBREVIATIONS

Ml	: Microliter
μM	: micromolar
nM	: nanomolar
HIV	: Human Immunodeficiency virus
AIDS	: Acquired immunodeficiency syndrome
CD	: Cluster of differentiation
CCR5	: C-C- type chemokine receptor
CXCR4	: C-X-C chemokine receptor 4
ART	: Antiretroviral therapy
DNA	: Deoxyribonucleic acid
PCR	: Polymerase chain reaction
LAV	: Lymphadnopathy associated virus
HTLV	: Human T-Lymphotropic virus
CDC	: Center for disease control
NACA	: Nigeria agency for control of AIDS
UNAIDS	: The joint United Nations programme on HIV/AIDS
PMTCT	: Prevention to mother to child transmission
MIPI	: Macrophage inflammatory protein
MCP2	: Monocyte chemo –attachment protein 2
RPM	: Revolution per minute
UV	: Ultra violet
BP	: Base pair
GP120	: Glycoprotein 120

Evaluation of *ccr5* gene variation frequencies in Nigeria and Zimbabwean populations living in north Cyprus

Basil Chukwuebuka Ndikom

Supervisors: Assoc. Prof. Umut Fahrioğlu

Assoc. Prof. Mahmut Çerkez Ergören

Department of Molecular Medicine

ABSTRACT

Aim: This study aimed at determining the *CCR5-Δ32* allele frequency in the Nigerian and Zimbabwean populations living in North Cyprus

MATERIALS: Chemicals :Agorose Biomax 100 mg, Ethidium Bromide(Serva, Heidelberg, Germany), Boric Acid (Serva, Heidelberg, Germany), Ethylenediamine tetraacetic acid (EDTA) (Serva, Heidelberg, Germany), Tris Base (Serva, Heidelberg, Germany),

KITS: Qiagen ,Thermo scientific 2x Master mix which include 0.05 U/µl*Taq* DNA polymerase, reaction buffer, 4 nM $MgCl_2$, 0.4 nM of each dNTP (dATP, dCTP, dTTP, dGTP) and 0.5 µM for forward and Reverse primer respectively

BACKGROUND: Cystine –cystine chemokime receptor 5 (*CCR5*) is the primary HIV co-receptor involved in the virus and its entry into the human cells. A variant of the *CCR5* gene known as *CCR5-Δ32*, which is a product of 32 base pair deletion in the gene. *CCR5-Δ32* plays a very important role in there infection, and progression of AIDS cause cells that possess homozygous *CCR5-Δ32* possesses no functional receptor site at their cell surface. This study was done to evaluate the *CCR5* gene variation frequency in the Nigerian and Zimbabwean population living in North Cyprus.

METHOD: The study population was made up of 103 Nigerians and 108 Zimbabweans. The sample was collected from Near East University Hospital; after an

informed consent was obtained from each of the participant .DNA was extracted from each sample using QIAGEN DNA extraction kit. Polymerase chain reaction was used in amplification of *CCR5* gene in each DNA in a Rotor-Gene –Q real time PCR cycler and there resolved on 4% agarose gel electrophoresis.

RESULTS: Out of the 103 Nigerians and 108 Zimbabweans sample assessed, all the sample were homozygous for the *CCR5* wild type gene (*CCR5* –wt) (100%), while none (0%) was homozygous for the *CCR5-Δ32* (Mutant gene), also no heterozygous was observed .

CONCLUSION: This study observed the absence of *CCR5-Δ32* deletion gene in the Nigeria and Zimbabwean population living in North Cyprus. This makes this population to lack the genetically advantage over HIV infection and rapid progression towards AIDS.

Keywords: CCR5, HIV, PCR, AIDS, CCR5 -Δ32

Kuzey Kıbrıs'ta yaşayan Nijerya ve Zimbabwe popülasyonlarında ccr5 gen varyasyon frekanslarının değerlendirilmesi

Basil Chukwuebuka Ndikom

Danışmanlar: Doç. Dr. Umut Fahrioğlu

Doç. Dr. Mahmut Çerkez Ergören

Moleküler Tıp Bölümü

ÖZ

Amaç: Bu çalışma Kuzey Kıbrıs'ta yaşayan Nijeryalı ve Zimbabwe popülasyonlarında CCR5-Δ32 alel sıklığını belirlemeyi amaçlamıştır

Malzemeler: Kimyasallar: Agorose Biomax 100 mg, Etidyum Bromür (Serva, Heidelberg, Almanya), Borik Asit (Serva, Heidelberg, Almanya), Etilendiamin tetraasetik asit (EDTA) (Serva, Heidelberg, Almanya), Tris Baz (Serva, Heidelberg, Almanya),

Kitler: Qiagen, Thermo bilimsel 2x 0.05 U / µTTaq DNA polimeraz, reaksiyon tamponu, 4 nM MgCl2, her dNTP'den 0.4 nM (dATP, dCTP, dTTP, dGTP) ve ileri ve geri primer için sırasıyla 0.5 uM içeren ana karışım

Amaç: Sistin –sistin kemokim reseptörü 5 (CCR5), virüse ve insan hücrelerine girişinde yer alan birincil HIV yardımcı reseptörüdür. Gende 32 baz çifti silinmesinin bir ürünü olan CCR5-P32 olarak bilinen CCR5 geninin bir varyantı. CCR5-Δ32, orada enfeksiyonda çok önemli bir rol oynar ve AIDS'in ilerlemesi, homozigot CCR5-Δ32'ye sahip hücrelerin hücre yüzeylerinde fonksiyonel reseptör bölgesine sahip olmamasına neden olur. Bu çalışma, Kuzey Kıbrıs'ta yaşayan Nijeryalı ve Zimbabwe nüfusunda CCR5 gen varyasyon sıklığını değerlendirmek için yapılmıştır.

Yöntem: Çalışma popülasyonu 103 Nijeryalı ve 108 Zimbabwe'den oluşmaktadır. Numune Yakın Doğu Üniversitesi Hastanesi'nden alınmıştır; katılımcının her birinden bilgilendirilmiş bir onay alındıktan sonra QIAGEN DNA ekstraksiyon kiti kullanılarak her örnekten DNA ekstrakte edildi. Polimeraz zincir reaksiyonu, her DNA'daki CCR5

geninin bir Rotor-Gene-Q gerçek zamanlı PCR döngüsünde çoğaltılmasında kullanıldı ve% 4 agaroz jel elektroforezinde çözüldü.

Bulgular: Değerlendirilen 103 Nijeryalı ve 108 Zimbabwe örneği örneğinden, tüm örnek CCR5 vahşi tip geni (CCR5 –wt) (% 100) için homozigotken hiçbiri (% 0) CCR5-Δ32 (Mutant gen için homozigot) idi), ayrıca heterozigot gözlenmemiştir.

Sonuç: Bu çalışma, Kuzey Kıbrıs'ta yaşayan Nijerya ve Zimbabwe nüfusunda CCR5-Δ32 silme geninin olmadığını gözlemledi. Bu, bu popülasyonun HIV enfeksiyonu ve AIDS'e hızlı ilerleme üzerinde genetik avantajdan yoksun olmasını sağlar.

Anahtar Kelimeler: CCR5, HIV, PCR, AIDS, CCR5 -Δ32

1. INTRODUCTION

Human immunodeficiency virus (HIV)/Acquired immunodeficiency syndrome (AIDS) which was first reported in early 1980s (Sharp et al., 2011) have been a major challenge to public health despite the campaign and advocacy in the rollback of HIV/AIDS. HIV is a retrovirus known to be the causative agent of AIDS. Due to the lack of an inactive retroviral vaccine against this virus, it has risen to be one of the most common public health challenges in the world (Chatterjee, 2010). The data obtained from Joint United Nations Program on HIV/AIDS (UNAIDS) and the World Health Organization (WHO) more than 37.9 million is infected with HIV infection in the world in 2018. According to the 2018 National Association of Control of AIDS in Nigeria (NACA), more than 1.9 million people are currently living with HIV with 130,000infected newly which makes Nigerian population to be ranked as the second largest HIV infected population in Africa behind South Africa, which has a population of the infected individuals at 7.1 million in the year 2018(NACA, 2017). Zimbabwe has 1.3 million people infected with HIV from the report of World Health Organization in 2018. HIV prevalence has been a great concern in the society because of its economic and health crisis (UNAIDS, 2019). HIV infection is mostly associated with progressive loss of the body's cellular immune system which makes the human body vulnerable to other life-threatening opportunistic infections, and the development of acquired immunodeficiency syndrome (Nsonwu-Anyanwu et al., 2017). Human immunodeficiency virus-1 (HIV-1) requires a primary receptor, CD4 receptor, and a chemokine co-receptor 5 (*CCR5*) or C-X-C motif chemokinereceptor 4(*CXCR4*)to enter the cell (Knipe et al., 2015). This chemokine and the natural receptor are the keys that lead to HIV-1 entering the human cell walls. The distinctive characteristics of HIV-1 infection is the gradual loss of CD4+ T cells, this leads to the loss of the body's immune system making the body vulnerable to opportunistic infection. HIV-1 which have the *CCR5*as its primary receptor are the predominant species isolated in the early stage of the infection, there has been significant heterogeneity in an individual's HIV susceptibility, the period of the time needed for the depletion of their CD4 lymphocytes and its progress to AIDS(Roy et al., 2016 and Buchbinder et al., 1994).Host genes collectively called AIDS restriction gene (ARGs): *CCR5* and *CXCR4* which are

needed in the binding of the infection to the cell receptors plays a very important role in the individual response to HIV-1 exposure, infection and its pathogenesis (Dean et al., 1996 and Samson et al., 1996). *CCR5* and *CXCR4*are both members of the G-protein coupled receptor (GPCR) family which is found mostly seen on the cell surface of monocyte, macrophages, dendrite cells and T cells (Lopalio, 2010). In the early period of the infection, *CCR5* HIV-1 is increased while the x4 strain appears in the late phase of the infection which causes a faster decline in the number of CD4 positive T-cells (Barmania et al., 2013). Both receptors have chemokines as their ligands and play an important role in multiple cellular processes such as development, angiogenesis, immune response, and leukocyte trafficking. Also, the *CCR5* receptor has the cc or beta chemokine as their ligands. The CCR5 wild type protein (CCR5-wt) consists of 32 amino acids long, with seven membrane-spanning regions, joined by three extracellular domains and three cytoplasmic domains. In addition to wild type, there is another allele with a 32 base pair deletion seen in the protein-coding region *(CCR5-Δ32).* This shorter protein encoded by this allele could not be observed on the cell surface (Solloch et al., 2017). *CCR5 -Δ3 2*variation has a significant effect on the entry of HIV-1 and its progression to AIDS. An individual who is homozygous for the *CCR5-Δ32*polymorphism are unaffected to HIV infection (Hutter et al., 2009). This resistance to HIV infection can only be seen on an individual with an R5 HIV strain (Alkhatib,2009). A heterozygous individual with *CCR -Δ32* allele shows a slower progression of the infection to AIDS when compared to a homozygous wild type individual (Angelis et al., 2007). HIV which enters into the human cells by its glycoprotein 120 receptor (gp120) and binds to the host cells receptor cluster of differentiation 4 (CD4+) on T lymphocytes, for its entry into the host cell there must be activation of the membrane fusion. The virus glycoprotein 41 (gp41) activates the fusion of the virus to bind to the host co-receptor which is either CCR5 or CXCR4 that facilitates the virus entry to the host membrane (Ferdousie et al., 2017). The *CCR5-Δ32*gene variation is mostly found in the European population, rare or no occurrence in African or Asian populations (Samson et al., 1996).

In this current study, I aimed to determine the CCR5-Δ32 gene allele frequency in the Nigerian and Zimbabwean populations that living in Northern Cyprus. Hopefully, this study will be a valuable addition to the literature and will also help the health

authorizes from the public health practice in North Cyprus and also countries of the populations that have been studied.

1.1 Human Immunodeficiency Virus (HIV)

Human immunodeficiency virus has been a major health challenge since it was first reported in 1980(Gilbert et al., 2003). Despite the intervention of many non-governmental organizations, governmental bodies, and other agencies, there is still a steep increase in HIV infection in Nigeria and Zimbabwe. HIV, which is the causative virus for AIDS has recorded a high mortality rate in African populations. HIV attacks the body's immune system and weakness the ability to fight infections and disease. The body's immune system which is made up of leukocytes or the white blood cells protects the human body from infections. CD4+ white blood cells which are helper cells or T-cells are the major targets of the HIV viruses (Cunningham et al., 2010).

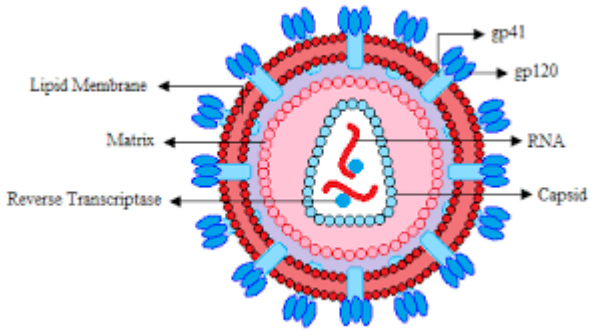

Figure 1.1: Structure of HIV (Adapted from Dawany, 2012).

HIV has two subtypes: HIV-1 and HIV-2.HIV-1 was the first virus that was discovered and called Lymphadenopathy associated virus (LAV) and human Lymphotropic virus 3 (HTLV-111). HIV-1 is more virulent and more infections than HIV-2, (Gilbert et al., 2003) and it is recorded globally.HIV-2 is commonly seen in West Africa (Reeves &Doms, 2002). HIV-1 was believed to have originated from southern Cameroon through the evolution of Siviz, a simian immunodeficiency virus (SIV) that infects wild chimpanzee species *Pantroglodteytroglodytes* (Zhu et al., 1998). The first well stated case of HIV in human was recorded in 1959, Belgian Congo (Zhu et al., 1998). Acquired immunodeficiency syndrome (AIDS) was first clinically recorded in the United State of America in 1981(Mandell et al., 2010). The first case of HIV were a cluster of injection of drug users and a homosexual man with unknown case of

impaired immunity system with expression of pneumocytes pneumonia which was a rare infection that occurs to an individual with compromised immune systems (Gottlub, 2006). After a while some homosexual men developed Kaposi's sarcoma which is a rare skin cancer (Friedman-kien,1981).These diseases caused the center for disease control and prevention (CDC) to investigate the outbreak. The CDC coined out a name for the infection by evaluating the infected communities. It was called the 4H disease which includes homosexuals, heroin users, hemophiliac and Haitians (Basvapathrumi &Anderson, 2007). In July 1982, after evaluating all the communities and seen that HIV/AIDS was isolated to the homosexual community, then the term AIDS was introduced. In 1983,two different teams Robert Gallo and Francouse Barre-Sinoussi and Luc Montagnier independently stated that a retrovirus may have infected AIDS patients(Gallo et al., 1983).In the report, Gallo examined a patient with AIDS, and he stated that AIDS has a very similar shape to other human T-lymphotropic viruses (HTLVs). He called this newly isolated virus HTLV-III.Francouse and Montagnier also examined a patient with swelling lymph nodes at neck as well a physical weakness which was few of the major symptoms expressed by AIDS patients opposed the finding of Gallo. She stated that the virus exhibit core proteins which are immunologically different from those Gallo recorded in HTL-1. She named the isolated virus lymphadnopathy-associated virus (LAV) (Basavapathrumi &Anderson, 2007). In 1986, after various research on two viruses (LAV and HTLN-III); it was well understood that they were the same virus, and renamed as HIV (Nobel Prize, 2008).

1.2 Symptoms

HIV exhibits different symptoms in infected individuals as well as depending on the disease. There are three different stages of the disease, and they manifest various symptoms: Acute HIV infection stage is the foundational stage in HIV infection that can be asymptomatic; in some cases it might show many symptoms. Within the first two to six weeks after infection the body’s immune system responds to the foreign body (virus). This response is called acute retroviral syndrome or initial/primary HIV infection. The common symptoms are normally like viral flu which may not last up to one week or two weeks. Other symptoms at this stage might include sore throat, headache, body rash, fever (raised temperature), swollen glands, joint aches and pains,

muscle pain, mouth ulcer (NACA 2019). These symptoms occur due to the production of HIV antibodies by the immune system. It is called Sero-conversion, and it occurs within 45days of infection and can take up to a few months to be complete. In stage II or asymptomatic stage/clinical latency/ chronic viral infection stage, sero-conversion has been completed, and the individual may be asymptomatic for many years. This stage can last up to ten years. The virus will be active, making copies, infecting new cells, and destroying the CD4 cells which cause a lot of destruction to the body's immune system. Stage III or the symptomatic HIV infection is started when the HIV infection has damaged the body's immune system, and it will progress to AIDS. The CD4 T-cell number falls below 200, which exposes the immune system to be vulnerable to opportunities infection, which includes pneumocystis pneumonia, Kaposi's sarcoma, and AIDS. Other symptoms include night sweats, swollen lymph nodes, shortness of breath, severe, long-lasting diarrhea, yeast infection in the mouth, throat, and vagina, purplish spots on the skin, fever, persistent cough, regular infections, extreme and unexplained tiredness, neurologic disorders (NACA 2019).

1.3 Transmission of HIV

HIV can only be spread through specific routes. In Nigeria which has a mixed epidemic, the groups that are most infected are the sex workers, homosexuals, drug users. These groups consist of 3.5% of the population, yet the population accounts for 32.0% of HIV infection (Rozembaum& Won, 1982). In sexual transmission, infection occurs when there is contact with infected sexual secretion during unprotected sexual intercourse with an infected individual. Sexual secretion includes vaginal secretions, rectal, genital or oral mucous membrane. Also, in prenatal transmission, this is the transmission of the virus from an HIV infected mother to the child through childbirth, pregnancy or breastfeeding. There have been accounts of HIV transmission through blood transfusion. Other routes of transmission include sharing of needles, syringes, and other injecting equipment. Also, health workers can accidentally be infected themselves when handling or treating patients with the infection. In Nigeria, homosexuality accounts for 23.0% of new infections while sex workers were recorded as14.4% in 2014(Garg et al 2012).

1.4 Prevalence of HIV

HIV/AIDS has risen to be a world /global pandemic and a major health threat. In 2018 more than 37.9 million are infected with HIV globally (Cohen et al., 2008). More than 21.0% of people are aware of living with the virus (UNAIDS 2019). Since the inception of the HIV/AIDS epidemic, more than 74.9 million people have been infected with 32 million people death cases relating to AIDS has been recorded. In 2018, 777,000 people had died of AIDS-related illnesses diseases. The major part of people living with HIV can be seen in low, middle income and developing countries In sub-Sahara African which is the worst-hit region that having 12.0% of the world population living with HIV infection meaning 23.8 million people living with HIV (WHO, 2017). Nigeria has the second highest number of HIV epidemic in the world (NACA, 2017). 1.5% less prevalence than other sub-Sahara African countries including South African (20.4%) and Zambia 11.3%.In 2018, 1.9 million people were currently living with HIV infection in Nigeria (UNAIDS. 2019). the USAID review in 2018 indicated that Nigeria accounted for two-thirds of the new infection in sub-Sahara African.

Table.1.1: The table indicates the HIV prevalence in Nigeria (adapted fromUNAIDS Data 2019)

	Nigeria (2018)
1.9 m	People living with HIV
1.5%	Adults HIV prevalence (15-49)
130,000	New HIV infections
53,000	AIDS –related deaths
55%	Adults on antiretroviral treatment
35%	Children on antiretroviral treatment

One of the worst-hit for HIV in sub-Sahara Africa is Zimbabwe. Approximately, 12.7% of the population, 1.3 million people, living with HIV (UNAIDS, 2019). The HIV epidemic is commonly driven by unprotected sexual intercourse and women and adolescent girls are the most affected gender in Zimbabwe (UNAIDS, 2018).

Table 1.2. The table illustrates HIV prevalence map in Zimbabwe (Source UNAIDS Data 2019)

Zimbabwe (2018)

1.3m	People living with HIV
12.7%	Adult HIV prevalence (age 15 – 49)
38,000	New HIV infections
22,000	AIDS-related deaths
89%	Adults on antiretroviral treatment
76%	Children on antiretroviral treatment

According to UNAIDS 90-90-90 program objectively states that by 2020, 90% of all the people living with HIV will know their status;90% of all the people with diagnosed HIV infection will receive antiretroviral drug and 90% of all people receiving antiretroviral therapy will have viral suppression.90-90-90 UNAIDS was revealed that only 67% of infected individuals were aware of their status, 53% of the infected individuals were receiving treatment while 80% of the infected individuals receiving treatment were virally suppressed in Nigeria. Six states that are mostly infected individuals were seen was Lagos, Benue, Kaduna, Kano, Oyo and Akwa Ibom (NACA, 2017). Its prevalence is high in the south which accounts for 5.5% and the southeast zone with the lowers rate of 1.8% (NACA, 2015). This is one of the main reasons of this study that studied different Nigerian ethnic backgrounds.

90-90-90 UNAIDS was impressive in Zimbabwe which indicated that 90% of people living with HIV were aware of their status, 95% of them were receiving HIV treatment and 87% of them were having there viral load suppressed (UNAIDS, 2019). Despite the challenges in the spread of HIV worldwide, there have been global efforts to make the treatment of HIV affordable and available to the developing countries. In 2018, 62% of people living with HIV infection were able to assess treatment, 62% of the people living with HIV were received retroviral treatment (ART). In Nigeria, the national strategic framework by the national agency for control of AIDS (NACA) has been a guide in the management of HIV in the country.

1.5 The C-C chemokine receptor type-5 *(CCR5)* Gene

C-C chemokine receptor type -5 (CCR5) or CD195 is a protein that can be seen on the cell surface of the white blood cells. They are one of the major factors in the immune system as acting as chemokines receptors (Jiao et al., 2019). The *CCR5* gene is located on the short (p) arm on chromosome three in humans.

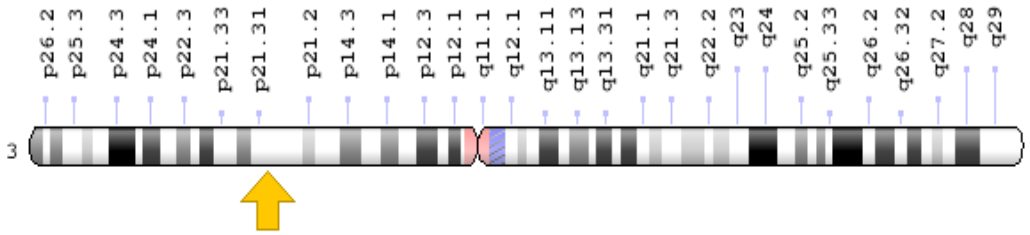

Figure 1.2: The *CCR5* gene location (This figure is adapted from Thomas, 2006).

The CCR5 protein is a member of the beta chemokine receptor family of integral membrane protein (Samson et al., 1996) which has the seven-transmembrane protein that is similar to G- protein-coupled receptors. It acts as a chemokine receptor in the c-c chemokine group. The c-c chemokine receptor ligands receptors includes macrophage inflammatory protein I alpha(MIP-I alpha), monocyte chemo attractant protein 2 (MCP2), macrophage inflammatory protein I beta(MIP-Ibeta), regulated on activation normal T expressed *CCR5* genes(RANTES) which are expressed on the macrophages, T cells, eosinophils, dendrite cells, microglia. It have also been detected in promyeloblastic cell line (Velasco-Velazquez, 2012).CCR5 is also selectively induced during cancer transformation but is not expressed in prostate or breast epithelial cells. More than 50% of triple-negative breast cancers express CCR5 (Velasco-Velazquez, 2012). The *CCR5*genealso serves as a co-receptor for the entry of macrophage-tropic strain of HIV.

1.6 Functions of the*CCR5*Receptor

Due to its major expression in the cell surface, it has many functions in HIV, where it acts as the major co-receptor with *CXCR4* for the virus to have entered into other cells. HIV-I envelope expresses a glycoprotein structure which enables the virus to have entered into the human cell(Alkinatib, 2009). These envelope glycoprotein's have two protein subunits which are the GP120 external subunit and the GP41 trans-membrane subunit (Alkinatib, 2009). The GP120 external subunit envelope protein has a chemokine mimic (Murphy, 2001).Even though it lacks the structure of a chemokine; it still has the ability to bind the *CCR5* and *CXCR4* chemokine receptors (Murphy, 2001). When the body is infected with HIV-I, the GP120 envelope glycoprotein subunit attach to the CD4 glycoprotein and the co-receptor (*CCR5* and *CXCR4*) which are expressed on the cell surface forming a heterotrimeric complex which stimulate fusogenic peptide. This allows the viral membrane to have entry into the membrane of the human cell (Murphy, 2001). This makes *CCR5* to be essential in the attachment of the R-5strain of HIV-I in the human cells (Lieberman-Blum et al., 2008). Thus, many investigations focused on the therapeutic agents' development on blocking *CCR5* function. An HIV drug called *CCR5* receptor antagonists is also be experimented to use to block the binding of GP120 envelope protein and HIV co-receptor *CCR5*(Lieberman-Blum et al., 2008).

In cancer, *CCR5* expression is induced in prostate and breast epithelial cells during transformation. This induction promotes migration, invasion, and metastasis. Some of *CCR5* inducing drugs like leronlimab and maraviroc have positive results of blocking of lung metastasis when used on breast cancer cell lines. Additionally, in preclinical structure, *CCR5* inhibitors were used in mice bone and brain metastasis (Sicoli et al., 2014). It has been also used to lower the penetrance of tumor cells connected with macrophages (Frankenberger et al., 2018). Maraviroc which is a *CCR5* inhibitor drug has also been used to increase inflammatory responses in stroke patients.*CCR5* have also shown to induced normal migration and connection in developing brains, which plays a very major role in stroke patient by decreasing the number of connecting/binding site on the neurons of the brain where the damage occurs (Berg, 2011).

1.7 TheCCR5-Δ32 polymorphic variation

CCR5-Δ32 is an allele of the *CCR5* gene. *CCR5 -Δ32* which is a 32 basespair (bp) deletion in the stop codon region of *CCR5* receptor locus, which causes the receptor to lose its function (Dean et al., 1996).The receptor site which is required for the entry of the M tropic strain of HIV-1 in the human cells. Individuals who are homozygous for the *CCR5-Δ32*allele do not express a functional *CCR5* receptor site on their cell surface, thus they are resistant to HIV-1 infection even when they are exposed to the virus (Liu et al., 1996). In a heterozygous individual with *CCR5-Δ32*polymophismhave a reduction in their functional *CCR5* receptor in their cell surface because there is a dimerization between the wild type and mutant receptors, which affects the distribution of *CCR5* to the cell surface(Benkirane, 1997).In an individual with heterozygote allele, they are resistant to HIV-1 infection that is related to wild type and also shows lower viral loads and slower progress to AIDS relative to wild types (Dean et al., 1996).In Europe, they have a frequency of 10% heterozygote and 1% homozygote *CCR5-Δ32*frequency. In pre-clinical research in mice, shows that the removal of *CCR5* improves their memory. This makes *CCR5* a power tool in suppressing neuronal plasticity, memory, and learning. In the studies of (Galvani, 2005, Lucotte 2010) it shows that*CCR5-Δ32*exhibit a district geographic distribution. They got the highest frequency in Nordic countries and the lowest frequency in the Sothern Europe. A higher frequency has also been recorded in Varangians in Russia which was a result of Viking dispersal in the 8th -10th century (Lucotte, 2001). The *CCR5-Δ32* allele have a high frequency in some European populations but is recorded to be absent in African, Asian, Middle Eastern and American Indian populations (Stephenes et al., 1998).

1.8 HIV and CCR5

CCR5 which came into the research limelight in 2009 when it was used in the treatment of the Berlin patient when he got leukemia treatment. He was transfused with a stem cell transplant using *CCR5-Δ32/ CCR5-Δ32* cell. After the transplantation, the HIV became undetectable from his blood and bone marrow without receiving Retroviral drug treatment (Corbyn, 2012; Gonzalez et al., 20011).DKMS (Germany, Poland, and UK) also collected samples from potential hematopoietic stem cell donors having

implemented the genotyping routine to newly register donors (Gupta et al., 2019; Hutter et al., 2009).Solloch et al., (2017) also studied *CCR5 -Δ32* allele frequency in 87 countries. Recently, Fahrioglu et al., (2019) investigated the frequency of the *CCR5-Δ32* gene polymorphism in the Turkish Cypriot population and giving only 3.0% allele frequency in only heterozygous state. Okoroiwu et al., (2015) studied *CCR5* profile in HIV infected patients who undergoing ART in Nigeria.

Moreover, new techniques have also been developed designing*CCR5*knowdown gene expression by gene therapy using zinc finger nuclease(ZFN), CRISPR/Cas9 and transcription activators like effector nuclease (TALEN) system (Symads et al., 2015). Yu et al., (2017) have also created a double knock out system for *CCR5* and *CXCR4* genes in circulating CD4 with the help of CRISPR/Cas 9. Worth to mention that this technique can lead a future in HIV prevention. Jian Ku He in November 2018 recorded his success when he successfully edited two human embryos which he disabled the *CCR5* gene. Lulu and Nana were born from first genetically edited embryos. However, they have both functional copies of *CCR5*as well as knockdown *CCR5* (Mosaicism), which makes them still vulnerable to HIV. There is a huge debate of this work as might be dangerous, unethical, and premature (Begley, 2018).

1.9 Aim of the study

There is still lack of information about the*CCR5 -Δ32* gene frequency in Nigerian and Zimbabwean populations in the literature. Therefore, in this current study, I aim to determine the *CCR5-Δ32* allele frequency in the Nigerian and Zimbabwean populations living in North Cyprus.

1.10 Significance of the study

The finding of this study will help in understanding the distribution of the *CCR5-Δ32* polymophism in the three different Nigerian ethnic groups and Zimbabwean population, also the study will help to public health organization, and other health allied bodies in North Cyprus to gain more insight and deep understanding of the distribution of the frequency.

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Suppliers

Thermo Scientific (Pittsburg, USA), Qiagen Ltd. (Crawley, UK), Eppendorf Scientific (Hamburg, Germany), Bio-Rad (HemelHemstead, UK), New England Biolabs (Hitchin UK), Philips Telecommunicatieeninformatie system (Amsterdam, Netherland),CHC LAB Co., Ltd. (Daejeon, Korea) Cleaver Scientific Ltd. (Warwickshire, UK), Wealtec Corp Ltd. (Nevada, USA), Centurion Scientific Ltd. (West Sussex,UK), HermleLabnet (Wehingen, Germany).

2.1.2 Chemical Reagents

2.1.2.1 Molecular Weight Markers

ThermoScientificGeneRuler 100bp (SM0241, ThermoScientific, Pittsburg, USA) 100-1000bp DNA Ladder

2.1.2.2 Oligonucleotides

200 nmol (MOPC) primers were ordered from Macrogen (Seoul, South Korea).

2.1.2.3 Human DNA

A venous peripheral blood was collected from 103 Nigerian (60male and 43female) and 108 Zimbabwean (56 male and female 52) in a 5µl EDTA vacuum tube at the Near East University Hospital from approval the Near East University Scientific Review Board (SBE/2020-153-20). Additionally, blood samples were donated by volunteers and each participant filled an informed consent form. Blood DNA samples were prepared under high containment to minimize the risk of contamination; in a class II laminar flow hood using designated pipettes. All solutions used for DNA preparation were UV treated to disable the amplifiability of any potential contaminating DNA.

2.1.2.4 Standard solutions

10x Tris-borate/EDTA (TBE) electrophoresis buffer were as described by Sambrook et al 1989

Thermo scientific 2x Master mix which include 0.05 U/µl*Taq* DNA polymerase, reaction buffer, 4 nM $MgCl_2$, 0.4 nM of each dNTP (dATP, dCTP, dTTP, dGTP) and 0.5 µM for forward and Reverse primer respectively.

2.1.2.5 Other chemical agents

AgoroseBiomax 100 mg, Ethidium Bromide(Serva, Heidelberg, Germany), Boric Acid (Serva, Heidelberg, Germany), Ethylenediamine tetraacetic acid (EDTA) (Serva, Heidelberg, Germany), Tris Base (Serva, Heidelberg, Germany).

2.1.3 Computers

Data and images were stored and processed using software packages Microsoft Office.

2.2 Methods

2.2.1 DNA Extraction from Blood

DNA was extracted using Qiamp DNA mini kit (Hilden Germany). All the frozen blood samples brought to room temperature (15-25°C). 20µl (w/v) of proteinase K was pitted into a 1. 5ml micro centrifuge tube, about 200µl of the whole blood was added into it. 100µlof Buffer AL (genomics lysis buffer) was added. Then, the samples were mixed by pulse –vortexing for 15seondsandwere incubated at 56°C for 10minutes.After the incubation samples were briefly centrifuged briefly, 100µl of 96% (v/v) ethanol were added to for each tube, then mixed by pulse –vortexing for 15seconds,. The mixture was transferred into a QIAamp Mimi spin column and centrifuged at 8000rpm for a minute; the flow-through and the filtrate/collection tube were discarded. The QiAamp Mimi tube was transferred to a new collection tube and 250µl of Buffer AW1 was added and centrifuged at 8000rpm for a minute, after then

the collection tube was discarded and the QiAmp mini tube was transferred into a new collection tube. 250µl of Buffer AW2 were added and centrifuges for 14.000rpm for three minutes. The spin column was transferred to a 1.5ml micro-centrifuge incubated at room temperature (24^0C) for two minutes. Lastly,50-100µl of Buffer AE(Elution Buffer) was added to the spin column and centrifuged at 8000rpm for a minute to elute the DNA.

2.2.2 Measuring DNA Concentration

The DNA concentration was estimated by measuring optical density at wavelength of 260 nm (OD_{260}) by Nanodrop (Thermo scientific, Pittsburg,USA).

2.2.3 The *CCR5* gene PCR Amplification

Generally, polymerase chain reaction (PCR) DNA amplification was carried in 50µltotal reaction volumes in 200 µl tubes on a RotarGene Real Time PCR (Qiagen,Hilden, Germany). All PCR reactions were carried out in a category II laminar flow hood to limit contamination; moreover, all reagents and plastic ware were PCR clean and all pipettes dedicated to PCR clean.

To amplify 5-10 ng DNA template, 25µlPCR Master Mix (2X) (Thermo Scientific, Waltham, Massachusetts, United States) which includes 0.05 U/µl Taq DNA polymerase, reaction buffer, 4 nM $MgCl_2$, 0.4 nM of each dNTP (dATP, dCTP, dTTP, dGTP) and 0.5 µM of each primer were mixed per reaction. The *CCR* gene region containing possible Δ32 variation were amplified using the following flanking primers 5'-CAAAAAGAAGGTCTTCATTACACC-3' and 5'-CCTGTGCCTCTTCTTCTCATTTCG-3'(Angelis et al., 2007).The PCR condition used for the amplification were follows 94°C for three minutes, 94°Cfor45 seconds, 55°C for 45 seconds 72°C for 15seconds, 72°Cfor 5minutues and repeated five cycles, then 35 cycles of 94°Cfor 30seconds, 60°Cfor 30seconds, 72°Cfor 30seconds and final step was 72°Cfor10 minutes.

2.2.4 Agarose Gel Electrophoresis

Sigma Agarose(Merck KgaA, Darmstadt, Germany)was used to make gels at a concentration of 3% (w/v) and sizes 20 cm x 20 cm. PCR products were run using horizontal submarine format with 0.5 x TBE (44.5 mMTris-borate (pH 8.3),1 mM EDTA) buffer containing 0.5ug/mlethidium bromide. ¼ volume of loading dye (Thermo Scientific, Pittsburg, USA) was added to each sample prior to loading between 1-5 µl into the well. The samples were electrophoreses alongside markers of known size using electrophoresis machine (Bio-Rad,Hemel Hemstead,UK). DNA bans were visualized using an ultraviolent trams-illuminator (DNR Bio Imaging system, Neve Yamin, Israel) Photographic records were taken

3. RESULTS

3.1 Introduction

With the recognition of chemokine receptor type 5 (CCR5), the entry receptor site for human immunodeficiency virus in to the human cells, efforts have been made in other to use the site as a turning point in the prevention and treatment of HIV infections. The deletion of the 32 base pairs in the *CCR5* gene (CCR5-Δ32) results in a truncated dysfunction prototype that does not expresses on the cell surface. In individuals with homozygous CCR5-Δ32 variation, it is extremely rare to get HIV infection and even if it occurs, it caused by viral strain that uses CXCR4 for viral entry (Gorry et al., 2002). In heterozygous individuals who have a wild type allele and a *CCR5 -Δ32*allele might be at lower risk for HIV infection (Samson et al., 1996), but may exhibit it attenuated course when infected with HIV infection (Cocchi et al., 1995), with lower levels of plasma uremia. In this study, we aimed to evaluate the *CCR5 -Δ32*allele frequency in the Nigeria and Zimbabwean population living in North Cyprus.

3.2 General characteristics of studied populations

3.2.1 Nigerian Cohort

Total 103 Nigerian subjects whose are currently residing in North Cyprus have been studied. This studied group was consisted of 60 male (58%) and 43 female (42%) Nigerians. A total mean age was 25.7. There was no any statistically difference *(p= 0.292)* between the mean age of male (25.2 ± 4.8) and the mean age of female (26.2 ± 4.8) (Table 3.1).

Table 3.1 The table illustrates a total mean age and the mean ages of male and females in Nigerian cohort.

Age (mean)	25.7	*Pvalue*
Male	25.2 ± 4.8	P= 0.292
Female	26.2 ± 4.8	

Nigerian population is from different ethnicities. Previously, the *CCR5-Δ32* gene variant has been studied in Nigerian population by Solloch et. al., (2017). However, they have not specified the ethnic origin of Nigerians in their cohort. Thus, we have divided our Nigerian cohort in three major ethnicities: Hausa, Igbo and Yoruba.

First group was 26 (24.5%) Hausa subjects with 16 (15.5%) males and 10(9%) females, second group was 45 (44.5%)Igbo subject with 27(26%) males and 19 (18.5%) females and the last group was 32 (31%) Yoruba individuals consisted of 17 (16.5%) male and 15 (14.5%) female subject. Table 3.1 shows the distribution of individuals according to their ethnicities in Nigerian cohort.

Table 3.2 The table shows the distribution of studied Nigerian individuals according to their ethnicities.

Ethnic Groups	Number of samples	Male (%)	Female (5)
Hausa	26	16 (15.5%)	10 (9%)
Igbo	45	27 (26%)	19 (18.5%)
Yoruba	32	17 (16.5%)	15 (14.5%)
Total	103	60 (58%)	43 (42%)

3.2.2 Zimbabwean Cohort

Total 108 Zimbabwean subjects whose are currently residing in North Cyprus have been studied. This studied group was consisted of 56 male (52%) and 52 female (48%) Zimbabwean. A total mean age was 24.6. There was no any statistically difference *(p= 0.291)* between the mean age of male (25.4 ± 4.8) and the mean age of female (24.6 ± 4.8)(Table 3.3).

Table 3.3 The table illustrates a total mean age and the mean ages of male and females in Zimbabwean cohort

Age (mean)	24.6	*Pvalue*
Male	25.4 ± 4.8	P= 0.291
Female	24.6 ± 4.8	

3.3 Genotyping

The region of the *CCR5* gene containing the Δ32 deletion was amplified using the following flanking primers by Angelis et al., (2007). The expected fragments from the wild type (WT) and the Δ32 allele were 189 and 157 bp, respectively (Figure 3.1). A homozygous WT individual will only display the 189 bp band, a heterozygous individual will display both the 189bp and the 157 bp band and a homozygous mutant individual will only display the 157 bp band.

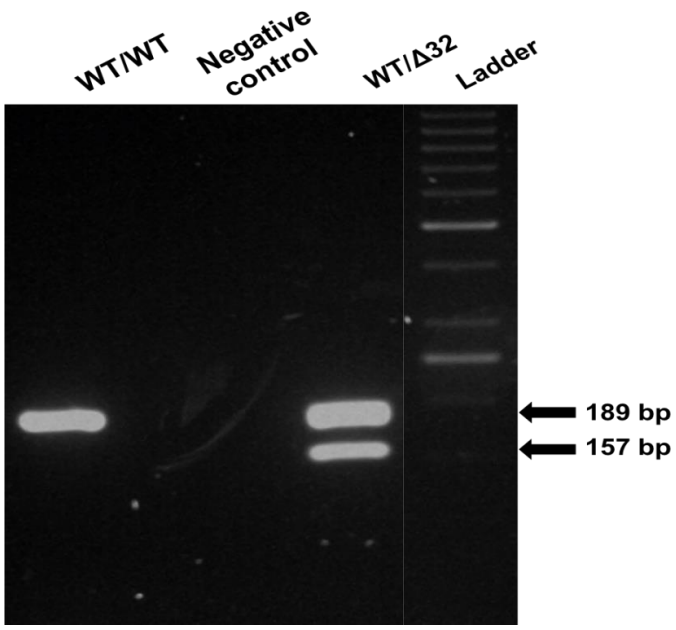

Figure 3.1 AgaroseGel picture showing WT and Δ32 allele bands, 189 bp and 157 bp respectively in a WT and heterozygous individual. The negative control has no bands as expected.

3.3.1 Genotyping results from Nigerian population

The subjects comprised 103 Nigerian of which of 60 male(58%) and 43 females (42%). Genotype distributions and allele frequencies of the *CCR5* gene Δ32 variant are shown in Table 3.2 There was no determined *CCR5*Δ32 allele in the studied Nigerian cohort. Therefore, Hardy-Weinberg Equilibrium could not be used for the allele distribution analysis (p= 0.00), X^2=0.00).

Table 3.4 Genotype distributions and allele frequencies of the *CCR5* gene Δ32 variant in the studied Nigerian Cohort

	WT/WT	WT/Δ32	Δ32/Δ32	X2	*p-value*
Observed	103	0	0	0	0
Expected	103	0	0		
WT allele freq.	1%				
Δ32 allele freq.	0%				

3.3.2 Genotyping results from Zimbabwean population

The subjects comprised 108 Zimbabwean of which of 56 male(52%) and 52 females (48%). Genotype distributions and allele frequencies of the *CCR5* gene Δ32 variant are shown in Table 4.2 There was no determined *CCR5*-Δ32 allele in the studied Zimbabwean cohort. Therefore, Hardy-Weinberg Equilibrium could not be used for the allele distribution analysis (p= 0.00), X^2=0.00).

Table 3.5 Genotype distributions and allele frequencies of the *CCR5* gene Δ32 variant in the studied Zimbabwean Cohort

	WT/WT	WT/Δ32	Δ32/Δ32	X2	*p-value*
Observed	108	0	0	0	0
Expected	108	0	0		
WT allele freq.	1%				
Δ32 allele freq.	0%				

3.4 Discussion

A total 103 Nigerians and 108 Zimbabwean aimed to determine the allele frequency of theCCR5-Δ32 gene variant in both populations. The entire assessed sample, were homozygote for the wild type CCR5 –wt .No single CCR5-Δ32 allele type has been observed neither homozygote nor heterozygote status.

The results indicated that the mutant frequency of CCR5-Δ32is low/absent in all the ethnic groups in the Nigerian and Zimbabwean populations.

4. DISCUSSION

4.1 Introduction

AIDS which remains one of the complex public health challenges in the world is a complex infectious disease that induces weakness of host immune responses, HIV infection and gene-environment interactions. In numerous studies reported both host genetic factors and viral genetics are the main important determinates of HIV-1 infection (Mc Laren and Carrington, 2015). HIV which is the virus that causes AIDS can be transmitted from one person to another through semen, vaginal secretion, infected blood, and mucousmembrane, pregnant woman to the baby during pregnancy, child birth, or breast feeding. AIDS is classified as a progressive deterioration of the immune system of the infected person. There is a progressive depletion of the CD4 T Lymphocytes which is the major target of the virus. The continuous rise in the population of people living with HIV makes it to be a great health challenge in the world. According to United Nations joint program on HIV/AIDS shows that more than 37.9 million are infected with HIV across the world in 2018, while in Nigeria population 1.9 million people are infected and 1.3 million infected in the Zimbabwean population.

Human immunodeficiency virus -1 enters into the immune cells cause macrophages and T cell posses CD4 proteins (Stephens et al., 1998). For the attachment and entry the virus into the target cells it requires a primary receptor CD4 receptor *(CXCR4)* and a chemokime co-receptor 5 (*CCR5)*. On attachment of the virus to the target cell, there is a cell interaction that is mediated by their host cell CD4 antigen and binds to the 20glycoprotein on the outer envelope of HIV. These helper cells are the main target of HIV cause they possess high number of CD4 molecules on their cell surface, these makes then to have high binding affinity (Tresoidi et al., 2002).Other cells such as langerhans cells, macrophages, dendrite cells, monocytes, and microglia brain cells possess CD4 on their cell surface.HIV can be grouped into two types, T –tropic or x4 strains, they are the HIV that infects T cells only and M-tropic or R5 strains are the HIV that infects both the macrophages and the T cells. *CCR5*is required for the entry of the virus into their macrophages which cause a conformational change in the 41 glycoprotein leading to the fusion of the virus to the cell membrane. At the cell

membrane the virus is taken into the cell and uncoiling of the particles exposes the viral genome (Easterbrook et al., 1999).

The enzyme reverse transcriptase releases complementary DNA from the viral RNA. The DNA forms an integrated part of the host cells genome, which is known as provirus. This provirus remains on the latent stage for a long length of time and causes no virus replication. Cytokines induces viral replication of the virus when they come in contact with the infected host cells. The viral DNA found in the nucleus transcript into the genomic RNA and messenger RNA (mRNA) which are moved into the cytoplasm. During this process translation of mRNA take place, with the assembly of viral particles and production of viral proteins. This leads to the infected virons been release for the host cells membrane and take up their envelope, were they spread to other cells.*CCR5 -Δ32*are mutate allele of *CCR5*having a 32 bp deletion which makes the cells to loss there binding site on the cell surface. These mutations are commonly found in the Europeans and Western Asian with higher frequencies in the Northern European (Rizzardi et al., 2002). Individuals that are homozygous carries of *CCR5-Δ32* mutations are resistances to HIV infection because they do not express a functional receptor site for*CCR5* used by the HIV-1 to enter CD4 cells. In individual that are heterozygous, they are associated with lower CD4 viral load and a slower progression to AIDS. Cause of their characteristic chemokime receptors gene *CCR5* and its mutation (*-Δ32*) have become the object of intense interest with their roles in the entry of HIV-1 into the target cells have been undefined. Deen et al., (1996)examined the progress of HIV to AIDS in the US population with difference exposure to HIV ranging from intravenous drug users, persons with hemophilia, and homosexuals, he observed the heterozygous individuals of *CCR5-Δ32* had two years delay in the progression to AIDS when compared with the those with homozygous wild type (Deen et al., 1996). This shows that heterozygous*CCR5-Δ32*(*CCR5-wt/ CCR5 -Δ32*) are not immune from HIV-1 infection but have a slow progression to AIDS.

CCR5 -Δ32 have also been employed as diagnostic tool in detection of diseases; Mamacio et al., (2007) used *CCR5 -Δ32* in the evaluating of pathogenesis of multiple sclerosis (ms). In patient with symptomatic west Nile virus infection *CCR5 -Δ32* allele is over expressed (Lim et al., 2006) and also associated with server

meningoencephalitis in tick borne encephalitis virus infection**(**Kindberg et al., 2006). In Turkish population *CCR5-Δ32*allele seems to be associated with resistance to crimca-congo hemorrhagic fever (CCHF) virus infection (Rustenoglu et al., 2017) .In autoimmune diseases such as rheumatoid arthritis increased leve3l of *CCR5* ligands such as *CCL5, CCL4,*and *CCL3* are seen in the synovial fluid (Patel et al 2001). The CCR5 variants are seen to be protected from disease (Pokorny et al., 2005). In atherosclerosis,*CCR5* also plays a role; they have been distributed as a non redundant, essential receptor for the horning of CD4 T cells that exacerbate atherosclerosis (Vandenseh et al., 2003)

In this study, the frequency of Δ32 allele in the all participated Nigerian and Zimbabwean populations living in North Cyprus resulted *CCR5* –WT homozygote wild type genotype.

Solloch et. al. (2017), studied the *CCR5-Δ32*allele frequency in 87 country populations including 160 Nigerian individuals. Our results concurred with their finding giving 0.00%CCR5-Δ32 allele frequency. However, Zimbabwean population was not included in their global study; therefore we have studied the population of Zimbabweans.

4.2. The frequency of the CCR5-Δ32 gene variation in Nigerian populations

Nigeria been the most populated country in Africa with a population of about 200 million people has three major ethnic groups. The Hausa that occupies the northern region of the country, the Yoruba which settle in the south west, and the Igbo's which are indigenous of the south east part of the country. In this study the Hausa made up 24.5% of the study population, the Yoruba's 31% while the Igbo's 44% of the population, from the results, No homozygous mutant of *CCR5* 32 was see , this concurred to the finding of Solloch et al., (2017) were he had no/absent level of *CCR5 -Δ32* in the Nigeria population. Also, Okoroiwu et al., (2015) had low/absent of *CCR5-Δ*32 in their study in calabar Nigeria. This shows that the distribution of *CCR5 -Δ32* in Nigeria ethnicity is not significant.

4.3. The frequency of the CCR5-Δ32 gene variation in Zimbabwean populations

Zimbabwean which is country located at the Sothern African region with a population of 14million people is ranked number 74 in the list of countries and dependency by population. In this study 108 Zimbabwean with male 52% and female 48%, represented the number of Zimbabwean living in North Cyprus. From the study no homozygous or heterozygous CCR5 32 was seen, this shows that the frequency of *CCR5 -Δ32* is low /absent in the population and this concurred to the findings that *CCR5 -Δ32* is low /absent in the Africa population.

4.4. The frequency of the*CCR5 - Δ32*allele in other populations

Solloch et. al.,(2017)observed that the *CCR5 - Δ32* allele were seen more frequent in the Northern European populations with a decline frequency observed in the southwards and eastwards, with no or rare occurrences in African, Asians, Americans and Oceania. Martinson et al.,(1997) and Okoroiwu et al., (2015)also recorded low/absent of the *CCR5 -Δ32*variation in the African region. In contrast, the *CCR5 - Δ32*allele has been reported to have a high frequency in North Europe. Solloch et al., (2017) stated that the Northern Europe especially the Baltic region of Sweden, Estonia, Finland, Belarus, and Lithuania have a high frequency in homozygote *CCR5 -Δ32*. Other cities with higher frequency of homozygote *CCR5 -Δ32* mutation includes the Russian cites of Moscow, northern coast of France, Volga Ural region of Russian and Ryazan (November et al., 2005).

The allelic distribution of the *CCR5* gene wild type in other populations were: 98.21% in Greek Cypriots (Salem et al., 2007), 75.56% in Russian (Salem et al., 2007), 91.22% in Jordanian (Salem et al., 2007),87.5% in Turkish(Martinson et al ., 1997), 97.16% in Syrian, 100% in Yemen (Martinson et al ., 1997) and 97.9% in Kuwaity, 100% in Sudanese (Su et al ., 2000) and 100% in Kenyan (Martinson et al.,1997). Recently, Umut Fahrioğlu and Mahmut Çerkez Ergören (2019) have studied the determination of genotypic distributions and allelic frequencies of the *CCR5* gene variations. They observed approximately 3.0% of allelic frequency of the *CCR5 -Δ32* variation within the Turkish Cypriot population with not any observed homozygous individual of *CCR5 -Δ32*genotype.Results of this study, it concurred to what Okoroiwuet al., (2015) observed in which they recorded low/absent of *CCR5 -Δ32* in the Nigeria population.

Also, it is in accordance with Solloch et al., (2017) where they observed rare or no occurrence of *CCR5 -Δ32* in Africans.

5. CONCLSION

Overall, this research showed that there is absence of the mutated genotype of *CCR5* (*CCR5 -Δ32*) in the Nigerian and Zimbabwean population which was studied. This makes the population to lack the genetic advantage against the HIV-1 infection, been vulnerable to the infection and have a quick progression to AIDS.

Due to the relative higher frequency seen the*CCR5*gene wild type form in the African continent, shows the mutant variant *CCR5-Δ32*,wasfairly recent in terms of human evolution (Galavin & Novermber, 2005 & Pveton et al., 2012).

The diversity of ethnicity in the Nigeria population have no related factor in the distribution of the *CCR5 -Δ32* mutation in the country. The significant absence of the *CC5R -Δ32*alelein the Nigeria and Zimbabwean population in this study shows that they will be vulnerable to HIV-1 infection because they lack resistance to the infection and progression of AIDS will be accelerated. This should serve as a greater awareness to the society cause the lack of genetic resistance can lead to the wild spread of HIV-1 in the population if exposed to the infection. There is need for greater prevention measures for HIV-1 infection to be placed in check. Turkish Republic of Cyprus been a small island with approximately 326,000 population and only 68 HIV /AIDS cases since 1997 and 2017 with only 5 deaths(Meryem et al., 20019) However, Cyprus is a big tourist destination and also a big home for the fast-rising African population especially Nigerian and Zimbabwean students, which has a high level of HIV epidemics. Lack of *CCR5 - Δ32* in this population will place a risk to the Turkish Cypriot population because the two populations can have an interethnic relationship and marriage between them. Due to the low population of the Turkish Cypriot population measures, must be put in check in other to control HIV and its spread in the island.

5.1 Future remarks

HIV/AIDS provides a very unique disease for the development of genome editing. CRISPR/Cas 9 nuclease system have been a major breakthrough in gene disrupt (Mandal et al., 2014) cause it guides multiple RNAs which can be used simultaneous

to potentiate the magnitude of gene disruption and also to induce specific deletion (Li et al ., 2015) in which they use Cas9 nuclease to cleave genome locus Ad5f35 Adenoviral vector, which was packed to carry Crispr/Cas 9 components and transuded primary CD4+ T cells . The positively traduced cell expresses disrupted *CCR5* which results to HIV-1 resistance, these makes Crispr/Cas9 as a potential tool for HIV-1 gene therapy. Since Crispr/Cas 9 genome editing is done directly in embryo this technique provides a more simple, efficiency and reduces the time needed to modify target genes. However, there are some limitation this technology because it is impossible to select the desired event lending to reduced the chance of identifying the desire allele, also the molecular mechanism used to insert DNA fragment (cDNAs) is controlled by DNA repair machinery which is activated by the double strand break initiated by Cas9.Since DNA repair system are not responsible to integrate DNA fragment in the genome, targeted alleles often carry additional modification like partial, or multiple integrations of the targeting vector , deletion and duplication (Pavlovic et al., 2016).

Since Crisp/Cas 9 genome editing technology have become a great tool in molecular genetics, its contribution with other technology have explore genetic opportunities for the generation of new for exploring the gene editing in humans.

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2. EDUCATION

YEAR	GRADE	UNIVERSITY	FIELD
2009 - 2014	(Second Class Upper Division)	Imo State University Owerri Nigeria	Medical Laboratory Science

3. ACADEMIC EXPERIENCE

PERIOD	TITLE	DEPARTMENT	UNIVERSITY
2009 - 20014	BMLS	Medical Laboratory Science	Imo State University Owerri Nigeria

4. FIELD OF INTERESTS

FIELDS OF INTERESTS	KEY WORDS
Molecular Medicine , Pathology, Laboratory Medicine	

5. WORK EXPERIENCE

Medical Laboratory Scientist Hosanna Rich Diagnostic Center Orlu Imo State Nigeria	2014-2015
Medical Laboratory Scientist at Dozie Diagnostic Center Orlu Imo State Nigeria	2015
Medical Laboratory Scientist at JAFAC Foundation (HIV& AIDS) Advocacy	2016-2017
Intern Medical Laboratory Scientist Federal Medical Center Makurdi Benue State Nigeria	2017
Medical Laboratory Scientist University Of Agriculture Makurdi Benue State	2017-2018
Chief Medical Laboratory Scientist Foundation Reproduction & IVF center Makurdi Benue state	2018-2019

PROGRAMME	ABILITY TO USE
Word, Excel, Power Point	Very good
SPSS	Very good