The roles of CCR5delta32 allele on HIV/AIDS epidemic in native Africans
Idris Abdullahi Nasir1*, Adamu Babayo2, Abdurrahman Effulaty Ahmad3, Emmanuel Chisom Okechukwu4, Iduda Nadia Ojeamiren5

1. Department of Medical Microbiology, University of Abuja Teaching Hospital, PMB 228 Gwagwalada, FCT Abuja, Nigeria.
2. Department of Medical Microbiology, Abubakar Tafawa Balewa University Teaching Hospital PMB 01117, Bauchi State, Nigeria.
3. Immunology Unit, Department of Medicine, Ahmadu Bello University Zaria, Nigeria.
4. Department of Medical Microbiology, Usman Danfodiyo University Teaching Hospital PMB 2370, Sokoto, Nigeria.
5. Department of Medical Laboratory Science, College of Medical Sciences, University of Maiduguri, PMB 1069 Maiduguri, Borno State, Nigeria.

ABSTRACT

Studying the replication pattern of Human immunodeficiency virus (HIV) is essential to understand modalities that could halt its survival in-vivo. The CC chemokine receptor 5 (CCR5) is exploited by HIV to gain entry into CD4+ cells. There are several polymorphisms in CCR5; the major coreceptor of HIV that has major influence on HIV transmission and progression to acquired immunodeficiency syndrome (AIDS). CCR5 genes that code for the CCR5 differ in humans, some individuals acquire mutant form this gene with 32 base pair deletion through mendelian inheritance pattern. Individuals who are homozygous for this gene are completely resistant to HIV infection while heterozygous individuals had extended life span while being infected for an average of 2 to 3 years. CCR5Δ32 allele is young in evolutionary time, yet it has reached relatively high frequencies in Caucasians but very low among native Africans. These properties indicate that the mutation has been under intense pressure selection. Surprisingly, there has not been any categorical explanation for this genetic selection. The absence or rare occurrence of CCR5 Δ32 allele among native Africans may be an explanation for high HIV transmission rate and burden in this race. There are very limited CCR5Δ32 studies from Africa thus, justifies the need for researchers to embark on more CCR5Δ32 projects in order to establish full range of mutant CCR5 genes that may exist in our societies.
Introduction

Human immunodeficiency virus (HIV) infection continues to be an expanding epidemic worldwide. The risk of HIV transmission varies and depends on multiple factors, in particular on the type of exposure: blood transfusions represent the greatest risk, with 90%, followed by vertical transmission with a 10-30% risk in the absence of any prophylactic intervention [1]. In contrast, the risk of transmission by sharing infectious needles is low, 1% similar to the 0.3% risk during accidental exposure in healthcare personnel [1].

In 2013 an estimated 35.0 million people were living with HIV (figure1). Sub-Saharan Africa, especially South Africa (national prevalence of 12.2%), has the highest global burden of HIV (70.8%). In Nigeria, an estimate of 3.2 million and about 220 thousands new cases was recorded in 2013 [1].

The global epidemiology of HIV infection has changed markedly as a result of the expanding access to antiretroviral therapy; by 2012, 9.7 million people in low income and middle-income countries had started antiretroviral therapy. The global prevalence of HIV increased from 31.0 million in 2002, to 35.0 million in 2012, because people on antiretroviral therapy are living longer, whereas global incidence has decreased from 3.3 million in 2002, to 2.3 million in 2012 [1].

For HIV to bind and enter target cells, it requires the presence of the CD4+ molecule that acts as receptor and another that act as coreceptor; usually chemokine receptors. Among the various arrays of chemokine receptors, CCR5 and CXCR4 are the main coreceptors for HIV entrance. Natural ligands for CCR5 receptor include macrophage inflammatory protein-1 alpha (MIP-1 alpha), macrophages inflammatory protein-1 beta (MIP-1 beta), monocyte chemoattractant protein-2 (MCP-2) and regulated on activation normal T expressed and secreted protein (RANTES) [2].

The CCR5 gene codes for a seven transmembrane protein similar to G protein-coupled chemokine receptor. Chemokines and their receptors form regulatory networks that control the development, recruitment and activation of lymphocytes. Thus, chemokine receptors play a central role in the immune response against many pathogens, particularly during inflammatory response [3]. CCR5 is the primary coreceptor in the initial phases of infection, and is thus fundamental in establishing HIV infection. The CCR5 receptor is expressed on various cell populations including macrophages, dendritic cells and memory T-cells in the immune system; endothelium, epithelium, vascular smooth muscle and fibroblasts; and microglia, neurons and astrocytes of the central nervous system. Interference with the expression of the CCR5 receptor in now...
Adults and children estimated to be living with HIV | 2013

Figure 1: Global prevalence of HIV infections (Sources: UNAIDS [1])

- Total: 35.0 million [33.2 million – 37.2 million]

It was almost simultaneous with the discovery of HIV coreceptors, a mutation in the gene that codifies for the CCR5 molecule which confers a high degree of resistance to HIV infection was also described [2, 3]. The mechanisms of human natural resistance to infectious diseases are essentially genetic or immunological. The main genetic mechanism so far reported to be responsible for resistance to HIV infection is the delta-32 mutation on the CCR5 gene [2, 3]. The CCR5Δ32 is estimated to be present in 15 to 20% of Caucasians individuals [3] however, other mutation in chemokine single nucleotide polymorphisms (SNIPs) have been more closely related to slow clinical progression than to resistance [4]. Intriguingly, CCR5Δ32 allele is young in evolutionary time, yet it has reached relatively high frequencies in Europe but very low among black Africans. These properties indicate that the mutation has been under intense pressure selection.

When HIV was initially discovered as the causative agent of AIDS, many expected a vaccine within a few years. This has however proven to be elusive; it has been more than 30 years since HIV was first discovered, and a suitable vaccine is still not in effect. In 2009, a paper published by Hutter et al [5] reported on a bone marrow transplant performed on an HIV positive individual using stem
cells that were derived from a donor who was homozygous for a mutation in the CCR5 gene known as CCR5 delta-32 \[5\]. The HIV positive individual became HIV negative and remained free of viral detection after transplantation despite having stopped ART \[5\].

The CCR5Δ32 allele probably originated from a single mutational event which occurred in the North of Europe in recent history some 2000-3000 years ago, with subsequent positive selection of the allele related to the epidemics of infectious diseases. The most probable selective pressures included both widespread infection with Yersinia pestis and smallpox, which was responsible for reduction of the population size \[3\]. The allele is the most common in the north of Europe in the region of the Baltic and North Seas, with a gradual decrease of frequency to the south. It is less common or virtually nonexistent in the genetically distinct populations of Asia and Africa. Heterozygous individuals have been identified in local populations in Egypt, Syria, and Sudan and recently in Cameroon but none in native South Africans \[6-8\].

To the best of our knowledge there are very few published studies on the prevalence of this mutation in African nations. In view of these, we present this article on CCR5 expression and HIV infection, evolution and global distribution of CCR5Δ32 allele and its possible roles in high occurrence and burden of HIV/AIDS in native Africans.

Method

This article was a critical review of published articles sourced through extensive internet search using PubMed and Scopus using these words “CCR5 coreceptor”, “evolution and distribution of CCR5Δ32”, “significance of CCR5Δ32 in HIV science” and CCR5Δ32 in Africa. For summary of results obtained from internet search, see table 1.

1. Inclusion criterion

Studies findings that strictly related to CCR5 chemokine coreceptor and CCR5 Δ32 mutation in HIV pathogenesis were used for this study.

2. Exclusion Criterion

Any study finding that was outside the scope of this topic was excluded.

Table 1: Summary of results from internet search

<table>
<thead>
<tr>
<th>Search engine</th>
<th>Number of hits (n)</th>
<th>No. included for study</th>
<th>No. excluded from study</th>
</tr>
</thead>
<tbody>
<tr>
<td>PubMed</td>
<td>238</td>
<td>25</td>
<td>213</td>
</tr>
<tr>
<td>Scopus</td>
<td>256</td>
<td>12</td>
<td>244</td>
</tr>
</tbody>
</table>
Review and Discussion

The CCR5 gene produces the CCR5 chemokine coreceptor, this alongside CD4+ receptor serve as entry port for HIV into host cells. HIV attaches to the CD4 receptor through gp120 thereafter, triggers a conformational changes in virus structure which reveals its gp41 and thus binds CCR5 (figure 2). Binding brings virus in close contact with host cell surface. Subsequently, pores are formed on host cell surface as a result of binding. The HIV fuses and then enters cell [9]. During early phase of infection, HIV infects macrophages using the CCR5 coreceptor, thus they are said to be M-tropic and R5 strains. Whereas, in late phase HIV infection towards AIDS progression, HIV infect cells through a different chemokine called CXCR4 found on T-lymphocytes. Hence, called T-tropic and X4 strains [9, 10].

In general, R5 strains seem to be more efficiently transmitted than X4 strains. The predominance of R5 strains during acute HIV infection is independent from the route of HIV transmission [11, 12]. Individuals who do not express functional CCR5 coreceptors because of a mutation in the CCR5-encoding gene are largely protected from HIV-1 infection despite the presence of functional CXCR4 coreceptors [13]. Many

![Diagram](attachment-and-fusion-pattern-of-hiv-on-susceptible-cells-source-kabat-et-al-[9])

Figure 2: Attachment and fusion pattern of HIV on susceptible cells (Source: Kabat et al [9])

Copyright©2012-2020 Published by Hongkong Institute of Biologicals Standardization Limited. All rights reserved.
mechanisms have been postulated to explain the preferential transmission of R5 strains. These include barriers at mucosal sites which may select against X4 strains but also specific humoral and cellular immune responses which may inhibit viral replication of X4 strains more effectively [14]. In 2006, it was postulated that the preferential transmission of R5 strains depends on the superimposition of multiple mechanisms rather than of one crucial 'gatekeeper' mechanism [15]. It should be noted that strains found in acute HIV infection are not exclusively R5. In a large cohort study on men who have sex with men from six major cities in the United States, X4 strains were found in 4 out of 195 samples collected within six months of HIV-1 seroconversion. Among 296 Spanish HIV-1 seroconverters, X4 strains (either pure or D/M) were recognized even in 17.2 % of the patients [16].

In individuals infected with HIV, the percentage of CD4+ CCR5+ T-cells is higher (13.2%) than when compared to uninfected individuals (6.2%) [17]. The highest percentage of expression was found in an individual with acute HIV syndrome, recorded at around 30-40%.

The variation in CCR5 percentages in HIV infected individuals did not correlate with genotype as three individuals with heterozygosity for CCR5Δ32, a CCR5 mutation known to reduce HIV infection had different levels of expression (2.7%, 13.1% and 17%). In contrast, the activation state of the CD4+ cells as measured by HLA-DR positively correlated with CCR5 expression [17].

In 1999, a study conducted by Husman et al [18] assessed CCR5 expression in terms of CCR5 genotype and HIV infection and progression. Individuals with wild-type CCR5 receptors had higher levels of CD4+ CCR5+ T-cells than those with heterozygous CCR5Δ32 genotypes, and this was observed in both HIV infected (wild-type:28%; CCR5Δ32 heterozygote: 21%) and uninfected individuals (wild-type: 15%; CCR5Δ32 heterozygote:10%). Furthermore, infected individuals in end stage HIV progression had higher percentages than individuals that had not progressed. The study postulated that the CD4+ CCR5+ T-cell percentage is directly correlated with HIV disease progression due to the constant immune activation associated with HIV. The presence of the CCR5 receptor on memory effector T-cells or mature activated T-cells supports the latter finding [18].

1. The CCR5Δ32 mutation

The CCR5Δ32 mutation was initially discovered in 1996 as a genetic mutation that confers protection to cells from infection by HIV [19]. Genetic analysis of the mutant gene by Liu et al revealed a deletion of 32 base pairs consisting of nucleotides
The deletion involves a frame shift mutation with the inclusion of seven novel amino acids following amino acid 174 and a stop codon at amino acid 182. The mutant allele contains 215 amino acids in comparison to the full-length 352 amino acid wild type CCR5. This allele has received much attention due to the fact that individuals homozygous for this mutation are resistant to infection by the HIV virus while those heterozygous for this allele who is HIV-positive have a delayed onset to AIDS of 2–3 years. Homozygosity of CCR5Δ32 in Caucasians was measured to be about 1%, with heterozygosity being anything up to 20%

2. Evolution of CCR5Δ32 allele

The origin of the CCR5Δ32 allele was dated back to between 2000 and 3000 years ago, as a unique mutation that increased over the years due to a selective pressure. Stephens et al. used haplotype analysis on the chromosomes of 192 Caucasian individuals and estimated the origin using a coalescence theory. They hypothesized that the Black plague was the strong selective pressure that caused the mutant allele boom.

Historical data however suggest that the Black plague is not the selective force. The distribution of CCR5Δ32 in a north to south gradient (figure 3) does not correspond to casualties of the plague. In fact, the distribution follows a south to north gradient. Moreover, the Black plague showed the greatest casualties and effects in areas with the lowest allele frequencies of CCR5Δ32, such as the Mediterranean region and China. In 2004, Mecsas et al. infected CCR5 deficient mice with the bacterial pathogen known to cause the Black plague. The experimental data demonstrated no differences in bacteria growth or survival of the deficient mice in comparison to CCR5 containing mice. The finding of ancient DNA in skeletal remains dating back 2900 years presents further evidence. The CCR5Δ32 allele frequency found in these remains was similar to that found in individuals ridden by the plague in the same region.

Smallpox was another pandemic hypothesized to be responsible for the CCR5Δ32 allele. The pandemic had severe casualties that exceeded those of the plague. Smallpox is a virus similar to HIV, as they are known to infect lymphocytes using chemokine receptors. Conversely, historical evidence refutes this theory as smallpox started outside Europe and did not affect anyone country more significantly than another. The discovery of ancient DNA with similar allele frequencies of CCR5Δ32 indicated that historic pandemics such as the plague and smallpox did not result in the allele increase.
Figure 3: Global distribution of heterozygous CCR5Δ32 allele (Source: Novembre et al\textsuperscript{[3]})

3. Geographical distribution of CCR5Δ32 allele

The CCR5Δ32 mutant allele is confined mostly to individuals of European descent, at gene frequencies of approximately 15%, and has a north to south latitude decline in frequency (figure 3). Martinson et al\textsuperscript{[29]} analyzed the distribution of the CCR5Δ32 mutation in more than 3000 individuals from various countries and found 2-5% gene frequencies in Middle East and some parts of the Indian subcontinent. Isolated incidences of CCR5Δ32 found in other regions were attributed to European gene flows into these areas. The highest frequency of the mutation was discovered in the Ashkenazi Jewish population at frequencies of 20.93%\textsuperscript{[3]}. In 2005, Novembre et al\textsuperscript{[3]} confirmed these results when they assessed the CCR5Δ32 frequency in various population groups worldwide\textsuperscript{[3]}. The mutant allele is absent in native black populations except the African American group whom might have acquired the mutation through admixture\textsuperscript{[2, 19]}.
Can CCR5Δ32 allele be a factor for high HIV transmission in Africans?

Sub-Saharan Africa has been hardest hit by HIV/AIDS with more than 15% of the general population HIV-positive in some countries \[30\]. In comparison, heterosexual epidemics in developed countries have not reached such severe levels. Factors contributing to the severity of the epidemic in economically developing countries include economic, health, and social differences such as high levels of sexually transmitted diseases and a lack of prevention programs. However, the overwhelming rate at which the epidemic has spread in sub-Saharan Africa has not been adequately explained \[31\]. The rate and severity of this epidemic also could indicate a greater underlying susceptibility to HIV attributable not only to sexually transmitted disease, economics, etc., but also to other more ubiquitous factors such as host genetics \[32\]. To exemplify the contribution of such a host genetic factor to HIV prevalence trends in Africa, we consider the well-characterized 32-bp deletion in the host-cell chemokine receptor CCR5 i.e. CCR5Δ32.

It is known that following infection with HIV, the rate of clinical disease progression varies between individuals. Factors such as host susceptibility, genetics and immune function \[33, 34\], health care and co-infections, as well as viral genetic variability \[35\], may affect the rate of progression to AIDS. Many studies have looked at the genetic viral distinctions that might explain delayed HIV progression. Considerable research investigating the viral genotype has focused on deletions in the nef gene of HIV seen in some nonprogressors. While many researchers have observed defective nef gene alleles in long term nonprogressors (LTNP) \[36\].

Other researchers have focused on human leukocyte antigen as another factor \[37\]. All these factors interplay to affect the acquisition of resistance or the progression into HIV/AIDS. However, CCR5Δ32 allele has been the major established genetic factor that determines rate of contracting, transmission of HIV and progression to AIDS. The quality of evidence we reported in this article can lead to possible explanation for high pandemicity of HIV/AIDS among native Africans.

Conclusion and Recommendation

The study of natural resistance to HIV infection and disease through CCR5Δ32 gene expression appears to offer numerous opportunities because there are many inconsistencies in the literature that need to be resolved especially factors responsible for racial and geographic distributions of these genes. While CCR5Δ32 individuals are immune to infection with R5 strains of HIV, safer sex and good injection practices are nonetheless imperative as these individuals are susceptible to infection spread by unprotected sex and needles. They
also can be infected with strains of HIV that use alternative receptor sites other than CCR5 to gain entry.

The lack or rare occurrence of CCR5Δ32 allele in native Africans may be a genetic explanation for high HIV transmission rate and ADIS burden among the black race; however, extensive researches need to be done to elucidate this phenomenon and establish the full range of mutant CCR5 alleles in our societies.

References


