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Occurrence of Stromal Derived Factor-1 Polymorphism In Kenyan Population

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Abstract

Genetic polymorphism in chemokine receptors and coreceptor genes influences susceptibility to HIV-1 infection and disease progression. The mutated Stromal Derived Factor-1 3A`/3A` (SDF-1 3'A) competes with the virus for the coreceptor site Cystein-X-cystein receptor 4 (CXCR4) on the CD4+ T-cells therefore down-regulating evolution of non-syncytial to syncytial induction during HIV-1 progression. Two hundred whole blood samples were collected from eight provinces of Kenya and analysed at the Kenya Medical Research Institute in Nairobi. Detection of SDF-1 gene polymorphism was done by extraction of proviral DNA from whole blood and the SDF-1 target gene was amplified by polymerase chain reaction using gene-specific primers. The different SDF-1 gene polymorphisms were detected by Restriction Fragment Length Polymorphism and gel electrophoresis. Chi-square test was used to test for the significance in the distributions of these polymorphism. The relationship between the frequency of SDF-1 mutation and HIV prevalence was analysed using Pearson's product moment of correlation coefficient (r). This study showed the presence of the stromal derived factor-1 polymorphism in Kenyan population with an average of 6.6% for the double mutant, 20.7% for heterozygous and 73.7% for the wild type. There was no correlation between the HIV-1 prevalence and the SDF-1 distribution in Kenya. These results will form a foundation for further research in Kenya given that double mutants had been found to resist HIV-1 infection or show good response to anti-retroviral drugs. The researchers can therefore incorporate genetics in the treatment of HIV-1.

Keywords: Stromal derived factor-1, Mutations, Coreceptors, HIV, PCR, RFLPRFLP

Introduction

Human immunodeficiency virus (HIV) the causative agent of Acquired immune deficiency syndrome (AIDS) appeared onto the scene in Kenya in 1981 and has since grabbed the headlines as far as diseases are concerned. Acquired immune deficiency syndrome is distinguished from virtually every other disease in history by the fact that it has no specific symptoms. The study of the slow but relentless decay of the immune system and pattern of infections that characterize the condition highlights the importance of Immunology in health and disease (Stine, 2005). The HIV infection and its ultimate progression to AIDS is controlled by many factors which include immune responses to HIV, viral cofactors and non-infectious cofactors. The virus has three varied impacts on immune cells which include direct viral killing of the infected cells, apoptosis and killing of the CD4+ T-cells (Stine, 2005). Human Immunodeficiency Virus (HIV) invades immune cells, especially the macrophages and T-cells because they possess CD4 proteins (Desiree et al., 2005; Stephens et al., 1998). The role of CD4 proteins as a component of the HIV receptor was known as from 1984 though it was discovered not to be sufficient to allow HIV to infect the cells (Dragic et al., 1998). Attachment and subsequent entry of the virus into the target cells requires both the CD4 proteins and a coreceptors such as CCR5, CXCR4, CCR2 and CCR3 (Desiree et al., 2005; Suresh et al., 2006; Tomasz et al., 2006). When HIV enters the host, it erodes and dysregulates the cytokine network of the immune cells.

The HIV infection and subsequent progression to AIDS in victims is associated with a myriad of immune abnormalities which include lymphopenia, polyclonal B-cell activation, impaired delayed hypersensitivity (DTH) reactions, alteration of cytokine expression, decreased cytotoxic lymphocytes (CTL) and natural killer-cell functions. Other reactions by the host may include decreased humoral and proliferative responses to antigens and mitogens, decreased major histocompatibility complex II expression, decreased monocytes chemotaxis and depletion of CD4+ T-cells. The patient's immune system is thus too weak to wade off any opportunistic pathogens. Deviating from the expectations above, three phenomena have indicated that natural resistance to HIV infection, while rare, does exist (Marmor et al., 2006; Shearer et al., 1998). First, there are individuals who have remained uninfected over long periods of time, though they are constantly exposed to HIV. This has been reported in some commercial sex workers who have unprotected sex with seropositive partners, HIV negative infants born to HIV infected mothers, health workers with accidental occupational exposure to HIV, Intravenous

drug users (IVU) sharing contaminated needles and hemophiliacs exposed to blood contaminated with HIV (Marmor et al., 2006). Secondly, there are individuals who have been infected with HIV but the disease has not progressed or has progressed very slowly (Long Term Non-Progressors) as compared to average experience. These people can survive for approximately 7 or more years with consistent low levels of HIV RNA with little or no loss of CD4+ T-cells. Lastly, there are discordant couples who are living together with one partner constantly exposed to the virus by but remain free from HIV infection (Marmor et al., 2006).

In 2005, UNAIDS reported that AIDS claimed 2.4 to 3.3 million lives of which 570,000 were children. As of January, 2006, UNAIDS and WHO separately estimated that 25 million more people were infected by HIV, which brought the total number of infections worldwide to 65 million since it was first identified in June, 1981. These statistics make HIV and AIDS the most destructive epidemic recorded in human history. No wonder, the Ministry of Health in Kenya has availed free anti-retroviral drugs to AIDS patients in government health facilities given that the treatment and management of HIV and AIDS is very costly (UNAIDS, 2006; WHO, 2006; UNICEF, 2006; Kenya AIDS Indicator Suevey, 2007).

Human immunodeficiency virus and AIDS challenge has stimulated many innovations in basic science and laboratory diagnosis for discoveries in chemotherapy and vaccine development. Studies have shown that repeated exposure to HIV does not always result into an infection (Van Rij et al., 2004; Suresh et al., 2006). The rate of HIV transmission is influenced by many factors. These factors include frequency and magnitude of exposure, inoculum's size, disease stage, CD4+ T-cells number, immune response of the patient, among others (Van Rij et al., 2004). The AIDS cohort studied to get the information about the influence of hosts' genetic factors to HIV infection further clarified that mutations of the Stromal Derived Factor-1 (SDF-1) had a recessive protective effect in individuals who had been infected with HIV for longer periods of time (Magierowska et al., 1999). This protection was twice as strong as the dominant genetic restriction of AIDS conferred by CCR5 and CCR2 chemokine receptor variants (Su et al., 1999; Winkler et al., 1998). Chemokines are chemo-attractant cytokines that are small peptides which are secreted by cells serving to regulate chemotaxis (movement of cells), adhesion and activity of the immune cells; however, they do not aid in the proliferation of the immune cells. The discoveries that chemokines can block replication and that their receptors play a significant role in fusion of the HIV to target cells raised expectations that chemokines might hold the key to understanding HIV pathogenesis and control of its spread (Su et al., 1999).

In India, studies on the distribution of SDF-1 polymorphisms were performed on three groups of people categorised as: exposed to the virus but are uninfected (EU), Healthy controls (HC) and HIV infected (HI). Among the exposed and uninfected individuals, the SDF-1 3'A mutant genotype frequency was 37%; the heterozygous genotypes (SDF-1 G/A) had a percentage frequency of 30.4% (17/55) and the wild type homozygous genotype (SDF-1 G/G) were 5.7% (2/35). Among the HIV infected controls, the SDF-1 3'A mutant genotype was found to be 30% with 26% (13/50) homozygous and 4% (2/50) heterozygous (Table 1.5). The HC had 25.3% mutants (SDF-1 A/A) with 20% (15/75) heterozygous (SDF-1 G/A) and 4% (3/75) homozygous wild type (SDF-1 G/G). The SDF-1 3'A allelic frequency was 0.21 in EU and 0.17 in HIV infected controls. The HC allelic frequency of the SDF-1 3'A was 0.14 (Suresh et al., 2006). In analysis of the SDF-1 3'A polymorphisms in the cohort of EU, the research group recorded high frequency of SDF-1 3'A genotype in EU individuals although it never showed statistical significance when compared to healthy individuals and HIV infected controls (Williamson et al., 2000; Mellors et al., 2002; Suresh et al., 2006). Among the non-Hispanic subjects, the viral suppression failure frequency on response to anti-retroviral therapy (ARV) was slightly higher in SDF-1 double mutant homozygotes with 29.4% (5/17) as compared to other subjects (O'Brien et al., 2000). In Moscow, a G to A substitution in the 3' untranslated region (3'UTR) was 63% of 76 for SDF-1 G/G, 30.8% of 37 for SDF-1 G/A and 5.8% of 7 for SDF-1 3'A/3'A. The allele frequency was therefore 0.2125, which was slightly higher than that obtained in Western European countries. This was attributed to high proportions of migrants from Asian region to Russia (Ryabov et al., 2002). The frequency of SDF-1 3'A in other populations in East Asia ranged from 0.029 to 0.366, though it was extensive in Oceania with a percentage frequency of over 37%. In Southeast Asian populations, the frequency of CCR2-64I was low while that of SDF-1 3'A was relatively high compared to percentages in the whites' population (Su et al., 1999).

Since inception, HIV and AIDS has no cure and over 65 million people have been infected with the virus with more than 25 million deaths reported world wide (Stine, 2005). The virus has become too elusive to contain, prompting countries, including Kenya, to declare it a national disaster (National AIDS Control Council, 2007; Ministry of Health report, 2007).

Predictably, it will remain devastating as long as cure or control is not found. Due to this challenge, alternative avenues of genetic minimization on the rate of HIV infection and progression to AIDS should be studied. Research on non-competitive mechanisms on blockage of CXCR4 dependant viruses and immune reconstitution should be put as priority besides vaccines and anti-retroviral therapeutic measures. Research scientists are therefore looking for a 'wonder' molecule to block the CXCR4 chemokine coreceptor thereby inhibiting CD4+ T-cell attack. However, there is a Stromal Derived Factor-1 (SDF-1) which is a natural ligand for CXCR4 coreceptor and can be used to block the CXCR4.

When the SDF-1 (a pre-B cell growth stimulating factor) binds on to the CXCR4 coreceptor, it down regulates the invasion of the CD4+ T-cells by the HIV (Yuan et al., 2000). The knowledge of the SDF-1-like mechanisms of HIV entry has resulted in the development of a new class of HIV therapy called entry inhibitors, which blockade the CCR5 or CXCR4 coreceptors and prevents HIV from continued infection of healthy cells (Marmor et al., 2006). There was also evidence that patients who are homozygous for the SDF-1 3'A allele had a more favourable therapeutic response to certain anti-retroviral drugs (O'Brien et al., 2000). This is true if SDF-1 3'A acts early in the course of infection through interaction with CXCR4 (HIV receptor) thus the hosts' genotype may also alter therapeutic responses to anti-retroviral (ARV) drug regiments. If validated, the findings could mark an initial step in the integration of the hosts' genetic information into treatment of viral infections in Kenya.

The SDF-1 occurs in its wild form with guanine/guanine (G/G) in its 3'untranslated region at position 801 of DNA strand. An adenine substitution sometimes does occur at the 801 causing the chemokine coreceptors have G/A (SDF-1 G/A) for heterozygous and A/A for the double mutant for example SDF-1 3'A/3'A. The mutants will have their SDF-1 proteins attaining the same configuration as gp120 on the HIV creating a competition for the CXCR4 between the two, an advantage to the host. This will slow down HIV attachment and therefore infection of the host CD4+ cells. Currently there is some information about the role played by mutant stromal derived factor-1 (SDF-1) to HIV spread and its interaction with host cells. However, data about its occurrence in Kenya is lacking and if present, data about its frequency and distribution is absent. This study therefore aimed at determining the occurrence, frequency and distribution of the SDF-1 polymorphism in Kenya.

The percentage distribution of SDF-1 3'A obtained may therefore give guidelines as to what type of ARVs that can be recommended for specific regions of Kenya. Although distribution of the SDF-1 polymorphism can be used to explain the disparities in HIV prevalence and guide on the specific regiment of ARV distribution in the country, information about its distribution in many African populations is scanty. Polymorphism within coding and regulatory regions of chemokine receptors and coreceptors has been discovered to have an impact on HIV related pathogenesis. In HIV infection in children, CCR5 was found to be the main coreceptor that is modulating perinatal transmission of HIV (Paxton et al., 1996; Buseyne et al., 1998 Hogan and Hammer, 2001). The presence of CCR5 D32, deletion of 32 base pairs from the coding region of CCR5 gene, leads to a distorted CCR5 protein which the virus fails to recognize as a receptor site. The benefit of carrying this deletion mutation was thought to result from decreased expression levels of the receptor in patients. This situation led to either resistance to virus infection for homozygotes (partial protection) or delayed disease progression in heterozygotes (Dean et al., 1996; Liu et al., 2004; Samson et al., 1996; Baroga et al., 1996; Winkler et al., 1998; Su et al., 1999). People who have inherited certain mutant genes or polymorphisms may have CD4 cells that are less vulnerable to infection by HIV. This is because the cells lack the co-receptors (CCR5 or CXCR4) or if present, they express them in a different way that cannot be recognized by the virus.

Heterozygotes for a D32 deletion (CCR5-wt/D32) are not protected against HIV infection but manifest slow progression to HIV and AIDS end points (Dean et al., 1996; McNicholl et al., 1997; Shearer et al., 1998; Kostrikis et al., 1999; Daar et al., 2005). Some of the polymorphisms in the promoter region have been found to raise the risk of perinatal transmission of HIV from the mothers to their babies (Kostrikis et al., 1998). A Guanine to Adenine substitution at position 180 affects the gene that codes for CCR2 which is a minor HIV coreceptor. This mutation causes Isoleucine to substitute Valine at position 64 (designated as CCR2-64I) which eventually slows down disease progression in adults (Smith et al., 1997; Kostrikis et al., 1998). Mutation in the 3'untranslated region of the gene that codes for SDF-1b (pre-B cell growth stimulating factor) lowers progression of HIV infection to AIDS. The SDF-1 3'A is a natural ligand for CXCR4, yet another HIV receptor protein, attaches onto the receptor preventing HIV from attachment and subsequent entry into the CD4+ T-cells (Crump et al., 1997; Winkler et al., 1998; Yuan et al., 2000). Despite the information above, subsequent studies have failed to confirm that SDF-1 3'A lowers disease progression

(Van Rij et al., 1998; Meyer et al., 1999; Ioannidis et al., 2001).

The CXCR4 (X4) viruses are frequently detected in individuals with advanced clinical manifestation and individuals homozygous for the SDF-1 3`A that have delayed disease progression. The SDF-1 3`A mutation may enhance expression of the SDF-1, thereby inhibiting the emergence of the X4 variants (Winkler et al., 1998). The model in which the SDF-1 3`A mutation favors replication of CCR5 (R5) viruses could also be used to explain this observation because M-tropic R5 viruses are more commonly transmitted from mother to infant than the T-tropic X4 viruses (Mangano et al., 2000).

Materials and methods

This was an exploratory study that was designed to run parallel with other research work going on at the Kenya Medical Research Institute-Nairobi (KEMRI-Nairobi) laboratories. The blood samples collected from the blood transfusion centres had therefore been cleared by the Kenya national ethical review committee. The samples were assigned random numbers before 25 samples from each province were randomly picked for processing. The basic minimum sample size was used to form a foundation for future studies on the SDF-1 polymorphism in Kenya. Data about the SDF-1 mutations was not available in East African region while that reported in South Africa by Desiree et al. (2005) of 1% for the Sotho and 2.8% for the Xhosa was too low to be used for sample size determination.

The world's average distribution of 15% reported by Yuan et al. (2000) was used to compute the 200 sample size used in the study. It was an unlinked study with no follow-up of persons from whom the blood samples were collected. The study population included indigenous Kenyans of African descent in the age bracket of 18 and 47 years, their sexes not withstanding. This group of people consented to participate in the study and the target age bracket was considered because it has the highest prevalence of HIV and AIDS infections in the country. A Polymerase Chain Reaction and Restriction Fragment Length Polymorphism were used for genotyping on 200 individuals sampled from the eight provinces of the Republic of Kenya. Amplification of a 302 base pair product of the SDF-1 gene was performed in a Perkin Elmer Gene AMP PCR system model using published primers of the following nucleotide sequence: Forward: CAACCTGGGCAAAGCC and Reverse: AGCTTTGGTCCTGAGAGTCC. Polymerase chain reaction (PCR) procedure was carried out in a 25 ml reaction volume. The reaction mixture contained 400 ng genomic DNA, 0.2 mM dNTP mix, 2 mM MgCl2, 25 pmol of each primer and 1.5 U Taq DNA polymerase (New England Biolabs). The reaction mixture was subjected to 35 cycles at 94°C for 30 seconds, 58°C for 30 seconds and 72°C for 2 minutes, to amplify the SDF-1 gene. A final extension step at 72°C for 5 minutes was included (Smith et al., 1997; Winkler et al., 1998; Su et al., 1999). Cleavage of the DNA products was done using 15 ml of the amplicon, 5 U Moraxella species-1 (Msp-1) restriction enzyme, Msp-1- specific buffer 2 and distilled water in a 20 ml reaction volume at 37°C for 2 hours.

The Msp-1 restriction map is shown below:

5'….CCGG….3' 5'….C ? CGG….3'

-------Msp-1-----?

3'….GGCC….5' 3'….GGC ? C….5'

After digestion, the products were genotyped by means of 3% agarose gel electrophoresis, stained using ethidium bromide and later visualized in a UV-radiation trans-illuminator. The gels were photographed and their pictures stored for analysis.

Results

Amplification and Restriction Enzyme analysis of the Stromal Derived Factor-1 gene

Stromal derived factor-1 gene polymorphisms were detected by use of PCR and restriction enzyme analysis. After the initial amplification, all the amplified samples were run on a three-percent agarose gel. The target amplicons of 302 base pair were observed. Restriction by an Msp-1 enzyme was done. This showed different allele polymorphisms of the SDF-1 when run on the 3% agarose gel. They were double mutant gene for the SDF-1 (SDF-1 3'A/3'A), heterozygous individuals (SDF-1 G/A) and wild type (SDF-1 G/G).

The Stromal Derived Factor-1 Polymorphisms in eight Provinces of Kenya The survey found no significant difference in the occurrence of SDF-1 3'A/3'A (p=0.058) across the provinces although Nyanza registered the highest frequency while Eastern had the lowest frequency. Nairobi registered the same frequency between the double mutants and the heterozygous individuals. All the other seven provinces showed highest frequencies of the wild type followed by the heterozygous and lastly the double mutant. The frequency of the heterozygous condition for the stromal derived factor-1 gene (SDF-1 G/A) was diverse in occurrence in all the eight provinces. There

was no significant differences (p=0.181) in the frequencies of heterozygous genes (SDF-1 G/A) observed across the provinces. Rift valley registered the highest frequency for the heterozygous gene followed by Coast, North Eastern, Eastern, and Central provinces while Nairobi province had the lowest frequency followed by Nyanza and Western provinces. The diversity observed in the distribution of the wild type gene (SDF-1 G/G) and heterozygous condition for the SDF-1 gene (SDF-1 G/A) was also noted in the double mutant type of SDF-1 3'A/3'A, however, there was no significant difference in the distribution of the SDF-1 polymorphisms across the eight provinces (p=0.136). The allele genotypic frequency of the SDF-1 3'A/3'A in all the eight provinces was below 12%. The highest frequency was experienced in Nyanza (11.1%) followed by both North Eastern and Rift Valley at 9.1%. Western province had 7.1% while other provinces had either 4% (Eastern) or about 4% which included Nairobi (4.2%), Coast (3.8%) and 3.9% for Central.

The Percentage Frequencies of the Stromal Derived Factor-1 Double mutant (SDF-1 3'A/3'A) in relationship to Human Immunodeficiency Virus prevalence in the eight Provinces of Kenya

The allelic genotypic frequency of the SDF-1 3'A in all the eight provinces was below 12%. The highest percentage frequency of 11.1% was experienced in Nyanza followed by both North Eastern and Rift Valley with a percentage frequency of 9.1% each. Western province had 7.1% as the other provinces of Nairobi, Coast, Central and Eastern had 4.2%, 3.8%, 3.8% and 3.7% respectively. The percentage HIV incidence as of 2007 was reported as 15.3% in Nyanza, 1.3% in North Eastern, 7.4% in Rift Valley, 5.7% in Western, 8.1% in Coast, 4.2% Central, 9.3% in Nairobi and 4.9% in Eastern provinces (KAIS, 2007).

There was no significant correlation between the HIV prevalence and SDF-1 double mutants occurrence (r = 0.324) in the eight provinces of Kenya. Some of the provinces showed comparatively high SDF-1 3'A/3'A occurrence than the HIV prevalence (Nyanza, Coast Nairobi, Eastern and Central) while North Eastern, Western and Rift Valley showed low SDF-1 3'A/3'A occurrence as compared to HIV prevalence. It is evident that HIV prevalence and distribution of SDF-1 3'A/3'A mutations does not follow the same trend in all the eight provinces. Interestingly, Nyanza province which registered the highest value of SDF-1 3'A/3'A mutation, had the highest HIV prevalence.

The Percentage Frequencies of the Stromal Derived Factor-1 G/A Mutation (SDF-1 G/A) in relationship to Human Immunodeficiency Virus prevalence in the eight Provinces of Kenya

Human immunodeficiency virus prevalence was higher than SDF-1 G/A percentage frequencies in the two provinces of Nyanza and Nairobi while its prevalence was lower in the other provinces (North Eastern, Coast, Western, Rift Valley, Eastern and Central). The study showed correlation between the occurrence of the heterozygous genotype (SDF-1 G/A) and the HIV prevalence (r = -0.334) in the eight provinces of Kenya. North Eastern province had the third highest SDF-1 G/A occurrence (27.3%) but had the lowest HIV prevalence (1.3%) which prompts the interest in the influence of the heterozygous individuals to HIV prevalence. Rift Valley had the highest range of values between the HIV prevalence and the percentage occurrence of the heterozygous condition (KAIS, 2007).

Discussion

Overview

This exploratory study focused on the analysis of the occurrence, frequency and percentage distribution of the SDF-1 polymorphism in the indigenous Kenyan population. The study aimed at identifying SDF-1 double mutants that could be used for future studies in understanding resistance to HIV infection, and other molecular aspects of HIV infection. Brumme et al. (2004) reported that double mutants for the SDF-1 gene (SDF-1 3'A/3'A) respond well and faster to specific regiments of anti-retroviral (ARV) drugs especially if the drugs are administered in early stages of infection. Identification of the double mutants in the Kenyan population could therefore guide on the distribution ARV regiments to specific regions of the country. The distribution of the half mutants (SDF-1 G/A) and double mutants (SDF-1 3'A/3'A) in populations varied from province to province like there is reported variations in HIV prevalence in different parts of Kenya. The study correlated HIV prevalence to SDF-1 mutations (SDF-1 3'A/3'A and SDF-1 G/A) to establish any influence of the later to the former. Correlation was found to be lacking. However, the SDF-1 mutations and the CXCR4 genetic markers (protective proteins) vary in frequency across the populations and recent studies have shown that their biological impact on the risk of HIV infection and development to AIDS is significant (Dean et al., 1996; Liu et al., 1996; Samson et al., 1996; Baroga et al., 1996; Winkler et al., 1998; Su et al., 1999).

The Distribution of the Stromal Derived Factor-1 Polymorphisms in Kenya

To study the distribution of HIV resistant allele of SDF-1 3'A in Kenya, 200 samples from healthy and HIV-1 infected individuals were collected and genotyped. The sample population constituted indigenous Kenyans of African descent in the age bracket of 18 and 47 years (age bracket with the highest percentage incidence of HIV and AIDS). Analysis of the frequency in the sampled population revealed that 145 out of 200 (72.7 %) were wild type, 42 (20.7%) were heterozygous and 13 (6.6%) were homozygous mutants for the SDF-1 gene.

Occurrence of the SDF-1 polymorphism in Kenya was found to be diverse. The highest percentage occurrence for the wild type gene (91.7%) was noted in Nairobi province while the lowest percentage occurrence for the same was observed in the Rift Valley province (54.5%). The province with the highest occurrence for the heterozygous gene mutant (SDF-1 G/A) was the Rift Valley (36.4%) while the lowest occurrence for the heterozygous gene mutant was experienced in Nairobi (4.2%). Nyanza province had the highest percentage (11.1%) population of homozygous mutant gene (SDF-1 3'A/3'A) while both Central and Coast provinces had the lowest percentage occurrence of 3.8% each. When approximated to a whole number, four provinces (Coast, Nairobi, Eastern and Central) had about the same percentage occurrences (4.0%) of the double mutant (SDF-1 3'A/3'A). Comparison of the occurrence of normal and mutant genes across the provinces revealed that Nairobi had the highest number of wild type and lowest number of mutants; this made the province have the highest range of values for the two conditions.

The Existence and Frequency of Stromal Derived Factor-1 Mutations in Kenyan Population

This study indicated the existence of SDF-1 polymorphism in Kenyan population with an average of 6.6% for the SDF-1 3'A/3'A (double mutants) and 20.7% for heterozygous genes. The average occurrence (6.6 percent) of SDF-1 3'A/3'A mutation in Kenyan population was lower than that of the world which was reported by Yuan et al., (2000) to be greater or equal to 15% (?15%). Studies done in the United States of America reported that the whites population had SDF-1 3'A/3'A mutation of about 37% while the blacks population had 11% both of which were higher than the results obtained in Kenya.

A survey carried out in Asia reported a higher frequency of an SDF-1 3'A/3'A mutation of about 37% as compared to Kenyan population. Though having a lower percentage occurrence of the SDF-1 3'A/3'A (6.6%) as compared to many populations of the world, Kenya still has a higher percentage occurrence of the double mutant gene as compared to Sotho and Xhosa of South Africa which had 1% and 2.8% respectively (Desiree et al., 2005). The SDF-1 3'A/3'A frequency occurrence of 6.6% obtained in Kenya was lower than 37% which was experienced in India (Suresh et al., 2006). Kenya had a lower percentage of 20.7% for the heterozygous gene (SDF-1 G/A) as compared to 25.3% reported in India (Suresh et al., 2006).

Nyanza province had the highest percentage frequency of the SDF-1 3'A/3'A (11.1%) while Rift Valley had the highest percentage frequency of the SDF-1 G/A (36.4%). The two provinces are inhabited by people with the same historical migration route. Having migrated from northern part of Africa, these individuals might have been exposed to same mutagens as the people in Europe and Asia who reportedly had a high frequency of the double mutants (Suresh et al., 2006). Despite the fact that mutations do occur spontaneously and they are mostly lethal, they can also be induced to generate beneficial genes to mankind. Generation of double mutants in a population of SDF-1 3'A/3'A resulting from artificial mechanisms could create individuals resistant to HIV infection. In this study however, it is rather difficult to conclusively say that either the double mutants or the heterozygous individuals have come as a result of spontaneous mutation or exposure to mutagens of some sort.

The Influence of Stromal Derived Factor-1 Double Mutant (SDF-1 3'A/3A) and Heterozygous (SDF-1 G/A) on Human Immunodeficiency Virus Spread

The percentage distributions of the SDF-1 mutant gene and HIV prevalence in all the eight provinces are so diverse. There was no specific trend established between the two variables in all the eight provinces. Analysis of the data obtained in this survey with the information adopted from Kenya AIDS indicator survey (KAIS) and NACC (2007) did not show a significant correlation between the SDF-1 3'A/3'A mutations and the HIV prevalence ($r = 0.324$). There were fluctuations in both HIV prevalence and SDF-1 mutations across the provinces. The patterns of fluctuations between the two variables differed between provinces, for instance, some provinces had high SDF-1 3'A frequencies but showed low HIV prevalence (North Eastern and Rift Valley) while other provinces showed low SDF-1 3'A/3'A incidences as compared to HIV prevalence (Western, Nyanza, Coast, Nairobi and Eastern). Central province had the percentage occurrence of SDF-1 3'A/3'A and HIV prevalence just about the same. Nyanza province had the highest percentage prevalence of HIV,

paradoxically; it also had the highest frequency of SDF-1 3'A/3'A (double mutant). On the other hand, only two provinces (Nairobi and Nyanza) showed a high SDF-1 G/A occurrence than HIV prevalence while the rest of the provinces had high HIV prevalence than the SDF-1 G/A occurrence. This study therefore showed no correlation between the SDF-1 mutation and HIV prevalence in the respective provinces of Kenya.

The study indicated that evolution of HIV infection from the fusion of the first virion to the CD4+ T-cells to AIDS and death is not influenced by SDF-1 polymorphisms alone; rather, there are so many other interacting factors to this course. These factors include immunological factors, infection route (with blood transfusion considered the surest way), age (below fourteen and above forty years considered most vulnerable) and genetic factors that lead to deficiency in or malfunctioning of HIV coreceptors (Mulrerin et al., 2003, Easterbrook et al., 1999). Other factors that influence the course of infection include gender and physiological factors. Genetic factors that cause variations in the functioning of cytokines and chemokines create a diverse range of HLA (which slows down disease progression) and synthesise a multi-drug transporters protein-1 (MDR-1) which protects the cells from the effect of anti-retroviral (ARVs) therapy therefore enhancing infection by HIV and progression to AIDS. More factors that have a bearing on HIV infection includes: nutrition, stress, lifestyles and a naturally occurring human protein (antiviral protein) that prevents viral replication. This protein is coded as APOBEC3G (Meyer et al., 2003).

It is therefore of prime importance to gain a prognostic significance of just a few of these elements for it makes possible to improve the management and long-term outcome of the individuals infected with HIV and AIDS. Host factors, although unalterable, remain important in considering disease development in patients and guidance to ARV therapeutic measures (Simone et al., 2007).

The Significance of the Survey

The findings of this survey contribute to the growing evidence that the presence and effects of genetic variant in the understudied African population are still important when predicting hosts susceptibility to HIV and AIDS within the sub-Saharan African region. There were variations in the distribution of SDF-1 polymorphisms in the eight provinces of Kenya when compared to the pattern of HIV prevalence reported in various literatures (Khamadi et al., 2005; NACC, 2005; KAIS, 2007). More studies on a cohort of SDF-1 3'A infected with HIV are thus required to assess the direct influence of the mutation to pathogenesis of HIV to AIDS. A large sample size could probably give a trend of the relationship between infection with HIV and progressive development to AIDS. Nyanza province could be used for this study as it registered the highest percentages in HIV incidence and SDF-1 3'A/3'A mutation.

Studies have shown that progression of HIV to AIDS has been strongly associated with hosts' SDF-1 gene polymorphisms. Persons with SDF-1 3'A (a subset of the SDF-1 double mutants polymorphism) represent a group of people that can enable the study of complex interactions between the hosts' chemokine (CXCR4) receptors (natural ligands for the SDF-1 3'A) and the viral determinant (gp120). This is because hosts' immunologic responses have been linked to slow progression to AIDS (Magierowska et al., 1999; Hendel et al., 1998). Polymorphism in SDF-1 may assist with a favourable course of HIV disease progression. The SDF-1? interferes with the T-tropic HIV strains from binding onto the CXCR4 on the CD4+ T-cells thereby down-regulating cell invasion hence protecting one from HIV infection or slowing down progression to AIDS once infected with HIV (Oberlin et al., 1996; Bleul et al., 1996; Amara et al., 1997).

It is important to note that besides SDF-1 3'A/3'A, progression of HIV to AIDS is controlled by an association of multiple parameters which includes viral load, CD4 count, host genetic markers (HLA genes) and chemokine receptors (CCR5 and CXCR4). More research has led to controversies about genetic influence to HIV infection and AIDS spread and this has been addressed in several literatures by several scientists (Desiree et al., 2005; Winkler et al., 1998; Magierowska et al., 1999). However, immune response to HIV infection and possibilities of resistance can still be clarified with further research.

Conclusion

The research findings in this study are important as they showed existence of the stromal derived factor-1 mutation in the Kenyan population. The overall frequency of mutations were 13/200 for the double mutants (SDF-1 3'A/3'A) and 42/200 for half mutants (SDF-1 G/A). However, Nyanza province had the highest frequency (3/28) for the double mutants while Rift Valley province had the highest frequency (8/22) for the half mutants. Overall percentage distribution of the SDF-1 polymorphism in Kenya was 6.6% (double mutants), 20.7% (heterozygous) and 72.7% for the wild type.

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