PHYLOGENETIC ANALYSIS OF HIV-1 ISOLATES FROM GEM SUB-COUNTY OF SIAYA COUNTY- KENYA

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ABSTRACT: As the HIV pandemic becomes increasingly complex and devastating in Africa, there is need to come up with better management strategies in terms of treatment, vaccine and better testing methods. However, this is getting hampered by the high diversity of Human Immunodeficiency Virus type 1 (HIV-1), which is brought about by high rate of replication and mutation. The occurrence of mutations leads to emergence of diverse subtypes and variants which are genetically related but distinguishable. Studies have associated the different subtypes with different clinical and public health consequences. In this study, molecular analysis of the Protease and Reverse Transcriptase gene sequences of the HIV-1 isolates from plasma samples collected from Gem sub county in western Kenya was done. Genes sequences generated were aligned with consensus sequences obtained from Los Alamos HIV Sequence database. Phylogentic analysis was then done using PAUP™ software, version 4.0. A total of 21 HIV-1 plasma isolate samples from Gem sub-county were taken for molecular analysis. The results showed several mutations in both Protease and Reverse Transcriptase gene regions. The phyllogenetic analysis revealed, 16 (76.2%) of the 21 isolates analyzed to be subtype A, subtype D were 4 (19.0%), and the remaining 1 (4.8%) was circulating recombinant form, CRF_AD. Since this study revealed three different HIV-1 subtypes in Gem, it would be necessary to conduct a future study to find out the effect of these subtypes on the transmission, pathogenicity and the rate of HIV-1 disease progression in Gem sub-county, western Kenya.

KEYWORDS: Mutation, Diversity, HIV Subtype, Antiretroviral, Protease, Reverse Transcriptase, Recombinant form.

1 BACKGROUND

Human Immunodeficiency virus (HIV) is a kind of virus that belongs to a group of viruses called retroviruses. It is believed that HIV originated in sub Saharan Africa during the twentieth century. [1] and it is now a pandemic, with an estimated 38.6 million people currently living with the HIV worldwide. [2] It has its genome in form of ribonucleic acid (RNA), and inside it are the three enzymes necessary for replication. These are surrounded by a coat of glycoprotein. This virus, like other pathogens needs a host cell to complete its life cycle. It starts its life cycle by first attaching to the host cell, usually the CD-4 T-lymphocyte cell, which is a cell of the immune system. After this attachment, it empties its content into the cell and with the help of its reverse transcriptase, it changes its genome, usually RNA into DNA and with the help of its intergrase enzyme, it integrates its genome into the host cell DNA. Once integrated, it is called a provirus.

When the host cell undergoes transcription it produces the viral messenger RNA (mRNA) which eventually get translated to viral protein which then gets chipped into sizes by protease enzymes into new viral particles which eventually burst the host cell. This process leads to killing of many of immune system cells thereby weakening the immune system, leading to a condition referred to as Acquired Immunodeficiency Syndrome (AIDS).[3] Once immune system is weakened, one remains
prone to opportunistic infections and tumors. Currently, there is no known vaccine or medicine that cures HIV/AIDS, save for the antiretroviral drugs that inhibit action of viral enzymes that are necessary for viral replication. Vaccine development has been hampered by the high rate of viral mutations that occurs naturally, enabling HIV to exist in an individual as a complex mixture of genetically related but distinguishable variants. If mutations occur in the gene region encoding molecular antiretroviral drug target, then this may cause ARV drug resistance.

In African continent, HIV prevalence varies. For instance, in southern African countries, more than one in every five pregnant women is HIV infected. In a few sub Saharan African countries, HIV prevalence in prenatal clinics in 2003 exceeded 10%, while in urban settings in southern Africa, antenatal sero-prevalence reached 40%. In West Africa, HIV prevalence in pregnant women remain generally stable at low levels, though in some urban areas it exceeds 10 percent, while in rural areas the rates are generally low.

In East Africa and parts of Central Africa, prevalence has fallen, for instance, in Addis Ababa, among 15-24 years old pregnant women, the prevalence fall to 11% in 2003 from 24% in 1995. Previous studies reveal that Asia is experiencing rapidly growing epidemics. Likewise, Eastern Europe is also experiencing rapid increase in prevalence, which is being attributed to rise in mother to child transmission. The risk of mother to child infection increases if a breast feeding mother is newly infected, owing to the initially high level of virus. It is therefore important that more attention should be directed to HIV/AIDS prevention and treatment.

HIV infection is most commonly due to unprotected sex with infected partner, but can also occur from receiving contaminated blood during transfusion or exposure to non sterile instrument or medical procedure. Most infected children under 15 years have contracted the virus by transmission from their mothers, an indication of the prevalence of infection in women of child bearing age. Mother to child transmission of HIV can occur before, during or after delivery, but only in rare cases does it occur during early stages of pregnancy. In Europe and America, the estimated rate of mother to child HIV transmission ranges from 14-25% while in developing countries, it ranges from 13-42%. In developed countries, antiretroviral therapy, elective caesarean section, and refraining from breastfeeding have been used to reduce the rate of mother to child transmission with some success. In developing countries, peripartum antiretroviral prophylaxis with one drug alone can reduce the rate of infection in breastfed infants assessed at 2 or 3 month of ARV administration to around 10% and with two or more drugs to about 7 percent at 6 weeks. Prolonged breastfeeding has been reported to expose infants more to the risk of HIV transmission. The risk of the mother to child transmission has been associated with the maternal viral load in plasma. A study also indicated the risk of mother to child transmission to depend on the subtype the mother harbors, where subtype D is easily transmitted than subtype A and C.

The current approach to prevention of mother to child transmission targets the late intrauterine and intrapartum periods, because this period is a relatively short interval of relatively high risk. An estimated 40 percent of overall transmission occurs in late pregnancy and during labour pain and delivery. Peripartum antiretroviral prophylaxis reduces transmission risk in the period of and around delivery. Studies have shown that antiretroviral therapy can reduce overall risk of HIV infection even in breastfeeding populations. Transmission through breastfeeding has been well documented with the initial reports indicating the possibility of transmission through breast milk in breastfed infants being of women who had been infected postnatally through blood transfusion or through heterosexual exposure. Another study conducted revealed infant infection through wet nursing.

There are two types of HIV responsible for infection, namely, HIV-1 and HIV-2. A major proportion worldwide is caused by HIV-1 virus which was first identified in 1983. The HIV type 2 was first detected in West Africa and is significantly present in this region since the beginning of AIDS epidemics two decades ago. HIV-1 has evolved significantly varying from one geographical region to another. HIV-1 is the most diverse and has evolved into three major groups, mainly, group O (Outlier), N (non M) and M (major). M group is the most divergent group and has evolved into nine different subtypes, subtype A, B, C, D, F, G, H, J and K.

All the subtypes originated from central Africa. In addition, strains of HIV-1 recombinants forms have been identified to be in circulation. Other forms which have been recognized are, A to H with subtypes A and B being the most prevalent. A study conducted in South Africa indicated that the virus diversity influences transmission and pathogenicity, and it associated subtypes B with male homosexual transmission and C with heterosexual transmission. Other studies also associate subtype A and G with longer AIDS free survival period, which is opposed to other non B subtypes. All groups of HIV-1 are found in Africa; while group M is found all over the continent, group N and O are restricted to Central Africa. Subtypes A, and D are prevalent in East Africa subtype A in West Africa, and C in South Africa, while recombinant subtypes viruses are found in Central and West Africa. In West and Central Europe, America and Australia, subtype B is the most prevalent form. Globally, subtype C is the predominant form causing much of heterosexual
infections worldwide. Unlike HIV-1, HIV-2 does not vary so much in geographical distribution, and almost all types of HIV-2 are found in West Africa and majority of HIV-2 characterized belong to the group A reported in West Africa region.

HIV genetic diversity has significant clinical and public health consequences; HIV mutagenic potential and diverse assortment of antigenic epitope has hampered HIV vaccine development effort. Many vaccine development attempts have concentrated on subtype B which is the dominant in the developed world which fund such vaccine development initiatives, raising the concern whether they will be effective in Africa where none subtype B dominate. The high genetic diversity also poses threat to testing and diagnosis of HIV. Most HIV tests detect the HIV antibodies, and originally, these tests relied on subtype B epitopes. For instance, when type O emerged in 1990s, it tested negative with kit manufactured based on subtype B model. Therefore, HIV diversity data is a very important tool in HIV and AIDS management and yet there is still relatively little information on the viral diversity in rural western Kenya, and the few studies on diversity in Kenya have been tilted to either antenatal clinic attendee or commercial sex workers in the urban centres thereby leaving out the general rural population. So it was necessary to carry out a study to determine the HIV subtypes circulating in Gem District, western Kenya. The findings of this study will be useful for better management of HIV/AIDS in Gem western Kenya, and other parts of the country.

2 MATERIALS AND METHODS

2.1 STUDY AREA

This study was conducted in Gem District, western Kenya, within Nyanza province. It is an area of approximately 403.1 km², and about 42 kilometers northwest of Kisumu town. Gem lies between latitude 0° 26′ to 0° 12′ north of Equator and longitude 33° 58′ east and 34° 05′ west of Prime meridian. It is an area were cultural practices, which fuel HIV transmission, such as wife inheritance and polygamy are common. It lies along the Kenya - Uganda highway, with trading centres along the highway.

2.2 STUDY POPULATION AND SAMPLING SCHEME

Study Population and Sampling Scheme According to 2007 Kenya AIDS Indicator Survey (KAIS), 7.1% adult (aged 18 years and above) Kenyans (representing 1,417,000) were HIV infected; Nyanza province in which Gem, western Kenya falls under had an average of 14.9% adult infection. Gem population was estimated at approximately 75,000, so working with 14.9% which was the average adult HIV prevalence in Nyanza province an estimated 11,175 adults were HIV infected in Gem, western Kenya, from which the study sample of 45 was drawn. The study sample of 45 tested HIV positive by Unigold™ Using the software Power and Sample Size program, 45 HIV positive adults were randomly selected to be able to reject the null hypothesis with a power of 0.95, given a standard deviation of 1.96 and type I error probability of 0.05.

2.3 INCLUSION CRITERIA

To be included in the study, one had to be an adult aged 18 years and above and tested HIV positive with Unigold™, and also, must have been resident of Gem for the last 6 months and willing to participate in the study.

2.4 EXCLUSION CRITERIA

Any persons below 18 years of age, and/or testing HIV negative with Unigold™ was excluded from the study. All persons not having been living in Gem in the previous 6 months, regardless of age and/or HIV status, were also excluded from the study.

2.5 ETHICAL CONSIDERATIONS

Informed consent was obtained from those individuals meeting inclusion criteria. Approval for this study was obtained from the ethical review committee at Kenya Medical Research Institute (KEMRI) and Human Investigational Review Board (HIRB) at Centre for Disease Control and Prevention (CDC), Atlanta, Georgia, USA. Since the process of drawing blood samples exposed the participants to some risk of pain, the process was carried out professionally and in a sterile manner to minimize the risk of infections.
2.6 **SAMPLE COLLECTION AND PROCESSING**

All the prospective study participants were requested to give 5 millilitres of blood and this was taken through venipuncture, put into anticoagulant tubes (EDTA tubes) and then put in cooler box and transported to the main KEMRI/CDC-HIV research laboratory for plasma separation. The amount chosen was sufficient for an extra aliquot to be stored as back-up during the experiments. The samples were centrifuged to separate plasma from whole blood and the plasma aliquoted in two 1.5 millilitres tubes (an extra tube as a back-up) and kept in a - 80°C freezer awaiting RNA extraction and nucleic acid analysis.

2.6.1 **QIAGEN™ RNA EXTRACTION**

During extraction, one tube of plasma of each sample was drawn from the freezer and thawed in ice (4°C) in the biosafety cabinet. An aliquot of 500 microlitres was made into another 1.5 millilitre tube. This was then centrifuged for one hour at 16400 revolutions per minute at 4°C in a refrigerated centrifuge (microcentrifuge, Eppendorf, USA). After spinning, 360 microlitres of the supernatant was pipetted out leaving 140 microlitre as the pellet. Then 560 microlitres of lysis buffer (in the QIagen™ RNA extraction kit) was added to the pellet, mixed by pulse vortexing and then briefly centrifuged. This was then incubated at room temperature for 1 hour to allow for maximum lysis of viral particles to occur. This was then followed by brief spinning and pulse vortexing. Exactly 560 microlitres of absolute ethanol was added and mixed by pulse vortexing followed by brief spinning to coagulate the viral nucleic acid. Then 630 microlitres of this sample was then added to the spin column, put in a 2-millilitre collection tube, and then span for 1 minute at 8000 revolutions per minute and at 25°C and the filtrate discarded. (The membrane in the spin column is meant to trap the viral nucleic acid as the filtrate passes.) This step was repeated with the remaining sample. Then 500 microlitres of wash buffer-1 in the kit, was added to the spin column to wash the already membrane trapped RNA. Same procedure was repeated with wash buffer-2 in the kit, and spinning at 8000 revolutions per minute done for 3 minutes at 25°C. Elution of the membrane trapped RNA was finally done with 60 microlitres of diethylpyrocarbonate (DEPC-treated) water in a separate 1.5 millilitres sterile RNase free tube. In cases where the RT-PCR reaction was not to be set immediately, the RNA extract was stored at -80°C.

2.6.2 **RT-PCR**

The protease and the RT regions from each patient sample were first amplified using RT-PCR. During this reaction, 10 microlitres of the RNA extract was added to the PCR reaction mix containing 1µl of supper script III one step enzyme (Gibco Chemicals, USA), 2 microlitres of 10 millimolar dNTP (Applied Biosystems USA), 10 microlitres of 5X PCR buffer (Applied Biosystems, USA), 0.5 microlitres of RNase inhibitor (Applied Biosystems, USA), 23.5 microlitres of DEPC treated water (Sigma Chemicals, USA) and a set of the following 1.5 microlitres of 8 micromolar of each primers: RT gen 4R, 5´-TAATTTTTAGGGAAAGATCTGGCCTCC-3´ bp2082-2108 and pro-out 5´-CTGTTAGTGGCTTGGTCTCT-3´ bp 3399-3420 (CDC, HIVR Laboratory, Atlanta, Georgia, USA). The reaction was performed under the following conditions: initial denaturation and subsequent reverse transcription at 65°C for 10 minutes, and 50°C for 45 minutes, respectively; denaturation of cDNA at 94°C for 2 minutes, 40 cycles at 94°C for 15 seconds, 55°C for 20 seconds, and 72°C for 2 minutes, and final extension of 72°C for 10 minutes. Deionized water was used as negative control for the reaction.

2.6.3 **NESTED PCR**

Since RT-PCR ended up with a little bit larger fragments, it was therefore necessary to do nested PCR to narrow the amplification to the specific fragment of interest. This second round of amplification was carried out in a 50 microlitres reaction mixture comprising of 10 microlitres of the RT-PCR product, 1 microlitres of 2.5 units of Taq polymerase (Applied Biosystems, USA), 2 microlitres of 10 millimolar dNTP (Applied Biosystems, USA), 10 microlitres of 5x PCR buffer (Applied Biosystems USA) and 24.5 microlitres of DEPC treated water (Sigma Chemical, USA) and a set of the following 1.5 microlitres of 8 micromolar of each primer: 215/219, 5´ TCAGAGCACAGAGCCACACACCCC-3´ bp 2136-2163 and PAF-4 5´CCTACTAATCTTGTAGTATTTGACACTCAGCT 3´ bp 3300-3334 (CDC, HIVR Laboratory, Atlanta, Georgia, USA), and the reaction performed with the cycling parameters of 94°C for 4 minutes followed 40 cycles at 94°C for 15 seconds, 55°C for 20 seconds, and 72°C for 2 minutes with a final extension step at 72°C for 10 minutes. Deionized water was used as negative control for the reaction.
2.6.4 **Gel Electrophoresis**

Gel electrophoresis was done by mixing 5 microlitres of the nested PCR product with 2 microlitres of the loading dye and loaded onto a 1% ethidium bromide stained agarose gel. This was followed with electrophoresis at 130V for 20 minutes. The loaded well that did not show any band as shown in Figure 2.0 were considered negative for HIV-1. Those that did show band as shown in Figure 2.0 at position 1200 basepairs as indicated by mass ladder control, were considered to be positive for HIV-1, and so were taken for purification prior to sequencing. At this stage, out of the 45 samples initially taken for amplification, only 30 samples amplified.

![Figure 1.0: Picture of a gel showing positive and negative samples](image)

A picture of a gel taken after electrophoresis of nested PCR product to identify the samples where gene amplification occurred. Each sample marked as S1-S8 was loaded into its well and electric current applied at a voltage of 130v for 20 minutes. Since the region amplified was 1200 basepairs in size, the samples in which gene amplification occurred; S5, S6 and S8 had bands corresponding to a 1200 basepair band in the ladder (control) as shown.

2.6.5 **QIAquick Purification of PCR product**

Due to the presence of nonspecific bands on some samples, the PCR products were purified with the QIAquick gel extraction kit (Qiagen, USA) as per the manufacturer’s instructions.

2.6.6 **Dilution of PCR Products**

For the samples that showed bigger bands, which meant high concentration of PCR product, dilution with DEPC-treated water was done either 1 in 4 or 1 in 10 depending on the size of the band (the bigger the band the more the DNA in the sample and therefore the higher the dilution). Dilution of the PCR product was done so as to have a less concentrated product recommended for sequencing reaction as this would give more distinct peaks during genetic analysis.

2.6.7 **Sequencing Reaction**

PCR products were sequenced using overlapping oligonucleotide primers. Sequencing reactions were performed using the Big Dye Terminator Cycle Sequencing Ready Reaction sequencing kit (Applied Biosystems, USA) according to the manufacturer’s instructions.
2.6.8 **Purification of the sequenced product**

This step was carried out to get rid of excess dDNTPs from the sequenced product and the purification at this step was done using Centri-sep Columns™ (Applied Biosystems, USA). The Centri-sep Column™ was first prepared by tapping it for the gel in it to settle at the bottom, the top was then removed. 0.8 ml of distilled or deionized water was added to the column and the top taken back, and this was allowed to settle for 1 hour at room temperature so as to fully wet the gel in the Centri-sep Column™. The column was then gently tilted on one side and tapped to remove any air bubble that could be trapped in the gel. Then, the top of the column was removed followed by the bottom seal and the column put in collection tube to collect the filtrate while spinning for 2 minutes at 300rpm. After this the filtrate was discarded and columns put in sterile 1.5ml tubes and the sample i.e. the sequenced product, added by gently pipetting from the top of the gel in the column and then briefly spinning to get out the purified sample as the filtrate.

2.6.9 **Speed Vac drying of the sequenced product**

The samples were then arranged in the Speed Vac™ (Applied biosystems, USA) for drying, and drying manually done at medium speed for 30 minutes.

2.6.10 **Denaturation**

The dried samples were then resuspended in the 20 microlitres of formamide and pulse vortexed and left to settle for 30 minutes after which the samples were put back to the optical tubes and loaded to the thermocycler set at 94°C for 2 minutes for the denaturation before sequence analysis.

2.7 **Sequence analysis**

The sequencing products were run on ABI 3100™ (Applied Biosystems) Genetic Analyzer and then assembled by Sequencher™ software (Gene Code, Ann Arbor MI). The PRO and RT gene sequences generated were then aligned with consensus sequences obtained from Los Alamos HIV Sequence database (http://hiv_web.lanl.gov) representing HIV-1 subtypes including A1, A2, B, C, D, G, F1, F2, K, H, J, and the circulating recombinant forms. Phylogenetic analysis was performed using PAUP™, Version 4.0, and maximum likelihood tree constructed and confidence of analysis tested using bootstrap values. The gene sequences were further analyzed by Similarity analysis using SimPlot™ to determine whether they were pure subtypes or recombinant forms. Similarity plot using SimPlot™ version 2.5 Software (S.Ray, http://www.med.jhu.edu/deptmed/sray) determined the percent similarity between Gem sequence and a selected group of reference sequences by moving a window of 400 basepairs fragment with 20 basepairs increment along the alignment. Similarity values were plotted at the midpoint of 400 basepairs fragment and similarity curve generated.

2.8 **Limitations of the study**

The study used in-house PCR method, which had just been developed by CDC/KEMRI HIVR Laboratory, Kisumu-Kenya, so was still not yet well refined and therefore could affect the PCR result. (It could amplify only up to 2000 viral copies per milliliters of plasma). The study was carried out under very strict management conditions that at times did not allow for repeat of certain assays that failed, like when some primers failed to anneal during sequencing reaction leading to failure of some samples to sequence.

3 **Results**

3.1 **Phylogenetic Analysis**

Phylogenetic analysis was done sing PAUP™ software and phylogenetic tree constructed as shown in Figure 2.0 Many of the sequences marked ‘Gem’ clustered next to subtype A reference sequences, confirming them as subtype A sequences. Some clustered next to subtype D reference sequences confirming them as of subtype D, while ones sequence, Gem.3001670 was next to subtype A, but with a very short branch as compared to other subtype A. Other analysis (similarity analysis) revealed it as a recombinant form (CRF_AD). The phylogenetic analysis result was extracted from the tree and tabulated as shown in Table 1.0, so as to show the subtype of each individual sequence analyzed phylogenetically. Out of 21 samples analyzed, 16 (76.2%) were subtype A, subtype D were 4 (19.0%), while circulating recombinant form (CRF_AD) was 1 (4.8%), as summarized in Table 2.
Figure 2.0: phylogenetic tree

The tree estimates the evolutionary past based on gene sequences. It consists of branches and nodes. The tree consists of both internal and terminal nodes as shown. The internal node corresponds to hypothetical last common ancestor of gene sequences arising from it, while the terminal node corresponds to the sequences from which the tree was derived. The length of the branches corresponds to amount of evolution (percent sequence difference) between the two nodes they connect. Thus the longer the branch, the more divergent the sequences attached to it. In Figure 3, the sequences analyzed are marked in the tree as ‘Gem’ in the branches, and amongst them are other sequences that act as reference sequences in the tree.
Table 1: Distribution of HIV-1 subtypes for individual Gem sequences

<table>
<thead>
<tr>
<th>Sample codes</th>
<th>Subtypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gem. 007 (control sample)</td>
<td>A</td>
</tr>
<tr>
<td>Gem.2000260</td>
<td>A</td>
</tr>
<tr>
<td>Gem.3002680</td>
<td>A</td>
</tr>
<tr>
<td>Gem. 2005360</td>
<td>A</td>
</tr>
<tr>
<td>Gem.2005470</td>
<td>A</td>
</tr>
<tr>
<td>Gem.3000040</td>
<td>A</td>
</tr>
<tr>
<td>Gem.300080</td>
<td>A</td>
</tr>
<tr>
<td>Gem.3000780</td>
<td>D</td>
</tr>
<tr>
<td>Gem.3001060</td>
<td>A</td>
</tr>
<tr>
<td>Gem. 3001150</td>
<td>A</td>
</tr>
<tr>
<td>Gem. 3001230</td>
<td>A</td>
</tr>
<tr>
<td>Gem. 3001670</td>
<td>CRF_AD</td>
</tr>
<tr>
<td>Gem. 3001990</td>
<td>D</td>
</tr>
<tr>
<td>Gem 3002400</td>
<td>A</td>
</tr>
<tr>
<td>Gem.3002500</td>
<td>A</td>
</tr>
<tr>
<td>Gem. 3003360</td>
<td>A</td>
</tr>
<tr>
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</tr>
<tr>
<td>Gem. 3004000</td>
<td>A</td>
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<td>Gem. 3004030</td>
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<tr>
<td>Gem.3004740</td>
<td>A</td>
</tr>
<tr>
<td>Gem. 3005280</td>
<td>D</td>
</tr>
<tr>
<td>Gem.3005690</td>
<td>D</td>
</tr>
</tbody>
</table>

Table 2: Summary of HIV-1 subtypes circulating in Gem, western Kenya.

<table>
<thead>
<tr>
<th>Subtype A</th>
<th>Subtype D</th>
<th>CRF_AD</th>
</tr>
</thead>
<tbody>
<tr>
<td>16 (76.2%)</td>
<td>4 (19.0%)</td>
<td>1 (4.8%)</td>
</tr>
</tbody>
</table>

3.2 RECOMBINATION ANALYSIS

Recombination analysis was done as a confirmatory test on the subtypes and the similarity curves plotted as shown. Similarity curve showing sequence Gem 2000260 as subtype A is shown in Figure 4, while Figure 5 shows a similarity curve for sequence, Gem 3005280, which was a representative of subtype D. Figure 6 shows similarity curve for sequence, Gem 3001670, which was found to be a recombinant form (CRF_AD). So the similarity analysis which was meant to confirm recombination among the subtypes showed only one sequence, Gem 3001670 (Figure 5) as the only recombinant form.
Similarity plot to determine percentage similarity between sequence, **Gem 2000260** and a group of selected reference sequences for subtype A, B, C, D, F, and K by moving a window of 350 basepairs fragments with steps of 10 basepairs increments along the alignment and similarity values plotted at the midpoint of 350 basepairs fragment.

![Similarity curve for sequence Gem 2000260](image)

Figure 4.0: Similarity curve for sequence Gem 3005280 (subtype D)

Similarity plot to determine percentage similarity between sequence, **Gem 3005280** and a group of selected reference sequences for subtype A, C, D, and H, by moving a window of 400 basepairs fragments with steps of 10 basepairs increments along the alignment and similarity values plotted at the midpoint of 400 basepairs fragment.

![Similarity curve for sequence Gem 3005280](image)

Figure 5.0: Similarity curve for sequence Gem 3001670 (recombinant CRF _AD)

Similarity plot to determine percentage similarity between sequence, **Gem 3001670** and a group of selected reference sequences for subtype A, C, D,H, O, and N by moving a window of 400 basepairs fragments with steps of 10 basepairs increment along the the alignment and similarity values plotted at the midpoint of 400 basepairs fragment.

![Similarity curve for sequence Gem 3001670](image)

Figure 6.0: Similarity curve for sequence Gem 3001670 (recombinant CRF _AD)

### Discussion

Phylogenetic analysis revealed that HIV epidemics in Gem sub-county is driven by subtype A, which was 76.2%, followed by subtype D which was 19.0%. There was also a small percentage of recombinant form (CRF _AD), 4.8%. The subtypes found in this study were similar to those found in another study in Kenya, except that it did not find subtype C and G which were
found in the previous study. The finding of this study were also in line with an other HIV-1 diversity study conducted in Kenya which found HIV-1 subtype A to be the dominant followed by subtype D. Other similarity analysis studies in Kenya have shown that most of the HIV-1 recombinant forms in Kenya have a portion of HIV-1 subtype A, so the recombinant form found in this study too was a combination of subtype A and subtype D. In this study some of the subtypes A in the phylogenetic tree were clustered around Ugandan subtype A strains which is an evident of the social interaction between the people of Gem subcounty and Uganda. The existence of HIV recombinant form in Gem confirms a possibility of co-infection. The existant of recombinant form of subtype A and D in this study therefore reveals coinfection by these two subtypes in this region. It may even involve other subtypes though not revealed by the study.

5 CONCLUSIONS

The study revealed that in Gem sub county, HIV-1 diversity is constituted by two subtype A and subtype D, with subtype A being the dominant. The result also showed that recombinant form (CRF_AD) also exists in small proportion. The findings of this study are in consistence with other studies that are so far conducted in Kenya except for the slight variations which may be attributed to sample origin.

Other studies conducted in other regions in Africa have shown that HIV diversity has influence on the transmission, rate of disease progression and pathogenicity. It is therefore advisable to carry out a future study to determine the effect of subtypes found in this study on the transmission, rate of HIV disease progression, and pathogenicity in Gem, western Kenya.

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