Studies on Human Immunodeficiency Virus Genetic Drug Resistance and Subtype Distribution in Northern South Africa.

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Summary of the study

HIV/AIDS is still the world's leading infectious cause of death, despite global efforts for prevention, better access to treatment and management of patients. At the end of 2010, about 33.3 million people were living with HIV/AIDS in the world. Sub-Saharan Africa is the most affected region in the global AIDS epidemic, with 22.5 million adults and children infected with the virus. Even though the use of antiretroviral treatment has significantly reduced HIV-related mortality in the industrialised world, only a small number of people in need of treatment in the developing world actually have access to it. Global initiatives aiming at universal access to treatment are still ongoing

Southern Africa has the highest HIV prevalences, with very serious health and socioeconomic consequences. The nine countries with the highest HIV prevalence in the world are found in the sub region. In 2009, National adult HIV prevalence exceeded 10% in nine countries of Southern Africa (Botswana, Lesotho, Mozambique, Namibia, South Africa, Swaziland, Zambia, Malawi and Zimbabwe). This has a negative effect on health care delivery, especially in a region that already has limited human and health infrastructure. Financial resources which should have been used for development are now being spent on the procurement of antiretroviral drugs. South Africa is one of the countries in this region which has been hit hardest by this pandemic. According to the South African National HIV survey conducted in 2009, the estimated prevalence was 29.3% among antenatal women aged 15 to 49 years old. The survey also estimated that the provinces most affected were KwaZulu-Natal (39.5%), Mpumulanga (34.7%) and Free State (30.1%). Limpopo is the seventh, with a prevalence rate of 20.7%. Western Cape Province has the lowest prevalence (16.9%).

Rollout of antiretroviral therapy in sub-Saharan African countries with high HIV prevalence has been identified by the World Health Organization as a global public health priority. Hence, the use of highly active antiretroviral therapy has been rolled out in recent years, through the massive financial support from programs such as the US President's Emergency Plan for AIDS Relief and the Global Fund for AIDS, Tuberculosis and Malaria.

Access to antiretroviral therapy is expanding in South Africa, including the Limpopo province. Antiretroviral drugs have been available to some communities for close to a decade in the Limpopo province (Northern South Africa). Within this time, there is the possibility of drug resistance emergence and transmission. In addition, resistance testing is not routinely performed prior to treatment initiation. Treatment is not accompanied by regular virological monitoring due to the high cost of viral load and drug resistance tests. As a result of this, patients stay on a standard first line therapy (which might be failing) for long periods, with the consequence of the emergence of high-level resistance.

There is paucity of information on the genetic resistance profile of patients in South Africa. Baseline HIV-1 genotypic drug resistance data are very important for resistance monitoring purposes, especially in a country such as South Africa, where large scale treatment programs have been initiated. It is very necessary to address the lack of knowledge about genotypic drug resistance in non B viral subtypes. The surveillance of antiretroviral drug resistance could be influenced by genetic diversity among HIV-1 subtypes. This information could impact on South African national policy on HIV prevention and monitoring, treatment, and vaccine research. Data generated will equally contribute immensely to the database of Southern African Treatment and Resistance Network, which is a group of African, European, and American researchers investigating issues peculiar to resistance and resistance development in HIV-1 subtype C which is becoming predominant worldwide.

Study subjects for these studies were recruited from the HIV/AIDS Wellness Clinic in Bela Bela and Phela O Phedishe (POP) HIV clinic in Mankweng Hospital, both in the Limpopo Province of South Africa. Bela Bela is located in the Waterberg district which has an estimated HIV prevalence of 27.5%. It is a holiday resort with hotels and camping sites. It is also a stop-over point for long distance truck drivers who patronize sex workers attracted to the luxury facilities of the area. The Bela Bela HIV Wellness Clinic receives patients from the urban and rural sectors of the area. The Clinic is run by the HIV/AIDS Prevention Group (HAPG) - a non-governmental organization established in 1996. On

the other hand, the POP HIV clinic is a component of the Polokwane-Mankweng Hospital Complex. The HIV Clinic has nineteen feeder clinics situated in nearby villages. Mankweng is located in the Capricorn district which has an estimated HIV prevalence of 24.2%. The development of drug resistance is one of the major drawbacks in ART programmes. At the time of sample collection, antiretroviral treatment had been available to the Mankweng and Bela Bela communities for 4 and 8 years, respectively. Besides, these were the first communities to benefit from antiretroviral treatment in northern South Africa. In the intervening time, there is a possibility of resistance emergence and transmission to antiretroviral (ARV) drug naive populations in these two sites. There is a paucity of data from these sites on the prevalence of drug resistance mutations.

This work is focused on three main investigated themes, contained in four separate chapters. The first theme deals with the determination of the prevalence of genotypic drug resistance substitutions in newly diagnosed, drug naive HIV-infected individuals in two different communities - Bela Bela and Mankweng in Northern South Africa. This theme is treated separately for each community and contained in two different chapters. Demographic and socio-economic characteristics of infected individuals, HIV-1 subtype distribution, intragenetic diversity of the *protease* (PR) and *reverse transcriptase* (RT) gene sequences; and HIV-1 genetic recombination, were also examined. Drug resistance mutations were identified and described using the Stanford HIV Drug Resistance Interpretation Algorithm and the International AIDS Society-USA guidelines or the Calibrated Population Resistance (CPR) tool contained in the Stanford HIV Drug Resistance database. Demographic and socio-economic characteristics of the subjects were collected using a study questionnaire. This enabled the collection of information on age, sex, place of residence, probable place and date of infection, marital status, house hold income, level of education, and number of dependants. Subtyping of HIV was done by phylogenetic analysis. Recombination analysis was done using the Recombination Identification Program and REGA HIV-1 subtyping tools. REGA employs phylogenetic analysis and bootscanning methods to assign genetic subtypes and recombination patterns respectively. Predicted amino acids of the PR and RT genes were aligned using the

BioEdit program. The intragenetic distances among the *protease* and *reverse transcriptase* sequences were determined by the Kimura 2- parameter model.

In Bela Bela where treatment became available to the community in 2001, major drug resistance mutations were detected in 2 out of 57 subjects (3.5%). One primary NNRTI mutation (Y181C) and one major PI mutation (L33F) were detected on two different patients. No primary NRTI mutation was recorded. The study group was made up of HIV-1 infected, treatment-naive subjects. The mean age of the subjects was 43.5 years (18 – 69 years). The most important risk factor for HIV transmission was sexual intercourse (88.6%). Phylogenetic analysis of the PR and RT genes showed that 56/57 (98.2%) of the viruses were HIV-1 subtype C on the PR; while 54/55 (98.2%) of the viruses were HIV-1 subtype C on the RT. One virus (08BBVCT31ZA) was HIV-1 subtype B on the PR and RT genes. Mean genetic distance for the PR sequences ranged from 0.0101 to 0.2035 and 0.0331 to 0.1377 for the RT sequences.

In Mankweng, 5 sequences (9.3%) harboured drug resistance mutations. Four of these (7.4%) were NRTI mutations and one (1.9%) was a PI mutation (M46I). No major NNRTI mutation was detected. The study group was also made up of HIV-1 infected, treatment-naive subjects. Phylogenetic analysis of the partial *pol* sequences showed that 52/54 (96.3%) of the viruses were HIV-1 subtype C. One virus (08MB08ZA) was HIV-1 subtype B, while another (08MB26ZA) was related to HIV-1 subtype J. HIV-1 subtype recombination analysis with REGA assigned the *pol* sequence to HIV subtype J (11_cpx) with a bootstrap value of 75%.

Apparently, the testing of patients for drug resistant viruses before the initiation of therapy may not be required in Bela Bela. However, the prevalence of drug resistance mutations observed in Mankweng is moderately high according to the World Health Organization classification for surveillance of transmitted resistance mutations, and relatively higher than previously reported from other parts of South Africa. According to WHO recommendations, this moderate drug resistance prevalence may necessitate routine drug resistance testing for all persons newly diagnosed with HIV or all persons

beginning HIV treatment in this community. This level also requires a review of the treatment program monitoring data for this community and the investigation of potential problems with regards to factors such as: continuous access to services, drug supply, drug quality, prescribing practice, compliance toxicity and/or adverse events, drug sharing and treatment failures. However, resistance in this population does not reflect current transmission of resistant HIV, because many newly diagnosed persons have been infected many years prior to diagnosis. Subsequent surveillance studies are needed to confirm these findings and to generate data relevant to sentinel surveillance policy, and treatment initiation guidelines.

The second aspect examined in this study focused on the genetic drug resistance profile in a cohort of HIV infected patients entering antiretroviral treatment programmes at two sites in Northern South Africa. Specific issues examined were the baseline demographic and socioeconomic characteristics of HIV-1 infected individuals in the cohort, baseline genetic drug resistance mutation profile of the patients prior to therapy, baseline CD4+ and viral load measurements, HIV-1 subtypes, intragenetic diversity of the *protease* and *reverse transcriptase* sequences and recombination analysis.

Results obtained revealed that the mean age of 112 individuals in the cohort was 40.5 years (range 22 – 59 years). The proportion of women and men was 78/112 (69.6%) and 34/112 (30.4%) respectively. Seventy of the respondents (62.5%) were single compared to 26 (23.2%) who were married. The most important risk factor for HIV transmission was unprotected sex (98.2%). In 88.4% of the participants, the most probable place of HIV infection was South Africa and a further 24/112 (21.4%) reported to have been probably infected in 2008. Probably older infections (2000 – 2007) were reported in 65.2% of the subjects. The mean viral load was 5.4 Log 10 copies/ml (range 3.0 - 5.7). The mean CD4+ count was 138 cells/mm^3 (interquartile range 51-197). Twenty six patients (23.2%) were under treatment for tuberculosis. Overall, 1/112 (0.9%), 11/112 (9.8%), 48/112 (42.9%) and 52/112 (46.4%) were classified as AIDS stage I, II, III and IV respectively according to the WHO system. Two (2.5%) primary RT drug resistance mutations (K103N and M41L) were detected in 2 different subjects. Phylogenetic

analysis of the PR and RT sequences showed that 79/80 viruses were HIV subtype C. One isolate (08BBCR06ZA) from Bela Bela clustered with pure A1 subtypes on the PR gene and pure C subtypes on the RT gene. Further analysis of the partial *pol* of 08BBCR06ZA (comprising the complete PR and 900 nucleotides of the RT) with recombination identification programs showed that the *pol* gene has a mosaic structure consisting of A1 sequences alternating with C sequences confirming that the isolate is a recombinant on the gene analyzed. Mean genetic distance for the PR sequences ranged from 0.0272 to 0.1311; and 0.0978 to 0.1014 for the RT sequences.

The results obtained from this baseline cohort study suggest that majority of the patients entering the ART programmes will obtain therapeutic benefit from the first line ART regimen. In addition, the level of resistant viruses appears to be low at the population level at the two treatment sites after eight years of access to ART. The identification of an A1/C recombinant may be a further indication of the gradual introduction of complex viruses in northeastern South Africa.

The third part of the study focused on the analysis of *integrase* gene sequences of viruses from treatment naive individuals in Northern South Africa. No *integrase* inhibitors are currently being used in South Africa, but with the rise in treatment failure, they could be introduced in the near future as salvage therapy. More understanding is needed on the significance of unknown mutational patterns associated with drug resistance loci in HIV-1 *integrase* gene. Furthermore, previous information gathered on circulating subtypes has largely been sought using the *gag* and partial *pol* genes. Given that HIV-1 is highly variable, it is necessary to attempt subtyping using the *integrase* (IN) gene so as to study the variability of this gene.

Integrase gene sequences from drug naive individuals from Bela Bela and mankweng communities were analysed for drug resistance substitutions, HIV-1 subtypes, intragenetic diversity, HIV-1 IN functional domains and HIV-1 genetic recombination. Using the Stanford HIV Drug Resistance Interpretation Algorithm and the International AIDS Society-USA guidelines, no major drug resistance mutations were detected in any

of the subjects. However, 6/89 (7%) minor mutations and polymorphisms were detected. Phylogenetic analysis showed that 88/89 (99%) of the viruses were HIV-1 subtype C on the IN gene. One isolate (08BBVCT28ZA) did not cluster with any reference subtype. The IN gene (864bp, HXB2 location: 4230-5093) and a partial *pol* fragment of sample 08BBVCT28ZA (1127bp, HXB2 location: 2253-3379) was further analysed for recombination using REGA and RIP recombination tools. The results revealed that the sequence is a mosaic of A1, C, F1, and F2 subtypes on the IN gene and HIV-1 subtype C on the partial *pol* fragment (PR and RT genes). This suggest that the isolate is a recombinant on the IN gene and HIV-1 C on the partial *pol* gene region.

The analysis of IN gene sequences revealed that there are no major Raltegravir associated drug resistant HIV mutations among the treatment naive population in Bela-Bela and Mankweng, South Africa. The significance of the polymorphisms observed is not yet known; however, more studies need to be carried out in order to generate baseline data in anticipation of the eventual use of *integrase* inhibitors in South Africa. The identification of a recombinant virus further indicates the introduction of hitherto undocumented variants in a region where C viruses are known to overwhelmingly predominate.

Overall, there is a difference in the prevalence rate of drug resistance mutations among newly diagnosed persons and those about to start therapy in Mankweng community. A moderately high prevalence (9.3%; 95%CI: 0-16.99) of resistance mutations was observed in newly diagnosed persons; while a low prevalence (2.5%; 95%CI: 0077-0.0863) was observed in HIV-1 chronically infected individuals about to start therapy. Drug resistance mutations may decay over time and this could have influenced the low prevalence observed in the latter group. The prevalence of drug resistance mutations in Mankweng community could be high.

Overall, this study has elaborated on the data on genetic drug resistance in northern South Africa, providing new information on drug resistance levels among drug inexperienced patients, and previously undocument genetic variants. In conclusion, it is recommended

that genetic studies are continued on a regular basis to update data on trends relevant for research and patient management.