GENETIC DIVERSITY, RESISTANCE PROFILE OF HIV AND RISK ASSESSMENT OF MOTHER-TO-CHILD TRANSMISSION IN PREGNANT WOMEN ON ANTI-RETROVIRAL THERAPY IN THE EASTERN CAPE, SOUTH AFRICA

By

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DECEMBER 2018

DECLARATION

I, the undersigned, declare that this thesis entitled, “Genetic Diversity, Resistance Profile of HIV and Risk Assessment of Mother-To-Child Transmission in Pregnant Women on Anti-retroviral Therapy in the Eastern Cape, South Africa” submitted to the University of Fort Hare for the degree of Doctor of Philosophy in Microbiology in the Faculty of Science and Agriculture, and the work contained herein is my original work with exemption to the citations and that this work has not been submitted to any other university in partial or entirely for the award of any degree.

Name: Oladele Vincent Adeniyi

Signature: 

Date: 29/03/2019

DECLARATION ON PLAGIARISM

I, Oladele Vincent Adeniyi, student number: 201515366 hereby declare that I am fully aware of the University of Fort Hare's policy on plagiarism and I have taken every precaution to comply with the regulations.

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Signature: ……. Date: …….

CERTIFICATION

This thesis entitled "Genetic Diversity, Resistance Profile of HIV and Risk Assessment of Mother-to-Child Transmission in Pregnant Women on Anti-retroviral Therapy in the Eastern Cape, South Africa" meets the regulation governing the award of degree of Doctor of Philosophy of the University of Fort Hare and is approved for its contribution to scientific knowledge and literary presentation.

09-04-2019

Prof. C.L. Obi

Main Supervisor

Date

DEDICATION

This final output is dedicated to the Glory of God Almighty for His mercies and protection over my family during the course of the journey of my PhD study.

My wife - Adekanbi Busola Adejoke; you are the pillar behind the completion of this programme. You stood firm and gave me confidence that this is achievable and must be completed.

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Finally, this research output is dedicated to the people living with HIV in the Eastern Cape and worldwide; for the opportunity to contribute to knowledge in this rapidly growing aspect of medicine.

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LIST OF ACRONYMS

3TC - Lamivudine

AAHIVM – American Academy of HIV Medicine

ABC – Abacavir

AIDS – Acquired Immune Deficiency Syndrome

ACTG – AIDS clinical trials group

AOR – Adjusted odd ratio

ART – anti-retroviral therapy

ATV - Atazanavir

AZT – Zidovudine

CAPRISA – Centre for the AIDS Programme of Research in South Africa

CDC – Centre for Disease Control and Prevention

CI – Confidence Interval

CRFs – Circulatory recombinant forms

D4T – Stavudine

DHHS – Department of Health and Human Services

DLV – Delavirdine

DNA – Deoxyribonucleic acid

DRMs – Drug resistance mutations

DRV – Darunavir

DTG – Dolutegravir

EACS – European AIDS Clinical Society

EDTA – Ethylenediaminetetraacetic acid

EFV – Efavirenz

EMTCT – Elimination of mother-to-child transmission

Env - Envelope

ETR – Etravirine

EVG – Elvitegravir

FACT – Follow-on African Consortium for Tenofovir studies

FDCs – Fixed dose combinations

FPV - Fosamprenavir

FTC - Emtricitabine

GFR – Glomerular filtration rate

GP - Glycoprotein

HIV – Human immunodeficiency virus

HPTN – HIV prevention trails network

HSRC – Human sciences research council

HTLV – Human T-lymphotropic virus

IDV – Indinavir

iPrEx – *Initiativa profilaxis pre-exposicion*

Lop – Lopinavir

LTRs – Long terminal repeats

LTNPs – Long-term non-progressors

MVC – Maraviroc

MHC – Major histocompatibility complex

MTCT – Mother-to-child transmission

MTN – Microbicides trial networks

MTN-02-ASPIRE – A Study to Prevent Infection with a Ring for Extended use

NAAT – Nucleic acid amplification test

NFV – Nelfinavir

NNRTIs – Non-nucleoside reverse transcriptase inhibitors

NRTIs – Nucleoside reverse transcriptase inhibitors

NtRTIs – Nucleotide reverse transcriptase inhibitors

NVP - Nevirapine

OR – Odd ratio

PCR – Polymerase chain reaction

PMTCT – Prevention of mother-to-child transmission

PrEP – Pre-exposure prophylaxis

r – Ritonavir

RAL – Raltegravir

RCTs – Randomized controlled trials

RNA – Ribonucleic acid

RNAse - Ribonuclease

RPV – Rilpivirine

RRE – Rev response element

RT – Reverse transcriptase

SADOH – South African Department of Health

SAMRC -South African Medical Research Council

SANAC – South African National AIDS Council

SIV – Simian immunodeficiency virus

SPSS – Statistical package for Social Sciences

SSA – Sub-Saharan Africa

START – Strategic timing of antiretroviral treatment

STIs – Sexually transmitted infections

SQV – Saquinavir

T-20 - Enfuvirtide

TAF – Tenofovir alafenamide

TAM -Thymidine analog mutation

TAR – Transactivational response element

TDF - Tenofovir disoproxil fumarate

TPV – Tipranavir

UNAIDS – The Joint United Nations Programme on HIV/AIDS

UNDP – United Nations Development Programme

UNICEF – The United Nations International Children's Emergency Fund

VL – Viral load

VOICE – Vaginal and oral interventions to control the epidemic

WHO – World Health Organization

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PREFACE

Background

South Africa implemented the World Health Organization Option B Plus strategy (life-long antiretroviral therapy - ART) in 2015 with the aim of achieving the global targets of eliminating mother-to-child transmission of HIV. However, there are no current data on the population-impact of this new strategy in HIV-infected pregnant women on the rate of virologic suppression at delivery and the early mother-to-child transmission rate. Despite the initiation of life-long ART in HIV-infected pregnant women, the rate and determinants of infant HIV transmission are not known, especially in the poor resource settings of the Eastern Cape, South Africa. Maternal anti-retroviral therapy (ART) is crucial for elimination of mother-to-child transmission (MTCT) of HIV. However, the inevitable risks of emergence of HIV drug resistance poses significant threat to achieving this goal of HIV-free generation and keeping mothers alive. Also, it is unclear if women with high viral load at delivery have acquired clinically relevant mutations, which could confer resistance to the ART, thus, further increasing the risks of mother-to-child transmission of HIV-drug resistance strains. In addition to the gaps identified in the prevention of mother-to-child transmission (PMTCT) context, the understanding of regional epidemics is crucial to the broader epidemiological profiling of HIV infections in the country. Despite the rapid influx of foreign nationals to South Africa and Eastern Cape Province, there has not been any molecular epidemiological studies profiling the HIV diversity in the Eastern Cape.

This study therefore documents the genetic diversity, resistance profile of HIV and risk assessment of mother-to-child transmission in pregnant women on anti-retroviral therapy in the Eastern Cape, South Africa. Findings from this study is expected to guide the department of health, programme managers and clinicians to improve the outcomes of care of pregnant women living with HIV in the country. Also, it is expected that the findings of the study will inform the formulation of treatment guidelines and prophylaxis in HIV-exposed infants.

This thesis is organised into six chapters namely, Introduction, Literature review and the experimental aspects comprising Chapters Three to Five and a chapter on General Conclusions and Recommendations. Each experimental chapter is presented as a

publishable unit, comprising Abstract, Introduction, Materials and Methods, Results, Discussions and References.

Chapter one provides an overview of the study. It presents the background and rationale of the study, statement of the problem, research questions, aim and objectives, study hypotheses and significance of the study.

Chapter two deals with extensive review of the literature on HIV epidemics globally, regionally and in South Africa. It also presents the review of the extant literature on the advances in HIV therapeutics and drug resistance. The structure, genomic analysis and diversities of HIV strains are presented with a focus on global, sub-Saharan African and South Africa. Also, the mode of transmission and prevention strategies are presented. In addition, a critical appraisal of the literature on the current epidemiology and trends on the mother-to-child transmission of HIV globally, in sub-Saharan Africa, South Africa and the Eastern Cape are presented. Also, the outcomes of the different World Health Organization strategies are presented as well as the resistance profiles to the first and second line anti-retroviral therapy regimens in the South African context are presented. Research gaps are identified to create the appropriate context for this study.

Chapter three presents the first paper (manuscript ready format). Analysis of the baseline data of an observational prospective cohort study of mother-infant pairs from the Eastern Cape Province, South Africa was conducted. The first aspect of the paper covers the results of the baseline characteristics, rate and correlates of the maternal virological suppression at delivery in parturient women already on anti-retroviral therapy. The second aspect presents the results of the rate and determinants of the early mother-to-child transmission of HIV (in-utero transmission) to the exposed infants in the cohort. Significantly high rate of virologic suppression and low rate of in-utero transmission were observed in the study setting.

Chapter four presents the second paper (manuscript ready format) on the HIV drug resistance among parturient women with virologic failure in the study setting. The paper highlights the baseline characteristics of parturient women with high viral load in whom the partial pol gene analysis of the viral isolates were sequenced and analysed for patterns of mutations. The clinically relevant mutations were presented.

The HIV diversities obtained from the pol gene were also reported. This paper showed that parturient women failing current anti-retroviral therapy are more likely to have acquired clinically relevant reverse transcriptase resistance mutations, which would still be sensitive to the protease inhibitors.

Chapter five presents the third paper (manuscript ready format) on the genetic characterisation of a near-full length genome sequence analysis of recombinant A1/C/D/K/B. The initial partial pol gene analysis (for drug resistance and subtyping) showed assignment of recombinant C/D, however, the near-full length genomic sequence analysis showed a mosaic pattern of A1/C/D/K/B unique recombinant form. This is the first report of this unique recombinant form from South Africa.

Chapter six provides the conclusions and recommendations of the study. High maternal virological suppression at delivery and very low mother-to-child transmission of HIV were achieved in the resource limited settings of Eastern Cape in South Africa. Few women delivering at high viral loads have acquired significantly high rate of drug resistance mutations. Intervention strategies focusing on addressing maternal lifestyle behaviours and ART adherence challenges require targeted research and programmatic re-engineering. An effective surveillance system for tracking all pregnant women on ART will assist in identifying those with virological failure and drug resistance during antenatal, labour and delivery for prompt interventions. A nationally representative drug resistance surveillance in pregnant women should be undertaken to guide future policies and management guidelines in the country. This study found subtype C as the predominant circulating viral strain in the Eastern with occasional presence of CRF02_AG and a mosaic pattern of recombinant A1/C/D/K/

ABSTRACT

Background

Despite the initiation of life-long ART in HIV-infected pregnant women, the rate and determinants of infant HIV transmission are not known, especially in the poor resource settings of the Eastern Cape, South Africa. Maternal anti-retroviral therapy (ART) is crucial for elimination of mother-to-child transmission (MTCT) of HIV. However, the inevitable risks of emergence of HIV drug resistance poses significant threat to achieving this goal of HIV-free generation and keeping mothers alive. Also, it is unclear if women with high viral load at delivery have acquired clinically relevant mutations, which could confer resistance to the ART, thus, further increasing the risks of mother-to-child transmission of HIV-drug resistance strains. In addition to the gaps identified in the prevention of mother-to-child transmission (PMTCT) context, the understanding of regional epidemics is crucial to the broader epidemiological profiling of HIV infections in the country. Despite the rapid influx of foreign nationals to South African and Eastern Cape Province, there has not been any molecular epidemiological studies profiling the HIV diversity in the Eastern Cape.

Objectives

In order to address the gaps identified, these specific objectives were investigated in this study:

1. To determine the rate and determinants of virological suppression in the cohort of pregnant women initiated on lifelong ART within the public sector PMTCT programme in the Eastern Cape.
2. To examine the rate and risk factors for early mother-to-child transmission of HIV to their infants despite maternal ART exposure.
3. To determine the true prevalence of virological failure and patterns of mutations in viral isolates from pregnant women on ART with high viral load at delivery.
4. To characterise the HIV clades and sub-clades in serum samples of parturient women in Eastern Cape.

Methods

This study was conducted in two phases. The first phase was nested in the East London Prospective Cohort Study; which was an observational, prospective cohort

study of 1709 HIV-infected pregnant women attending three maternity centres for delivery in the Eastern Cape, South Africa between 2015 to 2016. Maternal virological responses to ART and in-utero transmissions were analysed. Maternal viral load (VL) at delivery were assayed using HIV-specific quantitative polymerase chain reaction (PCR) and defined as suppressed if VL<1000 copies/ml. In-utero transmission was determined by using the HIV-specific qualitative DNA PCR at birth. Determinants of peripartum viral suppression and in-utero transmission were assessed in both bivariate and logistic regression analyses.

In the second phase of the study, genomic sequence analysis of the partial pol gene were carried out on 80 viral isolates from plasma samples of women with virological failure at delivery between January and May 2018 using standard protocols. The primary outcomes were clinically relevant major resistance mutations in the reverse transcriptase (RT) and protease genes. In the secondary analysis, subtyping of the viral isolates were conducted to identify the diversity of HIV in the region. A near-full length genome sequence of recombinant subtypes identified were undertaken to gain broader understanding of the complete genome.

Results

Out of the total 1463 mothers with available VL results; the overall rate of VL suppression was 82% (n=1200). Parturient women were at increased odds of not achieving virological suppression at delivery if they were younger than 25 years [Adjusted Odd Ratio(AOR)=1.47; Confidence Interval (CI)1.06-2.05, p=0.022], history of defaulting ART [AOR=2.98, CI 1.99-4.44, p<0.001], irregular pick-up of ART [AOR=1.61, CI 1.13-30, p=0.008], unemployed [AOR=1.66, CI=1.14-2.43, p=0.009] and smoked cigarette during pregnancy [AOR=1.99, CI 1.12-3.54, p=0.018] in logistic regression. The overall rate of perinatal transmission was 1.3% (20 of 1539 babies). The risks of perinatal transmission increased along maternal VL gradients: VL<20 copies/ml (0.5%), 20-999 copies/ml (0.6%) and VLs ≥1000 copies/ml (3.3%).

The prevalence of DRMs was 72.5% (n=58). The CD4 count demonstrated a negative linear association with the DRMs (p=0.002). Sub-type C accounted for nearly all the DRMs (98.3%) except one circulating recombinant form (CRF-C/D). The predominant DRMs were K103N (n=43; 74.1%), M184V (n=28; 48.3%) and K65R (n=11; 19%).

Among the parturient women on current treatment of EFV-based regimen; 79.1% already had K103N while nine patients on protease inhibitor-based regimen still harbors K103N. Other mutations conferring resistance to NNRTIs include: V106M (15.5%) and P225H (17.2%). The majority of the M184V mutations were observed in parturient women on first line regimen (n=23; 82.1%). The mean viral load (transmissibility risks) in DRMs was significantly higher than the wild type (174515 versus 52426).

Of the 80 viral isolates sequenced in this study, 78 were subtype C while the remaining two were; recombinants C/D and CRF02_AG. Further amplification of the full-length genomic sequence of the two recombinant genes achieved 50% success. Recombinant C/D demonstrated a mosaic form comprising of: recombinant C/D (in the *pol* gene), recombinant A1/C/D (in the *gag* gene) and predominant B subtype in the *env-rev* gene. The accessory genes consist of: recombinant B/K in the Vpu gene and others were subtypes; C (Vpr and Vif genes), and B (Nef gene).

Conclusion

High maternal virological suppression at delivery and very low mother-to-child transmission of HIV were achieved in the resource limited settings of Eastern Cape in South Africa. Few women delivering at high viral loads have acquired significantly high rate of drug resistance mutations. Intervention strategies focusing on addressing maternal lifestyle behaviours and ART adherence challenges require targeted research and programmatic re-engineering. An effective surveillance system for tracking all pregnant women on ART will assist in identifying those with virological failure and drug resistance during antenatal, labour and delivery for prompt interventions. A nationally representative drug resistance surveillance in pregnant women should be undertaken to guide future policies and management guidelines in the country. This study found subtype C as the predominant circulating viral strain in the Eastern with occasional presence of CRF02_AG and a mosaic pattern of recombinant C/D.

CHAPTER ONE

GENERAL INTRODUCTION

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1.1. Background and Rationale

The 2030 global target focuses on reducing the neonatal mortality rate to at least 12 per 1000 live births, under-five mortality to at least 25 per 1000 live births and to end the epidemic of AIDS (WHO, 2016). These objectives are laudable and attainable if gaps in the national and global responses to HIV/AIDS epidemic are addressed decisively. Sub-Saharan Africa (SSA) continues to experience the highest burden of mother-to-child transmission (MTCT) despite the precipitous decline in the incidences of paediatric HIV infections in developed countries (UNAIDS, 2013).

Besides India, twenty-one of the twenty-two priority countries targeted for global efforts towards the elimination of mother-to-child transmission of HIV are within the sub-Saharan African region (UNAIDS, 2013). Over 90% of the 3.2 million children under the age of fifteen years living with HIV in 2013 were living in sub-Saharan Africa. Despite the scientific successes in the development of the antiretroviral therapy (ART) in the prevention of mother-to-child transmission of HIV (Becquet et al., 2009; McIntyre, 2010), only 68% of pregnant women in sub-Saharan Africa had received ART (UNAIDS, 2013). The collective six-week MTCT in sub-Saharan Africa rose from 7% to 16% after breastfeeding (Mofenson, 2010). This is in stark contrast to <1% in many developed countries (Mofenson, 2010; Tudor et al., 2011).

South Africa has the largest HIV epidemic worldwide (7.1 million people living with HIV including 320,000 children); accounting for 19% of the global number of people living with HIV (UNAIDS, 2017). South Africa has the largest treatment programme globally; accounting for overall 20% of individuals receiving ART with 80% of the AIDS response funded by the government (UNAIDS, 2017). The journey towards elimination of mother-to-child transmission (EMTCT) began with 18 pilot sites in 2001 (Goga et al., 2015), which later cascaded to the nationwide implementation of evidence-based national policy and guidelines in 2010 (WHO Option A and B) and 2013 (WHO Option B Plus) (SADoH, 2010; WHO, 2010; WHO, 2013; SADoH, 2015). South Africa has recorded significant successes in the key indicators of EMTCT: over 95% of pregnant women are accessing antenatal care services including HIV testing, about 93% of HIV-infected pregnant women have received ART after diagnosis and the six weeks infant diagnosis reduced from 25 - 30% in 2001 to 2.6% in 2014 (Goga et al., 2016). All HIV-infected pregnant women accessing antenatal clinics are initiated on the fixed dose combination (Tenofovir/Emtricitabine/Efavirenz) or any three compatible ART

regimens. These achievements were largely attained through the government's commitment to achieving the global plans of elimination of new paediatric infections (UNAIDS, 2011), the Millennium Development Goals 2000 – 2015 (UNDP, 2000) and the sustainable development goals 2015 – 2030 (UNDP, 2015).

Despite these laudable successes, there were 12,000 new paediatric infections in South Africa in 2016 based on modelling estimates by the Joint United Nations Programme on AIDS (UNAIDS, 2017). In addition, the high prevalence of HIV infections of 23.8% among reproductive age women (15 – 49 years) (UNAIDS, 2017) and 30.8% amongst women who attended antenatal care in 2015 (SADoH, 2015) suggests that the battle towards EMTCT in every community, district, province and the country at large is not over. As such, intensified surveillance for pregnant and lactating women with high viral load on ART as high risk for MTCT should be implemented at all maternity services in the country. This strategy will allow for prompt intervention and should be incorporated into the current PMTCT guidelines in the country.

Besides the South African Medical Research Council PMTCT Evaluation Study Report 2012-2013 (Goga et al., 2015), there has not been any nationally representative study which evaluated the population-effectiveness of the current PMTCT programme. Though Goga et al., (2015) provided a reference data for the country, the study did not assess the maternal viral load during delivery or post-partum. Due to the significant loss to follow up of mother/infant pair, such additional data will help to identify high risk sub-groups with high viral load for intensified interventions and follow up. In addition, there is the likelihood that some pregnant women may be delivering their babies with high viral load; thus, increasing the risk for further transmission of HIV to their infants in the post-partum period. This highlights one of the risks of new infant HIV infections needing to be redefined in the context of universal ART access to all pregnant women. However, there is paucity of data on the rate and determinants of in-utero transmission of HIV to the exposed infants as well as high viral loads at delivery in South Africa.

While the replicative power of HIV may be slowed down by mutations, evidence suggests that resistance mutants are transmitted from mother-to-child (Benizri et al., 2008; Eastman et al., 1998; Siegrist et al., 1994; Welles et al., 2000; Zeh et al., 2011) and between sexual partners (Guimaraes et al., 2015; Stekler et al., 2018; Temereanca et al., 2013). The probable risks of transmission of HIV resistant strains to the infants and the pattern of mutations in mothers on ART who failed to achieve

viral suppression have not been extensively investigated in the South African PMTCT context. The overall implications of resistant mutations to the PMTCT of HIV including the choice of prophylaxis for HIV-exposed and ART regimen for the infected infants have not been investigated.

In addition to the identified gaps in the context of MTCT, there is an emerging concern about the evolution of HIV diversity in different regions of South Africa. Diversity of HIV strains in the sub-Saharan Africa region has been documented (Abecasis et al., 2013; Hemelaar et al., 2011; Zhang et al., 2010). South Africa has experienced an increasing influx of foreign nationals either for economic reasons, refugee seeking purposes or tourism in the past decade. A few studies of the general population in South Africa have reported non-type C and some recombinant HIV viral strains in the Western Cape and Limpopo Provinces (Bessong and Iweriebor, 2016; Jacobs et al., 2014; Nwobegahay et al., 2011; Wilkinson et al., 2015). The implications of sexual relationships among people with viral diversities in the evolution of different types of HIV, clades and sub-clades, and circulating unique recombinant forms is yet to be investigated in the Eastern Cape, South Africa.

Whether there are diversities in the HIV strains according to types, clades, sub-clades, and unique recombinant forms in both rural and urban Eastern Cape communities or not, together with the clinical relevance of such diversities if any, this will be crucial for molecular epidemiological profile of the HIV epidemic in the region. Also, the transmission of such diversified forms of HIV strains to infants is uncertain. Pregnant women as surrogate for sexually active individuals, and their infants provide unique opportunities to further understand the pathogenesis of mother-to-child transmission and the diversity of HIV in the Eastern Cape Province, South Africa.

1.2. Statement of the problem

With the widespread implementation of the WHO Option B+ strategy in South Africa in 2015 (WHO, 2013; SADoH, 2015), there is no current data on the population-impact of lifelong ART initiated in HIV-infected pregnant women on the rate of virologic suppression at delivery and the early mother-to-child transmission risks. The consequences of failure to consistently suppress HIV viral load during pregnancy, labour and delivery, and the breast feeding period for HIV-exposed infants poses a challenge to ongoing efforts in sub-Saharan Africa (SSA) to achieve elimination of

mother-to-child transmission. Despite the initiation of lifelong ART regimens in HIV-infected pregnant women, the incidence and timing of infant HIV transmission is not known especially in the poor resource settings of the Eastern Cape. Since the majority of pregnant women entering the antenatal clinics might have been initiated on triple ART for their own health in accordance with the ART guideline of the national Department of Health (SADoH, 2015; 2016), it is largely unclear if the timing of booking is still relevant in the context of PMTCT.

Also, it is unclear if women with high peripartum viral load have acquired clinically relevant mutations, which could confer resistance to the ART, thus, putting the infants at risks for transmitted resistant strains. Nevirapine is the backbone of ART prophylaxis in HIV-exposed infants in South Africa (SADoH, 2015). Also, Nevirapine is one of the core drugs for treatment of HIV infections in neonates especially in the first two weeks, when protease inhibitors (Lopinavir/ritonavir) are considered unsafe (Nuttal, 2014). In view of the low genetic barrier of Nevirapine, requiring only a single mutation to confer resistance on the class of non-nucleoside reverse transcriptase inhibitors, the far reaching implications of maternal ART resistance might impact on the effectiveness of infant prophylaxis and ART regimen in HIV-infected neonates. This will have deleterious effects on the morbidity and mortality of HIV-exposed infants in the country. All the identified gaps in the context of MTCT in the resource limited settings of Eastern Cape, South Africa justify the need for a new study.

In addition to the above, the understanding of regional epidemics is crucial to the broader epidemiological profiling of the HIV infections in the country. Over the last decade, there have been increasing reports of unusual evolutions in the HIV diversities in different parts of the country. Few researchers have documented the emergence of non-type C HIV strains and unique recombinants forms in the Western Cape and Limpopo province (Bessong and Iweriebor, 2016; Jacobs et al., 2014; Nwobegahay et al., 2011; Wilkinson et al., 2015). Also, there are regional variations in the HIV types and sub-types in the sub-Saharan Africa and worldwide (Abecasis et al., 2013; Hemelaar et al., 2011; Zhang et al., 2010). The overall impact of international travels for economic, tourist and other reasons suggests that there might be an emergence of new strains of HIV in different communities and provinces in the country. The Eastern Cape is an understudied province, with many foreign nationals residing in the various townships and cities. Also, there are many inter-ethnic marriages between the

predominant IsiXhosa people of the Eastern Cape and other African countries. However, there has not been any molecular epidemiological studies profiling the types and sub-types of HIV strains in the Eastern Cape. All the identified gaps within the context of PMTCT and the molecular epidemiology of HIV in the Eastern Cape province will be addressed in this study.

1.3. Research Questions

Given the identified gaps in the existing literature on HIV and mother-to-child transmission in the context of maternal lifelong ART, especially in the Eastern Cape, South Africa, the following research questions were explored in the study:

1. What is the rate of peripartum virologic suppression among pregnant women initiated on ART in the public sector prevention of mother-to-child transmission programme in the Eastern Cape, South Africa?
2. What is the rate of early mother-to-child transmission (in-utero transmission) of HIV to exposed-infants and their determinants?
3. To what extent is clinically determined virologic failure predictive of true virological failure as determined by genotypic resistance analysis in peripartum women with high viral load on triple ART regimens?
4. What are the prevailing HIV clades, sub-clades and recombinant forms in parturient women in the Eastern Cape?

1.4. Aim and Objectives of the Study

The overall aim of this study is to determine the rates and examine the risk factors of maternal virologic suppression and early mother-to-child transmission of HIV to the exposed infants in the Eastern Cape, South Africa. In addition, the study describes the molecular epidemiology of HIV infection and patterns of resistance mutations in pregnant women on ART in the Eastern Cape, South Africa.

The specific objectives formulated to guide the implementation of the study are:

1. To determine the rate and determinants of virologic suppression in the cohort of pregnant women initiated on lifelong ART within the public sector PMTCT programme in the Eastern Cape.

2. To examine the rate and risk factors for early mother-to-child transmission of HIV to their infants despite maternal ART exposure.
3. To determine the true prevalence of virologic failure and patterns of mutations in viral isolates from pregnant women on ART with high viral load at delivery.
4. To characterise the HIV clades and sub-clades in serum samples of parturient women in Eastern Cape.

1.5. Hypotheses

Null Hypotheses

The following hypotheses are formulated based on the existing literature and would be tested in the study:

1. At least, 90% of HIV-infected pregnant women on ART attending maternity facilities would have achieved virologic suppression at the time of delivery.
2. The rate of in-utero transmission (birth PCR) would be less than 1% in the mother-infant cohort.
3. Clinically relevant resistant mutations would be detected in over 75% of viral isolates from pregnant women delivering the index baby at high viral load.
4. Non-C HIV subtypes would be isolated in at least 5% of the viral isolates successfully amplified from the cohort.

Alternative Hypotheses

1. 1. Less than 90% of HIV-infected pregnant women on ART attending maternity facilities would have achieved virologic suppression at the time of delivery.
2. The rate of in-utero transmission (birth PCR) would be greater than 1% in the mother-infant cohort.
3. Clinically relevant resistant mutations would be detected in less than 75% of viral isolates from pregnant women delivering the index baby at high viral load.
4. Non-C HIV subtypes would be isolated in more than 5% of the viral isolates successfully amplified from the cohort.

1.6. Significance of the Study

Epidemiological data on the peripartum viral load in pregnant women already on ART will highlight the population effectiveness of the current PMTCT guideline and the progress towards the elimination of MTCT of HIV in the Eastern Cape. Findings of the

study might be relevant to similar settings within South Africa and other countries in the sub-Saharan African region. Also, it will be crucial to gain an understanding of the MTCT risks within the context of universal ART coverage in the Eastern Cape, the province with the worst PMTCT indicators (Pattison and Rhoda, 2014).

Genotypic resistance profiling of viruses from maternal HIV might stimulate researches and innovations on other biomedical strategies of achieving elimination of MTCT in the sub-Saharan Africa. Data on the true prevalence of virological failure in pregnant women on ART at the time of delivery might guide future ART prophylaxis and treatment in HIV-exposed and -infected infants, respectively. Findings from this research may generate hypotheses for modelling HIV-free survival of infants exposed to high maternal viral loads at the time of delivery, of which data is lacking. The molecular analysis of the types, clades and sub-clades of the prevailing HIV infection in the peripartum women may contribute to knowledge on the regional HIV epidemics in South Africa. Future studies on vaccine development, guidelines on MTCT, infant prophylaxis and ART regimen in HIV-infected infants in sub-Saharan Africa might be influenced by the outcomes of this study.

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CHAPTER TWO

LITERATURE REVIEW

(*Two review papers will be submitted for publications)*

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2.1 Introduction

The Centre for Disease Control and Prevention (CDC) published a report of three cases of atypical community-acquired pneumonia (Pneumocystis carinii pneumonia) combined with oral thrush and chronic ulcerating herpetic lesions around the anus in previously healthy homosexuals in 1981 (Hoffman and Rockstroh, 2012, pg 2). Pneumocystis jiroveci pneumonia, previously called Pneumocystis carinii pneumonia, a fungal infection, believed to be rare in immunocompetent individuals raised concerns of health authorities. In 1982, the New York Times reported several cases of new immune system disorders (n=335) and 136 deaths among homosexuals in the United States of America, thus, causing public health concerns. The CDC named this new immune system dysfunction as Acquired Immune Deficiency Syndrome (AIDS) in September 1982. Several cases of AIDS were also reported in Europe. A behavioural link between same sex practices among homosexuals and AIDS in the wake of the epidemic was considered the aetiology (Hoffman and Rockstroh, 2012, pg. 2).

The report of AIDS among haemophiliacs and an infant who had received blood transfusion raised the possibility of another aetiological agent of AIDS. In 1982, three cases of Pneumocystis pneumonia were reported by CDC among haemophiliacs. Also, a case of Cryptosporidiosis was reported in another haemophiliac in Pennsylvania, USA. Later in 1982, an infant developed AIDS after receiving blood a transfusion. These observations triggered the possibility of a virus as the aetiology of AIDS. The human T-lymphotropic retrovirus-1 (HTLV-1) was initially considered as the aetiological agent because of the propensity of the aetiological agent to be transmitted through sexual contacts, blood to blood and perinatally. However, initial experiments to isolate the HTLV-1 were partially successful; weak assay reactivity was observed, suggesting the possibility of co-infection of this virus (cross-reactivity) with the main aetiological agent. Later in 1983, HTLV-III was discovered as the main aetiology agent of AIDS, which was renamed as the human immunodeficiency virus (HIV) (Hoffman and Rockstroh, 2012, pg. 3). This chapter provides an extensive overview on HIV; origin and diversities, mode of transmission and preventive strategies, life-cycle and drug therapies, global and regional epidemiological updates.

2.2 Origin and Classification of HIV

There are two HIV types: HIV-1 and HIV-2 with completely different origins. Several hypotheses have been postulated on how humans came in contact with HIV. The zoonotic theory is the most popular and plausible origin of HIV due to the closely related genomic sequence of the simian immunodeficiency virus (SIV) and HIV. African hunters were believed to be first to be infected with HIV through direct exposure to infected blood during hunting or eating of the primates. Hence, there seems to be a consensus on the zoonotic theory of the origin of HIV through cross-species transmission events, which have been dated back to 1884 – 1924 using phylogenetic analyses.

HIV-1 and 2 were transmitted from apes and sooty mangabey monkeys, respectively. Three independent cross-species events occurring at different times and places have been documented leading to three HIV-1 groups: designated as M (Main), N (Non-M, Non-O) and O (Outliers). HIV-1 group M originated from the Congo River basin between 1915 and 1941, and has a worldwide predominance (Paterson et al. 2000). Very recently, there was a discovery of a putative group (P) of HIV-1, which originated from gorillas, native to Cameroon (Kuiken et al., 2011; Plantier et al., 2009). Groups N, O and P are mostly found in the West African region. Fig. 2.1 shows the graphical representation of HIV-1 groups and subtypes.

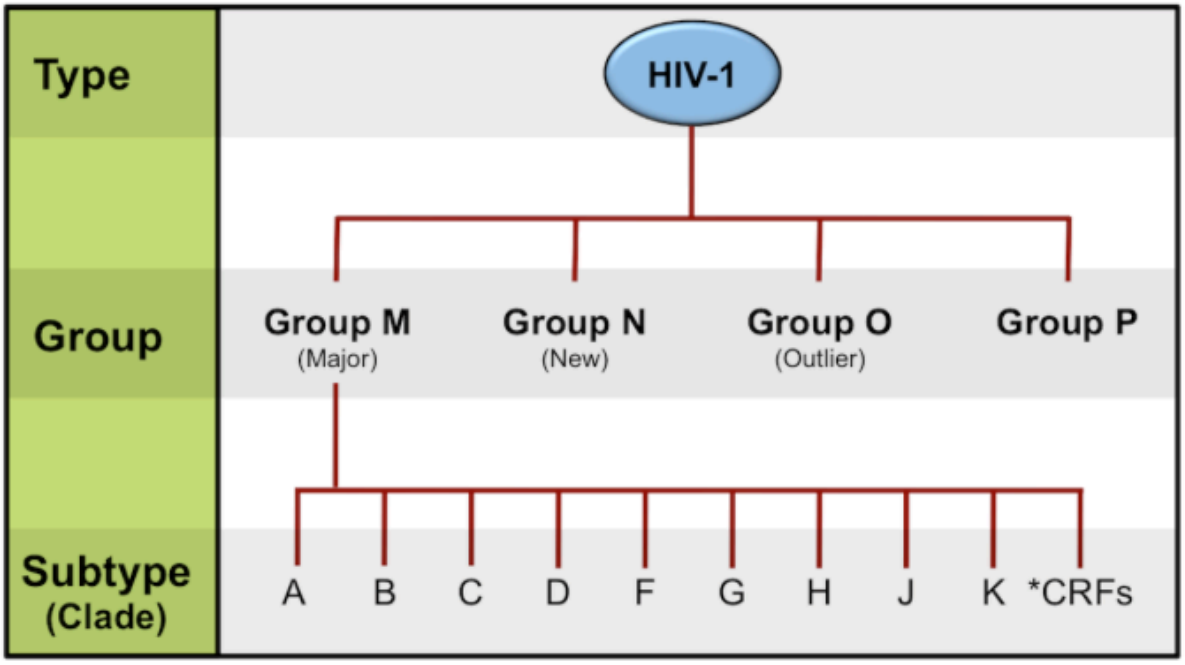

Fig. 2.1. Classification of HIV-1 to Groups and Subtypes (https://www.google.com/search?q=CLASSIFICATION+OF+HIV+TYPES+PICS.....)

The zoonotic origin of these HIV-1 groups; M and N have been traced back to the closely related viral sequences of simian immunodeficiency virus ($SIV_{cpz;}$ Pan troglodytes troglodytes) of the indigenous chimpanzees of the West-Central Africa. The viral sequences of HIV-1 group P, RBF168 and 06CMU14788, demonstrated a distinct lineage similar to the SIV sequences found in western gorilla ($SIV_{gor;}$ Gorilla gorilla gorilla). Thus, confirming the animal origin of this virus. However, the SIV origin of the outlier (group O) virus is still unclear. HIV-1 is readily transmissible and accounts for the world epidemic (Hemelaar et al., 2011; Patterson et al., 2000; Plantier et al., 2009; Vallari et al., 2011).

HIV-2 is a completely different strain of HIV with different primate origin. The viral sequences of HIV-2 are distinctly similar to the SIV_{sm} found in sooty mangabey monkey (Cercocebus atys) indigenous to West Africa (Lihana et al., 2012) and its transmission to man is presumed to have occurred between 1940 – 1945 in Guinea Bissau. Its spread must have been facilitated by the War of Independence with Portugal. HIV-2 is less pathogenic and has largely remained confined to the West

African region. However, few reports of HIV-2, resulting from international travels and sexual relationships, have been documented outside the region (Lihana et a., 2012).

2.3 Evolution and Classification of HIV

HIV-1 is characterized by numerous genetic diversities, which evolved from the process of mutational escape and recombination of genetic materials over several decades after gaining entry into humans (Hemelaar et al., 2011; Lihana et al., 2012). Mutational escapes confer a high degree of diversity to HIV-1 and protect the virus against the selective pressure of the antiretroviral therapy as well as the immune system activities. However, some mutations weakens the virus (Arien et al., 2005), thus, affecting the fitness or replicative capacity of the virus. Few studies have also reported the clustering of specific HIV strains to geographic locations and mode of transmission (Hemelaar et al., 2011; Lihana et al., 2012). In addition, the fast replication rate, genetic recombination of nuclear materials, and lack of proofreading mechanism in the reverse transcriptase enzyme makes the replication process to be error prone, thus contributing to the high diversities in HIV-1 (Lihana et al., 2012; Zhang et al., 2010).

There are nine subtypes or clades of HIV-1 group M described in the literature: A-D, F-H, J and K (Fig. 2.1) (Hemelaar et al., 2011; Lihana et al., 2012). These subtypes have distinct genomic sequences which differs from one another by a minimum of 25 -35%. Subtype C is the commonest HIV-1 worldwide with a prevalence of 52% (Hemelaar et al., 2011), while subtype B accounts for 11% of the world HIV-1 infections. Recombination between subtypes has been described as the mechanism behind circulating recombinant forms (CRFs) and unique recombinant forms (URFs) of HIV-1. The process involves two or more HIV-1 subtypes co-infecting the same cell within an individual, resulting in the incorporation of nuclear materials from the two viral genomes into the new virions. The subsequent coupling of the co-packaged viral genomes by the reverse transcriptase eventually results in producing a mosaic genome. The eventual transmission of the recombinant genome from an HIV-infected individual to another person then completes the process. About 98 HIV-1 CRFs and one HIV-2 CRF have been described in the literature

(https://hfv.lanl.gov/content/sequence/HIV/CRFs/CRFs.html. CRFs account for 16% of the global HIV epidemic (Hemelaar et al., 2011). The summary of the HIV-1 groups, subtypes and sub-subtypes are provided diagrammatically (Fig. 2.2).

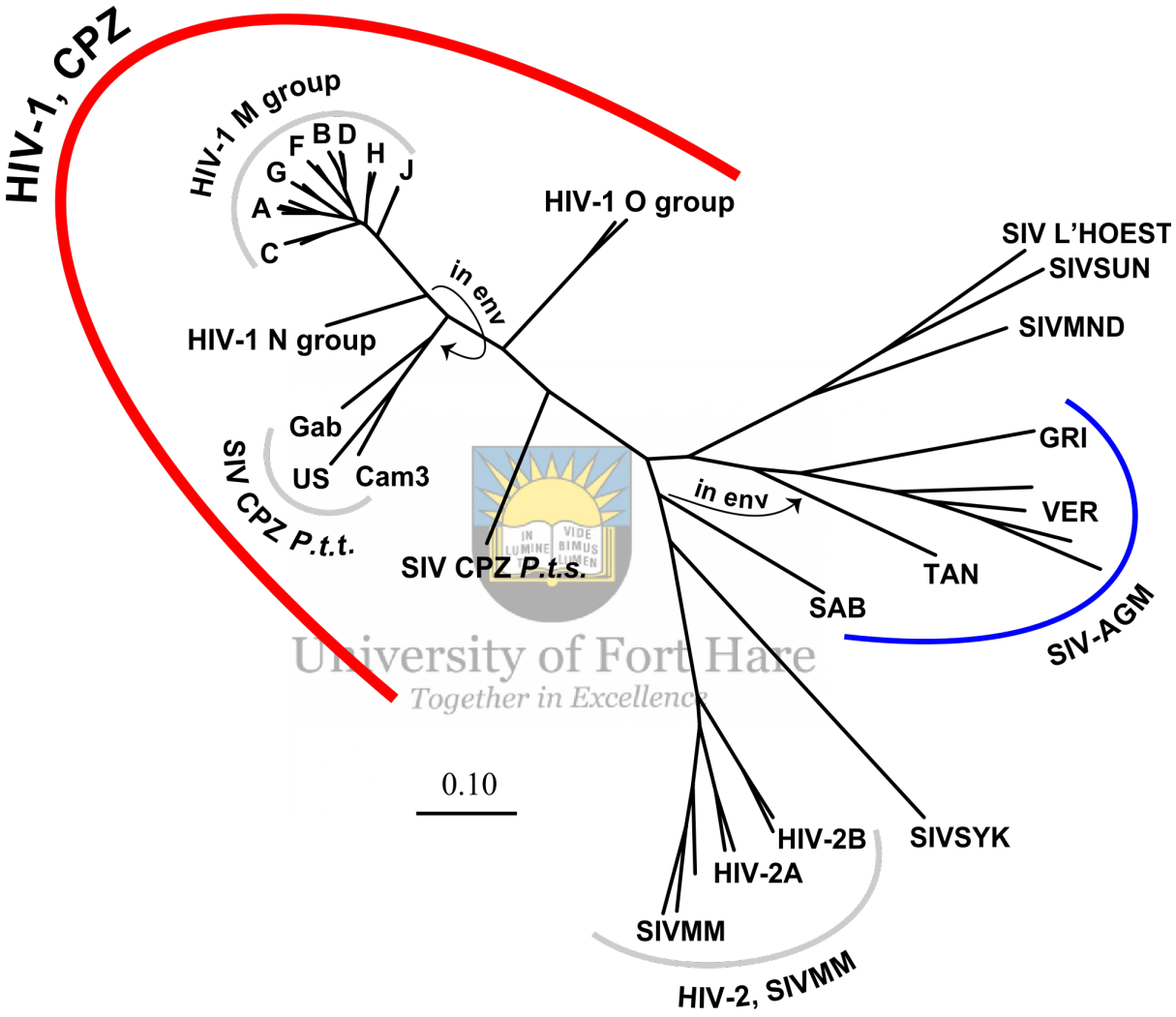

Fig. 2.2. HIV-SIV-Phylogenetic (Adapted from:https://www.google.com/search?rlz=1C5CHFA_enZA796ZA797&tbm=isch&sa=1&ei=vOBCXIK0Mamb1fAPxcea0AQ&q=phylogenetic....)

There is no subtype diversity among HIV-1 group N and group O, as all reported sequences till date are homogenous and have also not yielded distinct subtypes.

Group O seems to be largely confined to Cameroon, where it accounts for 1% of the HIV epidemic in the country. Group N and P have been reported in limited number of individuals residing in Cameroon (Abecasis et al., 2013; Vergne et al., 2003; Vessière et al., 2010). As such, more research studies are needed in this regard to gain a broader understanding of these viral groups. Findings from studies on HIV diversity will influence diagnostic modalities, virological monitoring, and treatment approaches within the clinical settings. With increasing migration of people from other sub-Saharan African countries to South Africa (StatsSA, 2015), it is not known whether HIV-1 groups O, N and P now exist in South Africa or not.

HIV-1 sub-subtypes have been documented following recombination events between viruses of different strains to form the circulating recombinant forms (CRF) and unique recombinant forms (Abecasis et al., 2013; Hemelaar et al., 2011; Zhang et al., 2010). Sub-subtypes are based on the distinct clustering of the genetic materials of certain subtypes which vary between 15 – 25%. For example: five different sub-subtypes of subtype A have been characterised and reported; A1, A2, A3, A4, and A5. Also, subtype F has two sub-subtypes: F1 and F2. Though HIV-2 consists of five subtypes (A, B, C, D and E), only groups A and B are predominant and have been reported in epidemic proportions in the West African region (Gao et al., 1994; Santiago et al., 2005). The remaining variants have been found in one or few people (Santiago et al., 2005).

2.4 Epidemiology of HIV and Subtype Distributions

2.4.1. Global

According to The Joint United Nations Programme on HIV/AIDS (UNAIDS, 2018), there were 36.9 million people living with HIV globally in 2017, bringing the total number of people infected with HIV since inception of the epidemic to 77.3 million. Adults account for the large majority of the people living with HIV (>95%) - about 35.1 million - while an average of 1.8 million children (aged less than 15 years) were living with HIV in 2017. AIDS-related illnesses claimed 940 000 lives in 2017, bringing the total of number of deaths from HIV to 35.4 million worldwide. There has been a

significant decline of 51% in the number of AIDS-related deaths since the peak in 2004; a decline from 1.9 million in 2004 to 940 000 deaths in 2017 (UNAIDS, 2018).

Also, there has been an accelerated expansion of access to anti-retroviral therapy (ART) globally; approximately 21.7 million people have been initiated on ART. Overall, 59% of all individuals currently living with HIV were accessing ART. Overall, 59% of adults and 52% of children living with HIV are already on treatment with ART. More women living with HIV (65%) have access to ART than men (53%). There have been significant reductions in the number of new infections globally; a 47% reduction since the highest peak in 1996. About 1.8 million new infections occurred in 2017 worldwide in comparison to 3.4 million in 1996. New infections among adults have also reduced by 16% since 2010; a reduction from 1.9 million to 1.6 million in 2017. Similarly, a dramatic decline of 35% in children infections have been recorded since 2010; from 270 000 to 180 000 in 2017. In relation to the UNAIDS 2020 agenda of 90:90:90 (UNAIDS, 2014); HIV diagnosis has improved remarkably; 75% of all individuals infected with HIV were aware of their status in 2017 worldwide. Of those who knew their HIV status, 79% were accessing ART; 59% of people living with HIV globally. Of the total on ART, 81% have achieved viral suppression; accounting for 47% of all individuals infected with HIV.

Regional variation in the HIV prevalence and incidence helps us to gain a better understanding of the global epidemic. There are 5.2 million people living with HIV in Asia and in the Pacific region (UNAIDS, 2018). Among these, there were 280 000 new infections and adults accounted for nearly all except 10 000 new infections in children less than 15 years of age. Of the people living with HIV in this region, 2.7 million people were accessing ART (53%). About 170 000 AIDS-related deaths occurred in Asia and the Pacific in 2017. In comparison, there were fewer people living with HIV in the Middle-East and North Africa. About 220 000 individuals were living with HIV in this region. Of this total, 18 000 new infections occurred in the region in 2017. Adults were responsible for nearly all the new infections except the 1 300 new infections in children. About 63 000 people were receiving ART (29%) in the region.

About 1.4 million individuals were infected with HIV in Eastern Europe and Central Asia and out of this total, 130 000 new infections occurred predominantly among adults in 2017 in the region. There were 34 000 AIDS-related deaths in the region by the end of 2017. Access to ART requires urgent attention of the government as only 36% of people living with HIV are accessing treatment (520 000). In comparison, more people live with HIV in the Western Europe and Central Asia; about 2.2 million were reported in the region in 2017. There were 70 000 new infections, mostly among adults. The number of people needing access to ART grew to 1.7 million individuals (78%) in 2017 (UNAIDS, 2018).

The Western and Central African region is home to 6.1 million people living with HIV, among whom 370 000 were new infections in 2017. Adults accounted for the majority; 310 000 new infections in those 15 years and above. There were 280 000 AIDS-related deaths in this region in 2017. Access to ART requires the attention of the health authorities; only 40% of those living with HIV have access to the effective ART; 2.4 million. The Eastern and Southern Africa are the regions most affected by the HIV epidemic; 19.6 million people were living with HIV in 2017. Of this total, new infections were 800 000 while 92 000 new infections were new infections among children. About 380 000 AIDS-related deaths occurred in 2017 in the region. However, significant progress in the provision of ART to those in need of treatment (66%) has been documented: an estimated 12.9 million people were already initiated on ART by the end of 2017 (UNAIDS, 2018).

2.4.2. South African HIV Epidemic

South Africa has the largest epidemic by country in the world; 19% of the people living with HIV worldwide are residents of this country (UNAIDS South Africa, 2017). The recently concluded South African National HIV Prevalence, Incidence, Behaviour and Communication Survey (2017) estimated that 7.9 million people are currently living with HIV in the country, representing a prevalence of 14.0% of the entire population. This represents an increase of 1.6 million over the last 2012 survey. Women are predominantly affected by the HIV epidemic; a prevalence of 17.3% of the entire women population are infected by the virus in comparison to the 10.6% of the men

population. By province, Kwazulu Natal has the highest burden of HIV; 18.1% of the residents of this province live with HIV. Mpumalanga and Free State provinces have an HIV prevalence of 17.3% and 17.0%, respectively. The prevalence of HIV in the Eastern Cape province is 15.3%, while Northern Cape province has the lowest prevalence of HIV (8.3%) (HSRC, 2017). The HIV prevalence increases sharply by the age of 25 years and reaches a peak level between the ages of 35 – 39 years.

About 231 000 people were newly infected with HIV in 2016; an incidence rate of 0.48%. Women were still acquiring new infections at a higher rate than men: 0.51% (122 000) versus 0.46% (109 000). The numbers of new infections are still high despite all the preventive efforts at the individual and governmental levels. The highest rates of new infections (1.51%) were observed among female young adults aged 15 – 24 years (66 000 new infections) and women in the reproductive age group (0.93%). By implication, the prevalence of HIV continues to increase and worse in two provinces, the Eastern Cape and Western Cape. The incidence of HIV has declined remarkably by 44% (378 700 new infections) from 2012 to 231 100 new infections in 2017. The country witnessed remarkable decline among women by 56%. There has been significant progress in ART coverage in South Africa: the 4 401 872 people currently on ART (62.3%), almost doubled the 2012 figures. More women have been initiated on ART compared to men: 65.5% versus 56.3%.

The current 90:90:90 dashboard for South Africa (UNAIDS, 2015) sits at; 85: 71: 86. This translates to 85% of HIV-infected individuals already having received their diagnosis and thus, being aware of their status. Among this group, 71% of those already diagnosed of HIV infection have been initiated on ART and 86% of these individuals receiving ART have achieved virological suppression. However, it should be noted that some of the individuals living with HIV have not been diagnosed as well as some of the people with HIV diagnosis who are yet to receive ART. As such, viral suppression at the population level is 62.3%, lower among males and younger age groups. This data has implications for HIV transmission within the population as the general norm suggests that viral suppression equals no transmission. It is therefore, imperative that more efforts be made to continue to expand the HIV care services;

from testing to ART initiation and viral suppression. According to the UNAIDS South Africa (2017), AIDS-related illnesses have claimed 110 000 lives in the country. Several key subsets of the population require attention of the authorities: these are sex workers (HIV prevalence of 57.7%) and men having sex with men (HIV prevalence of 26.8%).

2.4.3. Epidemiological trends in paediatric infections

Over 95% of HIV infections in children (aged 0 – 14 years) were transmitted from their mothers (UNAIDS, 2013). However, other less common modes of transmission are still being reported in different parts of the world; blood transfusion (Brown et al., 2017), sexual exposure (Mandalazi, Banda and Umar, 2013) and accidental causes (feeding with pre-masticated food and father-to-child) (Gaur et al., 2009; Ivy et al., 2012; Ezeonwumelu et al., 2018). Without interventions, between 25 – 45% of HIV-exposed infants will be infected (de Cock et al., 2000); largely during pregnancy, labour, delivery and the breastfeeding period. The United Nations General Assembly therefore set a target; to reduce new HIV infections in children by 95% in the year 2020. In addition, 22 countries were prioritised for global action on reducing mother-to-child transmission of HIV. Besides India, 21 of these countries are located in Africa. This is based on the fact that 95% of paediatric infections occur in sub-Saharan Africa. Significant progress has been documented in the efforts towards eliminating mother-to-child transmission of HIV globally, however, there are still variations in the rate of new HIV infections in children (UNAIDS, 2018). Of the 36.9 million people living with HIV in 2017, children accounted for 1.8 million (1.3 – 2.4 million) of the HIV epidemic globally. About 180,000 new infections occurred in children in 2017; this represents a 35% decline in paediatric infections from 2010 (270,000) to 2017 (180,000).

The success of the prevention efforts has been accredited to the rapid expansion of access of pregnant women living with HIV to the highly effective antiretroviral therapy (ART). According to the UNAIDS report (2018), about 80% (61 - >95%) of pregnant women living with HIV had access to ART either for prevention of transmission to their babies or for their own health (UNAIDS, 2018; WHO, 2013). This is a gigantic step forward in the commitment towards eliminating paediatric HIV infections. In

comparison to 80% in 2015, only 36% of pregnant women living with HIV in the 21 priority countries in Africa were initiated on ART in 2009 (UNAIDS, 2016). In addition, the majority of the priority countries have adopted the WHO Option B Plus strategy; 93% of the pregnant women were initiated on life-long ART for their health and invariably, prevention of transmission to their infants. Through the expansion of the life-long ART in these countries, AIDS-related maternal mortality has reduced remarkably; 43% reduction in AIDS-related deaths among women in their reproductive age group were documented between 2009 to 2015 (UNAIDS, 2016).

Since the WHO set the criteria for assessing elimination of mother-to-child transmission of HIV at the country level (WHO, 2014), several countries have received the validation. Cuba is the first country and subsequently, Thailand, Moldova, Belarus, and Armenia have received validation for elimination of mother-to-child transmission (WHO, 2016). The global targets of ensuring at least 95% antenatal care and HIV testing coverage among pregnant women and at least 90% ART coverage among HIV-infected pregnant women (WHO, 2014) are achievable in Africa with political commitment and governmental investments. So far, Uganda and Burundi have made giant strides toward attaining these targets; 86% and 84% ART coverage were documented in these countries respectively (UNAIDS, 2016). In order to meet the global targets of eliminating paediatric infections, all the poor performing countries and regions should be targeted for support. About 48% of pregnant women living with HIV accessed ART in Western and Central Africa in 2017. Consequently, 20.2% of exposed infants still got infected in the region. In comparison to Latin America and Caribbean, where three-quarter of women requiring ART were offered it. In Asia and Pacific, 56% were offered ART while Middle East and North Africa countries offered ART to 22% of HIV-infected pregnant women. The Eastern and Southern Africa have made tremendous progress towards eliminating mother-to-child transmission by initiating ART in 93% of the pregnant women living with HIV (UNAIDS, 2018). Though, 67% of births to HIV positive women occur in these regions, a decline in the incidence of paediatric infections has been recorded; 18.1% in 2010 to 9.9% in 2017 (UNAIDS, 2018).

With over seven million people living with HIV in South Africa, the largest epidemic in the world, paediatric infections are in a similar trend, with 320, 000 children currently

infected (UNAIDS, 2017). The South African government provides the funding for ART in the country for treatment and prevention efforts. These investments are directed towards achieving the global plans of elimination of new paediatric infections (UNAIDS, 2011), the Millennium Development Goals 2000 – 2015 and the sustainable development goals 2015 – 2030 (Buse and Hawkes, 2015). The prevention of mother-to-child transmission programme in South Africa started in 2001 with 18 pilots sites (Barron et al., 2013), the programme has cascaded to the nationwide implementation of evidence-based national policy and guidelines in 2010 (WHO Option A), 2013 (WHO Option B and B Plus) and recently, the 'test and treat strategy' (WHO, 2013; SADOH, 2010; SADOH, 2013; 2015; 2016). South Africa has recorded significant successes in the key indicators of EMTCT; over 95% of pregnant women are accessing antenatal care services including HIV testing, about 93% of HIV-infected pregnant women received ART after diagnosis and six weeks infant diagnosis reduced from 25 - 30% in 2001 to 2.6% in 2014 (Goga et al., 2015; Goga et al., 2017). All HIV-infected pregnant women accessing antenatal clinics are initiated on the fixed dose combination (Tenofovir/Emtricitabine/Efavirenz) or any three compatible ART regimens.

Despite the laudable successes, there were 12,000 new paediatric infections in South Africa in 2016 (UNAIDS, 2017). In addition, the high prevalence of HIV infections of 23.8% among reproductive age women (15 – 49 years) (UNAIDS South Africa, 2017) and 30.8% amongst women who attended antenatal care in 2015 (SADOH , 2015) suggest that the battle towards eliminating mother-to-child is not over in this country. As such, intensified surveillance of pregnant women and breastfeeding women with high viral load on ART as high risk for MTCT should be implemented at all maternity services in the country. This strategy will allow for prompt intervention and should be incorporated into the current PMTCT guidelines in the country. There are wide variations in the prevalence of HIV among pregnant women in the various provinces of the country. The prevalence of HIV in pregnant women ranges from 16% in the Western Cape Province to 40% in KwaZulu-Natal Province. The prevalence of HIV in the Eastern Cape Province is 11.6%, 20% and 29% for the entire population, among 20 – 64 year old individuals and among pregnant women, respectively. An estimated 300,000 babies are exposed to HIV annually in Eastern Cape Province annually through mother-to-child transmission (Shisana et al., 2012).

2.4.4. Geographical Distribution of HIV-1 Diversities

In order to gain a broader understanding of the molecular epidemiology of HIV-1, this review explores the global and regional distribution of subtypes, sub-subtypes and recombinants in different parts of the world. Firstly, it should be noted that there are dynamic changes in the patterns of HIV-1 diversity in any part of the world over time (Hemelaar et al., 2011; Kiwanuka et al., 2009; Lihana et al., 2012; Nwobegahay et al, 2011; Wilkinson et al., 2015). The changing trends of HIV-1 strains in any region or country are directly proportional to the new infections and deaths associated with each subtype and recombinant over time. In addition, the biological characteristics (transmissibility and disease progression) of each subtype also play significant role in the changing trends of HIV diversity in any region (Lihana et al., 2012; Nwobegahay et al, 2011; Wilkinson et al., 2015). Other factors relating to socioeconomic activities of the region such as transportation links, migration, urbanisation and population growth play important roles. Also, the founder effects of the virus and transmission networks are crucial for the dynamic changes in the circulating viral strains in any region of the world (Faria et al., 2014; Tebit et al., 2011). As such, periodic surveillance for the trends in the pattern of HIV strains in different regions of the world will help in gaining an understanding of new outbreaks of the HIV epidemic.

Subtype C accounts for 46.6% of all HIV-1 infections globally while subtype B accounts for 12.1% of all the global HIV pandemic (Hemelaar et al., 2018). Subtype A is responsible for 10.3% of all the global infections, followed by CRF02_AG (7.7%), CRF01_AE (5.3%), subtype G (4.6%) and subtype D (2.7%) (Hemelaar et al., 2018). Subtypes F, H, J and K occur to a lesser extent and account for 0.9% of the global HIV epidemic. Besides the CRF02_AG and CRF01_AE, other CRFs account for 3.7% of global infections, thus, bringing altogether the overall prevalence of CRFs to 16.7% altogether. Unique recombinant forms can be found in 6.1% of individuals living with HIV worldwide. Altogether, recombinant HIV strains account for 22.8% of the global HIV pandemic. Figure 2.3 shows the geographical distribution of HIV-1 groups, subtypes and recombinants.

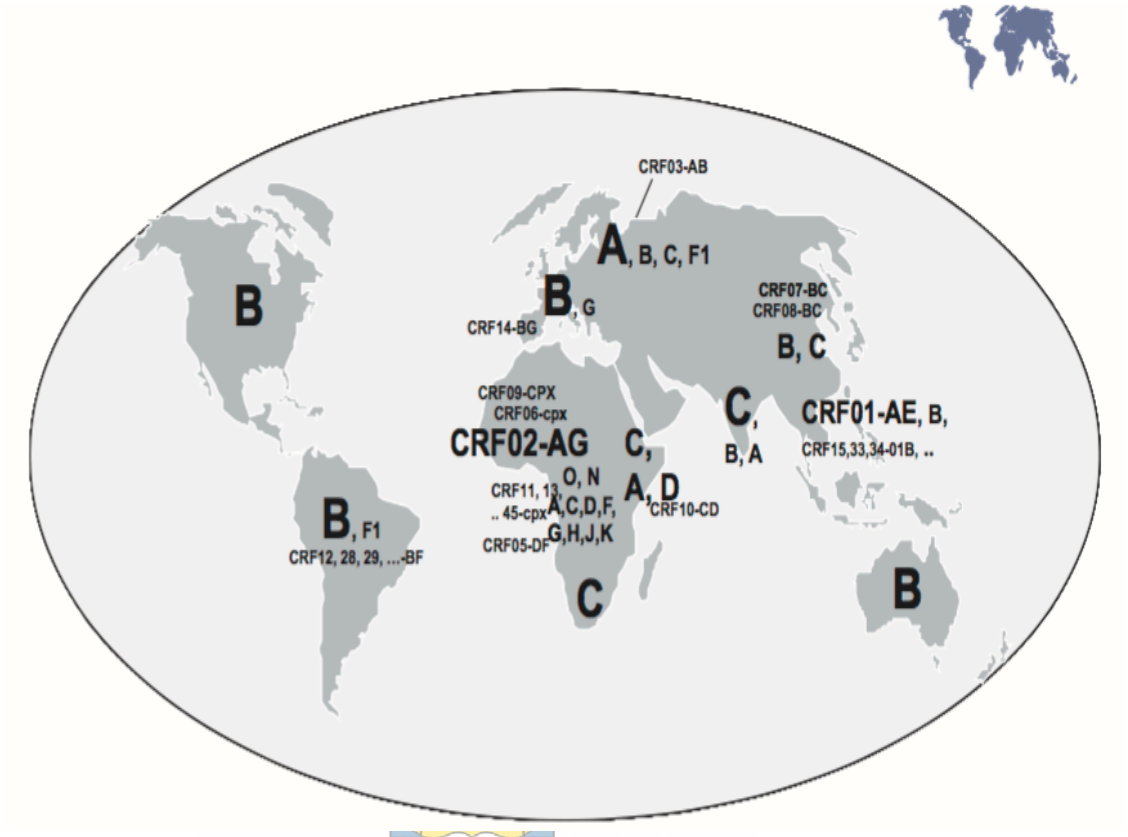

Fig. 2.3. Geographical Distribution of HIV-1 Groups, Subtypes and Recombinants (Obtained from Internet source: https://www.google.co.za/search?q=maps+showing+HIV+types+and+distribution&espv=2)

2.4.4.1. United States of America (USA)

HIV-1 subtype B is the predominant circulating viral strain in the USA (Bennet, 2005; Brennan et al., 2009; Carr et al., 2010; Delwart et a., 2012; Lin et a., 2006; Pyne et al., 2013; Walters and Trevelyn, 2011; Wheeler et al., 2007). Though the majority of these studies have reported HIV-1 diversities in different sub-populations of the USA, only two large datasets have reported on the entire sub-population in the country (Pyne et al., 2013; Wheeler et al., 2010). However, only one large dataset could be considered to be representative of the entire population of the USA (Pyne et al., 2013). Pyne et al. (2013) conducted a retrospective analysis of samples drawn from 24,386 people living with HIV and AIDS across 46 states between 2004 and September 2011. This study reported a predominant subtype B viral strain (96.73%). However, non-subtype B or recombinants were found in 3.27% of the samples. There was a progressive

increase in the prevalence of non-subtype B infections from 0% in 2004 to 4.12% in 2011. Among the non-subtype B strains, subtype C was the most prevalent (35.3%), accounting for 1.12% of the total sample analysed. Subtype A and CRF02_AG accounted for 0.61% and 059%, respectively. In addition, sub-subtype A1 to A4 as well as sub-subtype F1 and F2 were found in varying proportions. Recombinants accounted for 35.3% of the non-subtype B strains, of which CRFs were very predominant (86.2%). Unique recombinant forms were identified in 13.8% of the recombinants.

There are different mechanisms accounting for the introduction of the non-subtype B HIV-1 strains to the United States of America. Military personnel on foreign missions acquire HIV infections overseas (Brodine et al., 1995; Walter et al., 2000). Also, immigrants from different parts of the world travel with their HIV infections to the USA (Achkar et al., 2004; Gao et al., 1994; Lin et al., 2006; Sides et al., 2005). Emergence of non-subtype B HIV-1 strains have been reported in individuals without history of travels or relationship with immigrants in the USA (Robbins et al., 2003). Hence, periodic surveillance of new infections for HIV-1 diversity is mandatory in order to identify new outbreaks in a country.

2.4.4.2. South America

Countries in the South American region include; Brazil, Argentina, Ecuador, Colombia, Guyana, Uruguay, Venezuela, Chile and others. The HIV epidemic in the South American countries is caused by different viral strains. Brazil is the largest and the most populated country in the South American region, with HIV prevalence of 0.6% (Gräf and Pinto, 2013). The predominant HIV-1 strain is the subtype B; however, smaller proportions of subtype C, F1 and BF1 recombinants have been reported in the country (Machado et al., 2009; Stefani et al., 2007). Similar to Brazil, recombinant BF is the predominant HIV-1 strain circulating in Argentina (Dilernia et al., 2007; QuarlerI et al., 2004). Dilernia et al. (2007) found a predominant recombinant BF in the inner city of Buenos Aires. In addition, various recombinants in different proportions of subtype C, recombinants BC, BA and CRF06_$_{CPX}$ were reported (Dilernia et al., 2007). Similar to Dilernia et al. (2007), a pooled sample analyses from individuals living with

HIV-1 across five South American countries, namely, Argentina, Uruguay, Peru, Bolivia and Ecuador, yielded a similar pattern of results.

The BF recombinants were the predominant HIV-1 infection in Argentina and Paraguay. This is largely driven by the high proportions of intravenous drug users in the two countries. In comparison, countries with fewer intravenous drug users, such as Bolivia, Peru and Ecuador have subtype B as the predominant HIV strains in the population (Hierholzer et al., 2002). A study by Guimaraes et al. (2012), which described the phylogenetic analysis of the *pol* and *env* regions of two sets of HIV-infected samples aliquoted in 1996 and 2005. This study specifically described the temporal trends in the HIV-1 subtypes in the years 1996 and 2005 respectively. The results demonstrated that subtype B was the predominant HIV-1 strain (72.5%) in Bolivia during the study period which was closely followed by subtype BF1 recombinants.

2.4.4.3. Middle East, North Africa and Asia

Subtype B and CRFs (especially CRF35_AD) predominate in the Middle East and North African region. They both contribute 59.8% of the HIV infections in the region. The HIV epidemic in the Southeast Asia and East Asia is dominated by the CRF01_AE (Lihana et al., 2012). Southeast Asia has consistently had the highest proportion of CRF01_AE while East Asia has witnessed a steady increase in the proportions of CRF01_AE over time and now contributes 47.2% of the HIV epidemic in the region. Other CRFs also contributes to the epidemic in the region. CRF07_BC and CRF08_BC are the two predominant recombinants found in this region, where they combine with other CRFs to contribute 75.5% of the infections in the region. The predominant circulating viral strain in Central Asia is subtype A, where it accounts for over 50% of the epidemic with significant contributions by subtype B and other CRFs.

2.4.4.4. Europe

Subtype B is the predominant circulating viral strain in Western and Central Europe, where it accounts for 75% of the HIV epidemic in the region (Lihana et al., 2012). However, there seems to be an increase in the prevalence of CRFs and URFs in the region. In comparison, subtype A accounts for over 50% of the HIV epidemic in the

Eastern Europe. Subtype B and some CRFs also contribute to the epidemic in the Eastern European region.

2.4.4.5. Sub-Saharan Africa

As previously documented, sub-Saharan African is the region with the highest burden of HIV epidemic. HIV-1 diversity is a serious concern for vaccine development and as such, understanding of the variations in the viral strains in this region of the world will be critical for preventive efforts. Central Africa has the greatest amount of HIV-1 diversity in the world. It is characterised by many CRFs and URFs, and all HIV-1 subtypes are found in this region. The highest proportions of URFs (21.3%) are found in Central Africa, which accounts for 46.8% of all recombinants. It was observed over time that reduction in the prevalence of subtypes A, D, G, and F were accompanied by corresponding increases in the prevalence of subtype C. The genetic landscape of HIV diversity is different in the West Africa, East Africa and Southern Africa.

West Africa has the largest proportions of CRF02_AG (46.2%) and subtype G (26.8%) in the world. There are many URFs in this region where it accounts for 15.5% of the circulating viral strains and in combination with other recombinants, account for a total of 68.4% of the circulating HIV strains (Hemelaar et al., 2018). Subtype A is the predominant viral strains in East Africa, where it accounts for 53.4% of all the HIV infections in the region. To a lesser extent, subtype C accounts for 14.8%, subtype D accounts for 16.8% and URFs account for 12.6% of the circulating viral strains in the region. Subtype C is the predominant circulating virus in the Southern African region, accounting for about 90% of the circulating virus in the region. Though, other subtypes and recombinants have been reported in the region also.

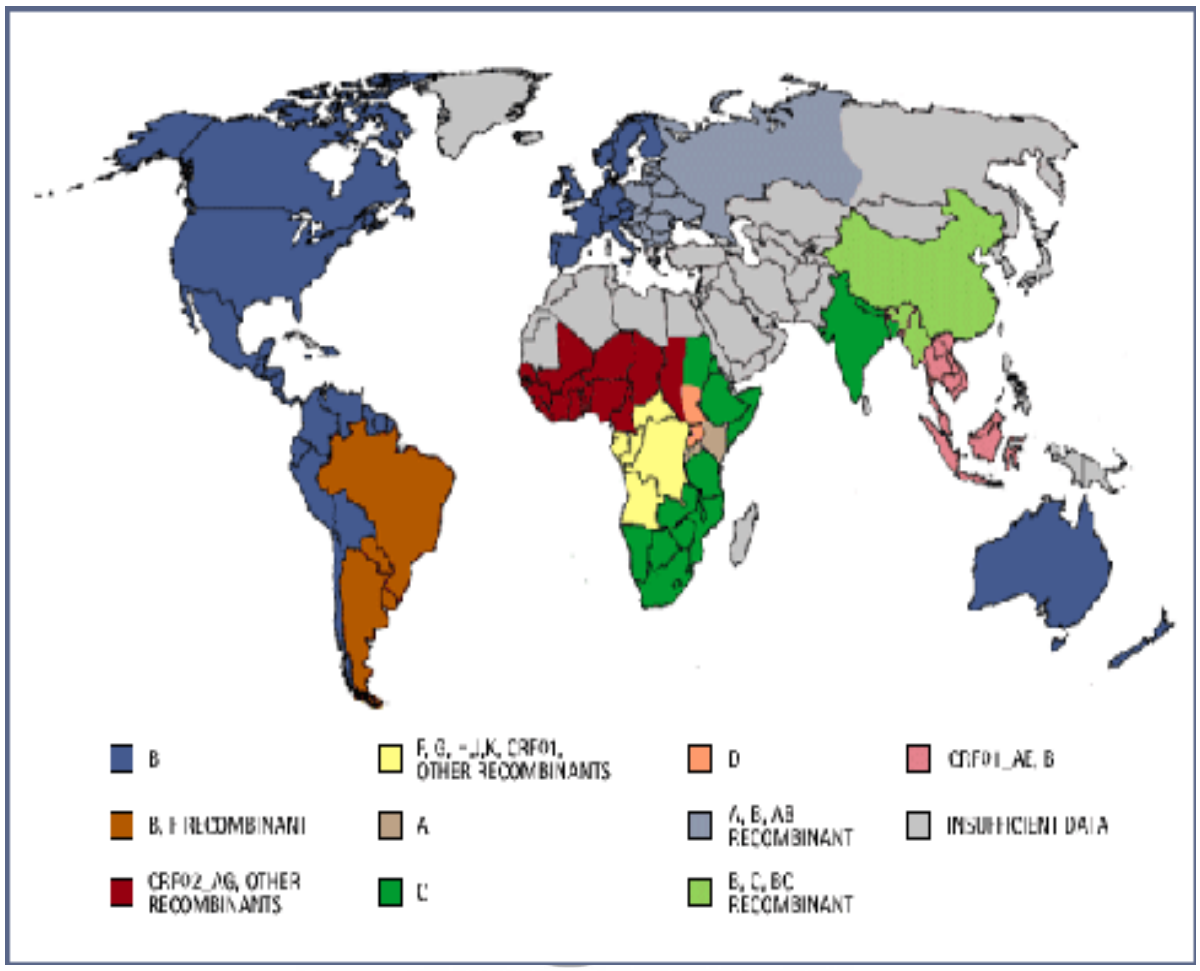

Fig. 2.4. Distribution of HIV-1 M subtypes across the world. [Internet source: https://www.google.co.za/search?q=maps+showing+HIV+types+and+distribution&espv=2....]

2.4.4.6. HIV-1 Diversity in South Africa

In the early 1980's, the HIV epidemic in South Africa mirrored the pattern seen in the developed world, which was predominantly subtype B among the men having sex with men (MSM) (Williamson et al.,1995). There were a few reported cases of subtype D (Loxton et al., 2005; Williamson et al., 1995). This changed quickly in the 1990's to high predominance of subtype C characterised by increasing heterosexual transmission (Van Harmelen et al., 1997). There had been report of subtype B were reported among MSM and a few reported cases of heterosexual transmission and mother-to-child transmission of subtype B (Williamson et al., 1995).

Since the year 2000, HIV-1 subtype C has been the most predominant epidemic in South Africa (93.8 - 95%), however, there is an emergence of non-C subtype in the Western Cape Province fuelled by the increasing migration and the booming tourism industry (Jacobs et al., 2014; Wilkinson et al., 2015). Jacobs et al. (2014) detected the presence of B subtype together with unique recombinant forms (BC) in 4.6% of their study cohort. Wilkinson et al. (2015) reported the emergence of subtype A, B, G, and unique recombinants AC and AD . Similarly, HIV-1 subtype B and J were reported in the Limpopo Province by Nwobegahay et al. (2011). To date, there are no studies on the genetic characterisation of HIV strains in the Eastern Cape, one of the most populous with high HIV prevalence in the country. Whether similar patterns of non-type C HIV strains and unique recombinant forms exist in the Eastern Cape Province is uncertain.

The study of HIV diversity is crucial for laboratory diagnosis (Apetrei et al., 1996), monitoring of viral load and resistance (Luft, Gill and Church, 2011), screening of blood donor (Delwart et al., 2012), anti-retroviral treatment (Kantor, 2006; Zhang et al., 2010) and the course of clinical disease (Pai, Shivkumar and Caja, 2012). With increasing migration of indigenes of African countries to South Africa and sexual relationships, there may be epidemiological and virological transitions of HIV strains in South Africa and the clinical consequences of such changes, if any, yet to be investigated in the context of mother-to-child transmission. Therefore, periodic surveillance and investigation of the diversity of HIV in different regions of the country would be important for epidemiological, preventive and clinical practices.

2.5. HIV Transmission

Transmission of HIV requires exposure of mucosal surfaces of an individual to infected-effluents from another or through percutaneous inoculation of infected effluents (blood or body fluids) (Shaw and Hunter, 2012). There are three main modes of transmission of HIV: sexual contacts with HIV-infected partner, needle sharing practices among intravenous drug users and mother-to-child transmission during pregnancy, labour and delivery, and breastfeeding. There are some less common routes for HIV transmission; such as occupational exposure (percutaneous inoculation or aerosol splash of infected blood/effluents) within the workplace and blood

transfusion in resource constrained settings. There has also been anecdotal reports of transmission of HIV to three infants through pre-masticated food from their mothers in the USA (Gaur et al., 2009). In addition, contacts of open wound or mucosal to HIV-infected blood or body fluids, or transmission by human bites were considered to be extremely rare routes of HIV transmission (Deshpande, Jadhav and Bandivkedar, 2011; Hoffman and Rockstroh, 2012, pg. 4). However, certain daily routines which bring people together such as sharing toilets or drinking from the same glass cannot transmit HIV.

2.5.1. Sexual Transmission of HIV

Sexual contact, either anal or vaginal, is the predominant route of transmission of HIV globally. The risk of HIV transmission through oral sex is still not quantifiable. The seminal fluids and vaginal secretions serve as vehicles for transmission of HIV. Highest concentrations of the virus (high viral load) are found in the blood and seminal fluids/vaginal fluids of HIV-infected individuals. As such, transmission risks increases in an individual with AIDS or an advanced clinical stage of the HIV infection. There is a strong correlation between the plasma viral load and genital secretions. In addition, certain environmental factors influences transmission during sexual contacts; concurrent sexually transmitted infections, skin lesions, mucosal trauma, circumcision and sexual practices of the individuals.

Table 2.1 shows the average transmission risks according to specific sexual practices. Evidence suggests a strong correlation between level of viraemia and transmission risks (Fideli et al., 2001; Hoffman and Rockstroh, 2012, pg 4; Quinn et al., 2000). Higher viral loads occur in the acute stage and at the later stage of the HIV disease continuum. Hence, individuals tend to transmit at a higher frequency during these periods. However, HIV has been detected in about 20% of individuals with undetectable viral load in the blood, thus, suggesting the possibility of the risk of transmission despite having undetectable viral load in the blood. There is also a strong correlation between transmission risks and concurrent sexually transmitted infections STIs). Ulcer-forming STIs such as syphilis, genital herpes and chancroid as well as inflammatory STIs (gonorrhoea and chlamydia) increase the risk of transmission and acquisition of HIV during sexual contacts.

Unprotected anal sexual intercourse is generally considered as a high risk sexual behaviour; whether for insertive or receptive sex partner (Table 2.1). However, the risk is considerably higher for the receptive partner. The rectum has a thin layer of columnar epithelium which is prone to microtears during intercourse and serves as a portal of entry for the virus. Also, the insertive partner is at risk of acquiring the virus through the urethra, open sores on the penis, microtears and wounds on the inner fore-skin, which is a poorly keratinised squamous epithelium (Shaw and Hunter, 2012).

Table 2.1. Likelihood for HIV Transmission per Sexual Practice

Type of contact/partner	**Probability of infection per contact**
Unsafe receptive anal intercourse with HIV-positive partner	0.82% (95% CI 0.24 – 2.76) Range (0.1 – 7.5%)
Unsafe receptive anal intercourse with partner with unknown HIV serostatus	0.27% (95% CI 0.06 – 0.49)
Insertive anal intercourse with partner with unknown status	0.06% (95% CI 0.02 – 0.19)
Unsafe receptive vaginal intercourse	0.05 – 0.15%
Unsafe insertive vaginal intercourse	0.03 – 5.6%
Oral sex	Probability is unknown. Although, case reports of transmission after receiving seminal fluids in the oral cavity (https://www.cdc.gov/hiv/risk/oralsex.html).

CI=confidence interval; (Adapted from Hoffman and Rockstroh, 2012: 5)

Both men and women are at risk of contracting HIV during vaginal sexual intercourse due to the presence of the virus in the pre-seminal fluids, seminal fluids, and vaginal secretions. The squamous, non-keratinised epithelium of the vagina and ectocervix has a wider surface area which is exposed to infected semen and the receptive

capability of the vagina leads to prolonged contact time and increased risk of HIV acquisition in women (Shaw and Hunter, 2012) (Table 2.2).

2.5.2. Transmission through Needle Sharing Practices

Injection drug use is one of the commonest modes of transmission of HIV in the USA, Western and Eastern Europe. The practice of sharing injection paraphernalia among drug users exposes them to large amount of infected blood. In addition, injection drug users usually confirm the correct placement of the needle in a vein by aspirating blood into the syringe. The needle and syringe serve as a reservoir for transmission of HIV to other users. While some countries such as the USA and Western Europe have implemented the needle exchange programme, Eastern Europe still criminalises drug use. As such, injection drug use continues to fuel the HIV epidemic in the region (Hoffman and Rockstroh, 2012: 5).

Beside the injection sharing practices in this group, other lifestyle behaviours that increase their risk for HIV have also been documented (Hoffman and Rockstroh, 2012: 5). Exchange of sex for drugs or money, sex with other injection drug users, multiple sexual partners, history of STIs and reduced frequency of use of condom or condomless sex are some of the lifestyle behaviours reported among injection drug users. In addition, there is greater risk of excessive use of alcohol among injection drug users.

2.5.3. Vertical transmission (Mother-To-Child)

Without any preventive interventions, the rate of mother-to-child transmission in a non-breastfeeding populations ranges from 14 - 23% in developed countries, however, the rate is higher in breastfeeding populations (25 – 45%) in the resource-limited settings (De Cock et al., 2000). Evidence suggest that there are complex interactions of maternal, foetal and viral factors leading to transmission of HIV from mothers to infants. Three distinct time-lines have been mapped for mother-to-child transmission of HIV; in-utero, intra-partum and during breastfeeding (Bryson et al., 1992). The risk factors for mother-to-child transmission of HIV differ in each period. As such appropriate interventions can be offered to further mitigate the risks of transmission of

HIV in addition to the highly efficacious ART. Scientific interventions have therefore targeted these time periods with significant degree of success.

2.5.3.1. In-utero transmission: Mechanisms and risk factors

Pre-natal (in-utero) transmission of HIV in a non-breastfeeding population is defined as the detection of HIV DNA by polymerase chain reaction (PCR) on a sample from an infant within 48 hours after delivery (Bryson et al., 1992). This eliminates the probable contribution of intra-partum transmission of HIV to the exposed infants. The exact mechanisms of HIV transmission during the pre-natal period remain inconclusive; though earlier study reported variable transmission risks from the first to third trimester, with increasing gradient of transmission risks (Mock et al., 1999; Langston et al., 1995). The plausible theory of cell-to-cell migration of HIV-infected maternal mononuclear cells into the fetal circulation (micro-transfusion) was advanced by Langston et al. (1995).

Breakdown in the placental integrity as a result of inflammation commonly associated with chorioamnionitis is associated with increased risk of transplacental HIV transmission (Mandrelbrot et al., 1996; Mwanyumba et al., 1999; Smulian et al., 1999). Chorioamnionitis is associated with foetal complications which are risk factors for HIV transmission: pre-term labour, prematurity and neonatal sepsis (Smulian et al., 1999; Van Dyke et al., 1999). A host of maternal factors have been identified as risk factors for in-utero transmission. For example; high maternal viral load (Garcia et al., 1999; Marinda et al., 2011; Dinh et al., 2015), severe maternal disease, low CD4 count < 200 cells/μl (Charurat et al., 2009) and co-infections with herpes simplex infection or systemic infections such as hepatitis B (Drake et al., 2007). Maternal systemic infections tend to stimulate HIV replication and increase the level of viraemia, thereby leading to increased risk of transmission. By implication, any strategies targeting treatment of maternal infections would have impact on mother-to-child transmission.

HIV-seroconversion during pregnancy, delivery and post-partum is an independent determinant of mother-to-child transmission; this relates to the high level viraemia during the primary infection, which consequently increases the risks three-fold (Dinh et al., 2015; Marinda et al., 2011; Moses et al., 2008).

2.5.3.2. Intrapartum transmission: risk factors

The risk of HIV transmission during the labour and delivery can be quantified in a non-breastfeeding population as the interval between onset of labour to the end of the delivery (stuart et al., 2010). Intra-partum HIV transmission is measured during this period by assaying the blood samples of infants for qualitative DNA PCR in those who were negative for HIV in the first week of life and become positive with the same assay method between seven to 90 days after delivery (Bryson et al., 1992). Several factors are associated with increased risk of HIV transmission in the intrapartum period. The duration of labour is an independent risk factor for transmission of HIV; the longer the fetal mucosa and skin are exposed to cervico-vaginal secretions and/or blood, the higher the risk (odds ratio of 2% per additional hour of ruptured membranes) (International Perinatal HIV Group, 2001). Maternal viral load is another independent risk factor for HIV transmission irrespective of antiretroviral therapy; higher VL is associated with increased transmission (Dinh et al., 2015; Garcia et al., 1999; Marinda et al., 2011; Mofenson et al., 1999). In addition, high level of p24 antigenaemia, and CD8 lymphocyte counts and high maternal IgA levels were reported in earlier studies to correlate with increased risks of MTCT (Hutto et al., 1989). Furthermore, viral shedding of HIV in the genital tract independent of the serum have been reported in some studies (Kovacs et al., 2001; Neely et al., 2007), thus, confirming the probable risk of MTCT even in women on ART (Tuomala et al., 2003). In addition to the compartmental variations in the viral activities, longer duration of ART is needed to ensure sustained virological suppression in the genital tract (Tuomala et al., 2003). However, most studies are unable to define the optimal duration of ART exposure to ensure viral suppression in the genital tract. Though the French cohort demonstrated that the majority of pregnant women will achieve viral suppression after three months and shorter duration is associated with increased risk of MTCT from residual infections (Warszawski et al., 2008). Mandrelbrot et al. (2015) demonstrated that women who started ART before the onset of pregnancy and maintained viral suppression are more likely to achieve zero transmission.

The evidence for scheduled caesarean section as a prevention modality for MTCT pre-dated the ART era (Newell et al., 1994; Villari et al., 1993). Subsequently, many other studies continued to support the protective effect of caesarean section before the rupture of membrane and in women with high viral load (Dorenbaum et al., 2002;

Mark et al., 2012). Therefore, most HIV-infected pregnant women in the western countries were offered caesarean section as an additional prevention strategy. The PACTG 316 study reported zero transmission among 17 infants delivered by scheduled Caesarean section in mothers with viral load > 10,000 copies/ml (Dorenbaum et al., 2002). Burr et al. (2011) also reported a decreased risk of perinatal HIV transmission if scheduled Caesarean sections were performed in women with viral load > 1000 copies/ml. Interestingly, Mark et al. (2012) found a protective effect of caesarean section in women on ART even when they were virally suppressed (VL<50 copies/ml).

However, the International Perinatal HIV Group (1999) demonstrated that there is no significant difference in the risk of MTCT between vaginal delivery and Caesarean section if membrane had ruptured in HIV positive pregnant women prior to delivery. With large scale roll out of ART all over the world, the additional protection offered by caesarean section may be waning. Most authorities have recently recommended vaginal delivery in HIV-infected pregnant women in developed countries when they are virally suppressed (Aebi-Popp et al., 2013; de Ruiters et al., 2014; DHHS, 2016; EACS, 2016). In addition, WHO guidelines (2013; 2016) recommend vaginal delivery in pregnant women on suppressive ART. South African PMTCT guideline also supports vaginal delivery in pregnant women living with HIV. Therefore, ART initiation is the most crucial intervention in the prevention of mother-to-child transmission of HIV all over the world.

Within the South African context, it is expected that pregnant women should be tested for HIV on the first antenatal clinic visit and initiated on ART. Adherence counselling and ongoing support should continue at every clinic visits. Viral load monitoring should be performed every three months during pregnancy and breastfeeding period. This will ensure that those failing current ART regimen can be diagnosed early. However, there is no recent study evaluating the level of viraemia among pregnant women linked to MTCT in the country. A small study in the Western Cape Province by Myer et al. (2017) showed a very high rate of viral suppression and low rate of early infant HIV infections, but the study is not generalisable to the region. The investigators recruited pregnant women on ART, monitored the viral load and performed adherence counselling including interventions when necessary. This methological approach does not resemble the situation in the majority of health facilities in the country. Therefore,

larger studies in other parts of the country should build on the methodological challenges of the study to provide robust data that informs the PMTCT directorate on the population impact of the current policy.

2.5.3.3. Post-partum Transmission

Post-partum (breastfeeding -related transmission) is defined as initial negative PCR results obtained in the first four weeks of life, then followed by a positive result in a breastfed HIV-exposed infant (Stuart et al., 2010). Nduati (2000) demonstrated an increased risk of HIV transmission (44%) in a randomised controlled trial among the breastfeeding population. This study also found a significantly higher rate of transmission in breastfed infants compared with non-breastfed infants in the six-week DNA PCR results. The majority of the transmission occurred early in the breastfeeding period. The proportion of HIV-infected infants correlates with the duration of breastfeeding (Taha et al., 2007). For every one log drop in breast milk viral load, there is a two-fold reduction in transmission of HIV through MTCT (Rousseau et al., 2003).

Breast factors influence transmission of HIV. Breast abscesses and cracked nipples increase the risk of MTCT. There is increased permeability of the mucosal barrier of the mammary epithelium, which promotes sodium influx into the breast milk in mastitis (Salado-Rasmussen et al., 2015). High sodium level in the breast milk is used as a marker of inflammation and predictor of increased risk of breastfeeding transmission of HIV. HIV-seroconversion during breastfeeding is a red flag for transmission (Dinh et al., 2015; Dunn et al., 1992). Certain infant factors promote HIV transmission during the breast feeding period: prematurity, low birth weight, skin and mucous membrane lesions (thrush) (Charurat et al., 2009). In addition, mixed feeding is a strong independent risk factor for post-partum transmission (Coovadia et al., 2007).

2.5.4. Other Routes of Transmission

Advancement in technology since 1985 has revolutionised HIV screening and diagnostic tools used in blood banks. Blood and blood products are screened with HIV RNA Nucleic Acid Testing which reduces the window period of HIV in donors to about 11 days (Weusten et al., 2011). Hence, the risk of transmission of HIV from blood transfusion is very rare. However, the risk is not yet eliminated completely in some parts of the world, where HIV screening is not available. The risk of acquiring HIV following percutaneous inoculation is 0.3%. However, the risk of transmission of HIV

increases depending on whether hollow needle is involved. The amount of inoculum is greater with hollow needles in comparison with blunt needles (suturing materials during surgery). The risk of acquiring HIV from aerosols or splash of body fluids to the face is low. Numerous studies investigating the risk of transmission of HIV through insects have come to the conclusion that it is impossible (Hoffman and Rockstroh, 2012, pg. 6).

Table 2.2 highlights the contributions of the various routes of transmission to the global HIV epidemic. Overall, heterosexual transmission accounts for 70% of the HIV infections globally while the rest are related to men having sex with men, plus mother-to-child transmission.

Table 2.2. Routes of Transmission and Estimated Contribution

Anatomical Site	Type of Epithelium	Transmission medium	Estimated contribution to HIV epidemic
Female genital			12.6 million
Vaginal	Squamous, non-keratinised	Semen; blood	
Ectocervix			
Endocervix	Columnar, single epithelial layer		
Male genital			
Foreskin	Squamous, poorly keratinised	Cervicovaginal and rectal secretions, blood	10.2 million
Penile urethra	Columnar, stratified		
Intestinal tract			
Rectum	Columnar; single	Semen; blood	3.9 million
Upper GI	Various	Semen; blood	1.5 million
Placenta		Maternal blood, genital secretions (intra-partum), breast milk, maternal blood (intra-uterine)	960 000
Chorionic villi	Two-layer epithelium		960 000 480 000
Bloodstream		Blood products, sharps	2.6 million

(Adapted from Shaw and Hunter, 2012)

2.6. Transmission Prevention Strategies

In order to effectively prevent the spread of HIV at the population level, a multi-faceted approach consisting of biomedical, behavioural and structural level efforts are needed. These approaches will keep women in reproductive age group safe from acquiring HIV from their partners or through other modes of transmission.

2.6.1. Prevention of Sexual Transmission

About 70% of the world HIV epidemic occurs through sexual exposures. Therefore, prevention efforts directed towards mitigating the acquisition and transmission of the virus to significant others are relevant. The model of transmission prevention strategies proposed by Cohen et al. (2010) gives insight as to how to tackle the epidemic at individual and population levels. There are numerous opportunities to address sexual transmission of HIV along the continuum of those who are unexposed, exposed and infected (Fig. 2.5). A combination of behavioural, structural and biomedical strategies can be applied at each stage along the continuum of HIV prevention. Prevention strategies can be adapted to target individuals who are unexposed with behavioural and structural interventions, individuals who are exposed either pre-coitally, during coitus or post-coitally and lastly, infected individuals can be targeted for prevention of the spread of the virus (AAHIV, 2012, pg. 49).

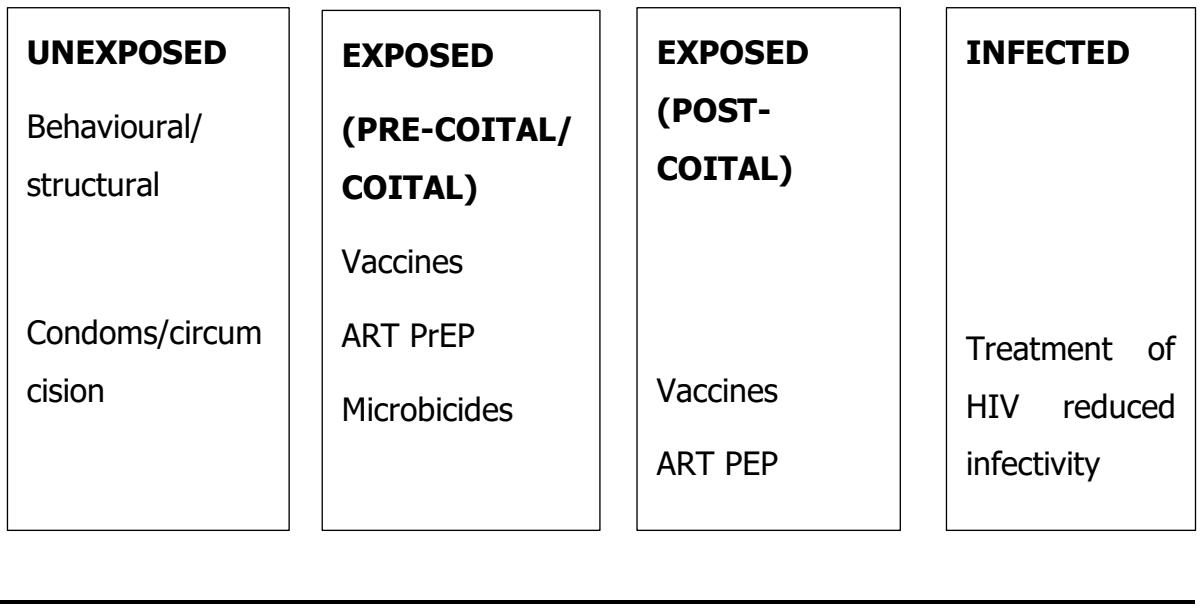

Fig. 2.5. Opportunities for Prevention Strategies (Adapted from AAHIVM, 2012, pg. 49)

2.6.2. Behavioural strategies

The ABC of behavioural prevention strategies were considered initially at the onset of the HIV epidemic. Abstinence from sexual exposure and delay of sexual debut, being faithful to one sexual partner and consistently using condoms were advocated by health authorities. However, none of the behavioural strategies were proven to have worked effectively in isolation. As such, prevention efforts that combines many interventions such as raising the knowledge and awareness of HIV at the population level, increasing HIV and other STIs screening including condom use and decreasing the number or concurrency of sexual partners were recommended. This report is supported by the findings from the systematic review and meta-analysis of behavioural interventions for HIV prevention in developing countries by Kennedy et al. (2010). With appropriate education and counselling of individuals living with HIV, there was a tendency for a change in behaviour towards embracing prevention of transmission to significant others. Individuals living with HIV were able to reduce the number of sexual partners, increase disclosure of HIV serostatus to sexual partners and insists on condom use.

2.6.3. HIV Counselling and Testing

HIV counselling followed by testing is the entry point to the care cascade for individuals living with HIV. Recent evidence supports treatment for all individuals diagnosed with HIV (The Insight START Study Group, 2015; TEMPRANO ANRS 12136 Study Group, 2015). Also, many health authorities have recommended the 'test and treat' strategy with a hope of saving lives but also to decrease new infections at the population level (European AIDS Clinical Society (EACS), 2016; Department of Health and Human Services (DHHS), 2016; SADoH, 2016; WHO, 2015). Counselling offered during HIV testing have been shown to increase the rate of condom use, increase disclosure of serostatus among sexual partners and also, decrease the number and concurrency of multiple partners (Kennedy et al., 2010; Johnson et al., 2008). Therefore, counselling and testing are crucial strategies toward preventing the spread of HIV at the population level.

2.6.4. Male Medical Circumcision

Evidence from well conducted randomised control clinical trials (RCCTs) from South Africa (Auvert et al., 2005), Kenya (Bailey et al., 2007) and Uganda (Gray et al., 2007) found reductions in the rate of HIV transmission in heterosexual relationships of 58%, 59% and 50%, respectively. A combined risk reductions of 56% in transmission risk attributable to medical circumcision was observed from the meta-analysis of the three RCTs (Mills et al., 2008). Based on the overall risk reductions from male circumcision, there may be secondary benefits to women immediately after wound healing. Circumcision may offer some protection against HIV acquisition in insertive sex among men having sex with men. However, evidence for this is conflicting. Also, circumcision offers protection against genital ulcers, herpes simplex-2, human papilloma virus and Trichomonas vaginalis (Weiss et al., 2010).

There are some biological mechanisms which can possibly explain the findings observed from the RCTs. The superficial layers of the foreskin contain a large density of CD4/CD8 and Langerhans/dendritic cells which increase entry of HIV. The foreskin is prone to microtears and abrasion during sexual intercourse (Donoval et al., 2006; McCoombe and Short, 2006; Patterson et al., 2002). In addition, the larger surface area of the uncircumcised foreskin is exposed to vaginal secretions (containing HIV) during unprotected sexual intercourse with an infected partner. However, there are still challenges with the implementation of medical male circumcision in the Eastern Cape, South Africa due to cultural demand.

2.6.5. Treatment of Sexually Transmitted Infections (STIs)

Mayer and Venkatesh (2011) succinctly summarised the biological and epidemiological interactions between HIV and other STIs (Fig. 2.6). STIs are associated with higher rate of shedding of HIV in the genital tract. Similarly, treatment of STIs has been shown to lead to decrease in the viral load in the genital secretions ((AAHIVM, 2012:52). Previous reports showed that individuals coinfected with HIV and any other STIs have higher risks of transmitting HIV to their sexual partners (Kissinger et al., 2009; Mayer and Venkatesh, 2011).

In addition, individuals who have STIs other than HIV are more susceptible to acquiring HIV. Both ulcerative and non-ulcerative STIs lead to recruitment of inflammatory cells (containing CD4 receptors) to the infected genital tracts, thus, enhancing acquisition and transmission of HIV. Therefore, ulcerative lesions are associated with higher risk of transmission (Mayer and Venkatesh, 2011). Evidence supports the treatment of acute STIs, leading to significant decline in HIV expression in the genital tract (Johnson and Lewis, 2008; Mayer and Venkatesh, 2011). However, there is concern about the post-treatment residual inflammation, which could last for weeks to months after treatment. Residual inflammation after acute STIs has been shown to be associated with higher viral loads in the genital tracts for prolonged periods and thus increases the risk of transmission after treatment.

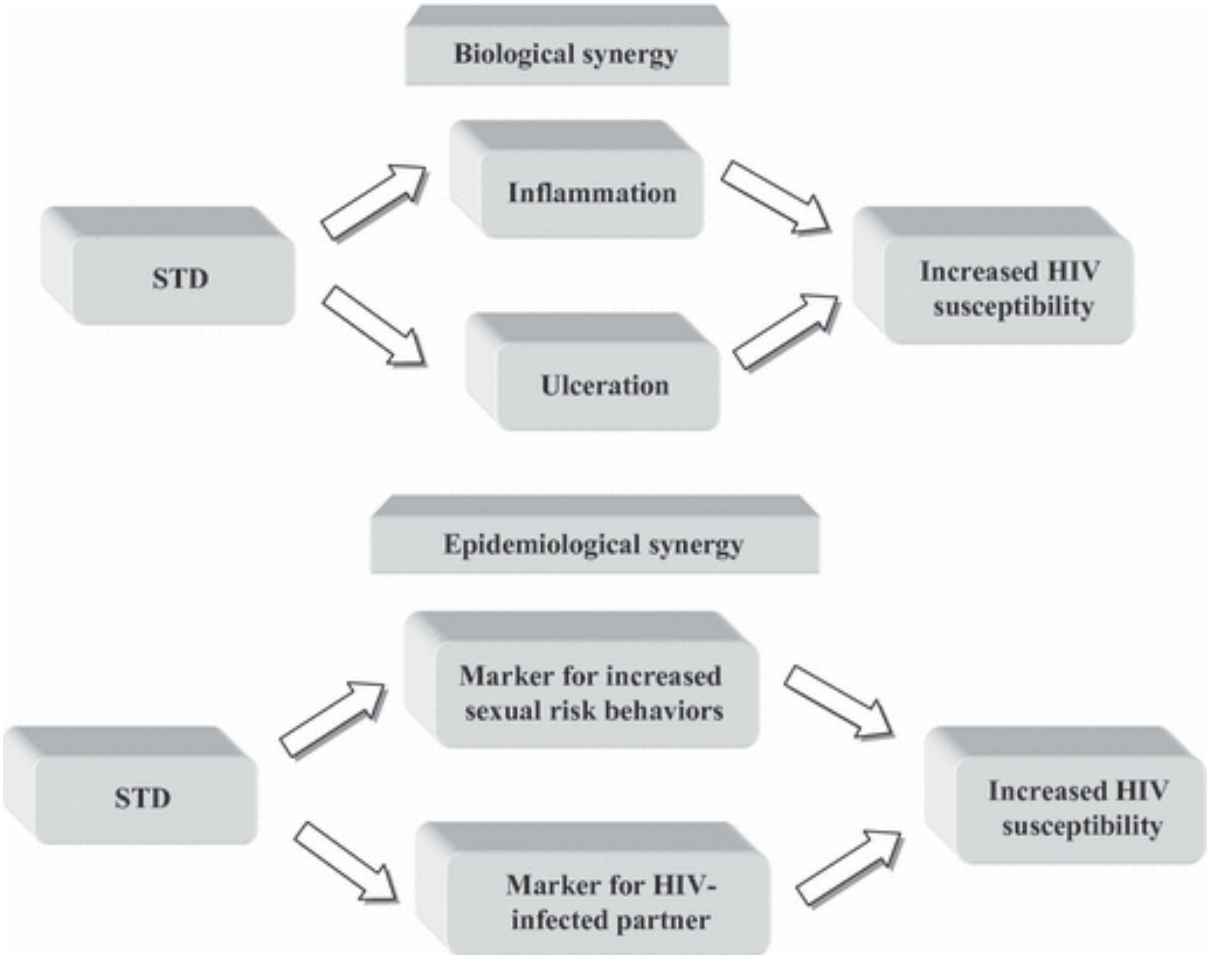

Fig. 2.6. Biological and epidemiological interactions of HIV and STIs (Adapted from Mayer and Venkatesh, 2011)

Within South African setting, screening for STIs has been incorporated into the primary health care services for individuals living with HIV (SADoH, 2015). Clinic visits' screening for STIs is incorporated into a package of service which includes HIV counselling and testing and tuberculosis symptom screening (SADOH STI Guidelines, 2015). Individuals diagnosed with STI will be offered early treatment, counselling for future risk reductions and contact tracing. The guideline essentially adopts the syndromic management strategy, which categorises STIs with similar signs and symptoms together. This approach has proven to be effective in resource constrained settings. However, the population impact of this new guideline in relation to HIV prevention in the country is unclear.

2.6.6. Structural interventions

For over two decades, prevention efforts have put emphasis on the individual-level behavioural interventions with some degree of success. However, it has become clearer that broader structural issues such as poverty and wealth, age, gender, power and policies impact on behaviours of individuals (Gupta et al., 2008). The physical, social, economic, political, cultural, organizational, community, legal, or policy of the country all constitute the structural factors which could impact on the vulnerability and risk of individuals to HIV infection (Adimora and Auerbach, 2010).

At the level of the individual, lack of income (poverty increases vulnerability) may drive a young woman to engage in transactional sex, thus, increasing her risk of contracting HIV. However, the prevailing cultural practice might be that it is acceptable for women to be dependent on men for financial support. Examples of structural interventions that have yielded good results are: microcredit programmes which improve the capacity of women to engage in business activities. Also, implementation of HIV testing during antenatal for pregnant women in South Africa (SADoH, 2010; 2013; 2015). Within the South Africa setting, the government has been pro-active in the provision of ART to the citizens; 80% of ART expenditure is provided by the government (UNAIDS , 2017). However, mandatory HIV testing needs to be implemented at all the health facilities in order to reach the first 90 of the UNAIDS target (UNAIDS, 2014). Therefore, it is pertinent to combine individual behavioural interventions with broader structural

interventions in order to achieve the goal of achieving sustainable decline in new HIV infections at the population level.

2.6.7. Biomedical Prevention Strategies

Significant successes were recorded with the first sets of biomedical strategies in the early 1990s. Condoms (either male or female) as physical barriers for prevention against HIV-infected genital secretions were recommended and reportedly used. The provision of clean injection equipment either for medical use at the health facilities and also, for needle exchange programme for injection drug users was provided. The prevention of mother-to-child transmission programme among others demonstrated the impact of biomedical strategies early in the HIV epidemic (UNAIDS, 2010).

a). Treatment as Prevention

Anti-retroviral therapy for prevention has emerged as the most important biomedical strategy to date. Irrespective of the routes of transmission, viral suppression with the highly efficacious ART will achieve significant reductions in the transmission risks of HIV. Earlier observational studies in different parts of the world showed that high viral load is the cornerstone of HIV transmission (Lingappa et al., 2010; Hallet et al., 2011). Following these observational data, the HIV Prevention Trials Network (HPTN052) further demonstrated the protective efficacy of ART in preventing transmission to sexual partners of individuals with a higher CD4 count (350 – 550 cells/mm^3) (Cohen et al., 2011). HPTN052 found a risk reduction of HIV transmission between serodiscordant partners of 96% (95% CI 73 – 99%). This report has translated to policy development on treatment for prevention (WHO, 2015; EACS, 2016; DHHS, 2016).

Evidence has shown unequivocally that ART reduces the extent of infectiousness of the HIV-infected individuals and thus, prevents the transmission of the virus at the population level. Very recently, The Insight START (2015) and Temprano (2015) studies have also added another line of evidence on the survival benefits of ART in all HIV-infected individuals. This new finding has led to changes in policy guidelines across the world on 'test and treat' approach for all HIV-infected individuals (WHO, 2015; EACS, 2016; DHHS, 2016; SADoH, 2016). Therefore, the UNAIDS 90:90:90 target (2014) holds true for population-level HIV prevention if at least 90% of all HIV infected individuals receive diagnosis, and at least 90% of those are initiated on ART

with consequent virologic suppression in at least 90% of those initiated on treatment. However, there are leakages at every stage of the care cascade all over the world. More importantly, expanding access to HIV testing is crucial into making in-roads to achieving the UNAIDS target.

b). Pre-Exposure Prophylaxis (PrEP)

In addition to reducing the degree of infectiousness in HIV-infected individuals, the use of ART as pre-exposure prophylaxis (PrEP) has gained recognition globally. Evidence supporting the efficacy and safety of TDF or TDF/FTC as pre-exposure prophylaxis (PrEP) either as peri-coital topical gel application or oral dosing are summarised below.

c). Topical ART as PrEP

The Centre for the AIDS Programme of Research in South Africa (CAPRISA) 004 study, a double-blind, randomised placebo controlled trial conducted in urban and rural Kwazulu-Natal, South Africa opened doors for topical TDF gel as a PrEP strategy (Karim et al., 2010). CAPRISA 004 demonstrated a risk reduction of 39% (95% CI 6 – 60%) following exposure to peri-coital 1% TDF gel. The results demonstrated adherence-efficacy relationship; women who self-reported more than 80% adherence, had efficacy of 54% in comparison to 38% and 28% observed in women who reported 50 – 80% and 30% gel adherence, respectively. Surprisingly, two other studies have failed to replicate the promise shown by CAPRISA 004 (Microbicide Trial Network (MTN)-003, 2013; Rees et al., 2015). The VOICE (Vaginal and Oral Interventions to Control the Epidemic study) and FACT (Follow-on African Consortium for Tenofovir Studies) 001 trials failed to show any effectiveness and were stopped prematurely. The participants in both studies did not adhere to the study protocols; less than 30% of the participants in the VOICE trial had demonstrable drug levels in their plasma and 20% of those in FACT 001 reported 80% gel adherence.

Dapivirine vaginal ring has shown good promise in the phase III clinical trials. The MTN-002-ASPIRE study, a double-blind placebo controlled clinical trial exploring long-

acting methods of PrEP with Dapivirine, a non-nucleoside reverse transcriptase inhibitor, found a risk reduction of 27%. The risk reduction was higher in participants older than 21 years with 37% efficacy (Baeten et al., 2016).A similar trend was demonstrated by the RING study, which reported a risk reduction of 31% (Hazard ratio, 0.69, 95% CI 0.49 – 0.99) overall, and 61% among women older than 25 years (Nel et al., 2016).

D). Oral Formulations as PrEP

The efficacy of oral TDF or TDF/FTC has been proven in many clinical trials (Grant et al., 2010; Mujugira et al., 2011; Thigpen et al., 2011). In the iPrEx study, oral TDF/FTC was offered as intervention in the randomised, placebo-controlled trial among men or transgender women having sex with men. A risk reduction of 44% (95% CI 15 – 63%) was obtained in the intervention group. The benefit is higher (73%) among the participants with over 90% adherence rate (Grant et al., 2010). The findings of the iPrEx study were corroborated by TDF2 study, a placebo controlled trial among heterosexual HIV-uninfected participants (Thigpen et al., 2011). The TDF2 study reported an efficacy of 63% among the participants who were retained in the study. Significantly, higher efficacy was obtained among men (80%) than women (49%).

The Partner PrEP study by Mujugira et al. (2011), a clinical trial comparing the efficacy of TDF alone, TDF/FTC combination and Placebo in East Africa, found either drug as effective for PrEP. The efficacy of TDF alone is 67% (44 – 81%) while the efficacy of TDF/FTC is 75% (95% CI 55 – 87%). Both TDF and TDF/FTC reduced HIV risk for men and women. The observed results in the Partners study were corroborated by the high adherence rate confirmed by plasma drug concentration levels (Bangsberg et al., 2012). Daily oral TDF as PrEP for men who injects drugs have also been added to the armamentarium of prevention strategy after the results of the Bangkok TDF study was released (Choopanya et al., 2013). Based on the convincing evidence summarised above, several health authorities have released guidelines on the use of oral Truvada (TDF/FTC) among key populations (men having sex with men, sex workers and serodiscordant couples) (WHO, 2015; EACS, 2016; DHHS, 2016). Structural

challenges and concerns about emergence of resistance strains to TDF are barriers to the uptake of oral PrEP in the developing countries.

e). Long-acting Injectable PrEP

The possibility of long-acting injectable preparations replacing oral formulations as PrEP is reassuring. According to the modelling study by Marshall et al. (2018), a coverage of 35% for HIV-uninfected individuals will achieve 44% risk reduction. This is higher than the risk reduction of 33% that would be achieved by oral PrEP. Carbotegravir, an integrase strand inhibitor, which has undergone phase 2a clinical trial has shown tremendous promise based on its safety profile and pharmacokinetic profile (Landovitz et al., 2018).

f). Post-Exposure Prophylaxis (PEP)

Post-exposure prophylaxis (PEP) involves the use of ART after an exposure in order to prevent infection. The evidence for the use of ART for PEP was derived from animal studies (Tsai et al., 1995; Van Rompay et al., 1992) and a case control study in humans (Cardo et al., 1997). Till today, there have not been any randomised controlled trials in humans to investigate the efficacy of PEP due to the complexity of the exposure, low event rate and the ethical restrictions on placebo controlled trials in the presence of proven efficacious interventions (Moorhouse et al., 2015). Notwithstanding, a recent systematic review and meta-analysis by Irvine et al. (2015), which included 25 animal studies showing PEP efficacy of 89%, when PEP is given within 72 hours of exposure in comparison with no interventions. Based on the available evidence from animal studies and observational data in humans including PMTCT studies, the WHO (2014) made recommendations on the use of ART for PEP.

PEP should be used within the context of both occupational and non-occupational exposures (AAHIV, 2012, pg. 56). The effectiveness of PEP is best achieved when it is offered as soon as possible after an exposure and not later than 72 hours. Once the decision to initiate PEP is made by the attending clinician, a minimum of three ART should be given. This approach will minimise the possibility of emergence of drug

resistance. A combination of TDF + FTC/3TC + RAL (Raltegravir) or TDF + FTC/3TC + ATZ/r in pregnancy should be given to adults for 28 days in the event of an exposure (Moorhouse et al., 2015). This recommendation by the Southern Africa HIV Clinicians Society on the choice of triple ART aligns with other international guidelines for PEP (WHO, 2014; CDC, 2016; EACS, 2017, pg. 17).

f). Vaccines

Without any shadow of a doubts, a preventive vaccine would be required to eradicate the HIV pandemic. Despite significant investments into vaccine research studies over the years, no single preventive or therapeutic vaccine has been successfully developed nor is one available for use today. The challenges with the development of an effective vaccine are due to the wide genetic diversities of HIV in different communities, regions and countries in the world. This is compounded by the rapid rate of replications of the virus with attendant emergence of mutations in each replicating cycle (AAHIVM, 2012, pg. 54). Early promises of candidate vaccines in clinical trials (Pitisuttithum et al., 2006; Buchbinder et al., 2008; Gray et al., 2011; Hammer et al., 2013; Rerks-Ngarm et al., 2009) have led to the possibility of the first human vaccine, which showed evidence for some protection (Rerks-Ngarm et al., 2009). The preceding phase 2 trial involving ALVAC-HIV (vCP1521) as primer and boosted with AIDSVAX B/E demonstrated cellular and humoral immune responses (Nitayphan et al., 2004).

The Thai RV144 study was a community-based, double-blind, randomised, placebo-controlled trial, conducted among HIV-uninfected heterosexual males and females aged 18 - 30 years in Thailand. In this study, each participant received four priming injections consisting of a recombinant canarypox vector vaccine (ALVAC-HIV) and two booster injections consisting of a recombinant GP120 subunit vaccine (AIDSVAX B/E). The vaccine demonstrated an efficacy of 31.2% (95% CI 1.1 – 52.1%) after excluding the seven participants who had HIV infection at baseline (modified intention-to-treat analysis). Despite this study holding promise for a human vaccine, it is still not good enough as a minimum of 50% protection is expected.

Encouraging data from the APPROACH Trial, a phase 1/2a multicentre, placebo-controlled trial, which evaluated the mosaic adenovirus serotype 26 (Ad26)-based HIV-1 vaccine candidates in East Africa, South Africa, Thailand and USA, found comparable and robust immune responses were found in both humans and rhesus monkeys (Barouch et al., 2018). A 65% protection was observed with this candidate vaccine molecule. This prompted a phase 2b clinical trials in sub-Saharan Africa (https://clinicaltrials.gov/ct2/show/NCT03060629), findings of which will be available in 2022. The HVTN 702, which is currently enrolling participants in South Africa to test the efficacy, safety and tolerability of ALVAC-HIV (vCP2438) plus bivalent subtype C GP120/MF59 will provide new insights on or before 2021 (https://www.avac.org/trial/hvtn-702).

2.6.8. ART for Prevention of Mother-To-Child Transmission

Initiation of ART in all individuals living with HIV, especially pregnant women is the standard of practice all over the world. The WHO Option B Plus strategy (WHO, 2013), which was adopted in South Africa in 2015 (SADoH, 2015) stipulates that pregnant women diagnosed with HIV should be initiated on ART for their own health's sake as a life-long treatment and also, for prevention of MTCT. As such, treatment with ART in the postpartum period is the standard of practice in South Africa. Table 2.3 highlights the use of ART as treatment and prophylaxis in the South African context.

The superiority of triple ART as the best prevention was demonstrated in a number of well conducted randomised control trials and observational studies worldwide (Cooper, 2002; Fowler et al., 2015; Kesho Bora Study Group, 2011; Myer et al., 2017; Mandrelbrot et al., 2015; Warszawski et al., 2008). Cooper (2002) found a gradient effect with different combinations of antiretroviral drugs: three drug combinations were more effective in the prevention of MTCT (1.2%, 95%CI: 0 -2.5%), than dual prophylaxis (3.8%, 95%CI: 1.1-6.5%) and followed by single therapy with zidovudine (10.4%, 95% CI: 8.2-12.6%). The majority of the studies found a significantly reduced risks of MTCT among women with viral load <1000 copies/ml. However, there are reports demonstrated that HIV transmission occurs in the context of suppressed viral load (VL< 400 RNA copies/ml) (Fowler et al., 2015; Tubiano et al., 2010). The risk of

HIV transmission is not completely eliminated with ART and is dependent on strict adherence to ensure maximal viral suppression (Voronin et al., 2014).

Clinicians should be concerned by the level of adherence in pregnant and breastfeeding women because of the correlation between viral suppression and adherence. Adherence in the post-partum period is greatly affected by a number of events, thus, leading to a drastic fall in the adherence levels post-delivery in comparison to the pregnancy period (Nachega et al., 2012). Adherence to ART among pregnant women living with HIV in high- and low-income countries is significantly below what is recommended for viral suppression and prevention of drug resistance (Leach-Lemens, 2012). Pooled analysis reports of adherence involving participants drawn from USA, Kenya, South Africa and Zambia showed that only 73.5% of pregnant women living with HIV reported good adherence during and after pregnancy (Nachega et al., 2012). The levels of adherence fell after delivery to 53% (95% CI 32.8 – 72.7%) in comparison to 75.7% (95% CI 71.5 – 79.7%) observed during pregnancy.

Adherence to therapy during the postpartum period has been a huge challenge for women (Kesho Bora Study Group, 2010). This is evidenced by the rate of infant infections diagnosed at six and twelve months among breastfed infants despite the mothers remaining on ART (Kilewo et al., 2009). The determinants of virologic suppression in pregnant women on ART have not been investigated in Eastern Cape Province, South Africa. Epidemiological data on the determinants of peri-partum suppression of viral load could be useful to inform strategies to effectively operationalise the WHO Option B Plus strategy in South Africa. More importantly, reliable data on adherence and its implication on MTCT will assist the South African Department of Health to strategize to design appropriate interventions for the region.

Table 2.3. ART in Pregnant and Breastfeeding Women in South Africa

Population	When to Start	Comments
Pregnant and breastfeeding women	Same day initiation in all pregnant and breastfeeding women regardless of CD4	Counsel on exclusive breastfeeding for the first six months, then complementary feeding can continue for the until 24 months
	Unbooked pregnant women tested positive in labour should be started on prophylactic ART during labour and initiated on lifelong ART immediately after delivery.	
First Line ART		
1st ANC visit	Drugs	Comments
ART-naïve Pregnant women or breastfeeding women	Start TDF + FTC (3TC) + EFV (fixed dose combination – FDC)	Contraindication to TDF; renal failure or Contraindication to FV: active psychiatric illness; give AZT and refer
Pregnant women currently on ART	Continue the ART Change to FDC if virally suppressed and no contraindication	Check viral load on arrival to ANC irrespective of when the VL was last done Virological failure on 2nd or 3rd line ART should not breastfeed the infants
2nd ANC visit (1 week later)		
Creatinine > 85μmol/l	Stop FDC, initiate AZT if Hb≥7g/dl	Refer
Contraindication to EFV	Continue AZT until reviewed	CD4 < 250 cells/μl; NVP 200mg daily for 2 weeks then 200mg BD; CD4 > 250 cells/μl; give Lop/r 2 tabs 12 hourly
Labour		
Unbooked and presents in labour Emergency caesarean section in unbooked woman with no ART	sNVP + sd Truvada and AZT 3-hourly in labour sNVP + sd Truvada for caesarean section Start FDC next day	All HIV-infected individuals qualifies for ART especially pregnant women Do creatinine and CD4 tests.
Post-partum		

Irrespective of the choice of feeding, all HIV-infected should be started on ART Breastfeeding women should be encouraged to start immediately	Life-long ART	
Second Line ART	Drugs to be started	Comments
Failing TDF-based regimen	AZT + 3TC + Lop/r AZT + TDF + 3TC +Lop/r	4 drugs if co-infected with Hep B
Failing AZT (or D4T)-based regimen	TDF +FTC (3TC) + Lop/r	
Diarrhoea or dyslipidaemia associated with Lop/r	Switch to ATZ/r	

ATZ/r=Atazanavir/ritonavir; AZT=Zidovudine; FTC=Emtricitabine; Lop/r=Lopinavir/ritonavir; TDF= Tenofovir; 3TC=Lamivudine

(Source: Adapted from the South African National Department of Health Guidelines, 2015)

2.7. Morphological Structure of HIV

HIV belongs to the class of viruses called retroviruses and the family of lentiviruses. Retroviruses are uniquely different from other viruses because of the presence of the reverse transcriptase enzyme, which catalyses the conversion of the viral RNA to DNA. The virus is able to use its RNA as a template to generate DNA copy, which can be integrated into the host cell genome (Bebenek et al., 1989; Boyer et al., 1992). In addition, the retroviruses have the adaptability for recombination when two different parental genomes co-infected the same cell. Lentiviruses (slow virus) cause infections that run a chronic course followed by prolonged period of asymptomatic disease phase with persistent replication. HIV-1 and HIV-2 are typical examples of lentiviruses. Both viruses have about 40 – 60% homologous genomic materials. However, they differ from each other in the molecular weight of their protein and accessory genes. Both HIV-1 and HIV-2 infect humans and become pathogenic in them. They replicate rapidly in CD4 cells, causing rapid destruction and depletion of the immune cells.

The HIV-1 particles are enveloped in the lipid bilayer and have a diameter of 100nm. The outer layer is derived from the host cell material during the process of budding. On the surface of each viral particle are 72 glycoprotein (gp) complexes (envelope proteins), integrated into the lipid bilayer, each consisting of an external gp 120 and the transmembrane protein – gp 41 (Fig. 2.7). Both gp complexes are loosely bonded, which allows the gp 120 to be shed spontaneously into the environment. Hence, gp 120 can be detected in the serum and lymphatics of HIV-infected individuals. On the inner surface of the lipid bilayer is the matrix protein (gp 17). Within the lipid envelope is the icosahedral shell of protein (p24), which further encloses a cone-shaped protein core – p7 and p9. There are two copies of HIV-1 RNA within the core of the virus. This is part of the protein-nucleic acid complex. The viral particle contains all the enzyme necessary for its replication: a reverse transcriptase (p66), an integrase (p32) and a protease (p11) (Hoffman and Rockstroh, 2012, pg. 21).

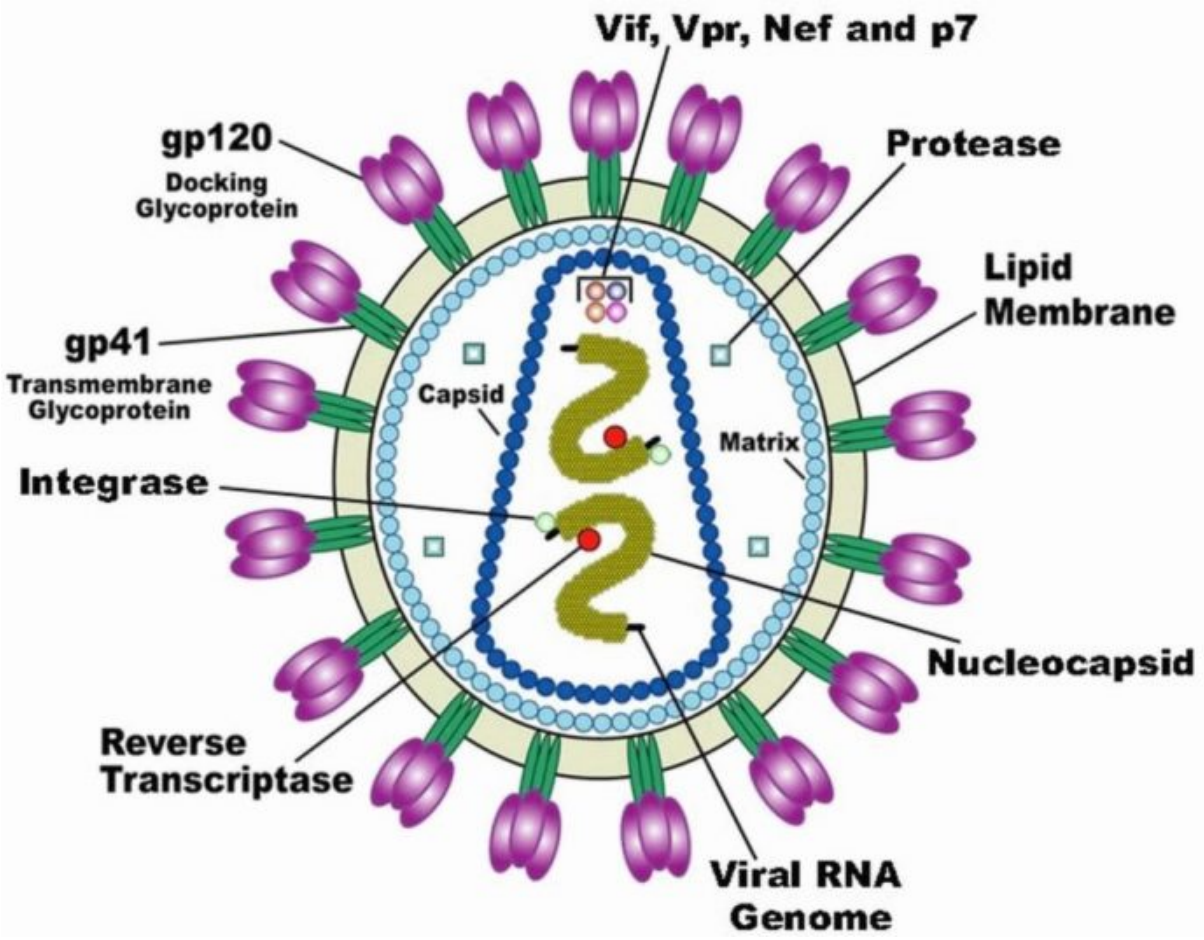

Fig. 2.7. Structure of an HIV virion (Adapted from https://www.projectinform.org/glossary/hiv-structure-function/)

2.8. Genomic organization of HIV: Genes and functions

HIV-1 genome is approximately 9.7 kilobases in length with long terminal repeats (LTRs) on both ends (Fig. 2.8) (Hoffman and Rockstroh, 2012: 22). The classical genomic sequence of HIV is denoted as (Fig. 2.8):

5'LTR-gag-pol-env-LTR 3'

LTRs represent the two ends of the HIV genome that are connected to the host cell DNA. LTRs do not code for any viral protein. Within the viral genome are many open reading frames which codes for important proteins of the virus. There are at least nine important genes coding for the various HIV proteins, divided into three classes:

1. The major structural proteins: gag (group-antigen), pol (polymerase) and env (envelope).
2. The regulatory proteins: tat and rev
3. The accessory proteins: vif, vpr, nef and vpu (Hope and Trono, 2000)

Structural proteins

Gag gene is very important for the replication process of the virus. It gives rise to a precursor protein – p55, which is expressed as unspliced mRNA. The cytoplasmic effect of the gag gene is triggered, leading to further recruitment of the two copies of viral genomic RNA and other important proteins needed to facilitate budding of the virions from the surface of the infected cells. After budding, the protease enzyme cleaves the p55 into four smaller proteins to ensure maturation; matrix protein (p170, capsid protein (p24), nucleocapsid (p9) and p6. The p17 is attached to the inner surface of the virus and stabilises the lipid bilayer. In addition, p17 plays a crucial role in the ability of the virus to infect non-dividing cells. P24 forms the conical core of the virus (Hope and Trono, 2000).

Pol gene codes for reverse transcriptase, integrase and protease enzymes. During maturation, Gag-Pol polypeptide precursors are cleaved by the viral protease to liberate the pol gene. The Pol gene is then digested to release the various enzymes. Reverse transcriptase enzyme catalyses the reverse transcription of RNA to double-stranded DNA, which is integrated into the host cell genome by the integrase. The

protease enzyme is responsible for cleaving long polypeptide chains of the virus into functional units. The pol gene is useful for understanding drug resistance and also, viral diversity.

Env gene is a 160 kD (gp160), which codes for the envelope proteins: gp120 and gp41, which are generated from gp 160. Envelope gp120 on the viral surface facilitates its binding to the CD4 receptors in the target cells. The gag and env genes both code for the membrane glycoprotein and the nucleocapsid. The p24 part of the gene codes for the viral capsid while the p6 and p7 code for the nucleocapsid. Analysis of the env gene is used to gain an understanding of HIV diversity because of its great variability.

Regulatory proteins

Tat, a 14kb protein, functions primarily as a transcriptional transactivator in the replication process of HIV. Tat binds to RNA only and is located within the nucleus and the nucleolus of the infected cells. As a regulatory protein, it binds to the transactivational response element (TAR), which is localized on the 5' LTR of the genome. Once binding takes place, there is activation of transcription of the proviral DNA to RNA in order of least 1000-fold. In addition, Tat is responsible for promoting the elongation of the RNA, thus, leading to the formation of a full-length transcript of the virions (Feinberg, Baltimore and Frankel, 1991).

Rev, a 13kb protein, serves as a nuclear export factor (Zapp and Green, 1989). Rev is localised to the nucleus and the nucleolus of the infected-cells, where it induces the transition from early to late phase of gene expression. It binds to the Rev response element (RRE) located in env gene. Once binding takes place, Rev facilitates the export of unspliced or incompletely spliced viral RNAs from the nucleus to the cytoplasm, which are normally found in the nucleus (Hope and Trono, 2000). This Rev-expression levels usually follows a negative feedback mechanism; lower levels of Rev signals minimal export of unspliced or incompletely spliced RNAs from the nucleus.

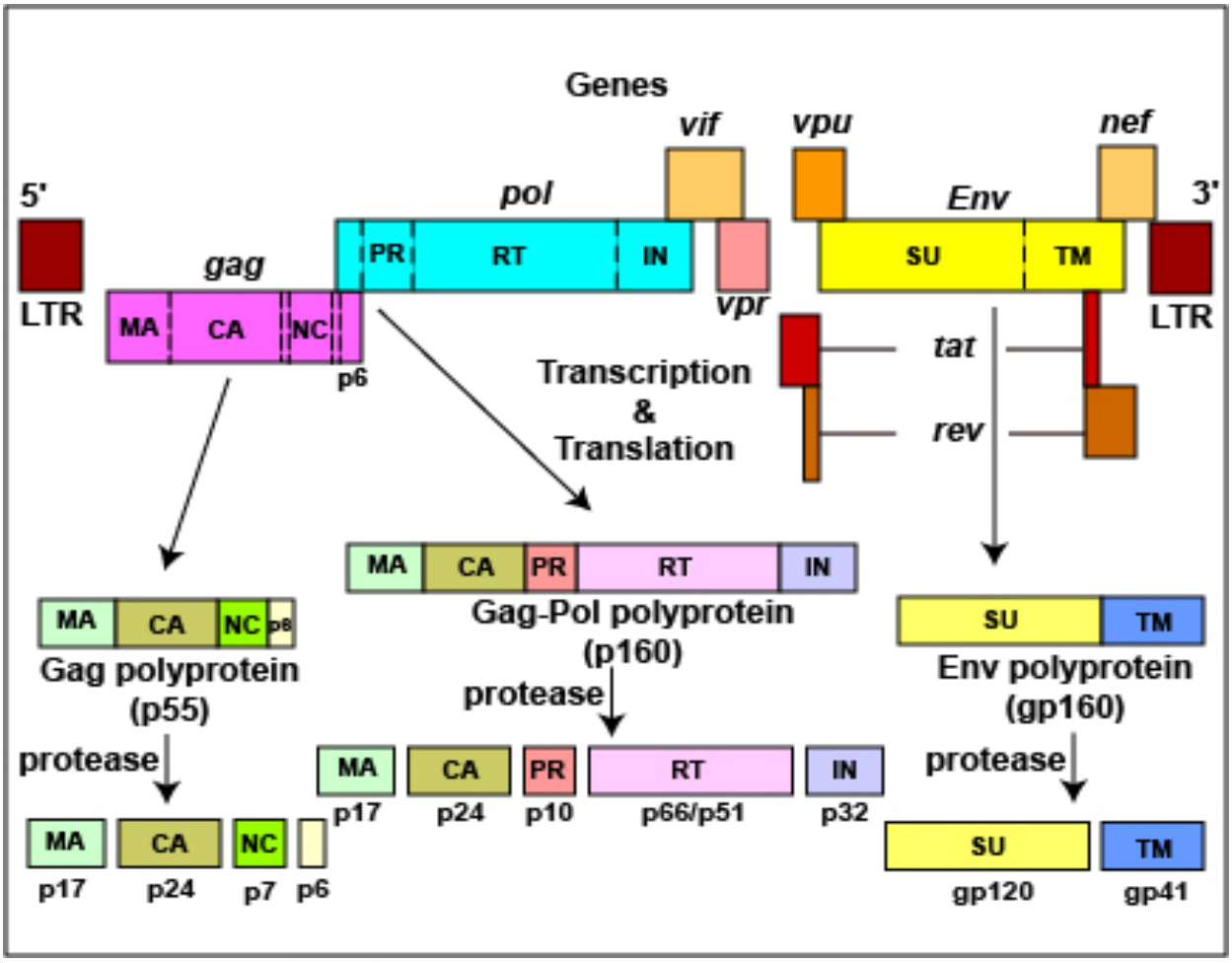

Fig. 2.8. Schematic representation of HIV-1 genome. All the important open reading frames are shown

(Adapted from: https://www.google.com/search?q=POL+GENE.....)

Accessory proteins

HIV-1 has four additional genes; nef, vpr, vpu and vif, encoding accessory proteins. However, HIV-2 lacks vpu which is replaced by vpx. The functions of each accessory protein are described below.

Nef, otherwise referred as to as the negative factor, is a 27kb myristoylated protein. This is the first detectable protein in the host following infection with HIV (Kim et al., 1989). It is critical for the survival of the virus upon gaining entry into the host cells. It provides an escape mechanism for HIV by downregulating the CD4 cells and HLA class I molecules from the surface of infected cells. Thus, evading recognition by the CD4 cells and evading attack by the cytotoxic CD8 cells. It has also been shown to

interfere with the activation of T-cells by blocking the transduction signal pathway (Hoffman and Rockstroh, 2012: 22). Nef is critical for replication and progression of the disease in an infected individual.

Vif is a 23 kb protein, very important for the replication of the virus in the peripheral blood lymphocytes, macrophages and other cell lines. It also promotes maturation of the virions. Evidence suggests that vif-incorporated virions are the only infectious strains produced (Hope and Trono, 2000).

Vpr is a 15kb protein, which helps the virus with transactivation and the replication process. Vpr facilitates the nuclear localization of the pre-integration complex, thus, enhancing the infectivity of HIV towards non-dividing cells (Heinzinger et al., 1994).

Vpu is a 16kb protein, which is located in the internal membrane of the virus (Sato et al., 1990). Its functions include; down-regulation of CD4 cells and facilitation of release of virions from the surface of HIV-infected cells. Without vpu, large amount of virions will cluster on the surface of the cells (Klimkait et al., 1990).

Vpx is an accessory protein found only in HIV-2 and the simian immunodeficiency virus. It is a 15kb protein, which helps in gaining entry into the host cells and facilitates the viral infectivity.

2.9. REPLICATION OF HIV (LIFE CYCLE)

The past two decades have witnessed significant progress in molecular biology, leading to improved knowledge on how HIV persistently infects the host cells and perpetuate itself in the body of the host (Engelman and Cherepanov, 2012). This review elucidates on how HIV gains entry, integrates its chromosomes, transcribes itself and matures out of the host cells. The life cycle of HIV starts when it engages the receptors on the surface of the target cells and ends with maturing virions budding out of the host cells (Fig. 2.9).

2.9.1. HIV Binding and Fusion

CD4 is the main receptor of HIV and can be found on the surface of about 60% of the T-lymphocytes, precursors of T-cells in the bone marrow and thymus, monocytes and macrophages, dendritic cells, eosinophils and microglial cells (Hoffman and

Rockstroh, 2012: 25). The first step in the life cycle of HIV is the entry of the viral particles into the host cells. The envelope spikes, consisting of the surface protein (gp120) and the transmembrane protein (gp41) play crucial roles in the presence of the chemokine-co-receptors (Zhu et al., 2006; Liu et al., 2008). The binding of the viral gp120 to the CD4 receptors on the surface of the host cell membrane (Fig. 2.9. step 1) triggers a conformational change which leads to fusion of the viral and host cell membranes (Fig. 2.9. step 2). Consequently, the virus elaborates its content into the host cell cytoplasm. This interaction of the gp120 and the CD4 receptors leads to formation of bridging sheets between the inner and outer domain of the gp120, thus exposing the chemokine co-receptors. The binding of the co-receptors leads to rearrangement in the transmembrane protein (gp41), which leads to fusion of viral and host cell membranes.

Two different chemokine co-receptors have been identified: CCR-5 and CXC-R4. The CCR5 co-receptors are utilised by monocytotropic HIV-1 isolates while CXC-R4 co-receptors are used by T-cell tropic viruses. Monocytotropic viruses grow easily in macrophage cultures and infect primary T cells while T-trophic viruses do not grow well in macrophage cultures but tend to infect primary T cells in the peripheral blood.

2.9.2. Uncoating and Reverse Transcription

The viral core is housed in a conical shaped shell, made up of the viral capsid protein. Inside the core are the replication machineries of the virus: reverse transcriptase and integrase enzymes and HIV RNA. The uncoating of this shell triggers the next sequence of events in the replication cycle of the virus (Engelman and Cherepanov, 2012). The conversion of viral RNA into the proviral DNA is mediated by the reverse transcriptase, which takes place in the cytoplasm of the host cell. The success of the reverse transcription is dependent on whether the infected cells are activated or not. The reverse transcription involves multiple steps, culminating in the formation of double stranded proviral DNA with LTRs at each end (Hoffman and Rockstroh, 2012: 30).

2.9.3. Integration

Following the transcription, a pre-integration complex is formed, which is transported into the nucleus where further replication steps take place. Integration of the proviral HIV DNA into the host cell genome takes place in the nucleus. The integrase enzyme is crucial for the success of this step. Integrase has two catalytic activities which are both crucial for the replication process; 3' processing and DNA transfer. Integrase strand inhibitors specifically targets the DNA transfer for blockage. It should be noted that cellular activation is crucial for integration to take place. Cellular activation is triggered by antigen contact, opportunistic infections and vaccines (Hoffman and Rockstroh, 2012, pg. 31). Proviral HIV DNA tends to accumulate in latently infected cells in macrophages and other cells in the body, thus, forming cellular (long-lived) reservoirs for the virus. The provirus becomes replication-competent once there is cellular activation and thus, presents challenge for cure science.

2.9.4. Replication

Integration signals the end of the early phase and the beginning of the late phase of the replication cycle. Following stimulation of the infected cells, cellular transcription factor – NF-KB is translocated into the nucleus where it binds to the LTR region of the provirus, thus, signalling transcription of HIV genes. The provirus makes use of the host enzyme – RNA polymerase to create copies of the viral genomic materials. mRNA are also produced by the RNA polymerase. The first genes to be produced are the regulatory genes; tat and rev. Tat then binds to the TAR (transactivation response elements), causing further transcription and elongation of the viral mRNA. Rev binds to RRE (Rev response element) and stimulates the production of structural genes (gag and pol) and inhibits the production of regulatory proteins. Smaller proteins are transported to the cytoplasm while singly spliced or unspliced proteins require rev to be exported through the nucleus. The proteins coded for by gag and pol genes then form the nucleus of the new virion while the proteins coded for by env forms the spikes, gp120.

2.9.5. Assembly

The large precursor polypeptide chains of HIV proteins are cleaved by the protease enzyme. GP 160 is cleaved into gp120 and gp 41 while gag proteins are cleaved into p24, p17, p9 and p7 gag proteins. After the long chains have been cut into smaller proteins, a new virus like particles then assemble the HIV-1 RNA, gag proteins and pol enzymes near the cell membrane and they bud from the membrane.

2.9.6. Budding

During the budding process through the cell membrane, the viral lipid bilayer may take some of the host materials such as HLA 1 and 2 and other proteins. As the viral particles bud from the cell membrane of lymphocyte into the extracellular spaces, the process is different for monocytes and macrophages, in which the virions accumulates in cellular vacuoles. The replication process of the virus is error prone due to the lack of a proof reading mechanism inherent in the reverse transcriptase enzyme. This results in 1 – 10 errors per genome and for each replication cycle. There are 10^9 virions produced per day with several mutations. The consequence of the resultant mutations includes the emergence of replication-incompetent viruses, several quasi-species of viruses being produced and resistance-associated mutations possibly emerging.

The understanding of the life cycle of HIV has been channelled into the development of drugs, targeting specific enzymes and other critical steps. In total, the following classes of anti-retroviral drugs have been produced for treatment of individuals living with HIV. Fusion inhibitors, CCR5-inhibitors, nucleoside (nucleotide) and non-nucleoside reverse transcriptase inhibitors, protease inhibitors and integrase strand inhibitors.

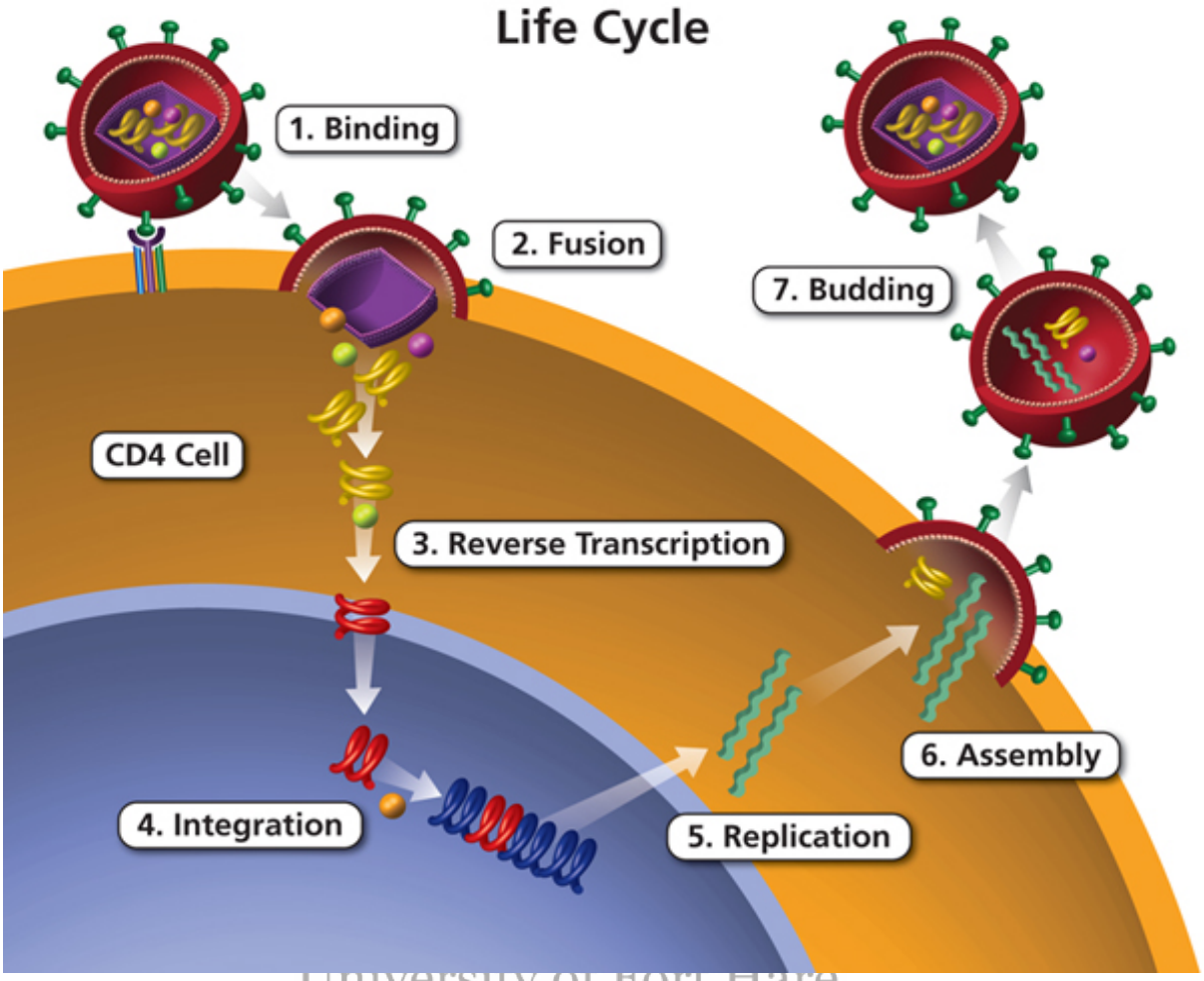

Fig.2.9. Life cycle of HIV (Adapted from https://aidsinfo.nih.gov/understanding-hiv-aids/glossary/1596/life-cycle)

2.10. Natural Course of HIV Infection

The role of mucosal transmission was elucidated succinctly by Wu (2008). Following exposure to HIV, the initial target cells are the dendritic cells (including Langerhans cells), CD4 T cells and macrophages which are abundantly available in the skin and mucosal epithelial surfaces (Hladik et al., 2007; Wu and KewalRamani, 2006). HIV gains entry through these intra-epithelial CD4 T cells in the form of a viral gp120-CD4 receptor fusion complex. Langerhans cells in the vagina engulfs the viral particle (in a process called endocytosis) (Hladik et al., 2007). Although, the epithelial cells of the vagina are believed to be involved in HIV transmission through transcytosis, this mechanism is rather inefficient in comparison to the other two mechanisms (Bobardt et al., 2007). Evidence shows that HIV replication commences within the dendritic cells

and multiplies rapidly in the presence of adaptor protein-3 (AP-3) (Garcia, Nicolic and Piguet, 2008).

The dendritic cells then present the HIV to the CD4 cells in a process is mediated by viral Nef (Wang et al., 2007). However, HIV-2 does not have the capacity to replicate within the dendritic cells and hence, is less pathogenic in comparison to HIV-1. HIV spreads from CD4 cells to uninfected CD4 cells using intracellular connections (nanotubes) (Sowinski et al., 2008). More CD4 cells are infected with HIV through cell-cell transmission, this process is far more efficient (>10,000) in comparison to infection by a plasma-free virus (Chen et al., 2007).

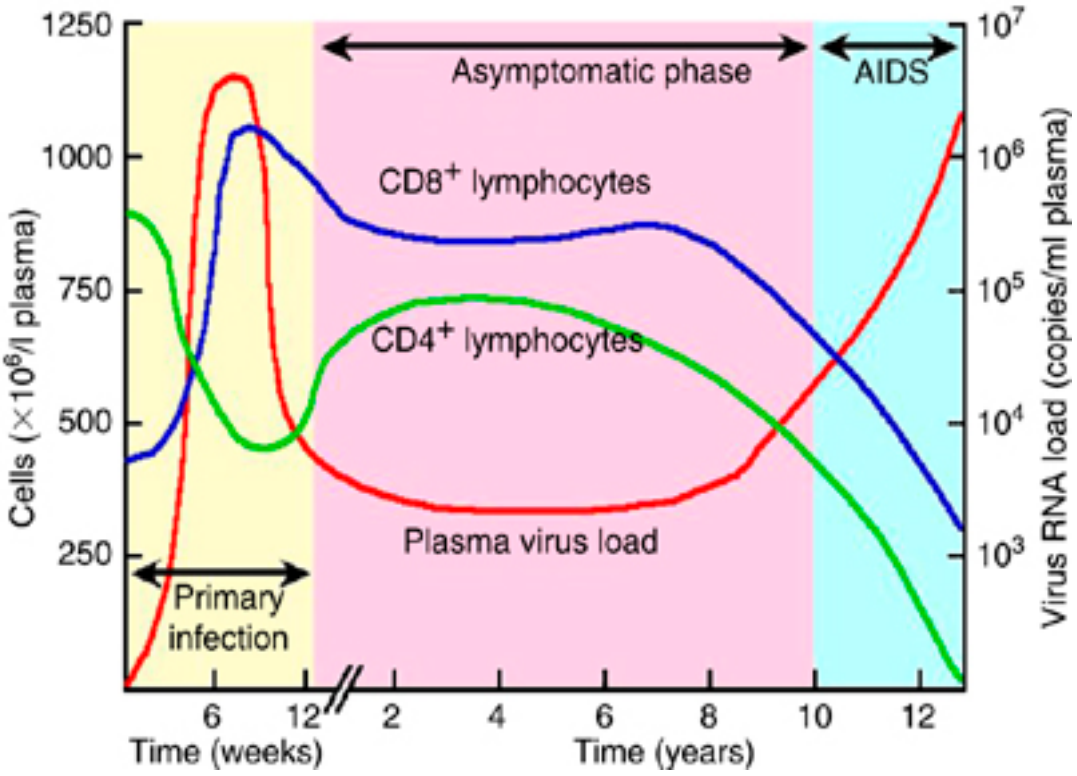

Fig. 2.10. Natural history and progression of HIV disease (Adapted from online source: https://www.google.com/search?q=natural+history+of+hiv+pics.....)

As shown in Fig. 2.10, acute retroviral syndrome develops in the HIV-infected individuals and lasts for less than four weeks after the infection. This is characterised by: generalised lymphadenopathy, fever, macular rash and fever. The symptoms are

non-specific and patients do not often present in the hospital. This brief period of acute HIV syndrome will be followed by a long period of clinical latency (asymptomatic). In the later stages, symptoms start to appear. The commonest symptoms include; herpes zoster, oral hairy leucoplakia and oral thrush (Hoffman and Rockstroh, 2012, pg. 7). The World Health Organization's clinical stages succinctly summarised the clinical conditions of the patients in the symptomatic stage of the HIV disease (Table 2.3) (WHO, 2007). These symptoms are an indication of cellular immune dysfunction. The median duration of HIV infection to the symptomatic phase is 8 – 10 years. Evidence showed that without any intervention at this stage, death will ensue. It should however, be noted that the natural progression of HIV infection varies from one person to another. This emphasises the critical role of the viral-host factors interaction with the environment.

Table 2.4. WHO Clinical staging of HIV disease in adults, adolescents and children

Adults and adolescent	**Children**
Clinical stage 1	
Asymptomatic Persistent generalized lymphadenopathy	Asymptomatic Persistent generalized lymphadenopathy
Clinical Stage 2	
Moderate unexplained weight loss (<10% of presumed or measured body weight) Recurrent respiratory tract infections (sinusitis, tonsillitis, otitis media, pharyngitis) Herpes zoster Angular cheilitis Recurrent oral ulceration Popular pruritic eruption Fundal nail infections Seborrhoiec dermatitis	Unexplained persistent hepatosplenomegaly Recurrent or chronic upper respiratory tract infections (otitis media, otorrhoea, Sinusitis, tonsillitis) Herpes zoster Linear gingival erythema Recurrent oral ulceration Papular pruritic eruption Fungal nail infections Extensive wart virus infection Extensive molluscum contagiosum Unexplained persistent parotid enlargement
Clinical stage 3	
Unexplained severe weight loss (>10% of presumed or measured body weight) Unexplained chronic diarrhoea for more than 1 month Unexplained persistent fever (intermittent or constant for more than 1month) Persistent oral candidiasis Oral hairy leucoplakia Pulmonary tuberculosis Severe bacterial infections (pneumonia, empyema, pyomyositis, bone or joint infection, meningitis, bacteraemia) Acute necrotizing ulcerative stomatitis, gingitivitis or periodontitis Unexplained anaemia (<8g/dl), neutropaenia (<0.5x10^9/L) and/or chronic thrombocytopaenia (<50x10^9/L)	Unexplained moderate malnutrition not adequately responding to standard therapy Unexplained persistent diarrhoea (14 days or more) Unexplained persistent fever (above 37.5°C, intermittent or constant, for longer than one 1 month) Persistent oral candidiasis (after first six weeks of life) Oral hairy leukoplakia Lymph node tuberculosis; pulmonary tuberculosis Severe recurrent bacterial pneumonia Acute necrotizing ulcerative gingivitis or periodontitis Unexplained anaemia (<8 g/dL), neutropaenia (<0.5 × 10^9/L) or chronic thrombocytopaenia (<50 × 10^9/L) Symptomatic lymphoid interstitial pneumonitis

	Chronic HIV-associated lung disease, including bronchiectasis
Clinical stage 4	
HIV wasting syndrome *Pneumocystis (jirovecii)* pneumonia Recurrent severe bacterial pneumonia Chronic herpes simplex infection (orolabial, genital or anorectal of more than one month in duration or visceral at any site) Oesophageal candidiasis (or candidiasis of trachea, bronchi or lungs) Extrapulmonary tuberculosis Kaposi sarcoma Cytomegalovirus infection (retinitis or infection of other organs) Central nervous system toxoplasmosis HIV encephalopathy Extrapulmonary cryptococcosis, including meningitis Disseminated nontuberculous mycobacterial infection Progressive multifocal leukoencephalopathy Chronic cryptosporidiosis Chronic isosporiasis Disseminated mycosis (extrapulmonary histoplasmosis, coccidioidomycosis) Lymphoma (cerebral or B-cell non-Hodgkin) Symptomatic HIV-associated nephropathy or cardiomyopathy Recurrent septicaemia (including nontyphoidal *Salmonella)* Invasive cervical carcinoma Atypical disseminated leishmaniasis	Unexplained severe wasting, stunting or severe malnutrition not responding to standard therapy *Pneumocystis (jirovecii)* pneumonia Recurrent severe bacterial infections (such as empyema, pyomyositis, bone or joint infection, meningitis, but excluding pneumonia) Chronic herpes simplex infection (orolabial or cutaneous of more than 1 month's duration or visceral at any site) Oesophageal candidiasis (or candidiasis of trachea, bronchi or lungs) Extrapulmonary tuberculosis Kaposi sarcoma Cytomegalovirus infection (retinitis or infection of other organs with onset at age older than one month) Central nervous system toxoplasmosis (after the neonatal period) HIV encephalopathy Extrapulmonary cryptococcosis, including meningitis Disseminated nontuberculous mycobacterial infection Progressive multifocal leukoencephalopathy Chronic cryptosporidiosis (with diarrhoea) Chronic isosporiasis Disseminated endemic mycosis (extrapulmonary histoplasmosis, coccidioidomycosis, penicilliosis) Cerebral or B-cell non-Hodgkin lymphoma HIV-associated nephropathy or cardiomyopathy

(Source: Adapted from**:** WHO case definitions of HIV for surveillance and revised clinical staging and immunological classification of HIV-related disease in adults and children. Geneva: World Health Organization; 2007. Available on www .who.int/hiv/pub/guidelines/HIVstaging150307.pdf.

The trend of viral replication measured as viral load is described in Fig. 2.10. Following HIV infection, the virus starts to multiply rapidly and unchecked by the host immune system for a period of time until it reaches the peak levels. Thereafter, the cytotoxic T cells are activated and other cellular immune systems, thus forcing a decline in the

viral load. Thereafter, the viral load is controlled by the host antibodies and other cellular immune system, and remains relatively stable. However, the virus continues to multiply and is cleared by the host immune system machinery, thus, causing ongoing inflammation in the patient. The viral set point (lowest point after the primary infection) is the most important determinant of HIV disease progression. Individuals with viral set point above 100,000 RNA copies/ml will progress to AIDS within two years while viral set point of less than 1000 RNA copies/mL may not develop AIDS in 12 years (Hoffman and Rockstroh, 2012: 7).

The dynamics of the viral set point are also important in understanding the decline in the immune system of HIV-infected individuals. Higher viral set points are synonymous with faster deterioration in the CD4 counts. The CD4 counts drops precipitously after the primary infection (Fig. 2.10), followed by a period of immune recovery which occurs after some months to the normal range values however, the pre-infection values are never attained in any individual. Subsequently, a gradual decline in the CD4 cells occurs until values drop below 200 cells/μl (normal values: >500 cells/μl), when AIDS-defining illnesses start to appear. A CD4 decline of ≥ 100 cells/μl per six months is associated with rapid progression to AIDS while a decline of < 20 cells/μl in six months would be associated with long-term non-progressors (LTNP) (Hoffman and Rockstroh, 2012: 7).

2.11. Determinants of Disease Progression (Host versus Viral Factors)

Early in the HIV epidemic, it was understood that there are variations in the time interval from infections to AIDS and overall survival varies from one individual to another (Sabin and Lungren, 2013). The viral load, which is a surrogate of the rate of replication of the virus is the most important determinant of disease progression. However, the predictive ability of viral load is enhanced when viral load is combined with the CD4 count of the individual. There are certain host-specific factors such as HLA-type and chemokine receptor polymorphism that affect the level of the viral set point in individual patients. However, the interplay between the host factors and the adaptive nature of the virus allows it to continue to perpetuate itself in the host cells. While some individuals progress to death in less than two years (fast progressors),

the majority live up 8 – 10 years (natural course) and a smaller proportion remain asymptomatic for a prolonged period of time (long-term non-progressors) (Fig. 2.11).

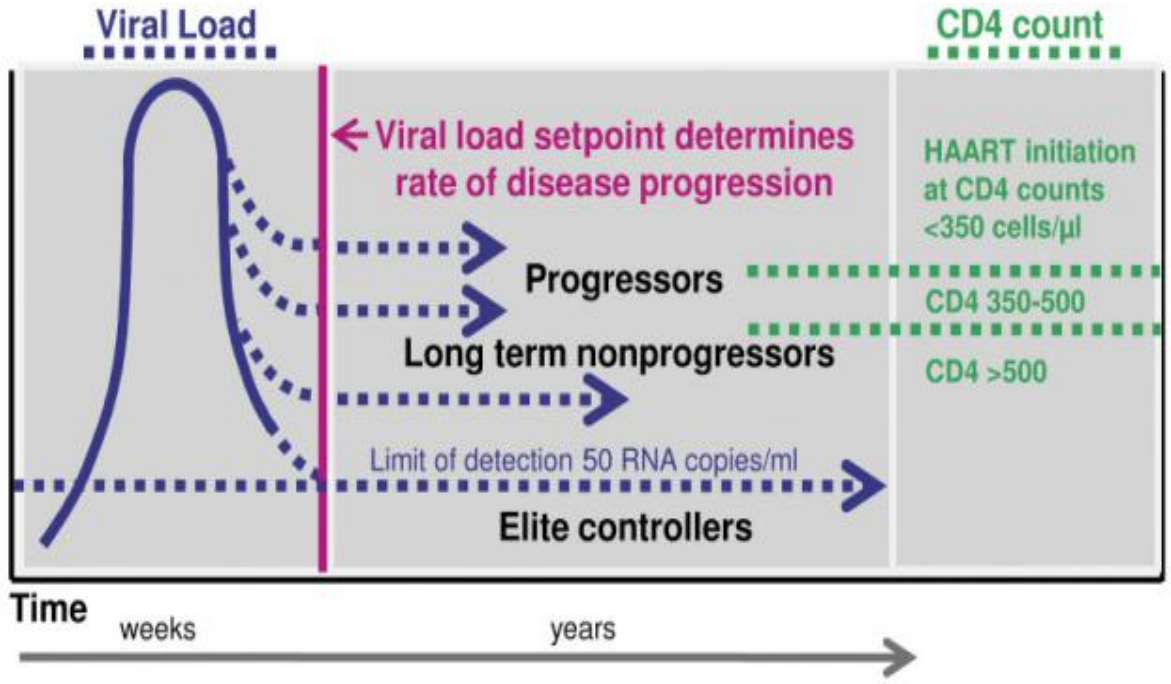

Fig. 2.11. Dynamics of disease progression (Adapted from online source: https://www.google.com/search?q=long+term+non-progressors+pictures.....)

While there are still gaps in the mechanism underlying the variations in the progression of HIV disease, it is understood that host, viral and environmental factors play significant roles in the outcomes of HIV infection. About 1 – 5% of individuals living with HIV are estimated to be LTNPs (Sabin and Lundgren, 2013). LTNPs tend to maintain high CD4 counts (> 500/μL) for several years. However, they eventually experience CD4 depletion and progress to AIDS. HIV controllers on the other hand rarely progress to AIDS (Hatano et al., 2009). They experience replication blips occasionally. Elite controllers are defined differently by different authorities. In USA, people who are able to maintain their viral load below the limits of detection assay (<50 RNA copies/ml) for at least a year without ART are termed elite controllers (Deeks and Walker, 2007), while in France, any individual who is able to maintain viral load ≤ 400 RNA copies/ml for at least five years is an elite controller (Lambotte et al., 2005).

Viraemic controllers have detectable viraemia but maintain viral load below 2000 RNA copies/ml (Okulicz et al., 2009). Immunological regeneration occurs in elite controllers and are able to maintain their CD4 count at the normal levels (Poropatich and Sullivan, 2011). Elite controllers are believed to spontaneously control the replication of the virus and account for less than 1% of individuals living with HIV. Evidence suggests that the CD4 cells of elite controllers are less susceptible to HIV in comparison to viraemic controllers and fast progressors (Pereyra et al., 2009). The severity of the primary HIV infection has been linked to the ability of the host to control the virus. Individuals with severe disease during seroconversion have been reported to progress rapidly in comparison to those with mild to moderate disease (Goujard et al., 2009; Miura et al., 2010).

The capacity of the adaptive immune system to preserve elite controllers has been linked to the major histocompatibility complex (MHC) class 1 allele, which are presented to CD8 T cells. Several HLAs have been identified and linked to blockage of replication: HLA-B*5701, HLA-B*5703, HLA-B*2705 and HLA-B*5801 (Migueles et al., 2000; Fang et al., 2004). These alleles are less likely to be found in fast progressors. Other host factors decrease the susceptibility of the target cells to HIV entry. The presence of 32 base pair deletion in the gene coding for CCR5 has been linked to protection against HIV entry, especially when they are present in the homozygote state. Also, HIV disease progression is delayed in individuals who are heterozygous for the 32 base pair deletion in their genes (Dean et al., 1996). Also, fast controllers are more likely to be co-infected with GB virus than controllers. Another major difference is the greater amount of cell-associated viral load between fast progressors and controllers. Also, the markers of mucosal immune activation are higher among LTNPs. Other host factors that play some roles in disease progression include; APOBEC and tetherin (Wang et al., 2012).

Certain viral factors also play some roles in the disease progression. The viral set point of the seroconverters has been linked to the viral load of the infecting partner (Hecht et al., 2010; Alizon et al., 2010). This suggests that HIV maintains its intrinsic replicative capacity after transmission from one person to another. The viral set point

is also affected by the presence of resistance mutations in the transmitted founder virus. Certain mutations affect the fitness of the virus (M184V) (Harrison et al., 2010). Also, the presence of numerous env diversities in the HIV has been linked to rapid progression of HIV disease (Rachinger et al., 2012). The sex of the seroconverters is equally important (Grinsztejn et al., 2011; Lingappa et al., 2013). Host factors such as HLA class 1 could be shared between partners. The pre-ART viral load in women has been found to be lower than that of men (Grinsztejn et al., 2011; Lingappa et al., 2013). Some environmental factors play crucial roles in the disease progression. Opportunistic infections as shown in Table 3 can influence the progression of HIV disease (Powderly, 1999). Opportunistic infections can activate the host cells, leading to further replication and decline in the host CD4 cells. In contrast, initiation of ART, can block the replication of the virus and thereby preserve the immune system of the individuals.

2.12. Advances in laboratory diagnosis of HIV infection

HIV diagnosis is the gateway to the care cascade. The UNAIDS global target aims to diagnose 90% of all individuals living with HIV (UNAIDS, 2014). It is very important to understand the viral dynamics in relation to the diagnostic modalities (Fig. 2.12). Immediately after primary infection up to the first ten days, HIV is undetectable in any form, that is, via RNA, p24 antigen or antibodies. This is called the 'Eclipse period'. Shortly after, the HIV RNA becomes detectable first at about 10 days after the primary infection. This occurs seven days earlier than the p24 antigen becomes detectable in the serum (Daskalakis, 2011). Thereafter, the p24 antigen appears and can be used for diagnosis five days earlier than the antibodies can be detected in the serum. This is the rationale for incorporating p24 antigen in the fourth generation enzyme-linked immunosorbent assays. The antibodies start to appear later at the third week; however, only about 60 – 65% of HIV-infected individuals can be diagnosed with screening for antibodies at four weeks, 80% can be diagnosed at six weeks, 90% can be diagnosed at eight weeks and 95% at 12 weeks (Cornet and Kirn, 2013).

The diagnostic tools for HIV have evolved since the first enzyme-linked immunosorbent assay (EIA) was licensed in 1985.

2.12.1. Enzyme-Linked Immunosorbent Assay (EIA)

This assay was based on the principle of antigen-antibody reaction. The first generation EIA targeted the immunoglobulin G (IgG) antibody to HIV-1 while the antigen incorporated was the viral lysate. The window period was detection averages 6 – 8 weeks (Owen, 2012). The EIA has a sensitivity of 99% and specificity of 95 – 98%. However, it does not detect any HIV-specific IgM and antigens. The second generation EIA was developed for use in 1987 in a quest to further reduce the prolonged window period of the first generation EIA.

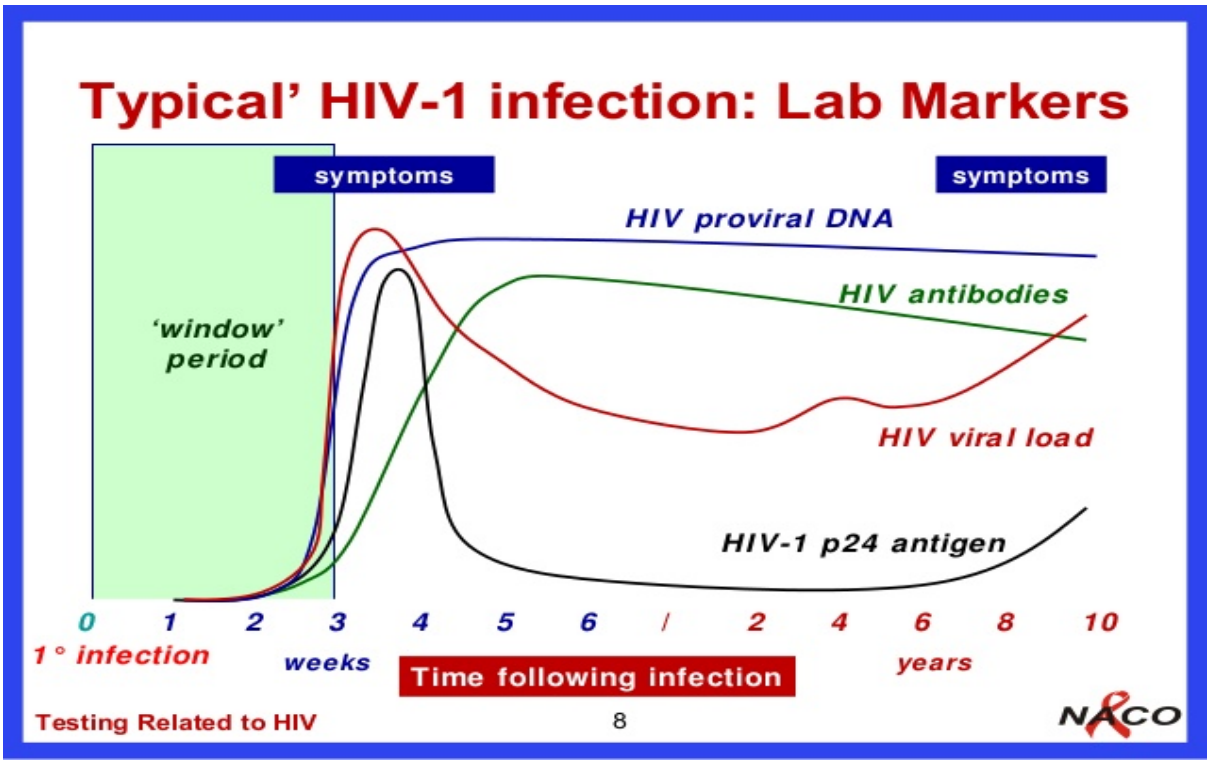

Fig. 2.12. HIV diagnostic modalities (Adapted from: https://www.google.com/search?q=natural+history+of+hiv+pics&rlz..../

The second generation EIA incorporated recombinant viral protein to the lysate antigen of the first generation EIA. This reduced the window period to about 4 – 6 weeks. The sensitivity and specificity also improved slightly; >99.5% and > 99%, respectively. The second generation EIA detects IgG antibodies to HIV-1 and HIV-2 in a single result (Owen, 2012). It however, does not detect HIV antigens. The third generation EIA was developed for diagnostics in 1991. It incorporates synthetic

peptides to the second generation EIA. It detects both IgM and IgG antibodies of HIV-1, HIV-2 and group O. The sensitivity and specificity improved slightly to > 99.5% and > 99.5%, respectively. It successfully reduced the window period to 2 – 3 weeks (Alexander, 2016). It however, does not detect any HIV antigens.

The fourth generation EIA was developed in 1997. It incorporated p24 antigen in addition to IgG and IgM antibodies to both HIV-1, HIV-2 and group O (Pandori et al., 2009). The window period is reduced to 2 weeks and is 5 – 7 days earlier than the third generation EIA (Bentsen et al., 2011; Pandori et al., 2009). The sensitivity and specificity also improved; > 99.8% and 99.5%, respectively. However, it does not differentiate the results of HIV-1 from HIV-2. In addition, fourth generation EIA may miss early HIV infection and is unsuitable for primary HIV infections. The fifth generation EIA was developed in 2015 and incorporates synthetic peptide to the recombinant molecule of the virus. The window period and specificity are similar to the fourth generation EIA, however, the sensitivity is 100% (Alexander, 2016). The main advantage of the fifth over the fourth generation EIA is that the fifth is able to separate the antigen-antibody results unlike the former. The Centre for Disease Control and Prevention has not released the algorithm for the use of the fifth generation EIA for HIV diagnosis.

2.12.2. Rapid tests

Point-of-care has revolutionised the diagnosis of HIV. It uses the plasma, serum, whole or capillary blood for the test. It works on the principle of immune-chromatographic methods, using lateral flow devices, immune-concentration, or agglutination techniques. The fourth generation EIA have been developed into rapid test for HIV since 2009. The sensitivity and specificity depends on the generation of the EIA used. The main advantage is that it is rapid; it can be performed within 20 – 30 minutes at the point-of-care.

2.12.3. Oral Tests

OralQuick has been approved by Food and Drug Administration of USA for self-testing of HIV. It uses the oral transudates for the test. It is suitable for self-testing at home which can be performed within 20 minutes. It has a sensitivity of 93% and specificity of 99%. However, there are still gaps on how to link patients who test positive to care (Alexander, 2016).

2.12.4. Nucleic Acid Amplification Tests (NAAT)

This is a PCR-based technology that specifically detects either the RNA or DNA of the virus. As explained earlier, the viral RNA is detectable in about 10 days after primary infection. As such, this modality of test is more suitable for acute HIV infection prior to the production of antibodies. However, it may miss about 5% of acute infections (Patel et al., 2010). The qualitative PCR test is also used for early infant diagnosis, within the first 18 months. However, the quantitative PCR (viral load) is unsuitable for HIV diagnosis. It should be noted that maternal antibodies are transferred through the placenta and lasts for a prolonged period in the child (Cormet and Kirn, 2013). Hence, HIV EIA is unsuitable for early infant diagnosis except for screening for maternal serostatus. The limitation of NAAT is that it can only diagnose HIV-1. False negatives can occur in children on ART prophylaxis. Also, NAAT is yet to be developed into a point-of-care technology. It is also expensive and requires expertise.

2.12.5. Supplemental HIV tests: Western Blot and Indirect Immunofluorescent Assay

Western Blot was used in the early years of the HIV epidemic as a confirmatory test. Viral proteins (lysate) are separated with electrophoresis according to their molecular weight on a membrane which is then incorporated onto a test strip. Test specimen from the patient can be incubated with the test strip. Antigen-antibody complexes are formed which are separated along the molecular weight of the antigens. The following viral proteins are used:

Env – gp41, **gp 120**, gp 160

Pol – p31/34, p39/40, p51/52, p66/68

Gag – p17/18, **p24**/25, p55

The first two to appear are; P24 and P120. A minimum of two to three bands are needed for a positive result (Rochstroh, 2012, pg. 15). The sensitivity and specificity are not superior to the third generation EIA.

Indirect immunofluorescence assay is of limited application in HIV diagnosis because it is very cumbersome and requires expensive equipment (microscope).

2.12.6. South African HIV Testing Policy

HIV tests are performed under a specific legal framework, which differs from one country to another. Since the year 2000, the South African Department of Health has implemented the voluntary counselling and testing policy which was expanded in 2010. The HIV counselling and testing policy consisting of the client-initiated and provider-initiated counselling and testing was implemented to meet the global targets. The policy adopted the voluntary testing approach, in which the patient is empowered through counselling to make an informed decision on whether to test or not. This is different from the opt-out testing policy recommended by CDC (Branson et al., 2006). Opt-out testing policy recommends that every individual seeking healthcare should have a HIV test done except if the patient expressly declines the test. However, the patient will be informed about HIV among other tests to be carried out. Individuals who perceived themselves to be at low risk of contracting HIV may be psychologically unprepared to receive the diagnosis. As such, such individuals may suffer psychological or social harm after the diagnosis (Galletly, Pinkerton and Petroll, 2008).

In South Africa, pre-test counselling is offered to the individual prior to testing followed by post-test counselling. HIV testing remains voluntary, however, the provider reserves the right to continue to offer counselling for the test at subsequent hospital visits.

Rapid test is approved for HIV screening in South Africa and another rapid test from another manufacturer is used as a confirmatory test. On rare occasions of discordant results, a third sample will be taken and sent to the laboratory for EIA to break the tie (Fig. 2.13).

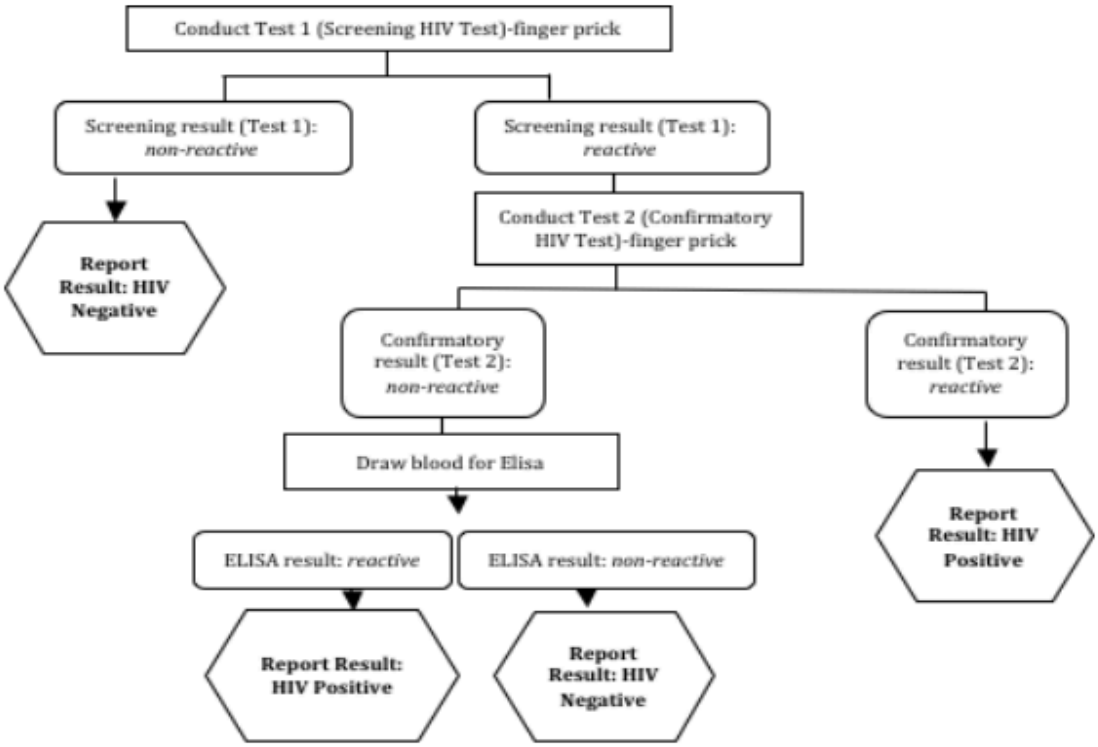

Fig. 2.13. Algorithm for HIV testing in South Africa (Adapted from: https://www.health-e.org.za/wp-content/uploads/2015/07/HCT-Guidelines-2015.pdf)

2.13. Management of HIV Disease

The discovery of the life cycle of HIV (Fig. 2.9) has opened doors for researches into drugs targeting specific aspects of the replicative process. Since the first drug - Zidovudine was licensed in 1987, several ART drugs have been approved by the US Food and Drug Administration (Table 2.5). All the currently available drugs for treatment of HIV can be categorised based on their mechanism of action into six classes (Table 2.5). The first drug showed promise of slowing the replicative process, delaying HIV disease progression to AIDS and prolonging life of people living with HIV. However, the promise was short-lived due to the evolution of resistance and failure. Hence, the use of triple drugs (2 NRTIs and one other drug from other classes) was found to be effective for treating HIV in 1996. Hence, the name; highly active antiretroviral therapy was coined. The use of ART has proven to be beneficial by blocking viral replication, leading to durable virological suppression (below limit of assay detection); immunological recovery, reducing morbidity and mortality,

enhancing survival and quality of life, and finally, preventing transmission of HIV from one person to another (SADoH, 2015).

Despite the promises of prolongation of life and survival, there is compelling evidence showing the increased risks of chronic inflammation, immunosenescence and non-AIDS associated illnesses (cancers and cardiovascular diseases) in individuals on ART (Deeks et al., 2012; Kiem et al., 2012). In order to get the full benefits from ART, there is a great need for good adherence (daily ingestion of drugs), which could potentially expose people to long years of experiencing adverse effects of these drugs (Table 2.4). In addition, there is a risk of HIV drug resistance in a non-complying patient, with rapid progression to AIDS defining illnesses. Above all, ART has been unable to eradicate the virus from cell and tissue reservoirs (Siliciano et al., 2003). The various classes of ART, their mechanisms of action and major side effects are discussed.

2.13.1. Nucleoside reverse transcriptase inhibitors (NRTIs)/Nucleotide reverse transcriptase inhibitors (NtRTIs)

The drugs in this class inhibit the action of the reverse transcriptase enzyme during replication (Fig. 2.9, step 3). They first undergo phosphorylation inside the host cells into the tri- or diphosphate metabolite. These drugs are either incorporated into the nucleotide analogue, leading to chain termination or compete with host nucleotide as viral substrate, thus blocking the conversion of RNA into double stranded DNA. These drugs are associated with severe mitochondrial toxicity. Tenofovir, abacavir, lamivudine and emtricitabine have less propensity to inhibit the mitochondrial polymerase gamma. Hence, they are better tolerated. Zalcitabine has been withdrawn from the market due to severe toxicities.

2.13.2. Non-nucleoside reverse transcriptase inhibitors (NNRTIs)

These drugs bind to the non-catalytic site on the reverse transcriptase enzyme, leading to conformational changes and thereby impair the action of the enzyme. These drugs require cytochrome P450 for metabolism and thus, are prone to drug-drug

interactions. The first generation NNRTIs – Efavirenz and Nevirapine - have low genetic barriers and are prone to developing drug resistance. Table 2.4 shows the common adverse effects associated with these drugs.

2.13.3. Protease inhibitors

The drugs in this class exhibit their action late in the replication cycle. The drugs bind to the protease enzyme and block the cleaving of long chain polypeptides of the virion. This prevents maturation of the virions. The drugs have high genetic barrier to resistance – they requires multiple mutations to accumulate before the drug can fail. Metabolic abnormalities such as dyslipidaemia, insulin resistance, lipodystrophy and hyperglycaemia are very common with these drugs. Ritonavir is used to boost the other drugs in the class due to its potent cytochrome C3A4 inhibitory activity. It is combined with two NRTIs and is used as a second line regimen in South Africa (SADoH, 2015).

Table 2.5. Available Antiretroviral drugs

Drugs	Abbrev.	Date of Approval	Major adverse effects
NRTIs (NtRTIs)			
Tenofovir D	TDF	26/10/2001	Proximal tubulopathy
Tenofovir A	TAF	1707/2018	Osteopaenia/pancreatitis
Abacavir	ABC	17/12/1998	Hypersensitivity reactions
Zidovudine	AZT	19/03/1987	Bone marrow suppression
Didanosine	DDI	31/10/2000	Pancreatitis/peripheral neuropathy
Stavudine	D4T	24/06/1994	Lactic acidosis/lipoatrophy
Lamivudine	3TC	17/11/1995	*Pure red aplasia
Emtricitabine	FTC	02/07/2003	Palmar hyperpigmentation
NNRTIs			
Nevirapine	NVP	21/06/1996	Hepatotoxicity/rashes
Efavirenz	EFV	17/09/1998	CNS changes/rashes
Delavirdine	DLV	4/04/1997	
Rilpivirine	RPV	20/05/2011	CNS side effects/rashes
Etravirine	ETV	18/01/1998	Rashes
Protease inhibitors			
Lopinavir	LOP	15/09/2000	Gastrointestinal/metabolic
Ritonavir	RTV	1/03/1996	Gastrointestinal/metabolic
Darunavir	DRV	23/06/2006	Rashes/caution in sulpha allergy
Atazanavir	ATZ	20/06/2003	Jaundice
Saquinavir	SQV	6/12/1995	Nausea/PR and QT prolongation

Fosamprenavir	FPV	20/10/2003	Rashes
Tipranavir	TPV	22/06/2005	Hepatitis/gastrointestinal
Indinavir	IDV	13/03/1996	Gastrointestinal/nephrolithiasis
Nelfinavir	NFV	14/03/1997	Gastrointestinal/hyperglycaemia
Fusion inhibitors			
Enfuvirtide	T-20	13/03/2003	Injection site reactions
CCR5 co-receptor antagonist			
Maraviroc	MVC	06/08/2007	Hepatotoxicity
Integrase strand transfer inhibitors			
Raltegravir	RAL	12/10/2007	Rhabdomyolysis/myopathy
Dolutegravir	DTG	13/08/2013	Headache/insomnia
Elvitegravir	EVG	24/09/2014	BMD loss/ renal tubular damage

BMD=bone mineral density; TDF=Tenofovir disoproxil fumarate; TAF=Tenofovir alafenamide;

(Adapted from: https://www.fda.gov/forpatients/illness/hivaids/treatment.....)

2.13.4. Integrase strand transfer inhibitors

The drugs in this class act in the replication cycle by blocking the integrase enzyme (Fig. 2.9, step 4). It prevents the bonding of the viral DNA to the host DNA, thus, impairing the process of integration. These drugs have been shown to rapidly bring down the viral load. Dolutegravir is a very potent drug with activity against HIV-1 strains resistant to both elvitegravir and raltegravir. Elvitegravir is given with cobicistat, which is an enzyme inhibitor without antiviral activity.

2.13.5. CCR5 Antagonist

To date, maraviroc is the only drug licensed in this class. Maraviroc prevents the entry of CCR5-tropic virus into the cell by selectively binding to the receptor. Doing this, prevents the interaction of the spike, gp120 with CCR5 receptor. It should, however, be noted that viruses using CXCR4 may gain entry into the cell or viruses using both receptors would still gain entry in the presence of the drug. Hence, a tropism test should be performed prior to initiating this drug in any patient.

2.13.6. Fusion inhibitor

Enfuvirtide blocks the fusion of the viral gp 41 and the host cell membrane (Fig. 2.9, step 2). This is the only injectable ART so far and is given subcutaneously. Injection site reactions such as pain, induration and nodules have been reported in patients using the drug as part of salvage therapy.

2.13.7. Fixed dose combinations (FDCs)

Combination of single tablets from different classes have ushered in new strategies of fixed dose combinations. This strategy has led to significant pill reductions for people living with HIV and thus, enhanced drug adherence. The following FDCs have been licensed for treatment of HIV by the US Food and Drug Administration.

TDF (300mg) + FTC (200mg) + EFV (600mg) = Atroiza/Atripla

AZT (300mg)+ 3TC (200mg) = Combivir/Lamzid

ABC (600mg) + 3TC (300mg) = Kivexa

TDF (300mg) + FTC (200mg) = Truvada

AZT (300mg) + 3TC (150mg) + ABC (300mg) = Trizivir

TDF (300mg) + FTC (200mg) + ELV/c (= Stribild

Tenofovir alafenamide TAF (10mg) + FTC (200mg) + ELV/c (150/150mg) = Genvoya

TDF (300mg) + FTC (200mg) + RPV (25mg) = Complera

2.13.8. South African HIV Management Guideline

Based on the advances in HIV therapeutics over the past two decades, the South African government always responds to new evidence by providing the best care possible for her citizens. This reflects in the introduction of Tenofovir (replacing the

stavudine, a toxic thymidine analog) and many fixed dose combination therapies in South Africa. Table 2.5 succinctly describes the adult ART guideline in the country.

Table 2.5. ART Regimen for Adolescents ≥ 15 years and adults

FIRST LINE REGIMEN		
Population	**Drug regimen**	**Comments**
• Adolescents >15years and weight >40 kg • All HIV-infected adults	TDF + FTC (3TC) + EFV (Fixed dose combination)	NVP should replace EFV in: • Neuropsychiatric adverse effects impairing functioning • Severe psychiatric co-morbidity
• All patients on D4T	All patients on D4T should be switched to TDF	Ensure that patient is virally suppressed and has normal creatinine VL > 1000 copies/ml; manage as virological failure
Adolescents < 15 years or weight < 40kg	ABC + 3TC + EFV	Follows paediatric treatment
Contraindications	Substitution Drug	Comments
EFV: ➢ Psychiatric co-morbidity ➢ EFV intolerance ➢ CNS adverse effects impairing daily functions	TDF + FTC (or 3TC) + NVP or Lop/r	CD4 < 250 in females and <400 in males, NVP can be safely given; 200mg daily for 2 weeks, then 200mg BD If CD4 ≥ 250 in females and ≥ 400 in males, use Lop/r 2 tablets 12 hrly
TDF: ➢ GFR < 50ml/min	ABC + 3TC + EFV (or NVP)	Avoid nephrotoxic drugs or in baseline renal disease MDR-TB treatment

SECOND LINE REGIMEN		
First-line virological failure	**Drugs**	**Comments**
➢ Failing TDF-based regimen	AZT + 3TC + Lop/r AZT + TDF + 3TC + Lop/r (Co-infection with Hep B)	VL>1000copies/ml, intensify adherence to ART and repeat VL in 2 months (1 month in pregnancy) VL remains > 1000 copies/ml after 2 months, switch to second line regimen
➢ Failing AZT or D4T-based regimen	TDF + 3TC (or FTC) + Lop/r	
➢ Lipid abnormality (TC>6 mmol/L) or diarrhoea associated with Lop/r	Switch Lop/r to ATZ/r	
➢ Anaemia and renal Failure	Switch to ABC	

ABC=Abacavir; ATZ= Atazanavir; EFV=Efavirenz; D4T= Stavudine; 3TC=Lamivudine; MDR-TB=Multidrug resistance Tuberculosis; r=ritonavir; TDF=Tenofovir; GFR=Glomerular filtration rate

2.14. Pol Gene Analysis

Advancement in sequencing analysis has improved the knowledge of HIV diversities. The env gene has great variability and is preferentially analysed to gain broader understanding of HIV diversity in many region of the world (Pasquier et al., 2001; Lessells, Katzenstein and de Oliveira, 2012). In recent times, other genes which have wide variability have been used for analysis of the genetic diversity of HIV. The study by Pasquier et al. (2001) found a higher degree of agreement between the env gene and the RT gene for HIV subtyping. However, some level of discordance between the

RT and env genes were found, which shows that at least a minimum of two or more gene regions should be evaluated for subtyping. The study by Pasquier et al. (2001) though characterised the env, RT and PR gene, but the env gene did not reveal all the CRFs shown in the RT genes. This is largely due to the fact that env gene analysis was limited to the C2/V3 gene region. As such, inter-subtype recombination in the viral genes was underestimated. In addition, the study showed that the RT gene is good for identifying recombinant genomes even better than the Env gene. Also, a Canadian study found a recombinant A/B; with the RT and PR gene showing subtype A while the integrase (IN) gene showed subtype B (Brenner et al., 2011). A complete genomic (full-length) sequence analysis should be performed to gain a better understanding of all the types, subtypes, inter-subtypes and CRFs of HIV in any region.

The pol gene (Fig. 2.14) which encodes the enzymes of replication of the virus (reverse transcriptase (RT), protease (PR) and integrase (IN)) have been shown to be reliable in the sub-typing analysis of HIV (Pasquier et al., 2001; Tebit and Arts., 2011). Also, this gene has gained prominence in its application for monitoring drug resistance in the genome of the replication enzymes; RT, PR and IN. The RT gene of HIV is fairly conserved and serves as a target point for the cytotoxic T cells. Most studies tend to perform partial genomic sequencing of different genes of the virus. Depending on the purpose for the analysis, partial sequence of the pol gene may be sufficient for resistance monitoring because all the enzymes which are prone to mutagenesis are encoded in it. However, partial sequencing of any of particular gene would be insufficient to fully understand the HIV diversity of any region.

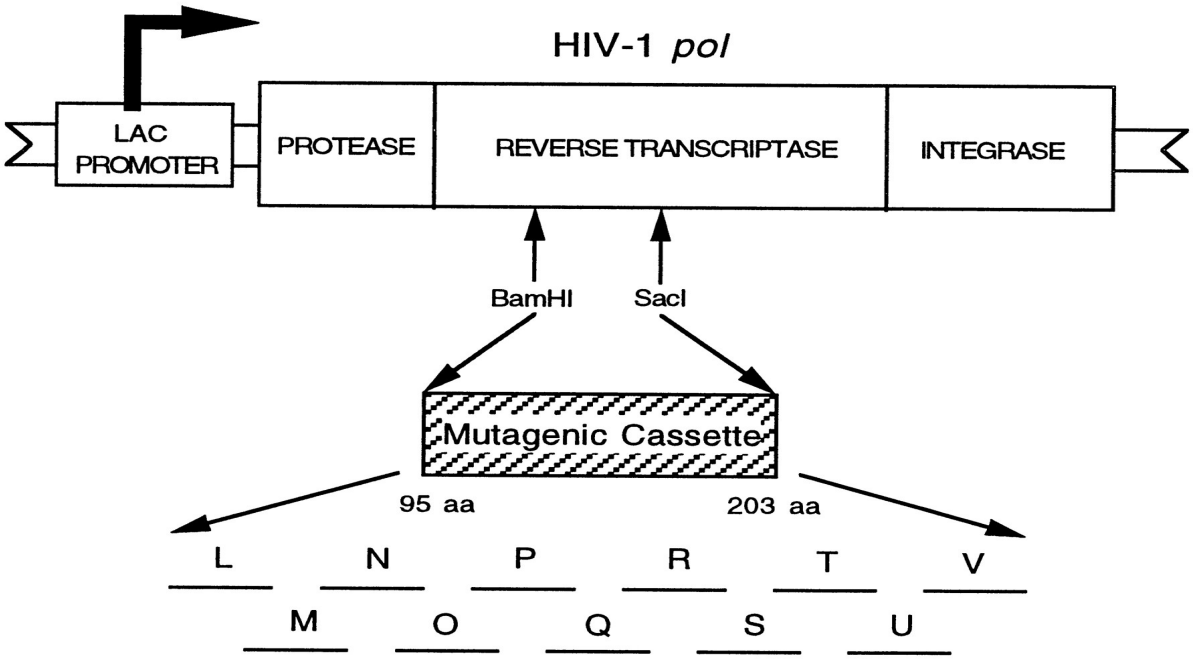

Fig. 2.14. Schematic representation of the Pol Gene: Protease, reverse transcriptase and integrase region of the gene shown (Adapted from: https://www.google.com/search?q=POL+GENE+OF+HIV+IN+PICS&rlz.....)

2.15. HIV Drug Resistance

Evidence from randomised controlled trials support the treatment for all individuals living with HIV (Insight START Study Group, 2015; TEMPRANO Study Group, 2015). Based on the evidence, the majority of national authorities recommended ART for every individual living with HIV all over the world (SADoH, 2016; DHHS, 2016; EACS, 2016; de Ruiters et al, 2014; WHO, 2015). Evidence for the regional access to ART is provided in Fig. 2.15. So far 21.7 million people have been initiated on ART globally. About 56% of people living with HIV (7.1 million) in South Africa are currently accessing ART, thus, SA being identified as having the largest ART programme in the world (UNAIDS, 2017).

With accelerated expansion of ART to all individuals infected with HIV, there is a public health concern on the inevitable emergence of drug resistance. The complex interactions of patient-, disease-, drug-, and health system-related factors impact unfavourably on the adherence to ART (Adeniyi et al., 2018; Loeliger et al., 2016; Mills

et al., 2006; Shubber et al., 2016). The naturally occurring mutations emanating from each cycle of replication of the virus and the overarching effect of selective pressure exacted by the ART are central to the emergence of resistant mutations seen in clinical practice.

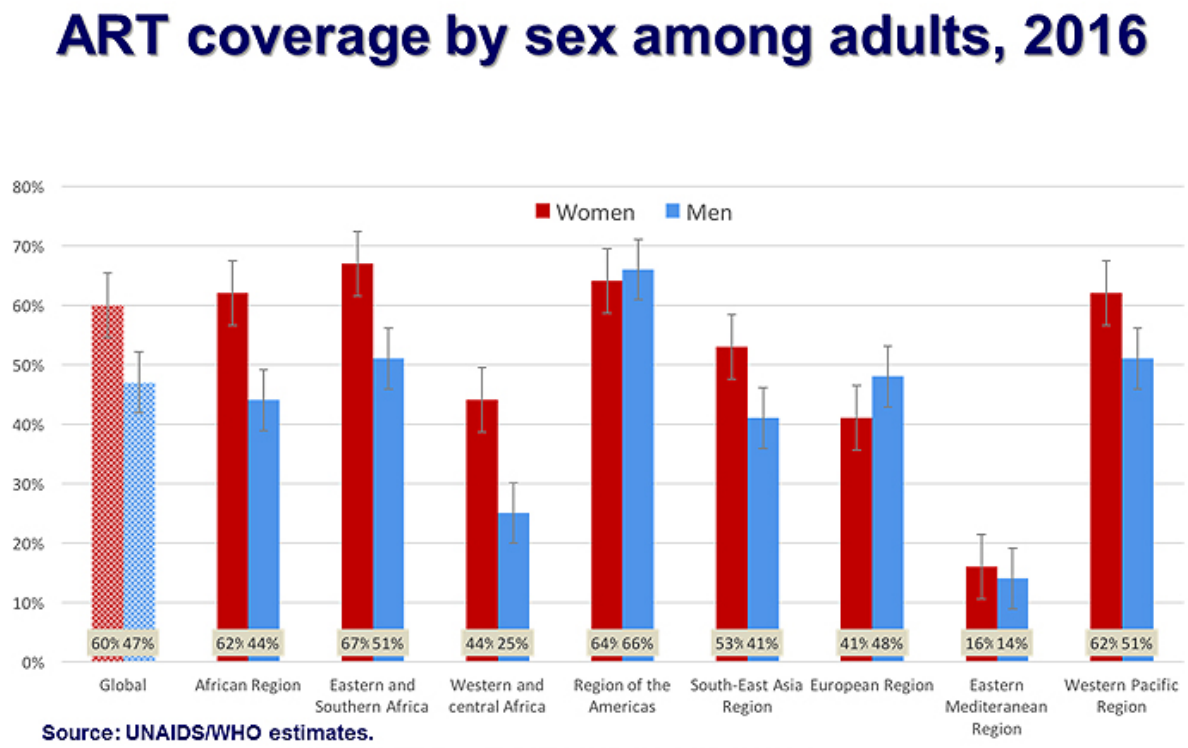

Table 2.15. Graph showing the regional ART coverage (Source: UNAIDS/WHO estimates)

During the replication cycle, there is a high turn-over of about 10 million virions per day. This is coupled with the inherent profile of the reverse transcriptase enzyme which is error prone due to the lack of proof reading mechanism. By implication, there is a high mutation rate with production of mutant viruses every day, even without ART exposure. However, the risk of developing resistant mutations is increased in the presence of ART. Antiretroviral drugs selectively exact pressure on the virus, thus, leading to the emergence of resistance-associated mutations. In the presence of one or more mutations conferring resistance to specific drugs, then, the viral mutants tend to gain replication advantage over the wild type virus in the context of ART. HIV drug resistance is the main reason for virological failure in patients on treatment (Hoffman

and Rockstroh, 2012, pg. 303). There are slight variations in the mechanism of development of resistance mutations to different classes of ARTs and individual drugs.

2.15.1. Reverse Transcriptase Inhibitors' Resistance

NRTIs undergo intracellular phosphorylation to become phosphorylated metabolites which are then incorporated into the proviral DNA building blocks of the virus. This leads to cease command on the prolongation step. There are two mechanisms underying NRTIs' drug resistance.

Sterical inhibition: Mutations in the viral genome lead to failure of reverse transcriptase enzyme to recognise the differences between NRTIs and dNTPs. In the presence of these mutations; M184V, Q151M, L74V, and K65R, the virus preferentially select the dNTPs instead of NRTIs.

Phosphorolysis: It is a process whereby the NRTIs which have been incorporated into the growing chain of the DNA are excised from the genome. There are several mutations that have this effect on NRTIs; M41L, D67N K70R, L210W, T215Y, and K219Q. K65R is associated with decreased excision of the NRTIs from the genome where it has been incorporated, thus, leading to stability of the mutations. While K65R could potentially lead to decrease in NRTIs susceptibility, it is associated with an increase in susceptibility of AZT (Hoffman and Rockstroh, 2012, pg. 309).

NNRTIs generally bind to the hydrophobic pocket of the reverse transcriptase enzyme, thereby inhibiting the enzymatic action of RT. However, mutations at the hydrophobic pockets of the RT will impair the action of the NNTIs. The first generation NNRTIs (Nevirapine and Efavirenz) require only one mutation to fail while the second generation NNRTIs require accumulation of mutations to fail. K103N mutation is the most prominent for NNRTIs. This is associated with 20- to 50- fold increased risks of resistance to EFV and NVP. Y181C/I is associated with 30-fold increase in NVP resistance. Others are G190A, which causes high level resistance to NVP and intermediate resistance to EFV; G190S and Y188C/L/H both result in high level resistance to NVP and EFV.

V106A is associated with 30- fold increase risk of resistance to NVP. V106M is more readily seen with the subtype C virus and is associated with NVP and EFV resistance. It is therefore, very important that resistance monitoring be performed in patients with

failing regimen. Second generation NNRTIs (Etravirine) have high genetic barriers and are effective against K103N, Y188L and/or G190A.

2.15.2. Protease inhibitors resistance

PR inhibitors: Protease plays crucial roles in the maturation of the non-infectious virions by cleaving the long polypeptides chains (precursor gag-pol-polyprotein) to functional units. Protease inhibitors require multiple mutations before they can fail. Hence, they are regarded as having high genetic barriers. There are major and minor mutations associated with protease inhibitors specific resistance. Major (primary) mutations are located within the active catalytic site and reduce the enzymatic activities of the drugs. However, the minor (secondary) mutations are located outside the catalytic site of the enzyme. While major mutations are selected early, the minor mutations are selected late after major mutations. For example: D30N, V32I, M46IL, I47VA, G48VMALSTQ, I54VAMLTS, L76V, V82ATFSMLC, I84VAC, N88S and L90M are major PR mutations . Major mutations impair the viral fitness while minor mutations tend to compensate for the fitness. Some degree of cross-resistance has been recorded with PR inhibitors. Ritonavir boosted PRs rarely develop resistance mutations. Lopinavir/r is associated with V82A followed by V32I, M46M/I and I47A. Atazanavir is associated with I50L in combination with A71V, K45R, and/or G73S. Darunavir is a second generation PR inhibitor with activities against PR resistant viruses. Darunavir resistance rarely occurs due to the high genetic barriers of the drug. The following mutations confer resistance to boosted Darunavir: V11I, V32I, L33F, I47V, I50V/L, I54L/M, T74P, L76V, I84V and L89V.

2.15.3. Integrase inhibitors: these block the insertion of the viral cDNA into the host cell genome. The drugs bind to the catalytic site of the enzyme, forming DNA/integrase pre-integration complex which is transported into the nucleus. The presence of integrase-specific mutations confers resistance to this class of drugs. The two steps of action of integrase enzyme are potentially affected by the mutations.

Dual class or triple class resistance have been reported previously. Some drugs require a single mutation for them to fail. For example: 3TC, EFV and NVP. Resistance

to 3TC (M184V) has been shown to be beneficial, by impairing the viral replication capacity and fitness. There is cross-resistance between 3TC and FTC, which have similar patterns of mutations. Also, NVP and EFV have similar patterns of resistance mutations.

Thymidine analog mutations (TAMs): These are mutations specific to the drugs derived from thymidine nucleotides, AZT and D4T. Examples of TAMS are: M41L, D67N, K70R, L210W, T215Y and K219Q. Two pathways have been described for TAMs; TAM-1 (M41L, L210W and T215Y) and TAM-2 (D67N, K70R, T215F and K219Q/E). TAM-1 pathway is generally associated with AZT and D4T.

K65R mutation confers resistance to the following drugs: TDF, ABC, DDI, 3TC, FTC and D4T. However, K65R does not confer resistance to AZT. K65R is antagonistic toward TAMs and does not occur together in the same genome. While K65R impairs the replicative capacity of the virus, TAMS lacks this capacity. Both M184V and K65R are associated with significant impairment in the replicative capacity of the virus.

2.16. Genotypic resistance test and its application in clinical practice

There are two standard resistance assays: phenotypic and genotypic resistance test. Within the clinical context, the genotypic resistance test is more applicable to detect the mutations conferring resistance to any of the specific ART. Conventional (population-based) genotyping is performed when the resistant mutations account for at least 20 – 30% of the circulating virus in the patient. In addition, the blood sample should be drawn when the patient is on the treatment or at least within four weeks of stopping the treatment. The viral load should be at least 500 – 1000 RNA copies/ml. However, ultrasensitive methods (singe genome sequencing or allele-specific real-time PCR) can detect minority viral mutants. The commonly used genotypic resistance assays are: HIV-1 TruGene (Siemens Healthcare Diagnostics) and ViroSeq (Abbott Molecular/Applera Corp. of Applied Biosystems and Celera). The genotypic test offers an indirect assessment of resistance and complex resistance mutations may be difficult to interpret.

Genotypic resistance works on the assumption that changes in the amino acid sequence of the viral genome can be compared with the wild type sequence. Sequencing of the pol gene encoding the enzyme: RT, PR and IN are used for

therapeutic purposes in clinical practice. Also, the analysis of the env – gp120 and gp41 are used for subtyping while analysis of other gene regions – gag and RNase H have limited application in clinical settings. It is recommended that patients entering care for HIV should have genotypic resistance testing done (DHHS, 2016; EACS, 2016; de Ruiters et al., 2014). However, this recommendation is not applicable in resource limited settings. In the resource poor countries, genotypic resistance tests are not accessible. However, patients with second line virological failure after addressing adherence challenges and having been on a boosted PR inhibitor-based regimen for at least 18 months do then qualify for a genotypic resistance test in South Africa (SADoH, 2015).

2.17. HIV Drug Resistance and Mother-To-Child Transmission

All pregnant women living with HIV in South Africa receive life-long therapy on arrival for care at the health facilities. Irrespective of the type of ART, the monitoring of viral load every three months during pregnancy and breastfeeding period should detect virological failure early before the accumulation of resistance mutations (SADoH, 2013; 2015). However, individual and health system factors often lead to missed opportunities when pregnant women on a failing ART regimen remain on these drugs till delivery and post-delivery period. This increases the risk of transmission of resistance mutations to the infants. In addition, drug resistance can potentially compromise the efficacy of the infant prophylaxis and future therapy in the infant.

Based on the available HIV drugs in South Africa, clinicians and patients should be most concerned about reverse transcriptase and protease inhibitor-associated mutations. For example: pregnant women on a failing first line regimen (TDF/FTC/EFV) would potentially accumulate the following NRTI-associated mutations: M184V, Q151M, L74V, K65R, M41L, D67N K70R, L210W, T215Y and K219Q, and NNRTI-associated mutations: K103N, Y188L and/or G190A. Pregnant women on a failing second line regimen (AZT/3TC/Lop/r) will potentially acquire any or combinations of these protease inhibitor-associated mutations: D30N, V32I, M46IL, I47VA, G48VMALSTQ, I54VAMLTS, L76V, V82ATFSMLC, I84VAC, N88S and L90M in addition to any or combinations of the RT mutations.

NNRTIs mutations tend to appear first by a change of a single amino acid; for example K103N, P225H and Y188L. Thus, conferring resistance to Efavirenz and Nevirapine, and to a lesser extent, Rilpivirine and Etravirine (https://hivdb.stanford.edu/dr-summary/resistance-notes/NRTI/). These NNRTIs mutations are clinically relevant in the context of South African PMTCT programme in which the first line ART is Efavirenz-based (SADH, 2015). Also, Nevirapine is the cornerstone of infant prophylaxis in many parts of the world (SADoH, 2015; WHO, 2013; 2016; de Ruiters et al., 2014). Therefore, women on a failing Efavirenz-based ART and acquiring NNRTIs mutations are at greater risk of transmitting the mutant viral strains to their exposed-infants, irrespective of Nevirapine neonatal prophylaxis (Alvarez et al., 2016; Poppe et al., 2017; Karade et al., 2017).

The French cohort provided evidence for the transmission of parental viral resistance strains to the infants (Benizri et al., 2008). Even primary resistance strains in ART-naïve individuals were transmitted to their infants. This confirmed that both primary and acquired resistance mutations in the mothers can be transmitted to the infants (Benizri et al., 2008; Alcantara et al., 2012). With the rapid expansion of access to ART at the population level through the implementation of test and treat strategy in many parts of the world, HIV drug resistance becomes inevitable. By implication, both transmitted and acquired resistance would impact on the disease progression of HIV in pregnant women (Bhargava et al., 2014; Gregson et al., 2016; Gupta et al., 2018; Karade et al., 2017), and consequently, higher risks of mother-to-child transmission will ensue.

Within the South African PMTCT setting, it is unclear whether the emergence of drug resistance in pregnant women has any significant impact on the outcomes of mother-to-child transmission. It is also unclear if the existing primary resistance in ART-naïve pregnant women entering health facilities to receive the first line regimen (TDF/FTC/EFV) contribute to early virological failure, maternal morbidity and mortality in the country. In addition, there are no data on the prevalence and patterns of drug resistance mutations among pregnant women residing in Eastern Cape and South Africa with virological failure, requiring switch from first to second line regimen in the country. Genotypic resistance tests that form part of the baseline investigation in the developed countries, are not readily available in the resource poor settings (Gupta et al., 2018). These missing links in the literature require investigations in order to gain a

better understanding of the risks of mother-to-child transmission of HIV in the context of maternal life-long ART.

Conclusion

In conclusion, research studies in HIV have gained prominence in the past three decades and so much knowledge has been heralded over this period. There are still many research gaps in HIV diversity, therapeutics and drug resistance. This chapter provided a comprehensive review of the literature on HIV epidemics globally, SSA and South Africa. In addition, the mode of transmission and prevention strategies, structure, genomic analysis, and life cycle were reviewed with a view to gaining new insights into the current knowledge on all aspects of the biological concepts on HIV. The chapter narrows down on the advances on HIV therapeutics globally and in South African setting, and on drug resistance. In addition, an extensive review of the epidemiological trends, mechanisms of mother-to-child transmission and prevention strategies with anti-retroviral therapy are provided with a view of creating appropriate context for new studies in the field. The next three chapters present the findings of this study (as publishable units) with a view of filling some of the research gaps identified in the literature.

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CHAPTER THREE

High Rate of Peripartum Virological Suppression and its Significant Implications for Elimination of Mother-to-Child Transmission of HIV in Resource-Constrained Settings of the Eastern Cape, South Africa: Population-Based Cohort Study

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ABSTRACT

Background: Initiation of life-long antiretroviral therapy (World Health Organization Option B Plus strategy) to all HIV-infected pregnant women will potentially lead to sustained viral suppression, further decline in mother-to-child transmission and improved maternal and child health. There are no nationwide population-effectiveness surveys on the Option B Plus implemented in South Africa in 2015 and specifically, in the resource limited settings. This study assessed the maternal virological suppression at delivery and early infant diagnosis as measures of the effectiveness of the public sector elimination of mother-to-child transmission programme in the resource limited settings of the Eastern Cape Province, South Africa.

Methods: Drawing from the baseline data of the multicentre, prospective cohort study which enrolled a total of 1709 HIV-infected mother/infant pairs in the Eastern Cape between 2015 and 2016, maternal virological responses to ART and in-utero transmissions were analysed. Maternal viral loads (VL) at delivery were assayed using HIV-specific quantitative polymerase chain reaction (PCR) and defined as suppressed if VL<1000 copies/ml). In-utero transmission was determined by using the HIV-specific qualitative DNA PCR at birth. Determinants of peripartum viral suppression and in-utero transmission were assessed in both bivariate and logistic regression analyses.

Results: Out of 1463 mothers with available VL results, the overall rate of VL suppression (VL<1000 RNA copies/ml) was 82% (undetectable viral load 56.9% and low viraemia 25.2%). The analysis shows that age 24 years and below [AOR=0.54, CI=0.38-0.78,], unemployment [AOR=0.54, CI=0.37-0.79], smoking during pregnancy [AOR=0.50, CI=0.26-0.96], short duration on ARV treatment[AOR=0.52, CI=0.33-0.82], lower CD4 counts [AOR=0.10, CI=0.06-0.16], were associated with a lower likelihood of having an undetectable viral load. Women who had never defaulted their ARVs [AOR=3.08, CI=1.88-5.05] had an increased odds of having an undetectable viral load compared to those who defaulted. The overall rate of early MTCT was 1.3% (20 of 1539 babies). There was a positive linear association between early MTCT risk along the gradient of maternal viraemia; VL< 20 copies/ml (0.5%), 20-999 copies/ml (0.6%) and VLs ≥1000 copies/ml (3.3%), respectively. Parturient women were at increased odds of transmitting HIV to their infants if they had VL > 1000 copies/ml (AOR=8.84; CI=2.33-33.56).

Conclusion: High maternal virological suppression at delivery and very low MTCT were achieved in the resource-constrained Eastern Cape, South Africa. Intervention strategies focusing on maternal lifestyle behaviours and ART adherence challenges require targeted research.

Keywords: Antiretroviral therapy; HIV; In-utero Transmission; Mother-to-Child Transmission; South Africa

3.1. Introduction

The World Health Organization (WHO) set two-impact criteria for assessing elimination of mother-to-child transmission (EMTCT) of HIV: a MTCT rate of less than 2% in non-breastfeeding women and less than 5% in breastfeeding women, and a case rate of new paediatric HIV infections of less than or equal to 50 per 100,000 live births (WHO, 2014). In addition, three-pronged process criteria were established: a minimum of 95% antenatal care and HIV testing coverage among all pregnant women, and at least 90% antiretroviral therapy (ART) coverage among HIV-infected pregnant women (WHO, 2014). Over the last decade, significant progress has been documented across different regions and countries of the world (WHO, 2016; UNAIDS, 2016). This reflects largely on the expansion of access to the highly efficacious ART for all HIV-infected pregnant women, either as maternal prophylaxis (WHO Options A or B) or as maternal lifelong ART (WHO Option B Plus) (WHO, 2013)

South Africa has the largest HIV epidemic worldwide (7.1 million people living with HIV including 320,000 children) (UNAIDS, 2017). The country has the largest treatment programme globally; accounting for 20% of individuals receiving ART worldwide with 80% of the AIDS response funded by the government (UNAIDS, 2017). Also, the country has recorded significant successes in the key indicators of EMTCT. Over 95% of pregnant women are accessing antenatal care services including HIV testing, about 93% of HIV-infected pregnant women received ART and six weeks infant diagnosis reduced from 25-30% in 2001 to 2.6% in 2014 (Goga et al., 2015). All HIV-infected pregnant women accessing antenatal clinics are initiated on the fixed dose combination (Tenofovir/Emtricitabine/Efavirenz) or any three compatible ART regimens (SADoH, 2015). These achievements were largely attained through the government's commitment to achieving the global plans of elimination of new paediatric infections (SADoH, 2015), the Millennium Development Goals 2000 – 2015 and the sustainable development goals 2015 – 2030 (UNAIDS, 2011; UNDP, 2000; 2015].

Despite the laudable successes, there were 12,000 new paediatric infections in South Africa in 2016 based on modelling estimates by the Joint United Nations Programme on AIDS (UNAIDS, 2017). In addition, the high prevalence of HIV infections of 23.8% among reproductive age women (15 – 49 years) (UNAIDS, 2017) and 30.8% amongst women who attended antenatal care in 2015 (SADoH, 2015) suggests that the battle

towards EMTCT in every community, district, province and the country at large is not over. As such, intensified surveillance of pregnant women, who are Son ART but with high viral load, should be given greater priority in maternity services in the country should be given greater priority. This strategy will allow for prompt intervention and could be incorporated into the current PMTCT guidelines in the country.

Besides the South African Medical Research Council PMTCT Evaluation Study Report of 2012-2013 (Goga et al., 2015), there has not been any nationally representative data on the population level effectiveness of the new PMTCT guideline (World Health Organization Option B Plus). Even though Goga et al. (2015) provided a reference data for the country, the study did not assess the maternal viral load during pregnancy, delivery or post-partum and in-utero MTCT of HIV. As such, there are no data to track the progress towards attaining the last 90% (viral load suppression) and EMTCT. While there is a study on PMTCT outcomes in the Western Cape (Myers et al., 2017), a resource-rich province, there are no data on PMTCT outcomes in resource-constrained settings of South Africa. Such data would help identify high risk sub-groups with high viral load, which could be targeted for intensified interventions and follow-up. This study specifically addressed the identified gaps and provided reliable epidemiological data on the population-effectiveness of the WHO Option B Plus strategy in the Eastern Cape Province, South Africa, a resource-constrained setting. This observational study specifically, assessed the maternal virological suppression at delivery and early MTCT risks. In addition, the correlates of high maternal viral load at delivery and early MTCT risks were identified.

The East London Prospective Cohort Study (Adeniyi et al., 2017) is an ongoing longitudinal observational study involving three large maternity services serving a combined population of 1,674,637 people in the Amathole/Buffalo City districts of the Eastern Cape Province, South Africa (StatsSA, 2011). The population in the two districts resembles the rural, semi-urban and urban demographics found across the entire breadth of the Eastern Cape (Adeniyi et al., 2018; Goga et al., 2012). The HIV prevalence at the population level is 12.7% and among pregnant women is 30.2% (SADoH, 2015). According to the 2010 review in the Saving Babies Report, Eastern Cape Province underperformed in the PMTCT indicators and worse still, Amathole/Buffalo City districts (8.89% transmission rate), had the worst PMTCT

outcome (Pattinson and Rhoda, 2014). Also, the South African PMTCT Evaluation Study Report of 2012-2013 showed that the Eastern Cape slightly lags behind the national MTCT rate (Goga et al., 2015). Hence, this study was implemented in these districts to monitor and evaluate the outcomes of the implementation of the WHO Option B Plus Strategy.

3.2. Methods

3.2.1. Study Design and Settings

We conducted an analysis of the baseline data of the East London Prospective Cohort Study (Adeniyi et al., 2017), a longitudinal cohort of HIV-infected pregnant women who attended three of the largest maternity centres in the Amathole/Buffalo City districts (Fig. 3.1) of the Eastern Cape Province, South Africa between September 2015 and May 2016. The East London Prospective Cohort Study aimed to generate reliable epidemiological data on the PMTCT outcomes in the Eastern Cape and factors impacting the outcomes. The selected maternity services represent the three tiers of healthcare services in the Eastern Cape and South Africa. Frere Hospital is an urban academic, tertiary hospital affiliated to Walter Sisulu University. This hospital delivers an average of 400 pregnant women monthly and receives referrals from regional hospitals, district hospitals as well as midwife obstetric units (MOUs) across the region. Cecilia Makiwane Hospital is a regional academic hospital located in the semi-urban township of Mdantsane and provides both level one and two obstetric services. This hospital delivers an average of about 500 pregnant women monthly. Bhisho Hospital is a district hospital (level one) located in the rural community of Bhisho. It delivers an average of 250 pregnant women from Bhisho and neighbouring, deep rural, antenatal clinics.

Fig. 3.1. Map of Eastern Cape Province showing the East London and Bisho area (Adapted from: https://www.google.com/search?q=amathole+district+map&tbm.....)

3.2.2. Inclusion and exclusion criteria

All HIV-infected parturient women who delivered their index infant at the study settings between September 2015 and May 2016 were considered eligible for the study. All HIV-uninfected mothers who gave birth over the study period were excluded from the study. Also, HIV-infected pregnant women who were yet to deliver their babies were ineligible for the study.

3.2.3. Sample size and Study Procedure

A sample size of 1709 participants was estimated to be required for the study after adjusting for possible missing responses. Our sample size estimation was based on a confidence level of 95%, 80% statistical power, 85% viral load suppression prevalence (Bedelu et al., 2007), and 2.2 effect size. The participants' sampling from the three study settings was proportional to the size of deliveries per month. Eligible participants (HIV-infected pregnant women on ART) were recruited consecutively into the study. All eligible participants took part in the study. Participants were recruited serially within

24 hours of vaginal delivery and 72 hours of Caesarean section at the post-natal wards. Some participants who had engaged in HIV care prior to conception had been initiated on ART based on CD4 count ≤ 350 cells/μL and WHO clinical stage III/IV (SADoH, 2013) or ≤ 500 cells/μL (SADoH, 2015). Trained research assistants completed the interviewer-administered questionnaire on an electronic platform (designed purposely for this study) synchronised with the East London Prospective Cohort Study PMTCT Database across the three centres. Relevant data on socio-demographics (age, level of education, parity, employment status and type of residence) and lifestyle behaviours (cigarette smoking and alcohol use during the index pregnancy) were obtained from interview and maternity records. Relevant clinical data, such as duration of treatment, duration of the disease, awareness of HIV status pre-conception, gestational age at the first antenatal clinic visit, timing of ART and disclosure to partners, were obtained from interviews. Adherence challenges to current ART were explored by documenting the history of default of ART and on-time pick-up of ART from the medical records, and self-report of adherence (or the absence thereof).

3.2.4. Peripartum Viral Load Monitoring

The research nurses assigned to each study sites drew about 5ml of venous blood from each parturient woman for viral load (VL) testing within 24hours of vaginal and 72 hours of Caesarean section delivery. All viral load assays were conducted by the National Health Laboratory Services using the Abbort Real Time HIV-1 assay (Abbott Laboratories, Chicago, Illinois, USA). All parturient women with high viral load results were treated as emergencies by the district clinical specialist team who provided care in accordance with the ART guideline (SADoH, 2015).

3.2.5. Early Mother-to-child transmission (In-utero transmission)

The current guideline recommends birth PCR in all HIV-exposed infants in order to diagnose in-utero transmission for prompt initiation of triple regimen (SADoH, 2015). The research nurse performed the heel prick for dried blood spot samples from all HIV-exposed live infants. All birth PCRs were performed centrally by the National Health Laboratory Services using the Roche Cobas AmpliPrep/Cobas TaqMan (CAP/CTM) HIV-1 assay (Roche Diagnostics, Branchburg, NJ, USA). Results were

retrieved within two weeks of the tests and were utilised in the management of the HIV exposed-infants.

3.2.6. Definitions

For the purpose of this study, peripartum virological suppression (treatment success) was defined as VL < 1000 copies/ml while high viral load was defined as VL ≥ 1000 copies/ml. This definition was supported by many studies which had reported risk reductions from vertical transmission below this cut-off (Fowler et al., 2015; Goga et al., 2015; Kesho Bora Study Group, 2011; Warszawski et al., 2008). The South African National Health Laboratory Services uses viral load assays with the lower limit of detection of less than 20 copies/ml. Hence, viral suppression was further categorised as; undetectable (VL < 20 copies/ml) and low level viraemia (VL 20 – 999 copies/ml). The clinical implications of the low level viraemia in vertical transmission are unclear; hence, this study further explored the correlation of early MTCT risks at this level of viral load. Some parturient women had no VL results; mostly, their blood samples were rejected by the laboratory due to clotted samples or unsuitable specimens and a few participants were discharged without blood samples for VL.

Early MTCT was defined as infants with positive birth PCR results. A repeat sample of the dried blood spot for confirmatory DNA PCR test was ordered for each positive result and antiretroviral prophylaxis would be stopped in order to commence triple ART regimen in accordance with the South African Paediatric ART Guideline (Nuttal, 2015; SADoH, 2015). Infants without valid results (rejected by the laboratory) due to insufficient samples or clerical errors were linked to the nearest primary health care facilities for repeat PCR tests.

3.2.7. Patient and Public Involvement

The patient and the public were not involved in the conception, design and implementation of the study protocol. Notably, the results of the outcome measures; maternal peripartum viral load and the birth PCR were utilised in the care of the patients.

3.3. Ethical considerations

The Walter Sisulu University Ethics Committee (Protocol number: 098/2014) granted ethical clearance for the implementation of the study. Additional ethical approval was obtained from the Research Ethics Committee of the University of Fort Hare (Protocol number: OBI021SADE01/2016) to analyse stored plasma samples from the participants. The Eastern Cape Department of Health and respective clinical governing bodies of each hospital gave permission before the investigators commenced data collection. Participants received information sheets written in both English and IsiXhosa languages, detailing the process and objectives of the study. Each participant gave a written, informed consent for her voluntary participation in the study. This was considered important because of the proposed 24 months follow-up period. Legal guardians completed consent forms for a few participants who were below the age of 16 years, who also gave their assent to be recruited into the cohort study. Participants' privacy and confidentiality of medical information were protected during and after the study.

3.4. Data Analysis

Data of HIV-infected mother/infant pairs were analysed using the IBM Statistical Package for Social Sciences Version 24.0 (IBM, Chicago, IL, USA). Analysis was performed on 1463 participants with viral load results and 1539 babies with birth PCR results. Descriptive statistics such as means (± standard deviations) and proportions were used to describe the maternal socio-demographic characteristics, rates of peripartum viral suppression and early MTCT. Maternal viral load response to ART was categorised into two; suppressed (undetectable viral load and low level viraemia) and high viral load (probable virological failure), which were compared with maternal demographics, lifestyle and clinical characteristics using the Pearson chi-square test and Fisher exact test for bivariate analysis. We fitted the adjusted and unadjusted multinomial logistic regression models to identify the risk factors for undetectable viral load and low viraemia. In the unadjusted model—the baseline model, we examine the independent effect of each demographic factor, lifestyle behaviours variable, and clinical characteristic on undetectable viral loads and low viremia relative to high viral loads/probable virological failure. The adjusted multinomial regression model— a

multivariate model— was used to examine the net effects of each variable while controlling for important covariates. In the secondary analysis, the rate of in-utero transmission (early MTCT) was determined. Bivariate analysis (Pearson chi-square) was performed to assess whether there were differences in maternal profiles between those who transmitted HIV to their infants and those who did not. Also, we examined the associations between the MTCT rates and timing of ART, gestational age at booking and maternal demographic profiles using adjusted and unadjusted binary logistic regression. All reported p-values are based on a 2-sided test.

3.5. Results

3.5.1. Baseline Characteristics of the Participants

The ages of the participants ranged from 14 years to 44 years with a mean (standard deviation) of 29.63 (±6.2) years. High proportions of the parturient women were single (68.6%), had attained grade 7 – 12 education (87.2%), were unemployed (74.2%), had never smoked cigarettes (89.4%) nor consumed alcohol beverages (59.2%), and had two or more children (69.2%) (Table 3.1).

Table 3.1. Demographic characteristics of study participants

Variables	Frequency (n=1463)	Percent
Age		
19 and below	55	3.8
20-24	281	19.2
25-29	393	26.9
30-34	384	26.2
35-39	265	18.1
40 and above	85	5.8
Marital status		
Married	274	18.7
Single	1003	68.6
Cohabiting	163	11.1
Divorce/separated	23	1.6
Place of residence		
Rural area	492	33.6
Semi-urban	680	46.5
Urban	291	19.9
Level of education		
No formal education	4	0.3
Grade 1-6	83	5.7
Grade 7-12	1276	87.2
Tertiary education	100	6.8
Employment status		
Unemployed	1085	74.2
Employed	378	25.8
Smoking status		
Smoked during pregnancy	81	5.5
Quit smoking during pregnancy	74	5.1
Never smoked	1308	89.4
Alcohol use		
Drank during pregnancy	202	13.8
Stopped drinking during pregnancy	395	27.0
Never drank	866	59.2
Parity		
One	450	30.8
Two	520	35.5
Three	301	20.6
Four & plus	192	13.1

Most parturient women were aware of their HIV serostatus before their first antenatal care visit (80.8%) and pre-conception ART occurred in 58.1% (n=850) of the parturient women. Of those who initiated ART during the index pregnancy (613), the majority began in the second trimester (430) while 77 women were initiated in the third trimester. The majority of the women (97.7%) were currently on first line regimen

consisting of predominantly Tenofovir (TDF)/Emtricitabine (FTC)/Efavirenz (EFV) or Nevirapine (NVP) (others include: ABC/3TC/EFV or NVP; AZT/3TC/EF or NVP) while only 2.3% of the participants were on the second line regimen—AZT/3TC/Lopinavir/ritonavir or Atazanavir/ritonavir or TDF/FTC/Lop/r or ATZ/r. The majority of the respondents were on treatment for more than 13 weeks (85.5%) while over a tenth of them had low CD4 counts after child delivery (Table 3.2).

Table 3.2. Clinical characteristics of study participants

Variables	Frequency (n=1463)	Percentage
Timing of ART initiation		
Pre-conception	850	58.1
First trimester	86	5.9
Second trimester	430	29.4
Third trimester	97	6.6
Duration on treatment		
0-13 weeks	209	14.3
14-720 weeks	1254	85.7
Gestation age at first antenatal care booking		
First trimester	176	12.0
Second trimester	1061	72.5
Third trimester	226	15.4
Year at first diagnosis		
1998-2010	128	8.7
2011-2014	339	23.2
2015-2016	501	34.2
Not stated	495	33.8
CD4 counts after delivery		
1-199	162	11.1
200-349	354	24.2
Not available	85	5.8
350-499	334	22.8
500-3200	528	36.1
ART regimen **		
First line regimen	1297	97.7
Second line regimen	30	2.3
Disclosed status to partner		
Yes	1089	25.1
No	365	74.9
Self-reporting of adherence		
Yes	1091	74.6
No	326	22.3
Not stated	46	3.1
Regular ART Pick up		
Yes	1124	76.8
No	262	17.9
Not stated	77	5.3
Defaulted ARV		
No	1232	84.2
Yes	154	10.5
Not stated	77	5.3
HIV status at first antenatal care		
Positive	1182	80.8
Negative	79	5.4
Unknown	202	13.8

** missing data for 136 participants

3.5.2. Maternal Virological Suppression at Delivery and its Correlates

Of the total 1463 HIV-infected parturient women whose peripartum viral load results were available for analysis, the virological suppression rate was 82% (1200 of the 1463 mothers). Undetectable viral load occurred in 56.9% (n=832) and low viraemia in 25.2% (n=369). High viral load (probable virological failure) occurred in 17.9% of the mothers (n=262). In bivariate analysis (Table 3.3), there was an association between peripartum viral suppression and the following maternal demographic characteristics: age, marital status and employment status. Also, significant association exist between peripartum viral suppression and lifestyle behaviours: smoking status and alcohol use as well as clinical characteristics such as partner disclosure, history of defaulting ART, self-report of adherence and regular pick-up of ART duration on treatment, peripartum CD4 counts and years diagnosed with HIV($p<0.05$).

Table 3.3. Pearson Chi-square statistics showing the correlates of peripartum Viral load

Variables	Undetectable n=832	Low viremia n= 369	High viral load n=262	*p-value*
Age				
24 and below	159 (47.3.4)	98 (29.2)	79 (23.6)	<0.001
25 and above	673 (59.7)	271 (24.0)	183 (16.3)	
Marital status				
Married	183 (66.8)	58 (21.2)	33 (12.0)	0.004
Single	541 (53.9)	267 (26.6)	195 (19.4)	
Co-habiting	98 (60.1)	35 (21.5)	30 (18.4)	
Divorced/separated	10 (43.5)	9 (39.1)	4 (17.4)	
Place of residence				
Rural	280 (56.9)	110 (22.4)	102 (20.7)	0.219
Semi-urban	387 (56.9)	182 (26.8)	111 (16.3)	
Urban	167 (56.7)	77 (26.5)	49 (16.8)	
Education Level				
No formal education	2 (50.0)	1 (25.0)	1 (25.0)	0.116
Grade 1-6	55 (66.3)	12 (14.5)	16 (19.3)	
Grade 7-12	712 (55.8)	329 (25.8)	235 (18.4)	
Tertiary	63 (63.0)	27 (27.0)	10 (10.0)	
Employment status				
Unemployed	592 (54.6)	277 (25.5)	216 (19.9)	0.001
Employed	240 (63.5)	92 (24.3)	46 (12.2)	
Smoking status				
Smoked during pregnancy	35 (43.2)	16 (19.8)	30 (37.0)	<0.0001
Quit smoking during pregnancy	48 (64.9)	15 (20.3)	11 (14.9)	
Never smoked	749 (57.3)	338 (25.8)	221 (16.9)	
Alcohol use				
Drank during pregnancy	99 (49.0)	50 (24.8)	53 (26.2)	0.018
Quit drinking during pregnancy	226 (57.2)	103 (26.1)	66 (16.7)	
Never drank alcohol	507 (58.5)	216 (24.9)	143 (16.5)	
Trimester at booking				
First	87 (49.4)	43 (24.4)	46 (26.1)	0.011
Second	627 (59.1)	261 (24.6)	173 (16.3)	
Third	118 (52.2)	65 (28.8)	43 (19.0)	
Pre-conception awareness of HIV status				
Positive	688 (58.2)	275 (23.3)	219 (18.5)	0.006
Negative	43 (54.4)	22 (27.8)	14 (17.7)	
Unknown	101 (50.0)	72 (35.6)	29 (14.3)	
Timing of ART initiation				
Pre-conception	519 (61.1)	183 (21.5)	148 (17.4)	<0.001
First trimester	37 (43.0)	22 (25.6)	27 (31.4)	
Second trimester	235 (54.7)	128 (29.8)	67 (15.6)	
Third trimester	41 (42.3)	36 (37.1)	20 (20.6)	
Disclosure to partner				
Yes	641 (58.9)	217 (24.9)	177 (16.3)	0.010
No	185 (51.0)	97 (26.6)	82 (22.5)	
Defaulted ART				
No	731 (59.3)	318 (25.8)	183 (14.9)	<0.001
Yes	51 (33.1)	39 (25.3)	64 (41.6)	
No response	50 (64.9)	12 (15.6)	15 (19.5)	

Self-report of good adherence				
No	154 (47.2)	82 (25.2)	90 (27.6)	<0.001
Yes	656 (60.1)	277 (25.4)	158 (14.5)	
No response	22 (47.8)	10 (21.7)	14 (30.4)	
On-time pick-up of ART				
No	131 (50.0)	59 (22.5)	72 (27.5)	<0.001
Yes	667 (59.3)	290 (25.8)	167 (14.9)	
No response	34 (44.2)	20 (26.0)	23 (29.9)	
Duration on treatment				
0-13 weeks	79 (37.8)	85 (40.7)	45 (21.5)	0<0.001
14 to 720 weeks	753 (60.0)	284 (22.6)	217 (17.3)	
Peripartum CD4 counts				
1-199	44 (27.2)	49 (30.2)	69 (42.6)	<0.001
200-349	180 (50.8)	104 (29.4)	70 (19.8)	
350-499	209 (62.6)	78 (23.4)	47 (14.1)	
500-3200	362 (68.6)	116 (22.0)	50 (9.5)	
Not available	37 (43.5)	22 (25.9)	26 (30.6)	
Year diagnosed with HIV				
1998-2010	77 (60.2)	33 (25.8)	18 (14.1)	0.041
2011-2014	217 (64.0)	65 (19.2)	57 (16.8)	
2015-2016	281 (56.1)	141 (28.1)	79 (15.8)	
Not stated	257 (51.9)	130 (26.3)	262 (21.8)	

ART=Antiretroviral therapy

To identify the risk factors of undetectable viral load and low viremia, we estimated unadjusted and adjusted multinomial logistic regression models. The results are presented in Table 3.4. The unadjusted model examined the independent effect of demographic factors, lifestyle behaviours, and clinical characteristics on viral load suppression (undetectable viral loads and low viremia) relative to high viral load (probable virological failure). The results showed that maternal demographic factors (younger age and unemployment), lifestyle behaviours (smoked during pregnancy and drank alcohol during pregnancy, self-report of non-adherence, irregular pick-up of ART and history of defaulting ART), and clinical characteristics (duration on treatment and peripartum CD4 counts) were independently associated with undetectable viral load. However, in the adjusted multinomial logistic regression, only age 24 and below, unemployed, smoking during pregnancy, history of defaulting ART, short duration on ART, lower CD4 counts were associated with a lower likelihood of having an undetectable viral load. Women aged 24 years and below, and unemployed women were 44% less likely to have an undetectable viral load compared to women aged 25 years and above and women who are employed, respectively. Also, women who smoked during pregnancy were 50% less likely to have an undetectable viral load compared to women who never

smoked. Women who had no history of defaulting ART were three times more likely to have an undetectable viral load compared to women with a history of defaulting ART. In terms of clinical factors associated with having an undetectable viral load, short duration on ART (0-13 weeks of treatment) was associated with 48% lesser likelihood of having an undetectable viral load compared to longer duration on treatment. Women with CD4 counts less than 200 cells/ml were 90% less likely to have an undetectable viral load compared to women who had CD4 counts above 499. In the baseline model, women who were unemployed, smoked during pregnancy, drank alcohol during pregnancy, self-reported non-adherence to ART, failed to pick up ART regularly, defaulted ART in the past and whose peripartum CD4 counts was low had a lesser likelihood of having low viremia. However, in the adjusted model, only peripartum CD4 counts and history of defaulting ART were associated with having a low viraemia.

Table 3.4. Multinomial logistic regression model showing determinants of undetectable viral load and low viraemia

Variable	Undetectable viral load		Low viremia	
	UOR	AOR	UOR	AOR
Age				
24 and below	0.55 (0.40-0.75)***	0.54 (0.38-0.78)*	0.84 (0.59-1.19)	0.81 (0.55-1.19)
25 and above (ref)	1	1	1	1
Employment status				
Unemployed	0.53 (0.37-0.75)***	0.54 (0.37-0.79)*	0.64 (0.43-0.95)*	0.68 (0.45-1.03)
Employed (ref)	1	1	1	1
Smoking status				
Smoked during pregnancy	0.34 (0.21-0.57)***	0.50 (0.26-0.96)*	0.35 (0.19-0.66)*	0.51 (0.25-1.05)
Quit smoking during pregnancy	1.29 (0.66-2.52)	1.84 (0.85-3.99)	0.89 (0.40-1.98)	1.03 (0.44-2.46)
Never smoked (ref)	1	1	1	1
Alcohol use				
Drank during pregnancy	0.53 (0.36-0.77)*	0.90 (0.55-1.44)	0.63 (0.40-0.97)*	0.97 (0.58-1.63)
Quit drinking during pregnancy	0.97 (0.69-1.35)	1.13 (0.77-1.65)	1.03 (0.71-1.50)	1.24 (0.82-1.86)
Never drank alcohol (ref)	1	1	1	1
Disclosure to partner				
No	0.63 (0.46-0.85)*	0.83 (0.59-1.18)	0.77 (0.55-1.10)	0.85 (0.59-1.24)
Yes (ref)	1	1	1	1
Self-reporting of non-adherence				
Adhere	2.43 (1.78-3.2)***	1.28 (0.86-1.92)	1.92 (1.35-2.75)***	1.28 (0.83-1.98)
No response	0.92 (0.45-1.88)	1.26 (0.41-3.83)	0.78 (0.33-1.86)	1.04 (0.31-3.44)
Did not adhere (ref)	1	1	1	1
ART Pick up				
Regularly pick up ART	2.20 (1.57-3.07)***	1.37 (0.91-2.06)	2.12 (1.43-3.14)***	1.43 (0.91-2.24)
No response	0.81 (0.45-1.48)	0.64 (0.26-1.55)	1.06 (0.53-2.12)	1.16 (0.46-2.91)
Default in picking ART (ref)	1	1	1	1
History of defaulting in using ARV				
Never defaulted in use of ARV	5.01 (3.35-7.49)***	3.08 (1.88-5.05)***	2.85 (1.84-4.42)***	1.72 (1.02-2.89)*
No response	4.18 (2.11-8.29)***	3.16 (1.40-7.14)*	1.31 (0.56-3.09)	1.07 (0.41-2.76)
Defaulted in using ARV in the past (ref)	1	1	1	1
Duration on treatment				
Short duration on ARV (0-13 weeks)	0.51 (0.34-0.75)*	0.52 (0.33-0.82)*	1.44 (0.97-2.16)	1.37 (0.88-2.13)
Long duration on ARV (14 to 720 weeks) (ref)	1	1	1	1
Year diagnosed with HIV				
1998-2010	1.20 (0.68-2.13)	1.12 (0.59-2.15)	1.03 (0.54-1.94)	1.17 (0.59-2.33)

2011-2014	1.07 (0.73-1.57)	0.82 (0.53-1.27)	0.64 (0.41-1.00)	0.62 (0.38-1.00)
Not stated	0.67 (0.48-0.94)*	0.70 (0.48-1.03)	0.67 (0.46-0.98)*	0.66 (0.44-0.99)*
2015-2016 (ref)	1	1	1	1
Peripartum CD4 counts				
1-199	0.09 (0.05-0.14)***	0.10 (0.06-0.16)***	0.31 (0.19-0.50)***	0.31 (0.19-0.52)***
200-349	0.36 (0.24-0.53)***	0.36 (0.24-0.55)***	0.64 (0.41-1.00)	0.66 (0.42-1.05)
350-499	0.61 (0.40-0.95)*	0.63 (0.40-0.99)*	0.72 (0.44-1.17)	0.74 (0.45-1.23)
Not available	0.20 (0.11-0.35)***	0.19 (0.10-0.35)***	0.37 (0.19-0.70)*	0.36 (0.18-0.71)*
500-3200 (ref)	1	1	1	1

UOR= Unadjusted odds ratio; AOR=Adjusted odds ratio; CI=Confidence interval; ARV=Antiretroviral drugs; *p-value<0.05; ***p-value<0.01; ref: reference

3.5.3. Rate and Determinants of early MTCT (In-utero transmission)

Of the total 1539 babies with birth PCR results, 20 new paediatric infections were diagnosed, resulting in an early MTCT rate of 1.3%. However, the rate of in-utero transmission differed significantly along the gradient of the maternal viral load; 4 MTCTs occurred from mothers with undetectable viral load (0.5%), 2 MTCTs occurred from mothers with low level viraemia (0.6%) and 8 MTCTs occurred from mothers with high viral load (3.3%). Also, the rate of early MTCTs was highest among women with adherence challenges who self-reported their poor adherence (2.4%), had a history of defaulting ART (3.8%), and irregular pick of ARVs (2.3%).

The results of the regression analysis which examined factors associated with early MTCT of HIV is presented in Table 3.5. In the unadjusted logistic regression, only history of defaulting ART, self-reporting of non-adherence, high viral load, were independently associated with a higher odds of early MTCT of HIV. However, after controlling for important covariates, only non-suppression of viral load was associated with increased odds of transmitting HIV to their babies. Parturient women who failed to achieve virological suppression at delivery were about nine times more likely to transmit HIV virus to their babies compared to those who had an undetectable viral load.

Table 3.5. Adjusted and unadjusted binary logistic regression models showing the factors associated with early transmission of HIV to infants

Variables	Negative n(%)	Positive n(%)	UOR	AOR
Age				
24 years and below	348 (98.9)	4 (1.1)	0.86 (0.28-2.58)	0.66 (0.20-2.12)
25 years and above (ref)	1184 (98.7)	16 (1.3)	1	1
Employment status				
Unemployed	1139 (98.7)	15 (1.3)	1.05 (0.38-2.9)	1.47 (0.50-4.37)
Employed (ref)	400 (98.8)	5 (1.2)	1	1
Smoking status				
Smoked during pregnancy	92 (100.0)	0 (0.0)	N/A	N/A
Quit smoking during pregnancy	72 (98.6)	1 (1.4)	1.63 (0.13-7.61)	1.06 (0.11-10.64)
Never smoked (ref)	1375 (98.6)	19 (1.4)	1	1
Alcohol use				
Drank during pregnancy	211 (97.7)	5 (2.3)	1.71 (0.60-4.85)	2.00 (0.63-6.36)
Quit drinking during pregnancy	390 (99.5)	2 (0.5)	0.37 (0.08-1.65)	0.39 (0.08-1.91)
Never drank alcohol (ref)	938 (98.6)	13 (1.4)	1	1
History of defaulting ART				
Ever defaulted in use of ARV	176 (96.2)	7 (3.8)	4.62 (1.77-12.08)**	2.69 (0.79-9.12)
No response	85 (97.7)	2 (2.3)	2.73 (0.60-12.53)	3.23 (0.55-18.98)
Never defaulted in using ARV in the past (ref)	1278 (99.1)	11 (0.9)	1	1
Self-report of good adherence				
Did not adhere to ARV	326 (97.6)	8 (2.4)	2.58 (1.03-6.47)*	1.65 (0.48-5.64)
No response	57 (98.3)	1 (1.7)	1.84 (0.23-14.53)	0.62 (0.03-12.53)
Adhere to ARV (ref)	1156 (99.1)	11 (0.9)	1	1
Regular pick-up of ART				
Default in picking ART	257 (97.7)	6 (2.3)	2.32 (0.86-6.25)	1.45 (0.44-4.77)
No response	88 (97.8)	2 (2.2)	2.26 (0.50-10.26)	1.27 (0.14-11.69)
Regularly pick up ART (ref)	1194 (99.0)	12 (1.0)	1	1
Peripartum Viral Load				
High viral loads (VL ≥ 1000 copies/ml)	234 (96.7)	8 (3.3)	6.72 (2.01-22.51)*	8.84 (2.33-33.56)***
Low level viraemia (VL =21-1999 copies/ml)	347 (99.4)	2 (0.6)	1.13 (0.21-6.21)	1.39 (0.24-7.97)
Not available	172 (96.6)	6 (3.4)	6.86 (1.91-24.55)*	6.48 (1.34-31.31)*

Undetectable viral loads (VL<21 copies/ml) (ref)	786 (99.5)	4 (0.5)	1	1
Peripartum CD4 counts				
1-199	164 (99.4)	1 (0.6)	0.39 (0.05-3.16)	0.11 (0.01-1.04)
200-349	347 (99.1)	3 (0.9)	0.56 (0.15-2.11)	0.26 (0.06-1.14)
350-499	341 (99.1)	3 (0.9)	0.57 (0.15-2.15)	0.42 (0.10-1.75)
Not available	173 (97.2)	5 (2.8)	1.86 (0.60-5.75)	0.59 (0.14-2.47)
500-3200 (ref)	514 (98.5)	8 (1.5)	1	1
Duration on treatment				
Short duration (0-13 weeks)	221 (99.1)	2 (0.9)	0.66 (0.15-2.88)	0.75 (0.16-3.50)
Long duration (1-14 weeks) (ref)	1318 (98.7)	18 (1.3)	1	1

ART=Antiretroviral therapy; UOR=Unadjusted Odd Ratio; AOR=Adjusted Odd Ratio; *p-value<0.05; ***p-value<0.01; ref: reference

3.6. Discussions

The prevalence of HIV in South Africa is 12.6% (over 7 million) at the population level and 23.8% among reproductive age women (15 – 49 years) (UNAIDS, 2017). The prevalence of HIV is higher among pregnant women attending antenatal care (30.8%) than reproductive age women in the country. Despite the governmental investments on the provisioning of ART to pregnant women living with HIV in South Africa and other prevention strategies implemented across the country; about 12,000 new paediatric infections occurred in 2016 (UNAIDS, 2017). Based on this evidence, the South African government cannot rest on her laurels towards eliminating mother-to-child transmission of HIV. Therefore, the present study shares new data on the population-effectiveness of the newly implemented World Health Organization Option B Plus Strategy using maternal viral load and birth PCR as indicators.

Our study specifically evaluated the progress of the PMTCT programme in the resource constrained settings of the Eastern Cape, South Africa. The observed high rate of maternal viral suppression at delivery (82%) and significantly low early MTCT of HIV (1.3%) in this study clearly demonstrate the significant progress and population-effectiveness of WHO Option B+ in the study area. These important results support the clarion call to all African leaders to invest in universal access to lifelong ART for all HIV-infected pregnant women, irrespective of socio-economic class or geographical

locations (UNAIDS, 2011; 2016; WHO, 2013). Our findings in the Eastern Cape, characterised by a high rate of unemployment (StatSA, 2017), high antenatal HIV prevalence and poor PMTCT outcomes (Pattinson et al., 2014; UNAIDS, 2017), suggest that universal access to ART and viral load monitoring are the game-changers in the effort to eliminate new paediatric HIV infections.

This study adds to the body of evidence supporting viral suppression with ART being equal to significant risk reduction of HIV transmission. Beyond the reduction of 15-30% risks of in-utero or birth HIV transmission averted with ART in our study, further risks of MTCT attributable to intrapartum and breastfeeding would possibly be averted if the women could remain virally suppressed. Also, the potential prevention of the transmission to partners of these women supports the public health benefits of treating all individuals living with HIV. Evidence has conclusively shown that treating individuals infected with HIV to suppression will reduce the risks of transmission by about 96% (Baeten et al., 2012; Cohen et al., 2011; Das et al., 2010; Rodger et al., 2016). Hence, the 82% suppression rate in our study has the potential to translate to further prevention of HIV transmission to sexual partners and breastfed infants in the post-partum period.

This study uniquely combined patient interviews, routine medical records obtained accurately from the point-of-care and standardized laboratory data to generate new epidemiological data for future references on PMTCT in the region. Previous health facility and National Surveys on PMTCT outcomes in South Africa assessed the MTCT rates with the previous strategies implemented in the country (Akinsanya et al., 2017; Goga et al., 2012; 2016). These studies either reported six weeks (early MTCT) and/or the 18 months MTCT rates without examining the maternal viraemia. Hence, the endpoints were completely different from our study. A South African study reported the association between maternal viraemia and MTCT risks (Myer et al., 2017). Myer et al. (2017) reported a slightly higher maternal peripartum suppression rate of 91% but similar early MTCT rate of 1.3%. The difference in the maternal viral suppression might have been influenced by the serial monitoring and interventions offered to the pregnant women in the study (Myer et al., 2017). Our study, on the other hand, took a snap-shot of viral load at delivery in many centres in a resource constrained setting. Gaps often exist when viral loads are not monitored adequately or results of the viral load are not utilised promptly during the antenatal period to manage patients,

especially in resource constrained settings. Therefore, a point-of-care viral load test at delivery could be a game-changer in bridging these gaps and also, offer unique opportunity for interventions. In addition, this innovation will ensure that all pregnant women on ART have viral load results prior to leaving the health facilities after delivery.

The majority of studies examining the population impact of triple ART regimen (outside of South Africa) have focused on new paediatric HIV infections rather than combining maternal virological responses and MTCT outcomes (Okonji et al., 2012; Palombi et al., 2007; Warzawski et al., 2008). As such, it is challenging to compare the findings from the present study with previous studies, which adopted different methodologies, definitions of virological suppression and timing of early infant diagnosis. We found a high virological suppression rate (82%) at delivery among parturient women in the Eastern Cape, South Africa, a resource-poor setting. This fell short of the 90% target [(UNAIDS, 2016), which highlights the remaining gaps in the battle towards elimination of new paediatric HIV infections in the region. High maternal viral load (18%) is a concern knowing that a high proportion of the women in this study initiated exclusive breastfeeding. Therefore, parturient women with high peripartum viral load are at high risk for breastfeeding transmissions (Fowler et al., 2015; Okonji et al., 2012; Palombi et al., 2007). In order to ensure sustained viral suppression during pregnancy, labour, delivery and breastfeeding, the PMTCT directorate, programme managers and attending clinicians need to provide effective supervision at the health facility level. Future strategies should intensify surveillance of high risk pregnant women for prompt interventions.

Our study found that parturient women were more likely to have high peripartum viral load if they were younger than 25 years, had previously defaulted on ART, were not regular in picking up ART from clinics, unemployed and smoked cigarettes during pregnancy. Younger women were more likely to have lifestyle challenges (unmarried, alcohol use and cigarette smoking) which would affect adherence to their ART (Adeniyi et al., 2018; Mekuria et al., 2016; Mukuli et al., 2016). These constellations of socio-behavioural challenges affect adherence of ART during pregnancy and consequently, sub-optimal drug concentrations, further viral replications, high viral load and probable viral resistance occur (Hoffman et al., 2016; Maggiolo et al., 2017; Zeh et al., 2011). Previous studies have proven conclusively that a near perfect adherence is needed to ensure viral suppression (Myer et al., 2017; Palombi et al., 2007; Warszawski et al.,

2008). Clinicians should aim to achieve viral suppression during pregnancy by addressing all the socio-behavioural challenges that impact on ART adherence. This should be coupled with viral load monitoring and prompt action taken for those with virological failure. Most women entering maternal services will most likely be on ART in accordance with the test and treat strategy implemented in the country in 2016 (SADoH, 2016); hence, clinicians have a duty to check the viral load, optimise treatment and provide ongoing adherence counselling and support till delivery and during breastfeeding. In addition, clinicians should engage women living with HIV on their future pregnancy plans, family planning and contraceptive options which are freely available in the health facilities in South Africa. Routine screening of patients for socio-behavioural issues while on ART would further strengthen the PMTCT programme in the region.

Our study also demonstrated the importance of early initiation of ART in order to ensure durable viral suppression prior to delivery. Shorter duration on ART was associated with failure to achieve viral suppression. This result corroborates previous studies reporting the association between timing of ART and viral suppression (Mandelbrot et al., 2015; Myer et al., 2017; Warszawski et al., 2008). All the findings in this study demonstrate the importance of viral suppression or lack thereof, as the central pathway to achieving elimination of mother-to-child transmission of HIV. Therefore, appropriate strategies to ensure that all HIV-infected women commence ART and achieve suppression prior to and during pregnancy will be critical in the study settings in the Eastern Cape. Mandelbrot et al. (2015) reported that women who began ART pre-conception are more likely to achieve zero transmission to their infants. Clinicians should educate young women along this direction in planning for their future pregnancies.

Early mother-to-child transmission risks of 1.3% are a laudable achievement and justification for the investments of the South African government in universal ART. Previous studies from South Africa defined early infant diagnosis as positive PCR results obtained at six weeks or more (Akinsanya et al., 2017; Goga et al., 2012; 2016; Myer et al., 2017). This outcome measure has been shown to be influenced by the infant prophylaxis prior to drawing the blood for testing (Myer et al., 2017). Also, six week PCR results tend to include intrapartum and early breastfeeding transmissions. Since our data collection was conducted within 72 hours post-delivery, our result

reflects in-utero transmissions as defined in Bryson et.al. (1992). As such, there are limited studies with which to compare our findings, locally and internationally, despite the fact that a similar rate of 1.3% was reported by Myer et al. (2017). However, the actual impact of the timing of the PCR tests which differ between the two studies is difficult to quantify. Our findings in this study are comparable to reports from Europe and America as well as reports from clinical trials (Fowler et al., 2015; Kesho Bora Study Group, 2011; Mandelbrot et al., 2015; Shapiro et al., 2013; Warszawski et al., 2008). High maternal viral load at delivery is the most important determinant of early MTCT risks in this study. The early MTCT risks demonstrated a positive linear association along the gradient of maternal viral load; 0.5%, 0.6% and 3.3% in women with undetectable viral load, low level viraemia and high viral load, respectively. This result further confirmed that maternal viral load is the key driver for MTCT of HIV, which has been described in previous studies (Fowler et al., 2015; Kesho Bora Study Group, 2011; Mandelbrot et al., 2015; Myer et al., 2017; Shapiro et al., 2013; Warszawski et al., 2008).

3.7 Strength and Limitations

This multi-centre population-effectiveness survey reports on the outcomes of both maternal and infant health within the public sector PMTCT programme in the Eastern Cape, a province with limited resources in South Africa. The combination of numerous data sources laid credence to the validity of the data used in the study. The blood samples for the outcome measures were taken within 72 hours after delivery, thus, confirming that the results were not influenced by the intrapartum or immediate post-partum events. The lower limit of detection of viral load assay used in this study was less than 20 copies/ml. The clinical implication of this low level viraemia in MTCT risks requires further studies. In addition, the study provides reference epidemiological data for the Eastern Cape. The link between maternal viraemia to MTCT risks was elucidated in the study. Some of the women did not have viral load results (about 9% of the participants) especially the parturient women in the rural health facility where blood samples were not drawn prior to discharge. Also, blood samples that had clotted or insufficient were rejected by the laboratory.

3.8 Conclusions

High maternal virological suppression at delivery and very low mother-to-child transmission rate were achieved in our study setting— a resource-poor setting in South Africa. Younger women who are unemployed and smoked cigarette during pregnancy are more likely to have adherence challenges leading to high maternal viral load at delivery. In order to ensure sustained viral suppression during pregnancy, labour and delivery, PMTCT managers and attending clinicians need to provide effective supervision of care at the health facility level. Intervention strategies focusing on addressing maternal lifestyle behaviours and ART adherence challenges require targeted research and programmatic re-engineering. Also, retention in the care of lactating women will provide insightful understanding of viral load suppression and breastfeeding transmission in the context of lifelong ART. Future studies should explore the impact of implementing a point-of-care viral load test at delivery in the study setting.

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CHAPTER FOUR

HIV-1 Drug Resistance Surveillance among Parturient Women on Anti-retroviral Therapy in the Eastern Cape, South Africa: Implications for Elimination of Mother-To-Child Transmission

(Under review in Journal of Clinical Virology; Manuscript number: JCV-D-19-00062*)*

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Abstract

Background: Maternal anti-retroviral therapy (ART) is crucial for elimination of mother-to-child transmission (MTCT) of HIV. The emergence of HIV drug resistance poses a significant threat to achieving the goal of HIV-free generation and keeping mothers alive. In this study, we assessed the burden of HIV-1 drug resistance mutations (DRMs) within the context of the public sector prevention of mother-to-child transmission (PMTCT) programme in the Eastern Cape, South Africa. We also examined the potential transmissibility of mutant viruses within the cohort.

Methods: We conducted genetic analysis on viral isolates (n=80) from plasma samples of women with virological failure at delivery between January and May 2018 from two large maternity centres in the Eastern Cape using standard protocols. Partial pol gene sequences covering 1030bp of the gene were amplified and sequenced according to previously reported protocol. DRMs were determined by submitting the generated partial pol sequences to the Stanford drug resistance database for query on mutations associated with drug resistance in HIV viruses as well as to the IAS guidelines for DRMs interpretations. These curated algorithms provide an online software for determining genotypic resistance associated mutations in HIV pol sequences. We also examined the correlates of DRMs using bivariate analysis.

Results: The age of parturient women ranged from 16 – 43 years. The majority of the parturient women were in WHO clinical stage 1 (62.0%), currently on Efavirenz-based regimen (first line ART) (82.5%) and had been on ART for more than 12 months (65.0%). The prevalence of DRMs was 72.5% (n=58). The CD4 count demonstrated a negative linear association with the DRMs (p=0.002). Sub-type C accounted for nearly all the DRMs (98.3%) except one unique recombinant form (URF-C/D). The predominant DRMs were K103N (n=43; 74.1%), M184V (n=28; 48.3%) and K65R (n=11; 19%). Among the parturient women on current treatment of EFV-based regimen; 79.1% already had K103N while nine patients on protease inhibitor-based regimen still harboured K103N. Other mutations conferring resistance to NNRTIs include: V106M (15.5%) and P225H (17.2%). The majority of the M184V mutations were observed in parturient women on first line regimen (n=23; 82.1%). The mean

viral load (transmissibility risks) in DRMs was significantly higher than the wild type (174515 versus 52426).

Conclusion: We found a high prevalence of DRMs in women delivering their index babies at high viral loads in the Eastern Cape, South Africa. An effective surveillance system for tracking all pregnant women on ART will assist in identifying those with virological failure and drug resistance during antenatal, labour and delivery for prompt interventions. A nationally representative drug resistance surveillance in pregnant women should be undertaken to guide future policies and management guidelines in the country. Future studies should explore the possibility of designing a point-of-care reverse transcriptase-PCR for screening for common resistance mutations (K65R, K103N and Y188L) to guide appropriate neonatal prophylaxis and maternal therapy.

Key words: HIV-1 Drug resistance mutations; K103N mutations; M184V mutations, K65R mutations; Mother-To-Child Transmission, South Africa

4.1 Introduction

The Joint United Nations Programme on HIV and AIDS (UNAIDS) Super-Fast-Track Targets aim to eliminate mother-to-child transmission (MTCT) by reducing the number of new paediatric HIV infections to less than 20,000 per annum globally (UNAIDS, 2016). This UNAIDS' agenda aims to expand the Global Plan targets of a reduction of 60% in new paediatric HIV infections in 21 priority countries in sub-Saharan Africa (SSA) by the year 2015 (UNAIDS, 2014). Also, some countries – Cuba, Thailand, Armenia, Belarus, Armenia and Republic of Moldova received validation for eliminating MTCT of HIV (Ishikawa et al., 2016; WHO, 2016). These successes were achieved by expanding access to anti-retroviral therapy (ART) globally. Of interest is the remarkable progress in the 21 of the 22 priority countries, where access to ART increased from 36% in 2009 to 80% in 2015. So far, over 1.6 million new paediatric infections have been averted with ART worldwide since 1995 and 1.3 million of them were averted between 2010 – 2015 (UNAIDS, 2018).

Despite the successes in the efforts toward elimination of MTCT, about 180,000 (110,000 – 260,000) new paediatric infections still occurred in 2017 globally, predominantly in the sub-Saharan Africa (SSA) (UNAIDS, 2018). With the accelerated roll-out of ART at the population level especially in pregnant and breastfeeding women, evolution of HIV drug resistance becomes inevitable (Bhargava et al., 2014; Gregson et al., 2016; Gupta et al., 2018; Karade et al., 2017). It is crucial to ascertain the contribution of drug resistance mutations (DRMs) to ongoing MTCT in the SSA, home to the largest proportion of pregnant and breastfeeding women living with HIV.

Robust evidence from randomised controlled trials proved conclusively the efficacy of combination anti-retroviral therapy in the prevention of mother-to-child transmission (PMTCT) (Shapiro et al., 2013; Fowler et al., 2016). Findings from these studies informed the World Health Organization (WHO) recommendations (WHO, 2013; 2016) and many other PMTCT guidelines across the world (de Ruiter et al., 2014; Panel on Treatment of HIV-Infected Pregnant Women and Prevention of Perinatal Transmission, 2017; European AIDS Clinical Society, 2017; US Department of Health and Human Services, 2018). WHO Option B Plus recommends life-long cART to all HIV-infected pregnant and breastfeeding women irrespective of the CD4 count or clinical stage (WHO, 2016). Many countries in Africa have adopted this strategy in an effort to achieve the global target of eliminating mother-to-child transmission (MTCT)

(Namibian Ministry of Health, 2016; South African National Department of Health (SADoH), 2015; Ugandan Ministry of Health, 2016).

In South Africa, all pregnant and breastfeeding women arriving at health facilities are offered HIV testing and initiation of ART is given on the same day for those who test positive (SADoH, 2015). In addition, adherence counselling and support are offered as standard of care. The preferred first line cART in South Africa is a fixed dose combination of two nucleoside or nucleotide reverse transcriptase inhibitors (NRTIs) plus a non-nucleoside reverse transcriptase inhibitors (NNRTIs) (SADoH, 2015; WHO, 2013; 2016). The guideline is explicit on viral load monitoring to detect virologic failure defined as two viral loads > 1000 copies/mL taken a month apart after the initial three months exposure. Prior to switching to second line regimen, clinicians are expected to exclude drug interactions and toxicities, and intensify adherence counselling and support for at least a month.

In the event of virologic failure, a switch to second line ART consisting of ritonavir-boosted protease inhibitors (PIs) plus two nucleoside reverse transcriptase inhibitors is recommended (SADoH, 2015; WHO, 2013; 2016). This strategy aims to optimise the outcomes of second line ART prior to accumulation of drug resistance. Many studies support this algorithm-based approach for managing first line virological failure in the resource constrained settings (Kanters et al., 2017; Paton et al., 2014). Contrastingly, HIV-infected pregnant women and those in virologic failure in developed countries undergo a baseline genotypic resistance test in decision making for second line ART (de Ruiter et al., 2014; European AIDS Clinical Society, 2017; Panel on Treatment of HIV-Infected Pregnant Women and Prevention of Perinatal Transmission, 2017; US Department of Health and Human Services, 2018). Perhaps, this contributes to better PMTCT outcomes in those countries than in SSA.

The timing of suppression with ritonavir-boosted PIs especially in the presence of DRMS would be crucial for PMTCT. Virologic suppression in pregnant and breastfeeding women is crucial for elimination of MTCT. As long as pregnant women continue to have sub-optimal adherence to ART for numerous reasons (Adeniyi et al., 2018) and clinicians fail to intervene timeously (Barth et al., 2012; Johnston et al., 2013; Sigaloff et al., 2012) according to treatment guidelines (SADoH, 2015; WHO, 2013; 2015), HIV drug resistance could potentially threaten the goal of elimination of MTCT in South Africa and SSA.

Beyond the adverse effects of the ritonavir boosted PIs, which often worsen the adherence challenges (Adeniyi et al., 2018), accumulation of resistance mutations and treatment failure are inevitable (Johnston et al., 2013; Sigaloff et al., 2012). This could potentially render infant prophylaxis ineffective and thus, increase the risks of HIV transmission. Studies are therefore needed to gain understanding of the potential risks of transmission of resistant viral strains to the infants that are inadvertently exposed through their mothers during the pregnancy, labour and delivery, and breast feeding period. There is paucity of research studies on HIV drug resistance during pregnancy, labour and delivery, and the breastfeeding period especially in SSA.

Available data on HIV drug resistance mutations were obtained from the general population (Barth et al., 2012; Gupta et al., 2018; Sigaloff et al., 2012), but such data are rarely transferable to the pregnant and breastfeeding population. Data-driven evidence on drug resistance mutations is needed for this unique population because of the significant implications for MTCT. NNRTIs mutations tend to appear first by a change of a single amino acid; for example K103N, P225H and Y188L. In this way, they confer resistance to Efavirenz and Nevirapine, and to a lesser extent, Rilpivirine and Etravirine (Stanford University HIV Drug Resistance Database, 2018). These NNRTIs mutations are clinically relevant in the context of PMTCT as the majority of pregnant and breastfeeding women on ART in the SSA will commence Efavirenz-based regimen as the first line regimen (SADoH, 2015, Namibian Ministry of Health, 2016; Ugandan Ministry of Health, 2016). Also, Nevirapine is the cornerstone of infant prophylaxis in many parts of the world (SADoH, 2015; WHO, 2013; 2016; de Ruiters et al., 2014). Therefore, women on a failing Efavirenz-based cART, acquiring these NNRTIs mutations, are at greater risks of transmitting the mutant viral strains to their exposed-infants, irrespective of Nevirapine neonatal prophylaxis (Alvarez et al., 2016; Poppe et al., 2017; Karade et al., 2017).

Within the context of a failing first line-regimen in pregnancy and breastfeeding, parturient women could potentially develop major nucleoside reverse transcriptase inhibitors (NRTIs) mutations which are selected by Tenofovir, Abacavir, Zidovudine, Emtricitabine and Lamivudine. Though, the genetic barriers of some of these drugs differ, however, failure to identify and switch failing regimen would invariably lead to accumulation of resistance mutations and emergence of cross-resistance (Iweriebor et al., 2012; Paton et al., 2017; Gupta et al., 2018). This could then compromise future

therapy options for the pregnant women and prophylaxis for the exposed-infant. It is therefore crucial to investigate pertinent research questions within the South African PMTCT context:

(1) What proportions of pregnant women with virologic failure on current cART in the Eastern Cape have clinically relevant DRMs?
(2) What are the patterns of DRMs in a failing cART regimen in pregnant women in the Eastern Cape, South Africa?

This paper highlights new findings in the discourse of HIV drug resistance in the context of elimination of MTCT in resource limited settings. Findings on the frequency, patterns of resistance mutations and transmissibility risks of mutant viruses in parturient women with virologic failure might give insights into new strategies and innovations in the efforts towards eliminating MTCT.

4.2 Methods

4.2.1 Study design, Setting and Population

This DRMs Surveillance sub-study was nested within the larger East London Prospective Cohort Study (Adeniyi et al., 2018), a longitudinal cohort of HIV-infected pregnant women delivering at three of the largest maternity centres in the Amathole/Buffalo City districts of the Eastern Cape, South Africa. All pregnant women on cART attending the maternity centres for delivery of index babies at Frere and Cecilia Makiwane hospitals were included in this sub-study. Frere hospital is located in East London and provides tertiary care services while Cecilia Makiwane hospital is a regional academic centre which provides level one and two services for the entire Buffalo City Metropolitan and Amathole districts, boasting a combine population of about 1.7 million people (StatsSA, 2011). The study area is shown in the map of the central region of the Eastern Cape consisting of towns and communities in the Amathole and Buffalo City districts (Fig.4.1). The majority of pregnant women in the region access the maternity centres of these hospitals for their delivery irrespective of where they received antenatal care.

The antenatal prevalence of HIV in the central region is 30.2% (SADoH, 2015). All pregnant women receive HIV counselling and testing during their antenatal, delivery and postnatal period. Initiation of cART is fast tracked in this population with ongoing adherence counselling and support. Viral load monitoring is performed at three monthly intervals during the pregnancy and breastfeeding period to detect virological failure for immediate interventions. The clinicians (mostly nurses and the attending doctors) interpret the viral loads results based on the recommendations by the South African National Department of Health PMTCT Guideline (2015). Patients are offered interventions in accordance with the standard of care (SADoH, 2015); intensified counselling and adherence support, and/or switch of treatment to second line regimen. Viral load assays for the central region of the Eastern Cape are performed by the National Health Laboratory Services (NHLS), East London in accordance with the standard protocols. Turn-around time for viral loads ranges from two – seven days depending on the distance of the facilities to the NHLS, East London.

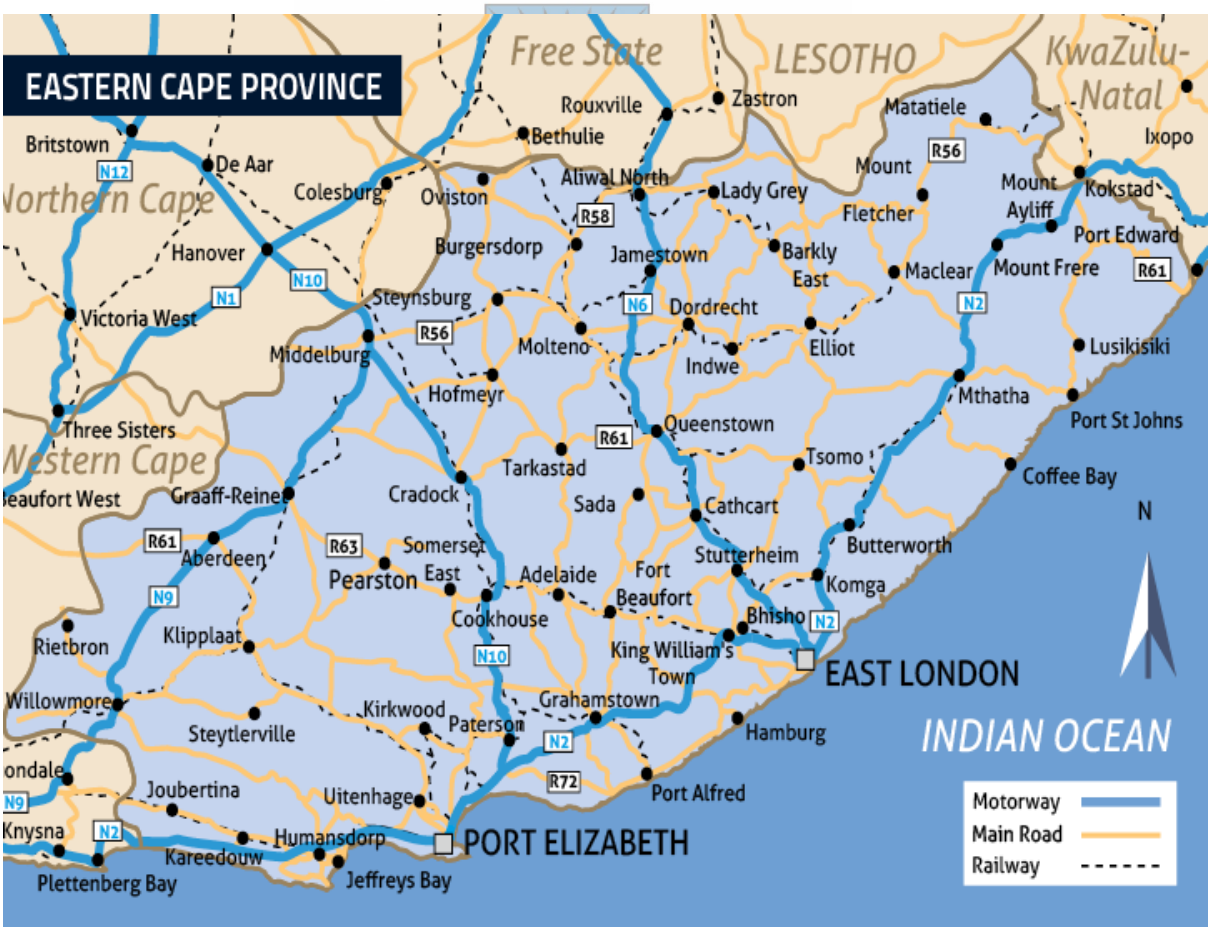

Fig. 4.1. Map of Eastern Cape Province showing the East London (Adapted from: https://www.google.com/search?q=amathole+district....)

Using the threshold HIV drug resistance surveys sampling recommendation (WHO, 2003), a minimum of 52 samples is required. Taking cognizance of insufficient samples and non-amplification, the sample number was increased to 102. Participants were included in this sub-study if they were older than 16 years, had been on cART for at least four months and the most recent viral load within two weeks of delivery (peri-partum viral load) was greater than 1000 copies/mL, considered as virologic failure. Relevant data on demographics (age, marital status, parity and employment status) were obtained through interviews.

We obtained information on the most recent CD4 count, current ART, prior switch of ART, total duration on ART including duration after switch of ART regimen. Self-report of adherence was categorized as good (if no missed pills/doses in the previous seven days), sub-optimal (if only one or two pills/doses were missed) and poor (if more than two pills/doses were missed). Each participant underwent clinical examination and the WHO Clinical Staging was documented. In total, 102 parturient women with virologic failure were recruited sequentially in both study sites to avoid bias selection. Venous blood samples (5 ml) were collected from each participant (N=102; Frere hospital cohort= 52 and Cecilia Makiwane hospital cohort= 50) into ethylenediaminetetraacetic acid (EDTA) vacutainer tubes between January to May 2018. Plasma was extracted and stored in RNase- and DNase-free tubes at -80^{0}C until use.

4.2.2. Viral RNA Purification and Reverse Transcriptase (RT)-PCR

The viral RNA was extracted and purified from the plasma samples using the QIAGEN Viral RNA Mini Kit (QIAGEN GmbH, Germany) in accordance with the manufacturer's instructions. Amplification of the polymerase gene was performed by using a one-tube RT-PCR followed by nested PCR in accordance with validated protocols (Bessong et al., 2005). In summary, a partial polymerase fragment of 1400 bp was generated using the following primer pairs: RT-RV, 50 -TAT TTC AGC TAT CAA GTC TTT GAT GGG TCA-30 and Pol1C 50 -GAA GGA CAC CAA TTG AAA GAC TGC AC-30.

For the RT-PCR step, 5'-CAA GGG GAG GCC AGG GAA TTT-30, and Pol2R, 50-TGA TGG GTC ATA ATA TAC TCC ATG-30 for the nested PCR. A 50μl of PCR mixture containing 5μl of RNA, 5μl of 10X buffer, 1μl each of the RT-RV and Pol 1C

primers (10 pmol/μl), 0.5 μl of 10mM dNTP mix, 0.25μl each of Taq polymerase enzyme (5U/μl), AMV RT (22U/μl), RNase inhibitor (40U/μl), 3μl of $MgCl_2$ (25 mM), and PCR-grade water was added to make up the final volume to generate the RT-PCR reaction. The thermal cycling conditions for the RT-PCR are as follows: 42^0C for 60 minutes, then an initial step of 95^0C for 3 minutes, followed by 30 cycles of 94^0C for 1 minute, 58^0C for 1 minute and 68^0C for 2 minutes, and a final extension time of 10 minutes at 68^0C.

We performed the nested reactions in 100μl reaction mixture with 5 μl of the first amplification product, 10 μl of 10X buffer, 2μl each of the second-round primers, 1 μl of 10mM dNTP mix, 0.5μl of Taq polymerase enzyme (5 U/μl), 6 μl of $MgCl_2$ and water to make the final volume. The thermal cycling conditions follow the same steps above except for the RT step. In order to detect contamination, a negative control was included in all PCR reactions. We confirmed PCR products for the expected band size (1084 base pairs) using 1% agarose gel electrophoresis followed by visualisation under ultraviolet transillumination.

4.2.3. Sequencing Analyses

The automated population-based sequencing was performed on both strands of viral DNA with the PCR nested primers using the dideoxynucleotide chain termination approach on an ABI PRISM 3130xl Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). Forward and reverse nucleotide sequences were assembled, edited and translated into predicted amino acid with the SeqMan Pro and Seqbuilder programmes included in the DNAStar software (DNASTART, INC, Madison, Wisconsin, USA).

4.2.4. Phylogenetic Analysis

HIV-1 subtyping for the obtained sequences was performed by using the REGA 4 HIV-1 Genotyping tool (de Oliveira et al., 2005). Phylogenetic analyses were conducted for 80 test isolates; protease (PR) and RT genes using neighbor-joining method included in the REGA 4 for sequences with unclassifiable subtypes. Reference sequences were obtained from the Los Alamos Sequence Database (http://hiv-web.lanl.gov) representing all HIV-1 subtypes: A-D, F-H, J and K.

4.2.5. Genotypic Drug Resistance Profiling

We performed analysis of the HIV drug resistance-associated mutations in 80 PR and RT genes by using the Stanford Genotypic Resistance interpretation algorithm (http://hivdb.stanford.edu/pages/algs/HIVdb.html). This program, which is based on subtype B consensus sequences, compares codons of query sequence with resistance-encoding nucleotides contained in the database. Using the Stanford HIV dbv7.0, HIV drug-associated resistance was categorised into five drug response levels based on net drug score: susceptible (≤9), potential low-level resistance (10-14), low-level resistance (15-30), intermediate resistance (31-59) and high level resistance (≥60) (Tang, Liu and Shafer, 2012).

4.2.6. Ethical Considerations

The DRMs Surveillance sub-study received ethical approval from the University of Fort Hare (Reference number: OBI021SADE01/2016) and Walter Sisulu University Ethics Committees (Reference number: 085/2017). The Eastern Cape Department of Health and the clinical governance of both hospitals granted permission for the implementation of this study. Participants received information on the purpose and process of the study in IsiXhosa or English depending on their preferences. Each participant provided written informed consent of her voluntary participation in the study. Participants younger than 18 years gave informed assent in the presence of a parent/guardian, who also provided written informed consent. Participants' rights to privacy and confidentiality were protected during and after the study.

4.2.7. Statistical Analyses

Analyses were limited to samples which were successfully amplified (n=80/102; 79.4%); 22 viral samples failed to amplify despite repeated attempts. We used descriptive statistics (mean, median and percentages) to describe the characteristics of the participants, frequency and patterns of resistance mutations. Using the bivariate analysis, we examined the associations between the resistance mutations and the clinical profile of participants (HIV sub-type, duration on ART and type of ART, CD4 count and viral load as well as demographic characteristics such as age, parity, marital status and employment status). We also examined the risk of transmissibility of the resistant mutants by comparing the mean viral load of the mutant viruses against one

another as well as the wild type virus. A p-value of <0.05 was considered statistically significant.

4.3 Results

4.3.1. Demographic and clinical characteristics of participants

The age of the parturient women ranged from 16 to 43 years with a mean (±standard deviation) of 30.2 (±6.2) years. The majority of the participants were single (86.3%), unemployed (78.5%), had a maximum of two children (62.4%), assessed as WHO Clinical Stage 1 (62.0%), currently on Efavirenz-based regimen (first line ART) (82.5%) and had been on ART for more than 12 months (65%) (Table 4.1). The median durations of treatment in those on first line and second line regimens were 21 months and 42 months, respectively. However, the median duration on second line regimen after switching therapy was 11 months. The mean CD4 count was 273 (interquartile range of 3 – 759) while viral load was 140941 (interquartile range of 1105 – 3320000).

Table 4.1. Clinical and Demographic Characteristics of the Participants

Variables	Frequency	Percentages
Age		
16-24	17	21.8
25-34	37	47.4
≥35	24	30.8
Duration on ART (months)		
≤6	16	20.0
7-12	12	15.0
13-24	12	15.0
≥25	40	50.0
Recent Viral Load		
1001-10000	18	22.5
10001-100000	43	53.8
>100000	19	23.8
Parity*		
1	30	39.0
2	18	23.4
3	23	29.9
4	6	7.8
Marital status		
Single	69	86.3
Married	11	13.8
Employment status		
Unemployed	62	78.5
Employed	15	19.0
Scholar	2	2.5
WHO Clinical Stage**		
I	44	62.0
II	11	15.5
III	14	19.7
IV	2	2.8
ART Regimen		
First Line	66	82.5
Second Line	14	17.5
Adherence***		
Complete	5	7.2
Sub-optimal	15	21.7
Poor	49	71.0
Recent CD4 Count****		
<200	23	34.3
200-349	24	35.8
350-499	14	20.9
≥500	6	9.0

*(Missing data were not documented); *Parity=3; **WHO Clinical Stage=9; ***Adherence=11; ****CD4 Count=13

4.3.2. Genetic Evidence for Drug Resistance in the Viral Isolates

Overall, HIV-1 drug resistance occurred in 58 (72.5%) viral sequences (Fig. 4.2). The prevalence of DRMs in parturient women on Efavirenz-based cART was 69.7% and protease inhibitors-based cART was 87.7%.

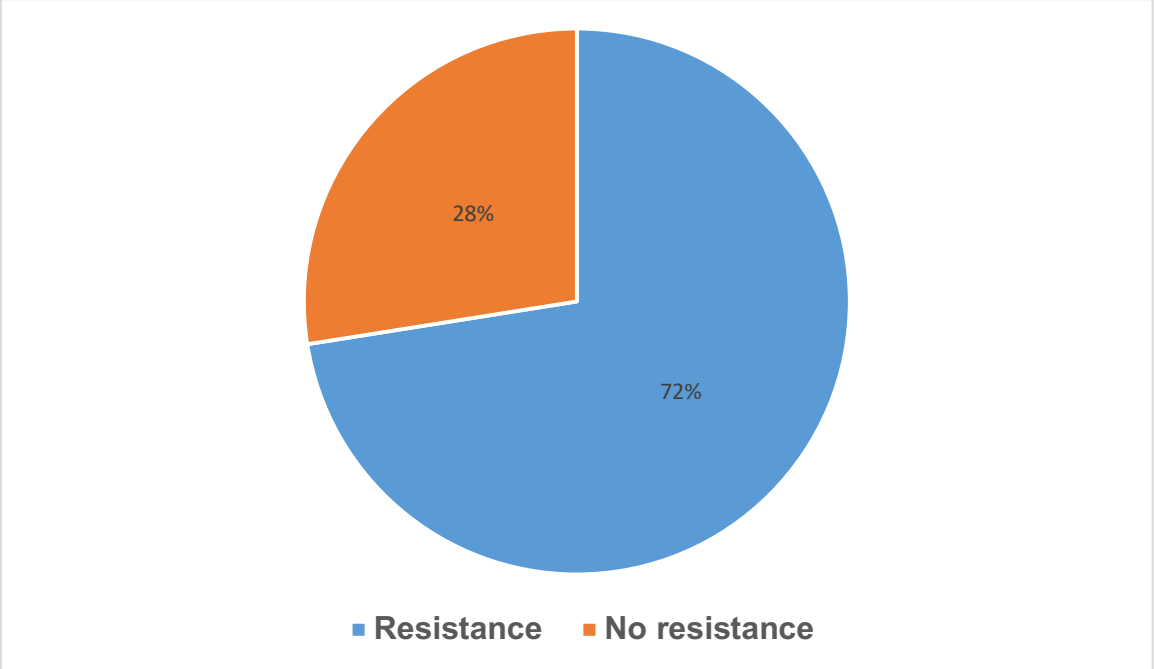

Fig. 4.2. Prevalence of HIV-1 Drug Resistance in the cohort

In the RT gene, the predominant mutation was K103N (n=43/58; 74.1%), which confers resistance to commonly used NNRTIs: Efavirenz and Nevirapine. The prevalence of K103N in participants who were still on first line regimen (TDF/FTC(3TC)/EFV) was 79.1% (n=34), while the prevalence in individuals already switched to second line regimen was 20.9% (n=9). The distribution of K103N in participants who were on second line regimen are: AZT/3TC/Lop/r= 6; ABC/3TC/Lop/r= 2 and ABC/3TC/ATZ/r= 1. Five of these participants on second line regimen had protease inhibitor-based regimen for about four months while the remaining four were exposed to PI-based regimen for 15, 17, 40 and 52 months, respectively. Other mutations conferring resistance to NNRTIs include: V106M (15.5%) and P225H (17.2%) (Table 4.2). A combination of multiple NNRTI-associated

resistance mutations conferring cross-resistance to Etravirine and Rilpivirine were also observed.

Major resistance mutations to NRTIs were observed in some of the viral isolates. M184V mutation conferring resistance to Lamivudine and Emtricitabine occurred in 28 (48.3%) of the participants. The majority of the M184V mutations were observed in parturient women on first line regimen (n=23; 82.1%). The remaining five participants had been switched to second line regimen, which still includes Lamivudine in the current regimen.

Also, K65R mutation conferring resistance to Tenofovir and Abacavir occurred in 11 participants (19%). The K65R occurred in 10 participants who were still on first line regimen (TDF/FTC/EFV) (90.9%) while the other participant had been switched to PI-based regimen (AZT/3TC/Lop/r) for 15 months at the time of the study.

In the PR gene, there were two major protease inhibitors-associated mutations; V82L and L90M found in the study. However, the mutations did not accumulate in any individual patient.

4.3.3. HIV-1 Sub-Type and Drug Resistance Mutations

Of all the viral isolates sequenced successfully in this study (N=80), sub-type C accounted for 97.5% (n=78) and the remaining two sub-types were: each of unique recombinant form (URF) C/D and circulating recombinant form (CRF02_AG). Sub-type C accounted for nearly all the DRMs (n=57; 98.3%). URF C/D harbors drug resistant mutations while CRF02_AG did not show any resistant mutation in its Pol gene.

Table 4.2. Drug Resistance Mutations in Viral Sequences

Drug Resistance Mutations	Frequency	Percentages (%)
Major NNRTI Mutations		
K103N	43	74.1
V106M	9	15.5
V108I	5	8.6
P225H	10	17.2
K101E	2	3.4
Y188L	4	6.9
Major NRTI Mutations		
M184V	28	48.3
K65R	11	19.0
K70R/E	4	6.9
K219Q	4	6.9
Major PI Mutations		
V82L	1	1.7
L90M	1	1.7

NNRTI=non-nucleoside reverse transcriptase inhibitor; NRTI=nucleoside reverse transcriptase inhibitor; PI=protease inhibitor

4.3.4. Dual and triple class failures

Dual class failures involving nucleoside and non-nucleoside reverse transcriptase inhibitors were mostly observed in some of the patients in the study.

AZT resistance: All the patients with intermediate (n=5) to high-level resistance (n=1) also harbored major NNRTI mutations; K103N and Y188L.

TDF resistance: High-level resistance occurred in 4 viral isolates, while 8 had intermediate level resistance and 6 had low level resistance. All the patients had major NNRTI mutations; K103N/R, Y188L, V108I and K103E.

Lopinavir (Atazanavir) resistance: Potentially low-level resistance occurred in 2 viral isolates. The patient with V82I mutation also had major NRTIs mutations (M184I and D67DN) and NNRTIs mutations (K103N and L100I). There was co-occurrence of L90M mutation with NRTIs mutations (K65R, M184V and K219KE) and NNRTIs (K103N, L100I and P225H).

4.3.5. Associations between HIV Drug Resistance and Baseline Characteristics

In the bivariate analysis, the resistance mutations did not demonstrate any significant associations with demographic parameters (age, parity, marital status and employment status) ($p>0.05$). However, the CD4 count demonstrated a negative linear association with the prevalence of DRMs ($p=0.002$). There is a high possibility of developing drug resistance with declining CD4 count. This association was not replicated by the WHO Clinical Stage, viral load, duration on cART and type of cART (Table 4.3).

Table 4.3. Bivariate Analysis of Clinical Correlates of HIV Drug Resistance

Variables	All	Resistance	No resistance	p-value
CD4 Count				
<200	23 (34.3)	23 (95.7)	1 (4.3)	0.002
200-349	24 (35.8)	13 (54.2)	11 (45.8)	
350-499	14 (20.9)	11 (78.6)	3 (21.4)	
≥500	6 (9.0)	2 (33.3)	4 (66.7)	
WHO Stage				
I	44 (62.0)	28 (63.6)	16 (36.4)	0.304
II	11 (15.5)	9 (81.8)	2 (18.2)	
III	14 (19.7)	12 (85.7)	2 (14.3)	
IV	2 (2.8)	1 (50.0)	1 (50.0)	
Current ART Regimen				
EFV-based regimen	66 (82.5)	46 (69.7)	20 (30.3)	0.189
PI-based regimen	14 (17.5)	12 (87.7)	2 (14.3)	
Duration on ART				
≤6	16 (20.0)	13 (81.3)	3 (18.8)	0.224
7-12	12 (15.0)	6 (50.0)	6 (50.0)	
13-24	12 (15.0)	10 (83.3)	2 (16.7)	
≥25	40 (50.0)	29 (72.5)	11 (27.5)	
Recent Viral Load				
1001-10000	18 (22.5)	14 (77.8)	4 (22.2)	0.097
10001-100000	43 (53.8)	27 (62.8)	16 (37.2)	
>100000	19 (23.8)	17 (89.5)	2 (10.5)	

ART=anti-retroviral therapy; WHO=World Health Organization; EFV=Efavirenz; PI=Protease inhibitors

4.3.6. Drug resistance mutations and individual ART

Though, there was a potentially low or low-level resistance to ATZ/r and LPV/r in two viral isolates, these level of resistance are not clinically relevant (Updated Stanford University HIV Drug Resistance Database, 2018). High level resistance was found against Zidovudine in only one parturient woman. Mutations conferring high level resistance to both Emtricitabine and Lamivudine were found in 36 viral isolates. High level resistance against Efavirenz and Nevirapine occurred in 56 and 57 viral isolates, respectively. Cross-resistance occurred within the NNRTI class; high level resistance against Rilpivirine (n=19) and Etravirine (n=5) were found in the study (Table 4.4).

Table 4.4. Drug resistance categories and ART

	High-level resistance	Intermediate resistance	Low level resistance	Potential low level resistance	Susceptible
*ATV/R Resistance (n=79)			1 (1.1)	1 (1.1)	77 (83.7)
DRV/R Resistance					80 (100.0)
LPV/R Resistance (n=80)			1 (1.3)	1 (1.3)	78 (97.5)
AZT Resistance (n=80)	1 (1.3)	5 (6.3)			74 (92.5)
D4T Resistance (79)	6 (7.6)	10 (12.7)	5 (6.3)		58 (73.4)
3 TC Resistance (n=80)	36 (45.0)				44 (55.0)
FTC Resistance (n=80)	36 (45.0)				44 (55.0)
ABC Resistance (n=80)	17 (21.3)	3 (3.8)	16 (20.0)	1 (1.3)	43 (53.8)
TDF Resistance (n=80)	4 (5.0)	8 (10.0)	6 (7.5)	1 (1.3)	61 (76.3)
EFV Resistance (n=80)	56 (70.0)	1 (1.3)		1 (1.3)	22 (27.5)
NVP Resistance (n=79)	57 (72.2)			1 (1.3)	21 (26.6)
RPV Resistance (n=80)	19 (23.8)	3 (3.8)	7 (8.8)	1 (1.3)	50 (62.5)
ETR Resistance (n=77)	5 (6.5)	10 (13.0)	2 (2.6)	12 (15.6)	48 (62.3)

ART=anti-retroviral therapy; ATV/R=Atazanavir/ritonavir; LOP/R=lopinavir/ritonavir; AZT=Zidovudine; D4T=Stavudine; 3TC=Lamivudine; FTC=Emtricitabine; ABC=Abacavir; TDF=Tenofovir disoproxil fumarate; EFV=Efavirenz; NVP=Nevirapine; RPV=Rilpivirine; ETR=Etravirine

4.3.7. Risks of transmission of resistance mutants

We examined the risk of vertical transmission of resistant viral strains using the mean viral load as the surrogate marker. There was a significant one-log difference in the mean viral load between resistant mutants and the wild type (174515 versus 52426). Similarly, there was a significant one-log difference in viral isolates with M184V mutations and those without the mutations (221326 versus 97656). However, there was no log difference in the mean viral load between resistant mutants with K103N mutation and those without the mutation (126144 versus 158136). Similarly, there was no log difference in the mean viral load between viral isolates with and without K65R mutations (278856 versus 118954).

By HIV sub-types: the mean viral load was highest in sub-type C (147438) in comparison with recombinant C/D (22585) and CRF02_AG (17700).

4.4. Discussions

In view of the accelerated scale-up of cART among HIV-infected pregnant and breastfeeding women in South Africa (SADoH, 2015), the emergence of HIV drug resistance mutations (DRMs) becomes inevitable (Bhargava et al., 2014; Gupta et al., 2018; Karade et al., 2018). The emergence of DRMs in the context of South African PMTCT programme could potentially become a serious public health concern in view of the high ante-natal seroprevalence of 30.8% and over 95% cART coverage in the country (SADoH, 2015; South African Global AIDS Response Report, 2015). Therefore, regional and national surveillance for DRMs among pregnant and breastfeeding women on cART would be crucial to inform future maternal therapy, neonatal prophylaxis and innovations to further mitigate the risks of MTCT of HIV. However, the extent of DRMs and its implications for elimination of MTCT in South Africa and other resource constraint settings have not been sufficiently investigated. Studies on DRMs within the context of WHO Option B Plus strategy in sub-Saharan Africa (SSA) are scarce. Available data from other African countries in the context of PMTCT are rarely generalizable because of the small sample sizes and different treatment practices (Machnowska et al., 2017; Poppe et al., 2017). It is very difficult to compare our results with other PMTCT-related studies in view of the variations in the

sensitivity assays of the sequencing analysis performed, sample sizes, study population and treatment practices.

The present study is the first from the Eastern Cape and South Africa to assess the prevalence, patterns and transmissibility risks of HIV DRMs from parturient women with peri-partum virological failure whilst on cART. This multi-centre HIV DRMs surveillance study utilised sequential selection of eligible parturient women in order to ensure unbiased sampling. This methodological approach followed the recommendations of the WHO (2003) guidelines for surveillance of HIV drug resistance and thus, validates the findings of the study. Though, the geographical sampling of parturient women in one region of the country might not be representative of the entire country, this study will stimulate interest in DRMs surveillance research in pregnant and breastfeeding population on cART. Indeed, the results will serve as reference data for future drug resistance surveillance in the region and South Africa.

4.4.1. Prevalence of DRMs in Parturient Women with Virologic Failure

Using the South African PMTCT guideline (2015) as criteria for defining virological failure in pregnant and breastfeeding women to select eligible participants, we found a high prevalence of DRMs of 72.5% in the cohort. The prevalence of DRMs in parturient women on Efavirenz-based cART was 69.7% and protease inhibitors-based cART was 87.7%. Though, it should be noted that the current cART in the cohort was predominantly Efavirenz-based regimen (82.5%), those on protease inhibitors-based regimen (17.5%) had been on first line regimen for about 31 months on average prior to the switch of regimen. It is interesting to note that none of these patients on current protease inhibitors-based regimen had developed clinically relevant resistance mutations to the drugs. However, they still harbor the reverse transcriptase resistance mutations despite having switched to second line regimen for about 11 months.

Our study provides new insights on the critical challenges posed by DRMs to the goal of elimination of MTCT. Women delivering their index pregnancy whilst on cART with high viral load would probably have acquired major resistance mutations to reverse transcriptase inhibitors. These DRMs might persist for prolonged period of time after switching to protease inhibitors-based regimen except adherence challenges are managed decisively prior to switching regimen and maintained throughout the

pregnancy and breastfeeding period. DRMs have great potentials to compromise the maternal cART, and increase the risks of maternal morbidity and mortality. In addition, it can potentially render neonatal prophylaxis ineffective and increase the risk of vertical transmission of resistant viral strains to the exposed infants (Alvarez et al., 2016; Poppe et al., 2017; Karade et al., 2018).

A recent Zambian study demonstrated a steady increase in the prevalence (from 21.5% to 40.2% in 2014) of DRMs in HIV-infected neonates whose mothers were on failing cART (Poppe et al., 2017). The study by Poppe et al. (2017) confirmed DRMs as an independent determinant of MTCT of HIV within the context of maternal life-long cART. Therefore, periodic surveillance for the emergence of DRMs in pregnant and breastfeeding women in the SSA region is imperative in the light of our findings. The genotypic resistance test is a useful investigation in decision making on the choice of an effective ART at baseline assessment or during switch of therapy (de Ruiter et al., 2014; European AIDS Clinical Society, 2017; US Department of Health and Human Services, 2018). This test could potentially be a game-changer in decision making on the most effective cART for HIV-infected neonates in the resource constrained settings if its cost becomes affordable.

4.4.2. Correlates of DRMs

We found a negative association between prevalence of DRMs and CD4 count of the patients. The prevalence of DRMs increased as the mean CD4 counts of the patients decreased. However, we did not found any significant associations between viral loads, WHO clinical stage and demographic characteristics of the patients with the emergence of DRMs. The lack of association between WHO clinical stage and the prevalence of DRMs is not surprising, given that clinical failure often lags behind both virologic and immunological failures (Misgena, 2011). It is also very clear from our results that DRMs are not associated with demographic profiles (age, parity, marital status and employment status). Therefore, clinicians should educate patients initiating cART about the relationship of adherence, drug resistance and decline in CD4 count, and the consequent risks of severe morbidity and mortality. We also observe a lack of association between DRMs and the types or duration of cART. This result should be

interpreted with caution in view of the eligibility criteria of the participants. Patients had been established on current cART for a prolonged period of time and had developed virologic failure.

4.4.3. HIV-1 Sub-types and DRMs

All the viral isolates with DRMs were HIV-1 sub-type C (98.3%), except one circulating recombinant CD. It should be noted that the predominant HIV-1 subtype in the study area had been sub-type C (97.5%) while recombinant C/D and CRF02_AG accounted for the remaining 2.5%. Previous studies in the general population had demonstrated that subtype C was and is the predominant circulating virus in South Africa (Nwobegahay, et al., 2011; Iweriebor, et al., 2012; Jacobs, et al., 2014; Wilkinson, et al., 2015). All these studies were conducted in other provinces rather than the Eastern Cape, home to 772,491 individuals living with HIV and accounting for 12.1% of the South African epidemic (SADoH, 2015).

There seems to be a consensus on the existence of naturally occurring polymorphisms within HIV-1 sub-types, which might impact on the response to cART and evolution of DRMs (Sunpath et al., 2012; Tang, Kanki and Shafer, 2012; Bansal et al., 2011; Kantor et al., 2006; Eshleman et al., 2005). HIV-1 sub-type C tend to develop DRMs more rapidly and frequently than other sub-types (Sunpath et al., 2012; Kantor et al., 2006; Eshleman et al., 2005). Though, we found three HIV-1 sub-types; C, CD and CRF02_AG in the Eastern Cape, we are cautious to compare the emergence of DRMs among the three sub-types because of the predominance of sub-type C in the region.

4.4.4. Non-Nucleoside Reverse Transcriptase Inhibitor-Associated Mutations

We found a predominant NNRTI-associated mutations: K103N, V106M, V108I, P225H, K101E, and Y188L in this study. These mutations confer varying degrees of resistance to Efavirenz and Nevirapine predominantly, and to a lesser extent, cross-resistance to Rilpivirine and Etravirine. Several non-polymorphic mutations (K103N, V106M and Y188L) tend to confer high-level resistance (30 – 50-fold) to Nevirapine and Efavirenz (Stanford University HIV Drug Resistance Database, 2018). However, P225H, an accessory mutation, confers high level resistance (>50-fold) to Nevirapine and Efavirenz only when it co-exists with a K103N mutation. Irrespective of the reverse transcriptase DRMs, evidence supports the practice of switching Efavirenz-based ART

to a protease inhibitors-based regimen (Paton et al., 2014; SADoH, 2015; WHO, 2016). However, it is worrisome that some pregnant women do not adhere to their cART (Adeniyi et al., 2018) and thus, increases the risk of emergence of NNRTI-associated DRMs which can be transmitted to their babies.

The high prevalence of K103N, Y188L and V106M mutations in parturient women poses serious threat to the PMTCT programme by rendering Nevirapine-based neonatal prophylaxis ineffective. Previous studies have confirmed the predominance of NNRTI-associated DRMs in HIV-infected infants whose mothers have failed Efavirenz-based ART (vertically transmitted NNRTI-associated mutations) (Kuhn et al., 2014; Poppe et al., 2017). Many national and international guidelines still recommend Nevirapine as neonatal prophylaxis within the context of maternal life-long ART (Namibian Ministry of Health and Social Services, 2016; SADoH, 2015; Uganda Ministry of Health, 2016; WHO, 2016). This is worrisome in view of the low genetic barrier of Nevirapine (Usach, Melis and Peris, 2013). Perhaps, the observed reduction in the rate of MTCT of HIV is attributable to the viral load suppression achieved by maternal ART rather than the Nevirapine prophylaxis. In the light of our results, supported by previous reports (Kuhn et al., 2014; Poppe et al., 2018), the rationale for the continued use of Nevirapine prophylaxis either alone or in combination with Zidovudine in HIV-exposed infants as prevention strategy needs further investigations. Recent data seems to support the use of Zidovudine prophylaxis alone without any significant difference in the rate of adverse events in HIV-exposed infants in comparison with Nevirapine prophylaxis (Powis et al., 2018).

Of significant interest is the persistence of the K103N mutations in parturient women after switching to protease inhibitors for a considerable period of 4 – 52 months. Failure to adequately manage adherence challenges prior to and after switching therapy is the plausible explanation for this result. Several reasons have been reported for the poor/sub-optimal adherence in the study setting (Adeniyi et al., 2018). This is coupled with health system failure to identify these high risk pregnant women prior to delivery. As such, archived resistant mutants continue to shed viruses into the circulation with resultant effect of high viral load and treatment failure. Ideally, no pregnant/breastfeeding woman should remain on a failing ART beyond one month after confirming virologic failure without prompt interventions (switch of regimen) in South Africa (SADoH, 2015). However, a complex interaction of patient and health

system factors often creates leakages in the cascade of care. This has thus led to some women remaining on a failing ART throughout their pregnancy, labour and breastfeeding period as seen in the study setting and elsewhere across SSA.

4.4.5. Nucleoside Reverse Transcriptase Inhibitors-Associated Mutations

We found several nucleoside reverse transcriptase inhibitors (NRTIs)-associated resistance mutations: M184V, K65R, K70R, K70E and K219Q in the study. M184V mutation was the predominant NRTI-associated DRM (48.3%) followed by K65R mutation (19%), classical thymidine analogue mutations (TAMs) (K70R and K219Q) and lastly, K70E. While many patients developed resistance to Abacavir (ABC) (21.3%), Lamivudine (3TC)/Emtricitabine (FTC) (45%) and Tenofovir disoproxil fumarate (TDF) (high level - 5%; intermediate – 10% and low level – 7.5%), Zidovudine (AZT) had the least resistance (high level – 1.3% and intermediate level – 6.3%). These results corroborate earlier reports by the EARNEST Study, which demonstrated that AZT is more durable than most of the NRTIs (Paton et al., 2017).

K65R mutation reduces susceptibility to TDF and Tenofovir alafenamide fumarate (TAF), ABC, Stavudine (D4T) and rarely, 3TC. However, evidence suggests that K65R increases susceptibility to AZT, except in the presence of Q151M, which rarely occurs together (Stanford University HIV Drug Resistance Database, 2018). Our result showed that Zidovudine is the least likely to develop high level resistance. As such, the recommendations of switching first line TDF-containing regimen to second line AZT-containing regimen (SADoH, 2013; 2015; WHO, 2013; 2016) is supported by our results. In addition, we found dual class failures involving mostly TDF and NNRTI-resistance, which further questions the rationale for Nevirapine prophylaxis in HIV-exposed neonates. Based on the evidence from our findings in this study and supported by previous studies (Paton et al., 2014; Kuhn et al., 2014; Poppe et al., 2017), Nevirapine may no longer have any role in neonatal prophylaxis especially, in the context of maternal virological failure. The two PIs-associated mutations in the viruses have no clinical relevance because multiple mutations are needed to compromise this class of drugs (Stanford University HIV Drug Resistance Database, 2018).

M184V mutation confers high level resistane to 3TC and FTC (100-fold), and low level resistance to ABC but increases susceptibility to AZT, D4T and TDF. It also impairs

the replicative capacity of the virus. The majority of patients with M184V mutation would have acquired major NNRTI-associated mutations due to the low genetic barriers of this class of drugs (Usach, Melis and Peris, 2013). Of interest is the presence of K70R in the cohort, which could potentially reduce the susceptibility of AZT and to a lesser extent, TDF and D4T. Also, K219Q could potentially reduce the susceptibility of AZT and D4T when present with other TAMs (Stanford University HIV Drug Resistance Database, 2018). However, we did not find the Q-complex (multi-nucleoside reverse transcriptase inhibitor) resistance associated mutations in the cohort.

4.4.6. Protease Inhibitors-Associated Mutations

There were two major protease inhibitors resistance-associated mutations; V82L and L90M found in different patients in the study. Protease inhibitors require gradual accumulation of mutations, leading to a progressive increase in the level of resistance (Molla et al., 1996). Also, the viruses isolates were still fully susceptible to Darunavir which is a component of salvage therapy in the South African cART guideline (SADoH, 2015). This is not surprising given that L90M does not affect the anti-viral activity of Darunavir and Tipranavir. However, the clinical significance of V82L is still unclear despite it reducing the anti-viral activity of Tipranavir (Stanford University HIV Drug Resistance Database, 2018). This confirms that the main reason for high viral loads in the patients on protease inhibitor-based regimen is sub-optimal adherence. It is therefore crucial for clinicians to screen for adherence challenges in patients with failing regimen and address them before switching to second line regimen. Adeniyi et al. (2018) reported several reasons why pregnant women in the study setting do not adhere to cART. This is particularly true for patients on second line regimen who may have to contend with the gastrointestinal adverse effects of Lopinavir in addition to nausea and vomiting associated with pregnancy.

4.4.7. Risks of Transmission of Resistant Viral Strains

Earlier studies have demonstrated a linear relationship between maternal load and risks of MTCT (Katzenstein et al., 1999; Garcia et al., 1999; Bailey et al., 1999; Warszawski et al., 2008); the higher the viral load, the higher the probability of MTCT and other modes of transmission. Using the mean viral load of the various HIV-1

subtypes as surrogate marker of transmissibility risks, HIV-1 subtype C had higher mean viral load in comparison with other subtypes. Thus, demonstrating greater risks of transmission of sub-type C. Similar reports have been documented extensively in the literature (Renjifo et al., 2004; John-Stewart et al., 2005; Brenner et al., 2006; Odaibo et al., 2006) and corroborate the predominance of sub-type C in the global HIV epidemic (Hemelaar et al., 2004; Buonaguro et al., 2007; Bhargava et al., 2014).

Also, we found higher mean viral load in individuals with DRMs in comparison with the wild type virus. Generally, resistant mutations tends to impair the replicative capacity of HIV, leading to reduction in the viral load (Buckheit, 2004; Machouf et al., 2006). However, it has been shown that the relationship between viral load and DRMs is non-linear. Machouf et al. (2006) described a V-shaped relationship between viral load and the number of DRMs. Viral load reduction occurs in patients with five or less number of DRMs while the viral load increases and become comparable to the wild type virus in patients with more than five DRMs. This is the plausible explanation for the result obtained in this study. This result has serious implications for the PMTCT programme in South Africa and all other resource constrained settings. As seen in the study setting, pregnant women on cART often come to deliver without any viral load or on a failing regimen for a prolonged period of time without any interventions. In fact, viral loads are performed in some instances without any interpretation and appropriate action taken on the results. The PMTCT directorate therefore needs to implement a monitoring system to track pregnant and breastfeeding women with high viral load for prompt interventions.

Contrary to previous studies (Wei et al., 2002; Deval et al., 2004), which showed a reduction in viral load in HIV strains with M184V and K70R mutations, we found a higher mean viral load in individuals with M184V mutations in comparison to those without this mutation. Also, there was no log-difference between the mean viral load in HIV strains harboring K65R and K103N, in comparison with those without these mutations. As previously explained, a heavily mutated virus (accumulation of DRMs) has the capability of overcoming the impairment in its replicative capacity (Machouf et al., 2006), thus, leading to a viral load comparable with the wild type virus.

4.4.8. Strength and Limitations of the Study

Though, this study provides a snapshot of the frequency, patterns and transmissibility risks of DRMs from women delivering babies at high viral loads in the Eastern Cape, a previously unexplored region of South Africa, the findings may not be representative of the entire Eastern Cape Province. We sequentially select women with virologic failure in order to prevent bias sampling. Notwithstanding that we selected the two largest maternity centres, serving the most populous, central region of the province for this study, a larger, multi-centre (across the entire province) HIV resistance surveillance study should follow to further elucidate our findings. In order to properly contextualize the findings of our study, perhaps, a baseline genotyping result would have shed light on the contribution of primary (transmitted) resistance to virologic failure in the cohort. As such, we were unable to quantify the risks of baseline DRMs to the PMTCT outcomes. Failure to use point-of-care non-B-sub-type resistance assays does not allow for immediate interventions based on DRMs results.

4.5. Conclusions

We confirmed a high prevalence of drug resistance mutations in women delivering their index babies at high viral loads in a resource constrained settings of the Eastern Cape, South Africa. Though, HIV-1 sub-type C is the predominant circulating virus in the region; thus, accounting for nearly all the drug resistance mutations. An effective surveillance system for tracking all pregnant women on cART will assist in identifying those with virologic failure and drug resistance during antenatal, labour and delivery for prompt interventions. A nationally representative drug resistance surveillance in pregnant women should be undertaken to guide future policies and management guidelines in the country. In addition, future studies should explore the possibility of designing a point-of-care reverse transcriptase-PCR for screening for common resistance mutations (K65R, K103N and Y188L) to guide appropriate neonatal prophylaxis and maternal therapy.

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CHAPTER FIVE

GENETIC CHARACTERIZATION OF THE NEAR FULL-LENGTH GENOME OF AN HIV-1 A1/C/D/K/B UNIQUE RECOMBINANT FORM FROM THE EASTERN CAPE, SOUTH AFRICA

(To be submitted for publication in AIDS Research and Human Retroviruses)

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Abstract

HIV-1 sub-type C is the predominant circulating virus in South Africa, a country with the world's largest HIV epidemic. There have been anecdotal reports of non-C subtypes of HIV-1 in different parts of the country in the past two decades. Though, Eastern Cape has the third largest HIV epidemic by province in South Africa, very little information exists on the genetic diversity of HIV in this understudied region of the country. This paper presents the genetic analysis of a mosaic recombinant variant of HIV-1 from Mdantsane, Eastern Cape, South Africa. Preliminary analysis of the partial *Pol* gene of the viral isolate showed inter-subtypes recombination of C and D, thus prompting a detailed analysis of the full-length genomic sequence which revealed a mosaic pattern comprising recombinant C/D (in the *pol* gene), recombinant A1/C/D (in the *gag* gene) and predominant B subtype in the *env-nef* gene. The accessory genes consist of: recombinant B/K in the Vpu gene and others subtypes: C (Vpr and Vif genes) and B (Nef gene). This is the first report of this mosaic pattern of HIV-1 in the Eastern Cape, South Africa. However, the clinical and epidemiological implications of this variant remains unclear.

Keywords: Near-full length genome**,** Unique Recombinant Form C/D, Eastern Cape Province, South Africa

5.1. Introduction

HIV is the worst epidemic to have ravaged the human species in the recent history. This RNA virus is characterised by extensive genetic diversity, leading to generation of variants that are distinct from one another (Bessong and Iweriebor, 2016; Hemelaar, 2011; Iweriebor et al., 2011; Ramirez et al., 2008; Taylor, 2008). Through the development of molecular biological tools, such as phylogenetic analyses, our knowledge of HIV has grown tremendously. Often, sub-genomic regions of viral isolates: *pol, gag and env* are analysed phylogenetically (Bessong and Iweriebor, 2016; Hemelaar, 2011; Iweriebor et al., 2011; Taylor, 2008). However, this approach could lead to missed information which can be detected in the full-length genomic sequence analysis (Hemelaar, 2011). Based on phylogenetic analysis, HIV is categorised into types, groups, subtypes (clades) and sub-subtypes. There are two HIV types: 1 and 2. So far, there are extensive literatures on HIV-1, which is responsible for the global HIV/AIDS epidemic while HIV-2 is found and remain confined to the West African region. HIV-1 is classified into four major groups: group M (Main), group O (outlier), group N (non-M/non-O) and group P (Abecasis et al., 2013; Hemelaar et al., 2011).

Group M, accounts for the majority of the global infections and can be genetically sub-divided into nine subtypes: A – D, F – H, J and K. Distinct genetic variability within the subtypes of about 15 – 20% (usually, genetic variability of 25 – 35% exists between subtypes), leads to sub-subtypes (Abecasis et al., 2013; Hemelaar et al., 2011). Sub-types A and F have been further sub-divided into sub-subtypes; A1 – A5 and F1 – F2 (http://www.hiv.lanl.gov). HIV-2 seems to be restricted to the West African region and has only two groups: A and B. HIV-1 group O is mainly found in Cameroon where it accounts for about 1% of the population living with the virus. Phylogenetic analysis of group O is yet to reveal distinct subtypes (Lihana et al., 2012). HIV-1 group N (non-M/non-O or new group) has been sequenced from humans from Cameroon. There are no subtypes described in the literature yet for HIV-1 group N. Group P was first described in 2009 and subsequently, in 2011 from Cameroonian descents (Abecasis et al., 2013).

Recombination of subtypes or sub-subtypes does occur in dually-infected individuals or individuals infected multiple times with viruses of two or more subtypes, leading to development of recombinant forms, with distinct characteristics (Hemelaar et al, 2011). Detection of identical recombinant virus from full-length genomic sequencing in at least three epidemiologically unlinked individuals is categorised as circulating recombinant form (CRF). When the recombinant form is restricted to a limited number of people, it is called unique recombinant form (URF). So far, there are at least 98 published HIV-1 CRFs and many URFs reported worldwide (http://www.hiv.lanl.gov). CRFs and URFs account for 20% of the global HIV epidemic, and thus, have become very important in the understanding of diversity of HIV (Hemelaar, 2011).

The global epidemic is driven by HIV-1 subtype C, which accounts for 50% of the world infections and 47% of new infections (Hemelaar, 2011; Lihana et al, 2012). HIV-1 subtype C predominates in the Southern African region and India. Recent data suggest an increase detection of subtype C in South America. HIV-1 subtype B accounts for 12% of the global HIV infections and is the predominant viral strain driving HIV epidemics in America and Europe. The Central African region HIV epidemic is unique in the heterogeneous nature of the viral strains without any subtype predominating in the region. Though, South Africa has the largest HIV epidemic, driven by subtype C, few anecdotal non-subtype C viral strains have been reported in different provinces in the country (Jacobs et al., 2008; Wilkinson and Engelbrecht, 2009; Papathanasopoulos et al., 2010; Iweriebor et al., 2011; Bessong and Iweriebor, 2016), albeit, no information exists about the Eastern Cape province, South Africa.

Molecular epidemiology of HIV in all the provinces in South Africa will advance knowledge on the circulating viral strains in the country. In addition, it is important to recognise new outbreaks and change in established epidemics in the various communities in any country. HIV diversity is also crucial for vaccine development, diagnostics, therapeutics and drug resistance (Lihana et al., 2012). There is no study on HIV diversity in the Eastern Cape, a previously unexplored province in South Africa. As such, this study presents the genetic characterization of the near full-length genome of a A1/C/D/K/B unique recombinant form from a pregnant woman in the Eastern Cape, South Africa.

5.2. Materials and Methods

5.2.1. Ethical Considerations

This study is a major component of the Drug Resistance Mutations Surveillance Study in the Eastern Cape, South Africa, which aimed at examining the patterns and frequency of clinically relevant mutations among pregnant women with virological failure. The study protocol received ethical approval from the Walter Sisulu University (Reference Number: 086/2017) and the University of Fort Hare Ethics Committees (Reference Number: OBI021SADE01/2016). The Chief Executive Officer of the Cecilia Makiwane hospital granted permission for implementation of the study protocol. All the participants in the larger study signed a written informed consent of their voluntary participation in the study before blood samples and demographic information were collected.

5.2.2. Patient information

Blood was drawn from a 29 year old para 3 woman (ADE/CMH/0032), single, unemployed resident of Mdantsane, Eastern Cape. She was diagnosed with HIV in 2013. She was initiated on Tenofovir disoproxil fumarate/Emtricitabine/Efavirenz in March 2013 and defaulted in September 2017. She was later re-initiated on the same ART regimen in November 2017. In May 2018 (after six months on the treatment), 5ml of venous blood was drawn within the context of a drug resistance mutations surveillance study. The CD4 count was 286 and the most recent viral load was 22585copies/mL. She admitted to poor adherence while on current treatment. The route of infection was heterosexual. Three millimetres of blood was placed in the EDTA tube and was centrifuged to generate the plasma. The plasma was stored at -80^{0}C and transported on dry ice from East London to the University of KwaZulu Natal, South Africa, where it was again stored at -80^{0}C until it was used for this study.

5.2.3. RNA and DNA Extraction

The preliminary study (Drug Resistance Mutations Surveillance Study in the Eastern Cape, South Africa) analysed the partial pol gene (protease and reverse transcriptase) sequence and revealed a sub-type assignment of recombinant C/D. This result prompted further investigation with the full-genome sequence in order to gain an

understanding of the full genomic sequence analysis of the viral isolate. RNA was extracted from the plasma sample using the QIAmp viral RNA mini kit (Qiagen, Valencia, CA, USA) in accordance with the manufacturer's instructions. Full protocol for RNA and DNA extraction was published previously by Rousseau et al. (2006).

5.2.4. Complementary DNA Synthesis

As previously described by Rousseau et al. (2006), a mixture containing 25μl of RNA, 1μl of 20mM dNTP and 2μl of 20mM oligo-dT was heated to 65^0C for 5 minutes. We combined 8μl of 5X buffer (250mM Tris-HCL [PH=8.3], 375 mM KCL, 15 mM $MgCl_2$ [Invitrogen, Carlsbad CA, USA]), 4μl of 100 mM DTT, 1μl of RNAse inhibitor (Invitrogen), 5μl of water and 1μl of Superscript III (200 U/μl; Invitrogen). The mixture was heated to 45^0C and added to the reaction. The reaction was then incubated at 45^0C for 90 minutes. We added 1μl of Superscript III to the reaction and incubated it for another 90 minutes. The reaction was then stopped by incubation at 70^0C for quarter of an hour. We added 1μl of RNAse H (2U/μl; Invitrogen) to the reaction and incubated further at 37^0C for 20 minutes. The final sample was stored at -20^0C until used.

5.2.5. Near-Full Length Genome PCR Amplification

The steps for synthesis of a near-full length genome from cDNA was described succinctly by Rousseau et al. (2006). Briefly, the sample was amplified in triplicate using nested PCR. In the first and second round reactions, a mixture of 5μl of 10X buffer 1 (Roche), 360μmol of dNTP, 15pmol of each primer, 1μl of cDNA template and 3.5Units Expand polymerase (Roche, Manheim, DE) in a reaction volume of 50μl was used for amplification. The first round primers were 1.U5Cc and 1.3'3'plCb; the second round primers were 2.U5Cd and 2.3'3'plCb (Table 5.1). We added the primers and nucleotides to ultra-thin-walled tubes (0.5ml tubes [GeneMate ISCBioexpress; Kaysville UT, USA), 96-well plates [IS 600, Island Scientific; Bainbridge, WA, USA] in a volume of 20 μl and kept on a cool plate (PCR-Cooler, Eppendorf; Hamburg, DE).

Thereafter, a single wax pellet (Ampliwax PCR Gem 50; Applied Biosystems, Foster City CA, USA) was added to the reaction and the tube was heated to 76^0C. Then, it

was cooled down on the cold plate to form a solidified wax barrier. The remaining reaction was added to each tube above the wax barrier. The thermal cycling reactions followed these steps: 94^0C for two minutes followed by 10 cycles of 94^0C for 10 seconds and 68^0C for 38 seconds, then 20 cycles of 94^0C for 10 seconds and 68^0C for 8 minutes 30 seconds with the cumulative addition of 20 seconds at 68^0C with each successive cycle, followed by a final extension of 20 minutes at 68^0C.

The PCR amplification process was performed in a RNAse-free environment; in a clean room, using sterile pippetes and materials. The cloned first round PCR product served as a positive control for the nested PCR. Spectrometry was then used to determine the copy number of the controls.

Table 5.1. Primer sequences used in the amplification process

Name	Position in HXB2	Primer sequence (5' – 3')
NFLG PCR Forward		
1.U5Cc 2.U5Cd	538-571(9623-9656) 519-551(9604-9636)	CCTTGAGTGCTCTAAGTAGTGTGTGCCCGTCTGT AGTAGTGTGTGCCCGTCTGTTGTGTGACTC
Reverse		
1.3'3'PICb 2.3'3'pICb	526–557 (9611–9642) 519–551 (9604–9636)	ACTACTTAGAGCACTCAAGGCAAGCTTTATTG TAGAGCACTCAAGGCAAGCTTTATTGAGGCTTA

(Source: Adapted from: Rousseau et al., 2006)

5.2.6. Sequencing of near-full length genome

The near full-length nested PCR product underwent purification first prior to sequencing with the use of the 454 Genome Sequencer FLX system using the steps described by Droege and Hill (2008). Subsequently, the sub-genomic regions were delineated by using the sequence locator software available in the HIV sequence database (http://www.hiv.lanl.gov/LOCATE/locate.html). Manual degaping was performed thereafter. The REGA HIV sub-typing tool version 3.0 was used to analyse the gag and pol sequences for recombination and circulating recombinant forms (Pena et al., 2012). This tool uses the phylogenetic and bootscanning techniques to assess the test sequence against a mirror of pure subtypes and CRFs (Bessong and Iweriebor, 2016). Recombinant breakpoints and sub-genomic subtype identity were confirmed by the REGA HIV-1 subtyping tool version 3.0 (www.bioafrica.net/subtypetool/html) and the jumping profile Hidden Markov Model (JPHMM) analysis (http://jphmm.gobics.de). Furthermore, phylogenetic analysis was conducted to assign subtypes to the near full length genome (Iweriebor et al., 2011).

Sequences were multiply aligned with Clustal X and phylogenetic trees generated by the neighbour-joining method. Using the pure subtypes A – D, F – H, J and K reference sequences contained in the Los Alamos database (http://hiv-web.lanl.gov). The complete protease, reverse transcriptase and integrase nucleotide sequences were submitted to the Stanford HIV Drug Resistance Interpretation Algorithm (http://hivdb.stanford.edu/pages/algs/HIVdb.html) for detection of mutations associated with drug resistance.

5.3. Results

The initial phylogenetic analysis of the *Pol* sequence [complete protease (PR) and the partial reverse transcriptase (1035 nucleotides)] indicated a subtype assignment of C and D. Also, the initial bootscanning analysis showed clustering of subtype C and D with several unclassified regions within the pol sequence of the virus. This observation prompted the full-length sequencing analysis. The near complete genome of sample ADE/CMH/0032 was successfully sequenced in order to elucidate the genetic composition of the virus.

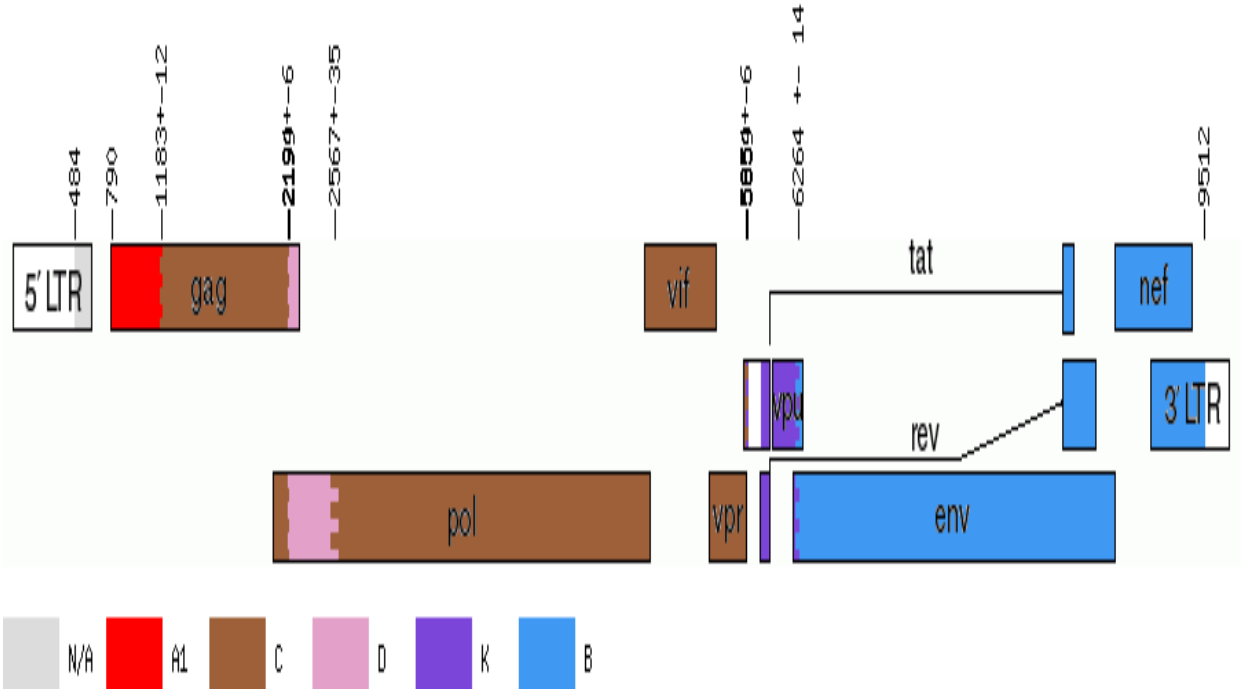
A near-full length genome of 8955 nucleotide long (position 790 – 9512 relative to HXB2 genome), and spanning across *gag* p17 to 3' LTR, was obtained. Jumping Profile Hidden Markov Model analysis showed that the sequence had six recombination breakpoints (Fig. 5.1). Sequence analysis of the viral structural proteins: gag, pol and env genes yielded different subtype assignments. Examination of the *gag* sequence indicated a subtype assignment of A1, C and D while the *pol* sequence indicated an alternating region of subtypes: C and D. However, the env sequence indicated a predominant subtype assignment of B and to a lesser extent, subtype K.

Examination of the viral regulatory proteins: tat and rev showed high level agreement in the subtype assignment. Both tat and rev showed subtype assignment of B and K. Analysis of the accessory gene regions (Vif, Vpr, Nef and Vpu) of ADE/CMH/0032 showed further variations in the subtype assignments of the viral sample. Both the Vif and Vpr showed subtype assignment of subtype C while Nef gene showed subtype B and Vpu gene showed a recombination of subtype B and K (Table 5.2).

Table 5.2. Subtype Assignment for ADE/CMH/0032

Complete Gene Region	JPHMM
Gag	A1/C/D
Protease	D
Reverse transcriptase	C/D
Integrase	C
Env	B
Vif	C
Vpu	B/K
Vpr	C
Tat	B
Rev	B
Nef	B

Genotypic analysis of the Pol gene sequence for drug resistance mutations using the Stanford HIV resistance database revealed no major resistance mutations in the protease and integrase region. However, the following resistance mutations were observed in the reverse transcriptase genes: D67wt/N, k70wt/R, L74I/wt, K103N, V108I, M184V, K219E and P225H. These mutations confer intermediate-level resistance to D4T and high-level resistance to: AZT, ABC, 3TC and FTC. However, low-level resistance to TDF was observed in the RT gene.



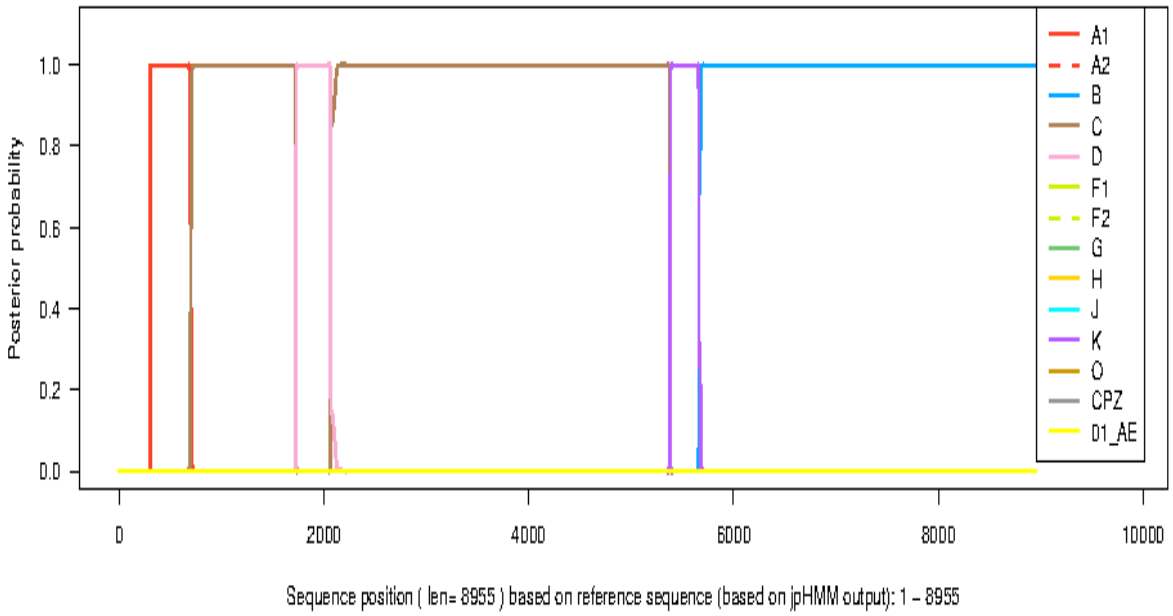


Fig. 5.1. Bootscanning analysis and representation of recombination breakpoints of ADE/CMH/0032 generated with JPHMM. Six recombination breakpoints are shown with numbers (relative to the HXB2 genome) identifying the breakpoints.

Phylogenetic analysis of the near full-length sequence saw the viral sample (ADE/CMH/0032) clustering with subtype C reference sequences with a bootstrap value of 43% (Fig. 5.2). However, it should be noted that this low bootstrap value is likely influenced by the fact that subtype C has a higher proportion of sequence than all the other subtypes in the recombinant virus.

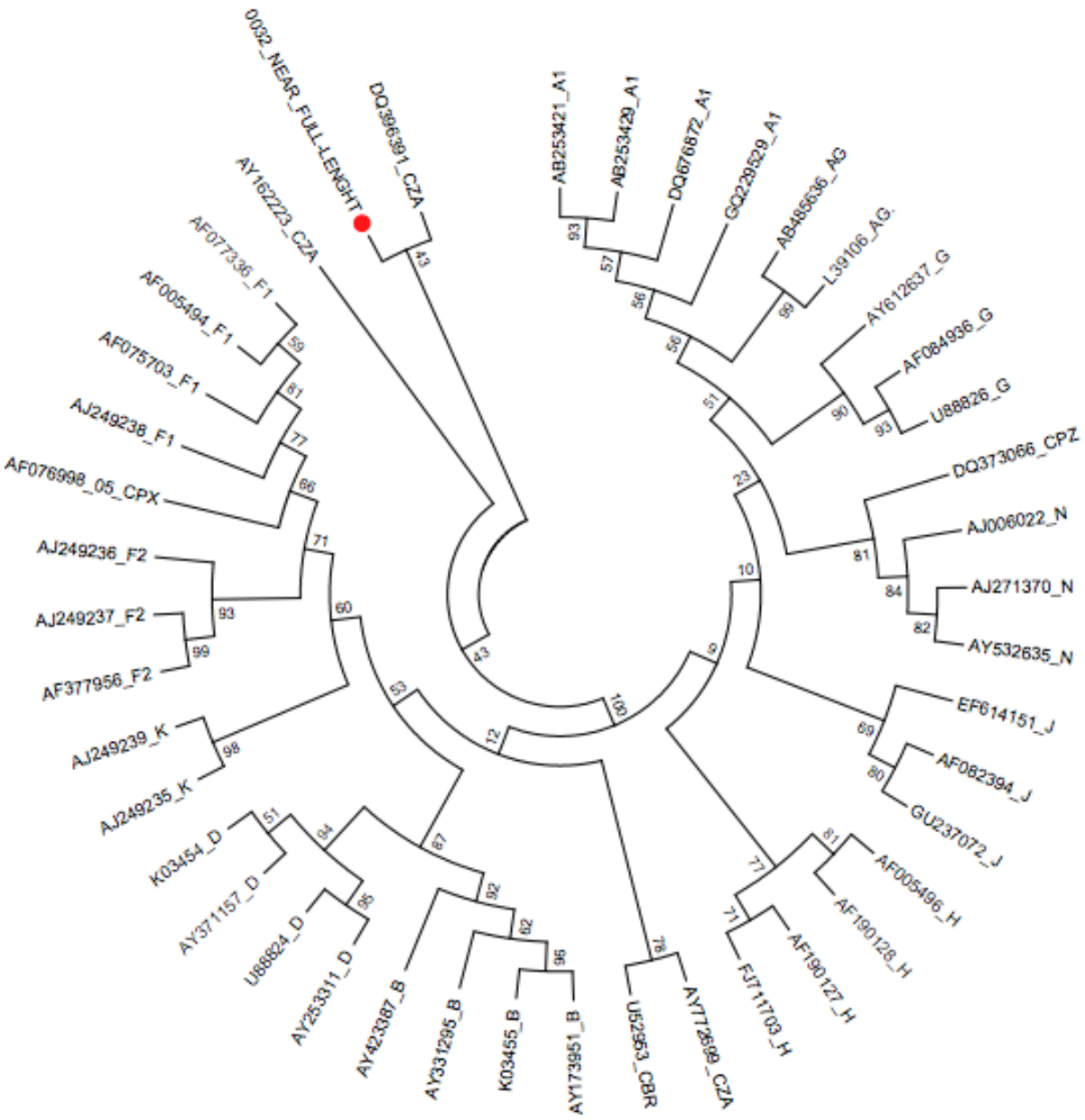


Fig. 5.2. Phylogenetic analysis of the near full-length sequence of ADE/CMH/0032. The test virus clustered with subtype C reference strain with a low bootstrap value of 43%

5.4. Discussions

The Eastern Cape Province has a HIV prevalence of 12.1% (with a total of 772 491 people living with HIV); highest prevalence is found between 15 – 49 years (19.6%) and contributes 16% of new HIV infections to the ongoing epidemic in the country (The Eastern Cape AIDS Council, 2017). Despite the contribution of the Eastern Cape to the overall HIV epidemic in South Africa, there is paucity of research reports on the

circulating viral strains in the province. For the first time, a near-full length genome sequence analysis of a recombinant strain A1/C/D/K/B identified from the sub-genomic analysis of the partial pol gene was conducted. The presence of some unclassified strains in the bootstrap analysis of the partial pol gene of the sample ADE/CMH/0032 prompted the investigation with near-full length genome sequence.

The variations in the recombination of the various subtypes heralded the report of a mosaic pattern rather than simply C/D recombination from the pol gene. This finding corroborated the superiority of the near-full length genomic sequence over the sub-genomic sequence analysis which tends to miss some important information (Bessong and Iweriebor, 2016; Iweriebor et al., 2011; Jacobs et al., 2014; Wilkinson et al., 2015). The finding of a mosaic viral strain which demonstrates variation in the structural gene regions; gag (recombinant A1/C/D); pol (recombinant C/D) and env (subtype B) is the first report of a unique recombinant form from South Africa. The viral strain also showed a predominant subtype B in the regulatory genes encoding Tat and Rev. In addition, the accessory genes are characterised by mosaic patterns: Vif (subtype C), Vpr (subtype C), Vpu (recombinant B/K) and Nef (subtype B). These variations at the sub-genomic regions support the recommendation that more than one sub-genomic region of the viral genome should be examined while investigating HIV diversity (Bessong and Iweriebor, 2016; Iweriebor et al., 2011; Jacobs et al., 2014; Williamson et al., 1995; Wilkinson et al., 2015).

The Bootscanning analysis of the near-full length genome with the Jumping Profile Hidden Markov Model showed an arrangement of six breaking points corresponding to the recombination alternating points among sub-subtype A1 and subtypes: B, C, D and K. This is the first report of this mosaic pattern in the Eastern Cape, and South Africa. Several studies in other provinces in South Africa have reported non-C subtypes, albeit, infrequently in Limpopo, KwaZulu Natal, Mpumalanga and Western Cape (Bessong and Iweriebor, 2016; Bredell et al., 2002; Engelbrecht et al., 1995; Iweriebor et al., 2011; Jacobs et al., 2014; Loxton et al., 2005; Msimanga, Vardas and Engelbrecht, 2015; Nwobegahay et al., 2011; Rousseau et al., 2006; Van Harmelem et al., 1997; Wilkinson et al., 2015). There are still many research gaps in the area of genetic diversity of the circulating HIV strains in various communities in the country. This data will be crucial for HIV diagnostics, therapeutics, vaccine development and

to monitor the emergence of new genetic variants at the population level in South Africa.

Of interest is the mechanism of acquiring this mosaic pattern by this 29 year old woman who had lived all her life in a rural township of Mdantsane, Eastern Cape. Though her CD4 count was 286 and viral load was 22585 copies/mL despite being on triple ART regimen since 2013, the impact of this mosaic pattern on the progression of the HIV disease and response to ART cannot be ascertained. Adherence to ART is crucial for suppression (Elul et al., 2013; Kalichman et al., 2014; Maggiolo et al., 2017), however, the patient confirmed defaulting ART for some time prior to re-initiation back on the same treatment she had defaulted. Also, the mode of transmission of the virus is believed to be heterosexual by virtue of the viral isolates being drawn from a parturient woman. Plausible explanation would include transmission of the mosaic pattern through sexual intercourse or the emergence of the mosaic pattern could have developed after several strains: A1, B, C, D and K had gained entry into HIV-infected host cells. The presence of sub-subtype A1 within the gag gene in a region where A1 is rare suggests the possibility of a mixed infection.

Also, the study used population-based (conventional) sequencing in analyzing the patient sample, thus, suggesting the possibility of the mosaic pattern being the majority virus in the patient. However, the presence of some of the viral strains identified in the various gene regions cannot exclude the possibility of these viruses being the minority variant. The process of recombination of C and D during the reverse transcription could have been possible due to the lack of a proof reading mechanism of the reverse transcriptase enzyme. The other strains (A1, B and K) could have been incorporated during the various steps of the replication cycle of the virus (Hoffman and Rockstroh, 2012, pg. 30). Also, the presence of subtype B within the genome of an heterosexual woman though first of its kind in the Eastern Cape, it must be stated that the HIV-1 subtype B strain has been reported among heterosexual women in other provinces in South Africa (Jacobs et al., 2014; Van Harmelem et al., 1995; Wilkinson et al., 2015).

Subtype B viral strain has been predominantly reported among men having sex with men in South Africa (Van Harmelem et al., 1995; Williamson et al., 1995). However, there have been anecdotal reports of subtype B among heterosexual men and women in the country (Jacobs et al., 2014; Nwobegahay et al., 2011; Wilkinson et al, 2015;

Van Harmelem et al., 1995). It is unclear if there were bisexual practices among the participants in these studies.

Despite the prolonged exposure of the patient to Tenofovir based regimen, there was no K65R mutation in the pol gene of the virus. The pattern of resistance mutations observed in this virus showed thymidine analog mutations (TAM-2 pathway: D67N; K219E), other NRTIs mutations (K70R; M184V; L74I/wt) and NNRTIs mutations (K103N; P225H). These mutations confer intermediate-level resistance to stavudine and high-level resistance to zidovudine, abacavir, lamivudine and emtricitabine. Though the exposure of the patient to thymidine analogue was not obtained from the medical records of this patient, its possibility cannot be excluded given the long years of the HIV infection. The current treatment with TDF/FTC/EFV for a prolonged period of time has led to the development of the emergence of resistance and treatment failure. K103N confers high level resistance to Efavirenz and Nevirapine because of their low genetic barrier. Also, M184V mutation confers high level resistance to Lamivudine and Emtricitabine. This mutation has been shown to impair the fitness of the virus (Hoffman and Rockstroh, 2012, pg. 31). The association of the viral subtype and drug resistance remains uncertain in view of the mosaic pattern and self-report of sub-optimal adherence of the patient.

This study concludes that several gene regions are indeed needed to gain full understanding of HIV diversity. In addition, a greater range of diversity was observed in the gag gene which showed inter-subtype recombination of A1, C and D as well as the accessory genes which showed heterogenous assignment of the virus to subtypes C (Vif and Vpr); B (Nef) and recombinant B/K (Vpu). The regulatory genes showed a homogenous assignment of the virus to subtype B (Tat and Rev). Though, this is the first report of a near-full length genomic sequence from the Eastern Cape showing a mosaic pattern of the viral isolate, the clinical and epidemiological implications of this viral variant remains unclear. More researches on the HIV diversity in the Eastern Cape communities should follow to gain a broader knowledge of the circulating viral strains in the province.

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CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

Table of content

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6.1. Introduction

This study sought to bridge specific research gaps in the context of mother-to-child transmission of HIV. This study documents the genetic diversity, resistance profile of HIV and risk assessment of mother-to-child transmission in pregnant women on anti-retroviral therapy in the Eastern Cape, South Africa. Findings from this study is expected to guide the department of health, programme managers and clinicians to improve the outcomes of care of pregnant women living with HIV in the country. Also, it is expected that the findings of the study will inform the formulation of treatment guidelines and prophylaxis in HIV-exposed infants. Above all, it is expected that this study will add to the body of knowledge on mother-to-child transmission and inform policy implementation on prevention of new paediatric HIV infections in the Eastern Cape Province, South Africa and sub-Saharan Africa.

6.2. Conclusions

The following conclusions are drawn from the study:

1). High maternal virologic suppression (82%) occurred at the time of delivery of index babies by pregnant women on anti-retroviral therapy in the Eastern Cape, South Africa.

2). A low rate of in-utero mother-to-child transmission (1.3%) was achieved in the Eastern Cape.

3.) Socio-behavioural factors such as younger age, unemployment and cigarette smoking predict high maternal viral load at delivery.

4.) Shorter duration on anti-retroviral therapy and adherence challenges are independent determinants of failure to achieve virologic suppression among pregnant women.

5.) Maternal peripartum viral load has a dose-response effect on the rate of mother-to-child transmission.

6.) Pregnant women with virologic failure at the time of delivering their index babies are more likely to have acquired clinically relevant resistance mutations (72.5%) within the viral genome.

7.) Pregnant women on a failing regimen would most likely harbour K103N mutation (74.1%), M184V (48.3%) and K65R mutation (19%).

8.) The viral mutants demonstrated significant transmissibility risks thus, confirming the potential risks to the exposed infants.

9.) Phylogenetic analysis of the partial pol gene sequence showed assignment of predominantly subtype C viral strain (98.3%; 78 of 80 viral isolates), and recombinants: CRF02_AG and C/D.

10.) Further analysis of the near-full length genome sequence of recombinant C/D revealed a mosaic pattern A1/C/D/K/B unique recombinant form.

6.3. Recommendations

a). For Pregnant women living with HIV

1). Detrimental lifestyle behaviour (smoking) that could negatively impact on adherence to ART should be stopped upon confirmation of the pregnancy.

2). Pregnant women should seek healthcare assistance with regards to their unsuppressed viral load.

b). For clinicians:

1). Against the backdrop of the findings on peripartum virologic suppression, intervention strategies focusing on lifestyle behaviours of pregnant women on anti-retroviral therapy and adherence challenges require targeted research. Clinicians should start screening all pregnant women on treatment for HIV for lifestyle behaviours which have been shown to be detrimental to their virologic suppression.

2). Parturient women who delivered their index pregnancy at high viral load should be targeted for intensified follow up after interventions have been given.

3). It is critical to ensure optimal follow up to determine the final mother-to-child transmission rate in the context of WHO Option B Plus strategy.

4). Clinicians managing pregnant women on anti-retroviral therapy should be pro-active in managing individuals with non-suppressed viral load in order to prevent the emergence of drug resistance mutations.

c). For Programme managers

1). Programme managers and clinicians should consider a point-of care viral load testing for immediate care of pregnant women during antenatal care and at delivery. This will potentially impact on strengthening services for these women and choice of prophylaxis to be offered to the exposed infants.

2). The HIV directorate should seek to monitor the final mother-to-child transmission rate in the context of WHO Option B Plus strategy at 18 – 24 months. This will provide overall impact of the implementation of the new strategy in the region.

3). On the basis of the high rate of drug resistance mutations in pregnant women with virologic failure, the PMTCT directorate of the National Department of Health of South Africa should be aware of the potential threats to the goal of elimination of mother-to-child transmission in the country.

4). Considering the findings on the phylogenetic analysis, it is recommended that future diagnostic, therapeutic and vaccine development for HIV in South Africa should incorporate the diversity of the circulating viral strains in the Eastern Cape.

5). Future studies should explore the possibility of designing a point-of-care reverse transcriptase-PCR for screening for common resistance mutations (K65R, M184V, K103N and Y188L) to guide appropriate neonatal prophylaxis and maternal therapy in the country.

RESEARCH OUTPUTS:

Publications and Conference presentations

MANUSCRIPTS FOR PUBLICATION

a). Manuscripts submitted for journal articles (under review/In press)

1. Adeniyi, O.V., Obi, C.L., Goon, D.T., Iweriebor, B.C., Selanto-Chairman, N., Carty, C., Gordana, A., Ajayi, A.I., Lambert, J.S. and Okoh, A.I., 2019. High Rate of Peripartum Virological Suppression and its Significant Implications for Elimination of Mother-To-Child Transmission of HIV in Resource-Constrained Settings of Eastern Cape, South Africa: Population Based Cohort Study. *Awaiting final editorial decision in BMJ Open.*

2. Adeniyi, O.V., Obi, C.L., Goon, D.T., Iweriebor, B.C., Lambert, J.S. and Okoh, A.I., 2019. HIV-1 Drug Resistance Surveillance among Parturient Women on Anti-retroviral Therapy in the Eastern Cape, South Africa: Implications for Elimination of Mother-To-Child Transmission. *Under review in Journal of Clinical Virology.*

b). Additional manuscripts to be developed from the thesis

3. Adeniyi, O.V., Obi, C.L., Goon, D.T., Iweriebor, B.C., Lambert, J.S. and Okoh, A.I., 2019. Genetic characterization of the near full-length genome of an HIV-1 A1/C/D/K/B unique recombinant form from the Eastern Cape, South Africa. *To be submitted for publication in AIDS Research and Human Retroviruses.*

4. Adeniyi, O.V., Obi, C.L., Goon, D.T., Iweriebor, B.C., Lambert, J.S. and Okoh, A.I., 2019. Update on the Molecular Epidemiology of HIV-1 Variants in South Africa: Two Decades in Review (1997 – 2017). *To be submitted to BMC Infectious diseases.*

CONFERENCE PRESENTATIONS

1.. Adeniyi, O.V., Obi, C.L., Goon, D.T., Iweriebor, B.C., Selanto-Chairman, N., Carty, C., Gordana, A., Ajayi, A.I., Lambert, J.S. and Okoh, A.I., 2018. Maternal and Foetal outcomes in the context of WHO Option B Plus strategy in South Africa. *Poster Presentation at the HIVR4P Conference, Madrid, Spain.*

2. Adeniyi, O.V., Selanto-Chairman, N., Goon, D.T., Lambert, J.S., 2017. Maternal and foetal outcomes in the context of WHO Option B Plus strategy in the Eastern Cape, South Africa. Findings from Prospective Cohort Study. Oral Presentation at the 8th SA AIDS Conference, Durban, South Africa.

APPENDICES


IN LUMINE TUO
VIDE BIMUS LUMEN
University of Fort Hare
Together in Excellence

Operational Definitions

In order to enhance the clarity of the thesis while reading, the following terminologies are defined below:

Phylogenetic analysis: This is conducted to establish the relationships between ancestral sequence and the descendant species. Each position in a genomic sequence can change independently from the other positions.

Genotypic resistance test: This involves the detection of specific genetic mutations in a patient's dominant viral isolate that are known to be associated with antiretroviral resistance. The PCR dideoxynucleotide sequencing is performed to amplify the reverse transcriptase and protease enzymes of the HIV virus.

Early MTCT: This is a measure of the effectiveness of the maternal ART in preventing MTCT of HIV. This is obtained by performing the HIV-specific qualitative DNA PCR test from the dried blood spots obtained from the heel of exposed infants immediately after delivery and corresponds to in-utero HIV transmission.

Virological suppression: This is the treatment target following ART initiation in an HIV-infected individual. The viral load is assayed from blood samples of individuals established on ART by performing the HIV-specific quantitative polymerase chain reaction (PCR) test result. The viral load is defined as suppressed if VL<1000 copies/ml. However, viral load below the limit of detection of laboratory assays is defined as undetectable viral load, which varies with the laboratory protocols.

Low level viraemia: This terminology has emerged in the literature and is defined as detectable viral load but less than 1000 copies/ml.

Probable virological failure: This is defined as VL ≥1000 copies/ml, a cut-off above which there is higher risk for MTCT. This diagnosis is made after a pregnant woman has been exposed to ART for a minimum of three months (NDOH, 2015) without any genotypic resistance test.

True virological failure: This describes the findings of clinically relevant mutations conferring resistance to the ART based on genotypic resistance test results.

University of Fort Hare
Together in Excellence

ETHICAL CLEARANCE CERTIFICATE
REC-270710-028-RA Level 01

Certificate Reference Number:	OBI021SADE01
Project title:	**Genetic diversity and resistance profile of HIV in peri-partum women; risk assessment of mother-to-child transmission in women on the highly active anti-retroviral therapy.**
Nature of Project:	PhD
Principal Researcher:	Oladele Vincent Adeniyi
Co-Investigator:	Prof A Okoh
Supervisor:	Prof L Obi

On behalf of the University of Fort Hare's Research Ethics Committee (UREC) I hereby give ethical approval in respect of the undertakings contained in the above-mentioned project and research instrument(s). Should any other instruments be used, these require separate authorization. The Researcher may therefore commence with the research as from the date of this certificate, using the reference number indicated above.

Please note that the UREC must be informed immediately of

- Any material change in the conditions or undertakings mentioned in the document
- Any material breaches of ethical undertakings or events that impact upon the ethical conduct of the research

FACULTY OF HEALTH SCIENCES
POSTGRADUATE EDUCATION, TRAINING, RESEARCH AND ETHICS UNIT

HUMAN RESEARCH COMMITTEE
CLEARANCE CERTIFICATE

PROTOCOL NUMBER	:	098/2014
PROJECT	:	PERI-PARTUM VIROLOGICAL SUPPRESSION RATE AND INCIDENT INFANT HIV INFECTION: IMPACT ASSESSMENT OF PMTCT PROGRAMME IN TWO DISTRICTS IN THE EASTERN CAPE PROVINCE
INVESTIGATOR(S)	:	OLADELE VINCENT ADENIYI
DEPARTMENT	:	OBSTETRICS & HIV UNIT
DATE CONSIDERED	:	04 MARCH 2015
DECISION OF THE COMMITTEE	:	APPROVED

N.B. You are required to provide the committee with a progress or outcome report of the research after every 6 months. The committee expects a report on any changes in the protocol as well as any untoward events that may occur at any time during the study as soon as they occur.

27/03/2015

Dr M J Ntsaba
CHAIRPERSON

Date

Walter Sisulu University
Academic Health Service Complex of the Eastern Cape
Postgraduate Education and Training
Faculty of Health Sciences
Walter Sisulu University
P/Bag X1, WSU, 5117, E.C.
Tel (047) 502 2100 / Fax (047) 502 2101

DECLARATION OF INVESTIGATOR(S)

(To be completed in duplicate and one copy returned to the Research Officer at Office L311, 3rd Floor, Old Library Building, NMD Campus, WSU)

I/We fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be contemplated from the research procedure as approved I/we undertake to resubmit the protocol to the Research Ethics Committee. I/We agree to a completion of a yearly progress report.

.....

.....

N.B. Please quote the protocol number in all enquiries.

Institutional Review Board (IRB) 00007448

HREC 1202009-020

FACULTY OF HEALTH SCIENCES
POSTGRADUATE EDUCATION, TRAINING, RESEARCH AND ETHICS UNIT

HUMAN RESEARCH COMMITTEE
CLEARANCE CERTIFICATE

PROTOCOL NUMBER	: 085/ 2017
PROJECT	: HIV RESISTANCE SURVEILLANCE IN PREGNANT AND POST-PARTUM WOMEN ON THE HIGHLY ACTIVE ANTI-RETROVIRAL THERAPY AND INFANT OUTCOMES IN BUFFALO CITY METRO/AMATHOLE DISTRICTS, SOUTH AFRICA (HVRS STUDY) - FOLLOW UP PROJECT
INVESTIGATOR(S)	: DR OLADELE VINCENT ADENIYI
DEPARTMENT	: FAMILY MEDICINE & RURAL HEALTH
DATE CONSIDERED	: 19 DECEMBER 2017
DECISION OF THE COMMITTEE	: APPROVED

N.B You are required to provide the committee with a progress or outcome report of the research after every 6 months. The committee expects a report on any changes in the protocol as well as any untoward events that may occur at any time during the study as soon as they occur.

DR Z VUNDLE
CHAIRPERSON

WALTER SISULU UNIVERSITY
ACADEMIC HEALTH SERVICE COMPLEX OF THE EASTERN CAPE
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19-12-2017
DATE

DECLARATION OF INVESTIGATOR(S)

(To be completed in duplicate and one copy returned to the Research Officer at Office L311, 3rd Floor, Old Library Building, NMD Campus, WSU)

I/We fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be contemplated from the research procedure as approved I/we undertake to resubmit the protocol to the Research Ethics Committee. I/We agree to a completion of a yearly progress report.

....

N. B. Please quote the protocol number in all enquiries.

Eastern Cape Department of Health

Enquiries:	Zonwabele Merlie	Tel No:	040 608 0830
Date:	18th June 2015	Fax No:	043 642 1409
e-mail address:	zonwabele.merlie@echealth.gov.za		

Dear Dr O Adeniyi and Dr N Selanto

Re: Peri-partum virological suppression rate and incident infant HIV infection: Impact assessment of PMTCT programme in Buffalo City Metro, Eastern Cape Province (EC_2015RP19_131)

The Department of Health would like to inform you that your application for conducting a research on the abovementioned topic has been approved based on the following conditions:

1. During your study, you will follow the submitted protocol with ethical approval and can only deviate from it after having a written approval from the Department of Health in writing.
2. You are advised to ensure, observe and respect the rights and culture of your research participants and maintain confidentiality of their identities and shall remove or not collect any information which can be used to link the participants.
3. The Department of Health expects you to provide a progress on your study every 3 months (from date you received this letter) in writing.
4. At the end of your study, you will be expected to send a full written report with your findings and implementable recommendations to the Epidemiological Research & Surveillance Management. You may be invited to the department to come and present your research findings with your implementable recommendations.
5. Your results on the Eastern Cape will not be presented anywhere unless you have shared them with the Department of Health as indicated above.

Your compliance in this regard will be highly appreciated.

SECRETARIAT: EASTERN CAPE HEALTH RESEARCH COMMITTEE

PMTCT RESEARCH DATA SHEET

NAME OF HOSPITAL:

IDENTIFICATION	FORM NUMBER	
	HOSPITAL NUMBER	

1. DEMOGRAPHY

- Age:

- Parity:

- Marital Status: Married-1 Single-2 Co-habiting-3 Divorce/separated-4

- Residence /location:

Rural - 1 Semi-urban - 2 Urban - 3

- Educational level: Illiterate - 1 Grade 1-6 - 2 Grade 7-12 - 3 Tertiary- 4

- Employment status: Unemployed-1 Employed-2

- Smoking status: smoked during pregnancy - 1 Quit smoking during pregnancy – 2 Never smoked – 3
- Alcohol use: Drank during pregnancy – 1 Stopped drinking during Pregnancy – 2 Never drank – 3

2. BOOKING SERVICES ASSESSMENT

- Gestational age at booking: ☐
- HIV status at booking: Positive-1 Negative-2 Unknown-3 ☐

Part A

- If positive, already on HAART at booking: Yes-1 No-2 ☐
- If yes, HAART Regimen: ☐
- Date of initiation: ☐
- Any change in Regimen: Yes-1 No-2 ☐
- Date of switch of regimen: ☐
- Indication for switch of regimen: ☐
- Viral load result: ☐

Part B

HIV positive, not on HAART

- CD4 count repeated: Yes-1 No-2
- Result of CD4 count:
- WHO Clinical stage:
- HAART initiated same day: Yes-1 No-2
- Which regimen:

Part C

If HIV status is unknown/ negative

HIV test done: Yes-1 No-2 Refused- 3

Result: Positive-1 Negative-2

- CD4 count result:
- If CD4 count < 100 cells/mm^3; Was CLAT done? Yes- 1 No-2
- If CLAT was positive: fluconazole treatment given: Yes-1 No-2
- HAART initiated: Yes-1 No-2

- Date of initiation:

Regimen given:

- If HAART is delayed: state reason(s) for delay: _____ _____ _____ _____

- Investigations done at booking:

- RPR:
- Hgb:
- Creatinine:
- CD4 count:

- Viral load:

- Weight at booking: Height:

3. ONE WEEK FOLLOW UP REVIEW: ASSESSMENT

- Did patient come for review of results at 1 week interval? ☐

  Yes-1 No-2

- If no, interval when patient came for review: ☐

- Any contra indication to FDC (TDF/FTC/EFV): indicate reason(s)

_____

_____

_____

_____

- Disclosure: patient disclosed to family members Yes-1 No-2 ☐

- What is the relationship with the person:

_____

_____

- Disclosed to sexual partner? Yes-1 ☐ No-2

4. MATERNAL HEALTH OUTCOME ASSESSMENT

- Adherence measures:

  - Self-reporting: Compliant – 1 Non-compliant – 2 ☐
  - On time pick up of HAART: Yes-1 No-2 ☐

(Please check antenatal clinic attendance to confirm the dates of pick up of ARVs)

- Defaulted ARVs: Yes-1 No-2 ☐

- Reasons for defaulting:
_____
_____
_____

- ARVs re-started ☐ date:

- Adverse effects experienced: indicate
_____
_____
_____
_____

- Co-morbid conditions/opportunistic infections treated during pregnancy:
_____
_____
_____
_____

- Any hospitalizations during pregnancy: Yes-1 ☐ No-2
- Length of hospitalization: ☐
- Diagnosis on admission: ☐

Other services provided during pregnancy:

- Pap smear: Yes-1 No-2 ☐ ☐
- Mantoux test: Yes-1 No-2

- INH prophylaxis: Yes-1 No-2 [ ]
- Bactrim prophylaxis: Yes-1 [ ] No-2 [ ]
- Any other prophylaxis: [ ]
- Any concurrent medications:

_____

_____

_____

5.LABOUR & DELIVERY CARE ASSESSMENT

- Gestational age at delivery: [ ]
- Mode of delivery: vaginal-1 [ ] c/s-2 [ ]
- Indication for c/s:

- If vaginal delivery:
- Epsiotomy was done: Yes-1 No-2
- Instrumental delivery was done: Yes-1 No-2
- Pre-labour rupture of membranes: Yes-1 No-2
- If yes, by how many hours:
- Total duration of labour:

Any other additional information about labour or delivery:

_____

_____

_____

_____

6. FETAL OUCOME ASSESSMENT

- Live fetus: Yes-1 No-2
- Still born: Yes-1 No-2
- Preterm baby: Yes-1 No-2
- Birth weight (kg)
- Head circomference (cm)
- Fetal length (cm)

Birth PCR done: yes-1 No-2

Result: Positive – 1 Negative 2 [ ]

Nevirapine initiation: yes-1 No-2 [ ]

- Was nevirapine given within 1 hour of delivery: Yes-1 [ ] No-2

- If answer is no any reason for delay:

_____
_____
_____
_____

- Interval of initiation of nevirapine:
- Congenital abnormality in fetus: Yes-1 No-2 [ ]
- If yes, indicate nature of abnormality:

_____
_____
_____

7.POST PARTUM CARE ASSESSMENT

- Contraceptive intervention: Yes-1 No-2 [ ]
- Indicate type:

_____

_____

- Haemoglobin: [ ]
- Viral load: [ ]
- CD4 count:
- Post partum intervention provided in respect of viral load result:

_____

_____

_____

_____

8. FEEDING OPTION FOR THE BABY

Exclusive breastfeeding – 1 Exclusive [ ] formula -2

Pasteurized milk – 3

Why did you choose that option of feeding?

_____

_____

_____

_____

9. FOLLOW-UP CARE PLAN ASSESSMENT

- Name of the nearest clinic for follow-up

- Follow-up reminder will be done via sms:
Cell number:

Alternative number:

Infant PCR at 10 weeks

Infant PCR at 12 months

HIV DRUG RESISTANCE AND SUB-TYPING REQUEST FORM

DR ADENIYI SAMRC/WSU HVRS STUDY

ALL RESULTS SHOULD BE COMPILED AND EMAILED TO:
vincoladele@gmail.com; +2779 311 0232; +2743 708 2127

CODE NUMBER:

PATIENT DEMOGRAPHICS

Requesting Clinic:	**CENTRE FOR SPECIAL CARE & RESEARCH, CMH**
Requesting Doctor:	**ADENIYI O.V.**
Collection Date:	
Collection Time:	
Tel No:	
Fax No:	

Patient code No	
DoB	
Tel Number:	
Grav	

E-mail:	

CLINICAL DATA

Last HIV VL(>1000) :		cp/MI	Date:	
Previous VL(>1000):		Cp/MI	Date:	
Last CD4 count:		cells/Ml	Date:	

IMPORTANT:
IT IS RECOMMENDED TO INCLUDE A RECENT VL RESULT (NOT OLDER THAN 2 MONTHS)

ARV L I S T S

Regimen 1 AZT ☐ ABC ☐ NVP ☐ SQV/r ☐ FPV/r ☐ Start Date: ☐

ddI ☐ TDF ☐ EFV ☐ IDV/r ☐ LPV/r ☐

D4t ☐ FTC ☐ ETR ☐ NFV/r ☐ ATV/r ☐ Stop Date: ☐

3TC ☐ TPV/r ☐ DRV/r ☐

Regimen 2 AZT ☐ ABC ☐ NVP ☐ SQV/r ☐ FPV/r ☐ Start Date: ☐

ddI ☐ TDF ☐ EFV ☐ IDV/r ☐ LPV/r ☐

D4t ☐ FTC ☐ ETR ☐ NFV/r ☐ ATV/r ☐ Stop Date: ☐

3TC ☐ ☐ TPV/r ☐ DRV/r ☐

Sample collection: 5ml whole blood must be collected in an EDTA anti-coagulant tube (purple top and inverted 8-10 times to mix. The blood must reach the lab within 6 hrs of collection to isolate the plasma and MUST be transported at 2-25 C. Note: Specimens with heparin are NOT suitable. If sending plasma, please freeze at -70 C and ship on dry ice.

Should you have further questions, do not hestate to contact the HUV Geno Dr . Kim Steegen at 011 489-8804 or kim.steegen@nhls.ac.za

Genotyping Laboratory between office hours (Monday to Friday :7:30am to 4:30 pm) at 011 489-8430 or contact the lab manager,

Lab Section

Received by:	
Date:	
Comments:	

PMTCT CONSENT FORM

I have read or had read to me in a language that I understand the above information before signing this consent form. The content and meaning of this information have been explained to me. I have been given opportunity to ask questions and am satisfied that they have been answered satisfactorily. I understand that if I do not participate it will not alter my management in any way. I hereby volunteer to take part in this study.

I have received a signed copy of this informed consent agreement.

.....	.....
Participant signature	Date
.....	.....
Investigator's signature	Date
.....	.....
Witness	Date

VERBAL PARTICIPANT INFORMED CONSENT (applicable when patients cannot read or write)

I, the undersigned,..... have read and have explained fully to the participant, the participant information leaflet, which has indicated the nature and purpose of the study in which I have asked her to participate. The explanation I have given has mentioned both the possible risks and benefits of the study. The participant indicated that she understands that she will be free to withdraw from the study at any time for any reason and without jeopardizing her position in the clinic. I hereby certify that the participant has agreed to participate.

.....	.....
Participants name	Signature & Date

Investigator's Name .....

Witness's Name .....

Signature.....

Date.....

(CONSENT FORM) IFOMU YOKUVUMA UKUTHABATHA INXAXHEBA KWINGQUBO YOKUKHUSELA UMNTWANA EKOSULELEKENI SISANDULELA NGCULAZA ESOSULELWA NGUNINA (PMTCT)

Ndiyifundile okanye ndizifundelwe ingcukacha ezikule fomu ngolwimi lwam endilivayo phambi kokuba ndityikitye ndacacelwa. Umxholo nokokuba ithetha ukuthini yonke lento ibhalwe kulefomu icacisiwe kum ndacacelwa. Ndilinikiwe ithuba lokuba ndibuze imibuzo yaye iphendulwe ngokundanelisayo yonke imibuzo yam. Ndiyaqonda yaye ndicacelwe ukuba ukungathabathi kwam inxaxheba koluphando akuyi kuthi kuphazamisane nendlela yokuphatha kwam nangoluphi na uhlobo. Ngako ke oko ndiyavuma ukuthabatha inxaxheba koluphando. Ndiyinikiwe eyam ifomu etyikityiwewo yokuvuma ukuthabatha ingxaxheba.

.....

Umtyikityo womthabathi nxaxheba Umhla

.....

Umtyikityo womphandi Umhla

.....

Umtyikityo wesizalwana Umhla

Ukuvuma ngomlomo ukuthabatha inxaxheba (Yenzelwe abathabathi nxaxheba abangakwaziyo ukufunda okanye ukubhala)

Mna, Ogama lingu,..... Ndimfundele yaye ndamcacisela Umthabathi nxaxheba ngokupheleleyo, iphepha elinengcukacha zophando elicacisa injongo nobume bophando endithe ndamcela ukuba athabathe inxaxheba kulo. Kuyo yonke ingcaciso endithe ndamnika yona ndizibalule nengozi eziqulathwe lupndo kunye namaqithiqithi oluphando. Umthabathi nxaxheba uthe wabonakalisa ukuyiqonda into yokuba unelungelo lokuba angarhoxa ekuthabatheni inxaxheba nanini na ethanda yaye futhi lonto ayizuchaphazela okanye iphazamisane nomsebenzi wakhe apha kwiziko lezempilo.

Ngako oko ndiyanqina ukuba umthabathi nxaxheba uzivumele enganyanzelwanga ukuthabatha inxaxheba.

.....

Umtyikityo womthabathi nxaxheba Umhla

Igama lomphandi .....

Igama lenqina .....

Umtyikityo.....

Umhla.....