SURVEILLANCE OF TRANSMITTED HIV DRUG RESISTANCE AMONG VOLUNTARY COUNSELING AND TESTING CENTERS IN GONDAR TOWN ETHIOPIA

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PREFACE

Ethiopia has been progressively expanding and intensifying the response to the HIV epidemic since enactment of the National HIV/AIDS Policy in 1998. In 2003, the Government of Ethiopia introduced the ART programme with the goal of reducing HIV-related morbidity and mortality, improving the quality of life of people living with HIV and mitigating some of the impact of the epidemic.

In Ethiopia ART was made available freely in 2005 and since then both the numbers of sites providing the service and patients receiving treatment have rapidly increasing. In June 2013, a total of 913 health facilities were providing ART while 308,860 patients were on treatment. With this rapid expansion of ART using the public health approach, emergence of HIVDR is an imminent threat for the national ART program. This is therefore, implementation of HIV drug resistance threshold surveys are of extreme relevance to the country as these may be used by decision makers for choice of therapeutic options, improvement of prevention and treatment programs and treatment monitoring strategies.

Thus, transmitted HIV drug resistance (TDR) survey was conducted from August 2011 to December 2012 among ART-naive adults who were seeking HIV diagnostic testing in VCT site in Gondar university hospital and Gondar Health center according to WHO recommendation. The results of this survey will therefore alerts national HIV program planners to identify possible sources of HIVDR transmission, through evaluation of prevention programs and intensification of HIVDR early warning indicators monitoring and adjustment of programmatic factors that lead to the emergence and spread of resistance to ART are needed.

EPHI will continue striving to produce such data for decision makers to improve HIV care and treatment program. As part of a future plan the TDR survey will be repeated at Gondar town in the same health facilities. TDR will also be conducted in Addis Ababa and on ANC clinics across the country to have national estimate of TDR.

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ACRONYMS

ABC	Abacavir
AIDS	Acquired ImmunoDeficiency Syndrome
ANC	AnteNatal Care
ATV/r	Atazanavir/ritonavir
AZT	Zidovudine
DNA	DeoxyriboNucleic Acid
DBS	Dried blood spot
DRAM	Drug Resistance Associated Mutation
EWI	Early Warning Indicator
EPHI	Ethiopia Public Health Institute
FDA	Food and Drug Administration
GFTAM	Global Fund to Fight AIDS, Tuberculosis and Malaria
HAART	Highly Active Antiretroviral Therapy
HIVDR	HIV Drug Resistance
HIV	Human Immunodeficiency Virus
LPV/r	Lopinavir/ritonavir
HAPCO	National HIV/AIDS Prevention and Control Council
NVP	Nevirapine
NNRTI	Non-Nucleoside Reverse Transcriptase Inhibitors
NRTI	Nucleoside Reverse-Transcriptase Inhibitors
PASER-M	Pharm Access African Studies to Evaluate Resistance Monitoring
PEPFAR	President Emergency Plans for AIDS Relief
PMTCT	Prevention of Mother to Child Transmission
PI	Protease Inhibitors
RT-PCR	Reverse Transcription Polymerase Chain Reaction
RNA	RiboNucleic Acid
TDF	Tenofovir
TDR	Transmitted Drug Resistance
VCT	Voluntary Counseling and Testing
WHO	World Health Organization

TABLE OF CONTENTS

ABSTRACT

Background: In Ethiopia, free access to ART has been expanded rapidly since 2005. With the rapid scale up and decentralization of the ART service, the emergency and transmission of HIV drug resistance (HIVDR) will be a major problem**.** In view of this, we evaluated the prevalence of transmitted HIVDR (TDR) in recently HIV-1 infected adults in Gondar town, Ethiopia using WHO threshold surveillance method.

Methods: A cross-sectional survey was conducted among antiretroviral-naive adults who were seeking HIV diagnostic testing in VCT site in Gondar university hospital and Gondar Health center, from August 2011 to December 2012 using the WHO recommended eligibility criterion. A total of 84 study participants fulfilling the inclusion criterion were consecutively enrolled, and blood specimen was collected. HIVDR genotyping was done using the in-house assay. Sequence was interpreted using the calibrated population resistance (CPR) tool of the Stanford university database according to the 2009 WHO surveillance drug resistance mutation (SDRM) list.

Result: Among the 84 participants, 70 (83.3%) were female and mean age was 21years (range: 18–24 years). Amplification and sequencing was successful for 67(79.8%) of specimens. Using the WHO-recommended truncated sequential sampling technique, among the first 47 sequenced specimens, 3 were found to harbor major HIVDR mutation associated to non-nucleoside reverse transcriptase inhibitor (NNRTI), suggesting moderate prevalence (5%–15%) of TDR in the study area. The mutation detected were K103N (n=2) and G190S (n=1). Including all sequenced sample in the analysis (n=67), 4(6%) were found to have major HIVDR mutations (K103N (n=2), G190S (n=1), and Y181Y (n=1)) associated to NNRTI. However, no nucleoside/tide reverse transcriptase inhibitor (NRTI) or protease inhibitor (PI) associated major HIVDR mutation were detected.

Conclusion: In comparison to previous studies done in the Gondar town, our result showed an increase in prevalence of TDR, which could be associated to the ART scale up. As the TDR HIV-1 may seriously affect the efficacy of first-line ART, the moderate levels of TDR observed in this study area indicate the need for continuous surveillance of TDR in Gondar town and in different region of Ethiopia to optimize treatment efficacy of the current ART and improve the drug resistance strategy within a country.

1. BACKGROUND

The Human Immunodeficiency Virus (HIV) induced acquired immunodeficiency syndrome (AIDS) pandemic, has been a major medical and public health problem globally. Since the start of the epidemic in 1981 around 78 million have become infected with HIV and an estimated 39 million people have died due to HIV/AIDS. At the end of 2013 an estimated 35 million people were living with HIV and 1.5 million HIV-related deaths has occurred [(1)].

Saharan Africa is the most affected region, with 24.7 million people living with HIV, were women accounts for 58% of the total number of people living with HIV in sub-Saharan Africa. In 2013, there were an estimated 1.5 million new HIV infections in sub-Saharan Africa. Eventhogh new HIV infections have declined by 33% from 2005 and 2013, Sub-Saharan Africa accounts for almost 70% of the global total of new HIV infections[(1)].

According to the EPP/Spectrum modelling in Ethiopia, there were an estimated 793,700 people living with HIV including 200,300 children in 2013. There were approximately 45,200 AIDS related deaths and about 898,400 AIDS orphans in the same year [(2).]

A first breakthrough in antiretroviral treatment (ART) was made in 1987 with the approval of zidovudine (AZT) by the US Food and Drug Administration (FDA). Treatment was initially started as mono-therapy, but was later replaced by combination therapy, which was proven to have higher clinical benefit [(3,4)]. Today the use of three or more antiretroviral (ARV) drugs from at least two of the classes is termed highly active antiretroviral treatment (HAART), is the recommended form of treatment. Since then treatment has further improved by the introduction of new regimens that are less toxic, have better efficacy (e.g. boosted Protease inhibitors (PIs)), and reduced pill burden [(5, 6).]

The introduction of HAART was the significant breakthrough in the battle against HIV/AIDS. Although antiretroviral drugs cannot eradicate HIV from infected cells, the therapy has resulted dramatic decline in morbidity and mortality associated with HIV, prolong survival, improve quality of life, restore and preserve immunologic function. Furthermore potent and durable viral

suppression decrease the number of viral copies and result in reduced probability of virus transmission, restricted viral evolution [7, 8].

The success of ART has motivated global political will to allocate antiretroviral drugs on the basis of need, irrespective of geographical location. The Global Fund to Fight AIDS, Tuberculosis and Malaria (GFTAM), the World Bank and the President Emergency Plans for AIDS Relief (PEPFAR) have developed drug financing policies favorable for ART provision in developing countries and provided significant financial support to build capacity for HIV/AIDS treatment and prevention, including provision and monitoring of ART [9, 10].

As a result, countries are expanding access to ART to people living with HIV/AIDS. At the end of 2013, 12.9 million people were receiving ART globally of which 11.7 million were in low- and middle-income countries, which represent 36% of the people living with HIV in low- and middle-income countries [10].

Even though it was lower than adults, the number of children younger than 15 years receiving ART in low- and middle-income countries have increased from 566 000 in 2011 to 762 921 in 2013. In 2013, an estimate of 1.3 million pregnant women living with HIV received ARV prophylaxis or treatment for prevention of mother to child transmission (PMTCT) (10).

The scaling up of ART has averted an averted 6.6 million AIDS-related deaths worldwide, including 5.4 million deaths in low- and middle-income countries in 1995-2012. AIDS-related deaths have fallen by 35% since 2005, when the highest number of deaths was recorded. In the past three years alone, AIDS-related deaths have fallen by 19%, which represents the largest decline in the past 10 years (10).

Furthermore the scaling up of ART is also contributing significantly to the ongoing drop in annual new HIV infections around the world, including among children. Expanding programmes for PMTCT and using more effective ARV regimens helped prevent more than 800 000 children from becoming newly infected between 2005 and the end of 2012(1).

1.1 ANTIRETROVIRAL THERAPY IN ETHIOPIA

Ethiopia has been progressively expanding and intensifying response to the epidemic since enactment of the National HIV/AIDS Policy in 1998. In 2001, the National HIV/AIDS

Prevention and Control Council (HAPCO) declared HIV a national emergency; this was followed by various interventions focusing on prevention, risk reduction, and behavioral change. In 2003, the Government of Ethiopia introduced its ART programme with the goal of reducing HIV-related morbidity and mortality, improving the quality of life of people living with HIV and mitigating some of the impact of the epidemic [(11-14).]

Free ARV treatment waslaunched in January 2005, which has given access to significant number of patients in a very short period of time. On the basis WHO recommendations, Ethiopia has implemented NNRTI based first line regimens for adults and protease inhibitor based second line regimens.The choice of ART combination was guidedby national treatment guideline and generally first line regimen consisted of two NRTI drugs, (tenofovir (TDF), lamivudine (3TC),zidovudine (AZT), abacavir (ABC) and one NNRTI, (nevirapine (NVP) or efavirenz). The second line therapy is based on protease inhibitor (atazanavir/ritonavir (ATV/r), lopinavir/ritonavir (LPV/r) along with two NRTI.

The number of eligible patients getting access to the antiretroviral drug is increasing and there is also an increasing number of ART centers across the country. Based on FHAPCO 2014 report, the total numberof facilities all over the country providing ART reached 913. According to this report, the total number of patients ever started ART was 439,301 and out of this 317,443 were receiving ART. Only 70.3% of individuals who ever started ART were currently on treatment indicating challenges in patients' retention [(2).]

Although HIV/AIDS-related mortality in sub-Saharan Africa has substantially fallen since the widespread distribution of ART, but this rapid scale-up of ART in is not without its challenges including inadequate capacity for treatment monitoring, limited options of ARV drug choices for those failing therapy, intermittent interruptions in drug supply, treatment adherence problems, and emerging HIV genetic diversity. These are factors that could fuel the emergence of HIV drug resistance [(15)].

Viral resistance is caused by mutation in the HIV genome coding for structural changes in the target protein that can affect the binding or activity of antiretroviral drugs. Because of the high mutation rate and high viral turnover of HIV and because of the lifelong treatment of the disease, it is expected that some degree of HIVDR will occur among persons in treatment even if appropriate regimens are provided and good adherence is supported [(16)]

HIV drug resistance can be acquired or primary. If HIVDR occurred before treatment in treatment-naïve persons, it is called primary resistance, or transmitted drug resistance (TDR), which might reflect direct infection from drug experienced individuals. When HIVDR develops in treatment-experienced persons, it is called acquired resistance. Transmission of drug-resistant HIV-1 variants from antiretroviral treatment-experienced persons has been documented to occur through multiple routes, including sexual intercourse, intravenous drug use and vertically from mother to child. Newly infected persons with transmitted drug resistance also act as a source for the onward transmission of resistant variants [(17)].

Both acquired and TDR are major public health concern. But TDR has the potential to more rapidly reverse the effectiveness of first line ARV at the population level especially when a standard regimen is used without any baseline monitoring of drug resistance [(18)],

Individuals with TDR start ART with a lower genetic barrier to resistance, a higher risk of virological failure and a higher risk of developing drug resistance, even to drugs in their regimen that were originally fully active [(19)]. Recent data suggest that up to 24% of patients receiving first-line ART in sub-Saharan Africa have virological failure within 12 months of initiation of first-line ART. Between 53% and 90% of these patients have viruses with clinically important HIV-1 drug resistance to NNRTIs and nucleoside reverse transcriptase inhibitors (NRTIs) [(20)] .Because the prevalence of transmitted resistance mutations can influence policy and guide treatment choices in the absence of individual resistance testing, the WHO has recommended the surveillance of drug resistance in areas where ART is being rapidly scaled-up in order to supply information that can help minimize the development and transmission of drug-resistant viruses and to assess the effectiveness of first-line regimens used in the area [(16)].

1.2 EPIDEMIOLOGY OF TRANSMITTED HIV DRUG RESISTANCE

In 1998, the first documented case of high-level resistance to zidovudine in a newly infected treatment-naive HIV patient was reported [(21)]. Since then, there have been numerous reports documenting transmitted resistance involving virtually all classes of approved antiretroviral drugs (NRTIs, NNRTIs and PIs), in developed countries, and the prevalence of these mutations have been steadily increase.

The prevalence of transmitted HIVDR depends on many factors, but one of the most important is ART use: that is, the extent to which ART is used in an area, how long it has been widely used and the numbers and percentages of those who are currently on a failing regimen.As the majority of ART patients are starting on highly potent regimens, the rise of drug resistance transmission is likely to be delayed in resource limited settings compared with resource-rich countries, where ART scale-up was initially implemented with resistance associated monotherapy and dual therapy. The effect of maintaining individuals on a failing regimen in the absence of viral load testing may contribute to increased transmission of resistant HIV strains as ART becomes more widespread [(22)].

Worldwide, the highest prevalence of resistance is observed in regions and populations with well-established use of antiretroviral therapy, including Western Europe, North America and regions of South America. In these settings, the use of mono and dual therapies in the pre-highly active antiretroviral therapy era, sequential functional monotherapy and the use of suboptimal regimens in the early HAART era and ongoing difficulties with adherence and tolerability have led to the accumulation of drug resistance in treatment-experienced patients and the subsequent spread of transmitted drug resistance [(23)].

The prevalence of transmitted drug-resistant HIV has been estimated to range from 2.1% to 51.5% in ART-naive recently and chronically infected patients across Europe in studies with varying time periods of study, populations, designs, methods, and definitions of resistance. Data suggest that 10–17% of ARV-naïve individuals treated in Australia, Japan, the United States of America and Europe are infected with virus resistant to at least one antiretroviral drug [(23)]. Recent stabilizing or declining levels of TDR in resource-rich countries are likely attributed to

declining incidence of acquired resistance, due to the use of more potent ART regimens, regimen individualization by use of pre-therapy resistance testing, and close viral load monitoring.

In middle and low-income countries, the levels of transmitted drug resistance are lower than in high-income countries, but this will likely change with time. There is evidence for a rapid increase in transmitted drug resistance in the years after rollout the rapid increase of availability of ART in middle- and low-income countries [(20)]. This is not surprising, because ART went from being virtually non-existent to being quite common in these regions. The prevalence of transmitted drug resistance in middle- and low-income countries is estimated to be around 7% [(23)].

In Africa, studies conducted during the early scale-up of ART reported low levels of TDR. Recently accruing evidence, however, points to increasing TDR among individuals with recent HIV infection in East Africa. A recent WHO HIV drug resistance report 2012 summarized the results from 72 surveys of transmitted drug resistance in 26 countries between 2004 and 2010. While the 72% (n=52) of surveys found a low prevalence (< 5%) of drug resistance-associated mutations in all three drug classes, none had reported high resistance (prevalence of resistance >15%). In this report it has been shown there was an increase over time in the percentage of surveys reporting moderate resistance in the globe from 18% in the period 2004–2006 to 32% in 2007–2010. Similarly, there was a significant increase in the percentages of surveys reporting moderate resistance in Africa from 17.6% in 2004–2006 to 40.7 in 2007–2010 [(23).]

There is a significant difference in the prevalence of resistance between regions where the study was done. Overall, 65% (13 of 20) of the surveys showing a moderate prevalence of TDR to any drug class were in the African Region, particularly in eastern Africa (30%, 6 of 20). Five (25%, 5 of 20) were conducted in the Western Pacific Region, one in Latin America and the Caribbean and another in the European Region. No survey from the South-East Asia Region showed resistance between 5% and 15% [(23)].

There was also evidence of an increasing trend in NNRTI resistance in Africa (43 surveys in 18 countries), while no significant trend was found in any other region for any drug class. Areas surveyed varied considerably over time, even within the same country, so caution is needed when extrapolating these results beyond the areas surveyed.

A separate review of studies specifically from South Africa, home to the largest ART program in the world, found no evidence of an increase in transmitted drug resistance between 2002 and 2010, and apart from 2002, the level was below 5% [24] . However, one study in the KwaZulu-Natal region of South Africa has reported approximately 6% of naive patients with Drug resistance associated mutation (DRAM) [25].

1.3 HIV DRUG RESISTANCE IN ETHIOPIA

In Ethiopia, HIV drug resistance threshold survey was conducted in 2005, from ANC clinics with the HIV sero-surveys conducted in the capital city, Addis Ababa, where ART was first started. Specimens were subjected to multiple transportation and freeze-thaw cycles (genotyping was conducted outside of Ethiopia, Central Virology Laboratory, Sheba hospital, Ministry of Health, Tel-Hashomer, Israel) which might probably account for the low amplification rate (52%). This study has revealed no major drug resistance for all available HIV drug classes [26].

In 2003, another HIV drug resistance study was conducted among 92 TB/HIV co-infected patient in Gondar town, Northern Ethiopia. Although the study did not follow the WHO threshold survey method, the result of the study has demonstrated the prevalence of HIV drug resistance to be 3.3%. This study indicated the presence resistance conferring mutation to reverse transcriptase inhibitors (V75I) in one person (1.1%) and G190A mutation which confers resistance to non-nucleoside reverse transcriptase inhibitors in two persons (2.2%) and also other minor mutations for protease inhibitors [27].

Another study done in Gondar University by Mulu et al in 2008/09, among 155 treatments naive patient reported HIVDR 12.9% (20/155) of antiretroviral drug resistance mutation using the IAS HIV DR algorithm. Major NRTI and NNRTI resistance mutations were detected in 2.6% (n=4) and 10.3% (n=16), respectively. But using the Stanford University HIV DR algorithm, antiretroviral drug resistance mutations were detected in 5.2% (8/155) of the patients. The HIVDR mutation detected in this study for NRTI (n=4) were, K219E (n=1), L210W (n=2), K65R (n=1) and for NNRTI Y188HY (n=1), K101E (n=1), G190A (n=1) and G190A (n=2). There was no major drug resistance mutation in protease region in both algorithms. Simultaneous

resistance to both NRTI and NNRTI was not observed. However, high rate of natural polymorphisms at RT &PR regions were reported by the study [28].

Another prospective cohort study was done among 265 ART-naive patient initiating ART in Jimma, Ethiopia. Virological failure was observed among 14 (5.3%) participants after 6 months of follow up , and genotyping of corresponding baseline samples of 12 participant with virological failure revealed 6 (50%) had HIV drug resistance mutation at baseline[29] .

JUSTIFICATION OF THE STUDY

Scale-up of antiretroviral therapy in low- and middle-income countries has been achieved by using a public health approach that involved national standard regimens and clinical monitoring in settings where laboratory infrastructure was not available. One concern with this simplified approach, often involving switching regimens based on clinical or immunological criteria only, strategy potentially allows for long periods of unrecognized viral failure, and the potential accumulation of resistant mutations in patients where ART is not fully controlling viral replication, which could lead to extensive transmission of drug-resistant HIV strains, thus potentially compromising the future efficacy of first-line regimens. Individualized resistance testing is routinely used in high-income countries prior to ART and at the time of virological failure in order to guide clinical management. However, this is neither affordable nor practical in many resource limited settings implementing a public health approach to ART, with standardized low cost first line ARV regimens and minimal laboratory monitoring.

In response to this concern, the WHO has developed population- based survey methods to assess whether significant levels of TDR in recently infected populations are emerging in resource limited setting?. This method does not indicate the true prevalence of TDR, but enables to make classifications of the prevalence in a population as low (< 5%), moderate or intermediate (5-15%) or high degree > 15%). The survey is implemented in specific geographical areas based on certain criteria; where ART service has been delivered for at least more than three years, the HIV epidemic is focal or generalized, and HIV sentinel sero-survey methodology is already in use and at least one round of surveys has already been conducted.

The periodic surveillance of the prevalence, pattern, and trends of HIV transmitted drug resistance are therefore recommended so that timely interventions can be implemented as needed. This practice will help in ensuring that the efficacy of antiretroviral drugs used for ART, prevention of mother-to-child transmission (PMTCT), treatment for prevention, and pre-exposure and post-exposure prophylaxis are preserved.

As with most other countries that have incorporated ART in routine HIV care, the rapid scale up in ART access in Ethiopia will most likely be accompanied by the emergence and transmission of HIV drug resistance. A previous survey conducted in 2003 in Gondar University before the ART expansion, has indicated TDR of 3.3%. Surveillance of TDR is important to alert HIV program managers about the level of resistance currently being transmitted, enabling them to undertake corrective action when possible. It also provides important public health information regarding the efficacy of current pre- and post-exposure prophylaxis and may predict population level efficacy of first-line regimens when recently infected populations require future ART.

2. OBJECTIVE OF THE STUDY

2.1. General Objective

- To undertake HIV drug resistance threshold survey in HIV Counseling and Testing clients at Gondar University Hospital

2.2. Specific Objectives

- To assess whether the prevalence of transmitted HIV drug resistance has reached or exceeded a threshold of 5% among HIV Counseling and Testing clients at Gondar University Hospital
- To provide information on currently circulating HIV-1 subtypes among HIV Counseling and Testing clients at Gondar university Hospital

3. MATERIALS AND METHODS

3.1 Study design

This study was a cross-sectional study which involved newly HIV diagnosed persons of age 18-25, who were recruited from HIV Counseling and Testing Center at Gondar University hospital and Gondar health center in Gondar town located at 700km far from Addis Ababa.

3.2 Study site selection

In resource constrained countries where ART scale-up has been in progress, a certain criteria for site selection to conduct the threshold survey have been established by the WHO which include;

- the HIV epidemic is generalized or focal
- HIV sentinel sero-survey methodology is already in use and at least one round of surveys has already been conducted
- HIV Counseling and Testing service is given at the facility or facilities in the study area.
- HIV testing is done at the facility
- Availability of minimum laboratory requirements at or in close vicinity of the study area/facility
- Serum/plasma specimens or DBS specimens collected for the last HIV Sentinel Sero-survey have met the minimum Laboratory Quality Control standards established for the country

Therefore, 15 selected geographical sites from different parts of the country based on the above recommendations were assessed. Each site assessed has given ART service at least for three years. In addition to this, this site selection has also been tuned by proximity of appropriate laboratory in the area and whether HIV drug resistance survey has previously been conducted.

Based on these criteria, we have selected Gondar health center and Gondar University Hospital, in Gondar town. Gondar was the second largest town in Amahara region, Northern Ethiopia,

with a projected population size of 213,673 (CSA, 2007) and it was one of the areas where ART has first been started. Gondar University Hospital—the study site for this project-- has provided ART for more than 8 years. The total number of HIV patients up on treatment during the study period in the hospital was 2525 and ever started ART was 3982. Gondar University Hospital is part of the national health system where previous HIV diagnoses and ART exposure can easily be traced. Furthermore, a study conducted at the hospital in 2003 [27] has indicated the presence of transmitted resistance in the area.

3.3 Study participants

The participants of this study were from HIV Counseling and Testing clients at Gondar University Hospital and Gondar health center. Participants were first approached by trained counselor nurses at the HIV Counseling and Testing center who normally provide the service. The nurses were guided by a pre-prepared questionnaire, (which contains the inclusion and exclusion criteria in a systematic way, see Annex. III), to recruit potentially eligible participants. If a participant fulfills the inclusion criteria and was not excluded by any one of the exclusion criteria, the participant was consented to participate in the survey. A participant who fails to meet any of the inclusion criteria or qualifies for any one of the exclusion criteria, the counselor automatically terminate the questionnaire and continue to provide the HIV Counseling and Testing service normally. Consenting was proceed by reading the participant information (Annex. I) which delineates why the participant might consider being part of the survey, and any risks from providing 10ml blood through venipancture. The subject who agreed to be part of the study, he/she signed the consent form prepared for this purpose (Annex II) and the nurse draw 10ml of blood and filled socio-demographic data in the participant data form.

The inclusion criteria for this group were;

- Age between 18-25
- If female, no previous pregnancy
- Has lived in the specified area for about a minimum of a year

The exclusion criteria for this study group is

- previous positive HIV testing history
- Record or self report of previous positive HIV diagnosis
- Previous exposure to ARV drugs
- Any indication of AIDS like symptoms
- For woman, more than one pregnancy

3.4 Sample size

The sample size for the survey follows the sequential sampling method selected by WHO for the surveillance of transmitted HIVDR in low-resource settings. According to the recommendation, even though a sample of ≤ 47 eligible individuals consecutively diagnosed with HIV in sites within a survey area are required for analysis, it is advised to collect 60-70 specimens where possible, to allow for amplification problems, and post-diagnosis determination of ineligibility. In this survey a total of 84 specimenswere collected.

3.5 Laboratory components

3.5.1 Specimen collection, processing and storage

Whole blood (10ml) was collected by venipuncture using EDTA vacutainer tubes (labeled with specific Survey Code) at the HIV Counseling and Testing center in Gondar University hospital and Gondar health center and was centrifuged within 4 hours to separate plasma and it was stored at -20^{o}C at Gondar hospital laboratory. Simultaneously, DBS specimens were also prepared. Specimens were shipped to Ethiopia Public Health Institute (EPHI) on dry ice for long term storage at -80^{o}C until genotyping. DBS were stored at -20^{o}C

3.5.2 Preparation of DBS

Dried blood spots were prepared by pipetting 75-100µl of EDTA anti-coagulated whole blood on to premarked circles (five spots per card) on No.903 Whatman filter paper cards (Whatman, formerly Schleicher &Schuell, Keene, NH). Each card was then dried overnight and was then individually be packaged into Bitran zipper-lock bags (Fisher Scientific Company, Pittsburgh, PA) containing a silica gel pack (Mini Pax Sorbent; Multisorb Technologies, Buffalo, NY).

3.6 Data Collection, Management and Processing

3.6.1 Data Collection and flow

Demographic data was collected at the HIV Counseling and Testing center by trained nurses, which were then passed to the database at the hospital's ART clinic. Sample transmittal form, which was identically coded with the specimen was filled at the laboratory with appropriate specimen information and was also passed to the database. Data clerks verified both forms; demographic data form and specimen transmittal form which were labeled with identical survey codes (see Annex IV, indicating data flow). Any discrepancies noted on the forms were corrected immediately. Any notations regarding missing or unusable etc. samples were also entered into records as well. Specimen transmittal forms and accompanying documents were filed and submitted to EPHI central laboratory.

3.7 Laboratory testing procedures

HIV testing: An antibody for HIV was detected using EIA algorithm format already being employed for HIV surveillance purpose. Initial screening was done using 4th generation HIV screening test (Vironostika, uniform Ab/Ag test, Biomureux).

3.7.1 RNA extraction from plasma and serum

Total HIV-1 RNA was extracted from 140µl patient plasma using QIAamp Viral RNA Mini kit (Qiagen, Hilden, Germany). Accordingly, 140µl patient plasma sample was mixed with 560µl lysis buffer and incubated for 10 minutes at room temperature to allow lysis and binding of the nucleic acid to the membrane. Unbound materials were removed by washing steps with wash buffer. Finally, the nucleic acids were eluted and resuspended in elution buffer, and stored at -80^0c until use.

3.7.2 Amplification by RT-nested PCR

A 1kb pair fragment of HIV-1 *pol* comprising amino acids 6-99 of the protease and 1-254 of the reverse transcriptase was amplified using an in-house RT-PCR and nested-PCR method. For each run, a control sample (positive control and negative control) was used. Briefly, 10µl of extracted nucleic acids was added to a 40 µl RT-PCR master mix containing SuperScript^{TM} III one step RT-PCR enzyme with Platinum *Taq*DNA polymerase high fidelity. Then 2µl of the first round reverse transcription polymerase chain reaction product was added to 48µl PCR mix containing primers and AmpliTaq gold LD DNA polymerase (Applied Biosystems, Foster City, CA). Cycling conditions for the reverse transcription polymerase chain reaction was 45 minute at 50^0C and 2 minute at 94^0C followed by 40 cycles of 15 sec at 94°C, 20 sec at 50°C, and 2 min at 72°C followed by extension at 72^oc for 10 minute. Cycling conditions for the nested polymerase chain reaction was 4 minute at 94ºC followed by 40 cycles of 15 sec at 94°C, 20 sec at 55°C, and 2 min at 72°C followed by extension at 72^oc for 10 minute.

3.7.3. Agarose Gel Electrophoresis

PCR products were checked for yield with 1% agarose gel electrophoresis, where ethidium bromide (EtBr) was used as a DNA stain, to confirm amplification of the region of interest. Results were accepted only if the result of both controls (negative and Positive) were correct. The purification of the PCR product was then done with QIAquick PCR Purification Kit (Qiagen, Hilden, Germany) according to the manufacturer recommendation.

3.7.4 Cycle Sequencing

Cycle sequencing was performed by using premixed Big Dye terminator sequencing reagents (Applied Biosystems, Foster City, CA), which are used to sequence the PCR products with 6 in house primers in both direction (forward and backward).One µl of the purified DNA template mixed with 19µl master mixes containing each primer with Big Dye terminator was put into thermocycler. Conditions for cycle sequencing (25 cycles) was 96°C for 10 s, 50°C for 5 s, and 60°C for 4 min.

3.7.5 DNA Sequencing and Genotype interpretation

ABI 3100 and ABI 3500xl Genetic Analyzer were used for sequencing dye labeled DNA fragments of the samples through capillary electrophoresis. Electrophenogram data was transferred to a different computer for sequence editing, assembly and analysis. Sequence assembly and editing were performed using Standalone ReCall HIV-1 sequencing analysis tool.

3.7.6 Quality assurance

Nucleotide sequence data generated from the survey specimens were analyzed together, for genetic relatedness to detect laboratory contamination using ,Bioedit version 7.0.0 (Ibis Biosciences, Carlsbad CA) and MEGA version 5.0 (The Biodesign Institute, Tempe AZ).

3.7.7 Transmitted Genetic Resistance Analysis

Transmitted drug resistance mutations were examined according to the calibrated population resistance (CPR) tool version 6.0 beta contained in the Stanford HIV Drug Resistance database (http://cpr.stanford.edu/cpr/servlet/CPR). The CPR tool analyzes sequences for resistance mutations from drug naive individuals, based on the WHO surveillance transmitted drug resistance mutation list of 2009 [30]. Apart from providing subtype assignment CPR also examines sequences for quality (unusual, bad and hypermutated sequences).

Rates and frequencies of ARV drug resistance associated mutations was calculated for each of drugs and drug classes using Stanford Genotypic Resistance interpretation Algorithm (http://hivdb.stanford.edu/pages/algs/HIVdb.html

3.7.8 Sub-type determination

Subtypes were screened using the Rega HIV Subtyping Tool (version 3.0; available at http://dbpartners.stanford.edu/RegaSubtyping/) .

3.7.9 WHO HIVDR threshold category

WHO TS binomial sequential sampling method was used to categorize HIVDR prevalence, accordingly, the specimens were listed in order of date of blood draw from the older to the newer specimens and the genotype results were examined. The specimens with major HIVDR mutations was recorded and compared to the WHO recommendations. A classification of HIVDR prevalence was made based on the total number of specimens with major HIVDR mutations.

A predetermined lower limit and upper limit were used for determining the prevalence.

According to this method, the prevalence of HIVDR will be classified as "**low prevalence**" (<5%), if the total number of specimens with mutations was less than the lower limit and as "**high prevalence**" (≥15%). if the total number of specimens with mutations was higher than the upper limit.

If a total of 47 specimens were genotyped and the total number of specimens with major HIVDR mutations was neither less than the lower limit nor greater than the upper limit, prevalence will be classified as "**moderate prevalence" (≥5% and** ≤15%).

According to this method of analysis classification can first be limited to the first 14 specimens:

If >5 specimens with HIVDR are found when 14-24 specimens have been genotyped, then the HIVDR prevalence will be classified as "high prevalence" (>15%).

If this is not the case, then analysis should continue until the 34th specimen:

If >6 specimens with HIVDR are found when 25-34 specimens have been genotyped, then the HIVDR prevalence will be classified as “high prevalence” (>15%)

If no specimen with one or more known mutations associated with HIVDR has been found after the 34th specimen is genotyped, the prevalence will be classified as “low prevalence” (<5%). If HIVDR prevalence cannot be classified on the basis of the initial 34 specimens, additional specimens must be analyzed, line until the maximum of 47 specimens has been analyzed.

If >8 specimens with HIVDR are found when 47 specimens have been genotyped, then the HIVDR prevalence will be classified as “high prevalence” (>15%).

If less than 2 specimen with one or more known mutations associated with HIVDR has been found after the 47th specimen was genotyped, the prevalence will be classified as “**low prevalence**” (<5%).

If the maximum survey sample size (47) is reached without limit being crossed then sampling stops and the population is classified as “**moderate prevalence” (5- 15%).** If prevalence is categorized, moderate or high prevalence then classification of prevalence to the particular drug or drug class can be made.

3.8 ETHICAL CLEARANCE

Scientific and ethical approval was granted by the Research and Ethical Clearance Committee of EHNRI, IRB of CDC and the National Health Research Ethics Review Committee of Ministry of Science and Technology of Ethiopia.

4. RESULT

Study Population

From August 2011 through December 2012, ARV-naive individuals with newly diagnosed HIV infection who were seeking HIV diagnostic testing in VCT sites in Gondar university hospital and Gondar health center were evaluated for inclusion in the survey.

A total of 84 samples (64 from Gondar university hospital, and 20 from Gondar health center) were found to be eligible and offered participation and subsequently enrolled. The mean age of participants was 21 years (range, 18–24 years); 70(83.3%) were female.

Prevalence of NRTI, NNRTI, and PI HIVDR Mutation

After analyzing the first 14-24 specimens since no specimen with major HIVDR associated mutation was found, the HIVDR prevalence could not be classified. When 34 specimens were genotyped, three specimens with major HIVDR associated mutations were found. Since the number of specimens exhibiting HIV drug resistance found after the analyzing the 34 samples was neither below the lower limit or above the upper limit the prevalence cannot be classified as "**low prevalence**" (<5%) or as "**high prevalence"** (>15%). Additional specimens were then analysed until the maximum of 47 specimens, as was recommended by WHO.

After analyzing the 47 specimens, only 3 specimens carrying major HIVDR associated mutations were found, which was lower than the upper limit (8) but higher than the lower limit (2). Thus, according to the WHO recommendations of sampling and classification plan, our study showed the HIVDR prevalence to be classified as "**moderate prevalence"** (5-15%)

Table 01: Sampling and classification HIVDR prevalence

Sample Number genotyped	Lower Limit	Running total of specimens with listed mutation	Upper Limit	Sample Number genotyped	Lower Limit	Running Total of specimens with listed mutation	Upper Limit
1	ND	0	ND	25	ND	0	6
2	ND	0	ND	26	ND	0	6
3	ND	0	ND	27	ND	**K103KN**	6
4	ND	0	ND	28	ND	**G190S**	6
5	ND	0	ND	29	ND	2	6
6	ND	0	ND	30	ND	2	6
7	ND	0	ND	31	ND	**K103KN**	6
8	ND	0	ND	32	ND	3	6
9	ND	0	ND	33	ND	3	6
10	ND	0	ND	34	1	3	6
11	ND	0	ND	35	1	3	7
12	ND	0	ND	36	1	3	7
13	ND	0	ND	37	1	3	7
14	ND	0	5	38	1	3	7
15	ND	0	5	39	1	3	7
16	ND	0	5	40	1	3	7
17	ND	0	5	41	1	3	7
18	ND	0	5	42	1	3	7
19	ND	0	5	43	1	3	7
20	ND	0	5	44	2	3	7
21	ND	0	5	45	2	3	7
22	ND	0	5	46	2	3	8
23	ND	0	5	47	2	3	8
24	ND	0	5	STOP	STOP	STOP	STOP

Since the WHO recommended categorization of TDR using prevalence corresponded to “moderate", a classification of prevalence to the particular drug or drug class can be made. Based on this, after the analysis of the first 47 genotyped RT sequences obtained, revealed no resistance mutations were identified for NRTIs and hence the prevalence of transmitted HIVDR associated with NRTI was classified as low level (<5%).

For non-nucleoside reverse-transcriptase inhibitors (NNRTIs), 3 major NNRTI associated resistance mutation (**K103N, G190S,** and **K103N)** were detected, and the prevalence of transmitted HIVDR for NNRTI was classified as **moderate level (5%–15%).**

In line with the WHO recommendations for classifying the prevalence of transmitted HIVDR analysis of the first 47 protease sequences obtained revealed no major transmitted HIVDR mutations associated to PI and, on the basis of the developed sampling and classification plan the prevalence of transmitted HIVDR for the PIwas classified as low (<5%) .

Prevalence of primary HIVDR mutation

Based on the analysis of the 67 samples amplified and sequenced using Stanford genotyping algorithm, there were 4(6%) samples with major HIVDR mutation. Among which one study participant (GONHIVDR055) (sex, female age, 20 years) harbored K103KN, which confers high level resistance to NNRTIs, NVP and EFV. Similarly other study participant GONDARHIVDR058 (age,18 sex, female) was found to have the same K103KN and also E138AE mutation which confers high level resistance to NNRTIs, NVP and EFV and low level resistance to rilpivirine. The third participant GONDARHIVDR056 (age,23 sex-male) was found to harbor G190S which confer a high level of resistance to high level resistance to NNRTIs, NVP and EFV and potential low-level resistance to rilpivirine and etravirine. The fourth study participant GONDARHIVDR088 (age =23, sex male) was found to harbor Y181CY which confers a high level of HIVDR to drug NVP and intermediate resistance to EFV, to rilpivirine and etravirine

With regards to PI, there was no major mutations characteristic of primary resistance to protease inhibitor detected. However, minor mutations have been observed in the protease region. The frequency of observed amino acid substitutions associated with secondary resistance mutations are shown in the figure below.

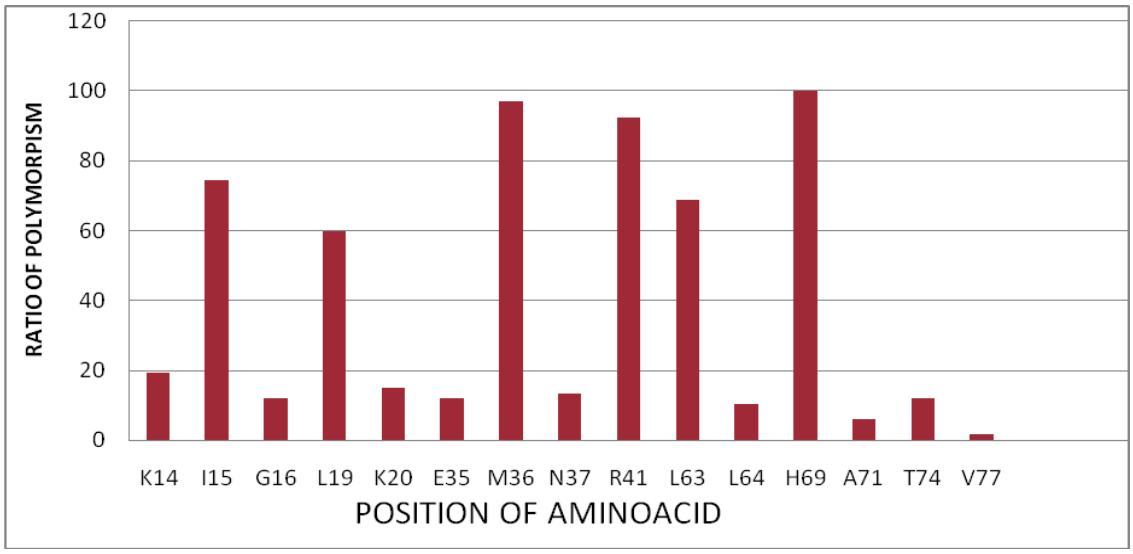

Figure 01: Frequency of minor protease inhibitors associated mutations

In this study there was no major NRTI associated mutation detected but several minor or secondary amino acid substitutions in reverse transcriptase were detected as was shown in the fig-2.

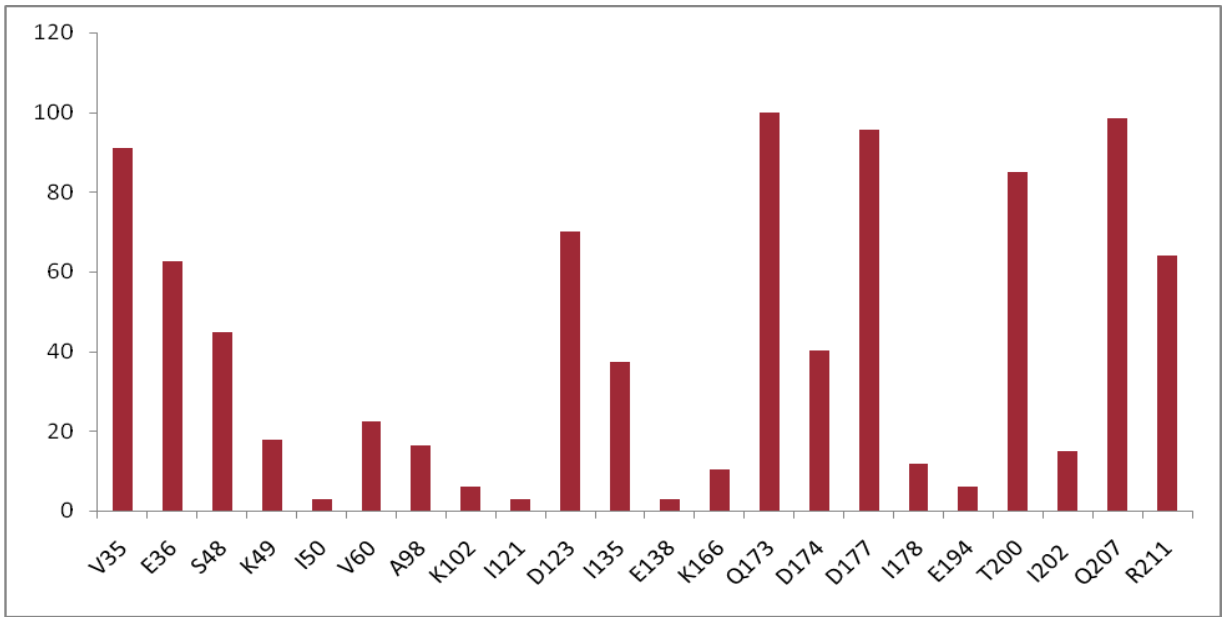

Figure 2: Frequency of minor reverse transcriptase inhibitors associated mutations (n=67), 2011.

Subtype

The analysis of the pol gens sequence using the Rega HIV Subtyping Tool (version 3.0; available at http://dbpartners.stanford.edu/RegaSubtyping/) revealed 95.5% (n=64) were found to be subtype C, 3 %(n=2) were CRF-A1 C, 1.5% (n=1) were subtype A.

5. DISCUSSION

In a context of rapid scale-up and decentralization of HAART services the emergence and transmission of drug resistance virus variants is inevitable. Studies have shown temporal trends in prevalence of transmitted HIV drug resistance to vary across cohorts and that pre-therapy resistance more than doubles the risk of first line failure and increase the further acquisition of drug resistant mutations in the first year of treatment [(18)]. Increases have been observed in different countries with wider access to ART. The PASER-M study in six African countries estimated that the rate of transmitted drug resistance increases at 38% a year after roll-out of antiretroviral therapy [(17)].

Rising drug resistant HIV in sub-Saharan Africa is a potential threat to the worldwide control of HIV/AIDS. National HIV treatment programmes should continue to expand access to antiretroviral drugs but also ensure quality in order to preserve treatment options for tomorrow. They need to ensure robust supply chains, improved diagnostic laboratory capacity, introduction of low cost viral load technologies, and the implementation of resistance surveillance.

With recent introduction and scaling-up of HAART in Ethiopia data generated from HIV drug resistance threshold survey studies are of extreme relevance to the country as these may be used by decision makers for choice of therapeutic options, improvement of prevention and treatment programs and treatment monitoring strategies.

Our study fulfilled almost all the criteria recommended in the WHO HIV drug resistance threshold survey method. Specifically, the median age of the patients in our study was 21 years, consistent with the HIVDR recommended threshold of less than 25 years. In addition, as recommend by the WHO: patients were consecutively included, the number of samples was > 47, and there was no known exposure to ARV drugs, including PMTCT. Furthermore, the survey was conducted in geographic areas, based where ARV drugs have been widely available for more than 3 years, and available to at least 20% of eligible individuals. Beside to this, in the study area there was a report of 3.3% prevalence of transmitted HIVDR a study done in 2003, before the scale up of ART [(27)].

Following the WHO protocol,by analyzing the first 47 samples revealed that the levels of TDR in the Gondar town to be classified as moderate level (5%–15%).But by including all successfully sequenced samples (n=67) the prevalence of TDR was found to be 6% (n=4). The HIVDR mutation detected in this study were all associated with NNRTI, and were, K103N (n=2), Y181C (n=1), and G190A (n=1). There was no major drug resistance mutation in protease (PR) and reverse transcriptase region. Simultaneous resistance to both NRTI and NNRTI was not also observed. However, high rate of natural polymorphisms at RT & PR regions were reported by the study.

Our results indicated that the levels of TDR appeared to be increasing with the scale up ART in Gondar town compared to the 3.3 % in 2003 and 5.2 % in 2008/09 reported Kassu *et al.*,2007 and Mulu *et al* 2014, respectively [27,28] .

An increasing ARV drug exposure in populations, following the roll-out of ARVs for treatment and prevention of mother-to child transmission, may cause a rise in TDR in the study area. Similarly, reports from industrialized countries with a long ART experience have shown increases in transmitted HIVDR to up to 25%. Also, recent studies from other African settings have reported this increase in HIV drug resistance mutation over time, as ART programs are scaled up, such as in Zambia where up to 15.3% HIV drug resistance mutation prevalence was observed in 2009 compared with 0.0% in 2002, although on a limited sample panel [23].

Similarly other data also showed an increase in the prevalence of transmitted HIV drug resistance with the roll out of ART in some settings. In Kampala, Uganda, the prevalence of transmitted HIV drug resistance increased from 0% (2006–2007) to 8.6% (2009–2010) [31]. The IAVI Early infection cohort conducted among the most at risk populations in East and Central Africa has also reported an increase in the prevalence of transmitted HIV drug resistance in Zambia, from 0% (2005) to 16% (2009) [32] Another study done in Yaounde, Cameroon, a showed steady increase in the prevalence of transmitted HIV drug resistance, from 0% (1996–1999) to 1.9% in 2001, 4.1% in 2002 and 12.3% in (2007) [33].

In Kenya, a cross-sectional study done in Nairobi in 2005 found 4/53 (7.5%) new clients had transmitted HIV drug resistance. The International AIDS Vaccine Initiative cohort reported an

overall TDR prevalence of 3.1% from three sites in Kenya 40. [34]. Another multisite cross-sectional study from the PASER group, conducted between 2007 to 2009, reports transmitted HIV drug resistance frequencies of 9/200 (4.5%) in Mombasa and 10/204 (4.9%) in Nairobi [35] . Beside to these, a cross-sectional survey among newly diagnosed ARV-naive adults attending four VCT centers from Mombasa in 2009–2010 reported an overall transmitted HIV drug resistance prevalence of 13.2%.

Based on the 72 transmitted drug resistance surveys done in 26 countries between 2004-2010, WHO has reported an increase in surveys reporting moderate prevalence according to this report, surveys reporting having moderate resistance (prevalence of resistance 5%–15%) has increased from 18% in the period 2004–2006 to 32% in 2007–2010. In the report it has also been indicated that, the increase was due to an increase in the percentages of surveys reporting moderate resistance in Africa from 17.6 % (3 out of 17) in 2004–2006 up to 40.7% (11 out of 27) in 2007–2010 [23] .

In another systematic review of 218 datasets from 2001-2011 containing data on 26,102 untreated adults (15 years or older), a similar increase in transmitted drug resistance over time was revealed. The increase was most pronounced in East Africa, where the prevalence of any drug resistance associated mutation increased from 0.9% to 7.4% after 8-9 years of program roll-out. The increase was less pronounced in Southern Africa, from 2.1% to 3.7% after 5-7 years

Even though the NNRTI prevalence rates observed in this survey is slightly higher than those observed in previous reports, the HIV drug-resistance mutations detected were consistent with the widespread use of this drug class as part of standard first-line ART, as well as single-dose NVP for prevention of mother to child transmission, the low genetic barrier for the development of resistance to this class.

This observation is consistent with results of a recent meta-analysis, coordinated by WHO, from resource-limited settings which shows a statistically significant increase in prevalence of NNRTI-resistant mutations in East Africa (36% per year) and Southern Africa (23% per year), the estimated prevalence of NNRTI resistance was 5.1% (3.1-8.2) 8 years after rollout in east Africa [23] .

In a study Pharm Access African Studies to Evaluate Resistance Monitoring (PASER-M) which is multicentre, prospective cohort from 11 regions in Kenya, Nigeria, South Africa, Uganda, Zambia, and Zimbabwe, by analyzing 2436 participants who had a pretreatment genotypic resistance result overall sample-weighted drug-resistance prevalence to be 5·6% (4·6–6·7). The drug class-specific resistance prevalence was 3·3% (2·5–4·2) for NNRTIs, 2·5% (1·8–3·2) for NRTIs, 1·3% (0·8–1·8) for PI, and 1·2% (0·7–1·7) for dual-class resistance to NRTIs and NNRTIs [(35)] .

The low occurrence of PI associated resistance mutation among the study participant might be associated with the limited use of the regimens and high genetic barrier required for the HIV drug resistance mutation.

Subtype

Beside to the determination of the prevalence of transmitted HIV drug-resistance mutations, our study also aimed to determine the HIV-1 subtype that circulates in the study area. Our result showed that the HIV/AIDS epidemic in the study area is dominated by subtype C which is consistent with previous reports by Kassu *et al*, 2007. In addition, we found, new circulation recombinant of A1 and C, and subtype A, which may suggest the possible transmission of HIV epidemic from neighboring countries such as Sudan and Kenya where subtype A is dominant [(27)].

Different HIV-1 subtypes are reported to impact on the viral fitness, immunogenicity and pathogenicity, which might have consequences for prophylaxis and therapeutic interventions. Studying the distribution of HIV subtypes within a given population provides the opportunity to study patterns and trends of the epidemic and track routes of HIV transmission.Considering Ethiopia as center for different international organization and country with different historical place for tourist attraction center the introduction of new HIV strain is possible. Thus, study on the molecular epidemiology of HIV is very important

6. CONCLUSIONS AND RECOMMENDATIONS

Expanded access to combination antiretroviral therapy during the past decade has remarkably improved the prognosis of HIV-1-infected individuals. But deficiencies in health systems, such as lack of virological monitoring and intermittent drug supply, have raised concerns about the rapid emergence and spread of drug-resistant HIV-1 strains. A study by Gupta et al., 2012, pointed out there is a rapid increase in transmitted drug resistance in the years after the rapid increase of availability of ART in middle- and low-income countries.

The consequences of increasing levels of transmitted drug-resistant HIV-1 variants are substantial, and include: increased treatment failure rates with greater morbidity and mortality, increased need for more expensive second-line regimens, and costs associated with further transmission of more highly resistant and potentially untreatable viruses.

In a context of rapid scaling-up and decentralization of ART services in Ethiopia , implementation of HIV drug resistance threshold surveys are of extreme relevance to the country as these may be used by decision makers for choice of therapeutic options, improvement of prevention and treatment programs and treatment monitoring strategies.

The finding of this study showed an overall prevalence of primary HIVDR resistance 6.0% and as per WHO recommendation the survey was classified as having moderate prevalence (5%–15%) of transmitted HIVDR resistance. Moreover, compared to the findings of other studies done in the Gondar town at different periods, there is an increase in prevalence of drug resistance mutation among untreated HIV-1-infected individuals with the ART scaled up.

As per the WHO recommendation, if moderate level of transmitted HIVDR is reported, it needs to be confirmed through repetition of the survey in this area and possibly in additional areas of the country. Besides to this, since the finding of this survey cannot be extrapolated to other areas (as different factors might have different effect on the emergency and transmission of HIV drug resistance), surveillance of transmitted HIV drug resistance in representative health institutions from different part of the country and among different target population is highly recommended.

Furthermore further study need to be done to identify possible sources of HIVDR transmission, through evaluation of prevention programs and intensification of HIVDR early warning

indicators monitoring and adjustment of programmatic factors that lead to the emergence and spread of resistance to ART are needed.

Even though the high level of NNRTI associated mutation detected in this survey is worrisome as the NNRTI are part of standard first line regimen in Ethiopia, there is no need of changing first-line regimens (from NNRTI-based to PI-based regimen) at the population level.

Beside to the effort of expanding access to antiretroviral drugs a lot has to be done to ensure robust supply chains, improved diagnostic laboratory capacity, introduction of low cost viral load technologies, and the implementation of resistance surveillance.

WHO recommendation for moderate prevalence classification (5-15%) of TDR

If TDR is classified as **moderate** (5-15%) for in a specific geographic area, extra quality assurance of laboratory and epidemiological data should be performed to ensure accuracy of survey results. If no sequence quality assurance issues are noted and appropriate survey inclusion/exclusion criteria were followed, surveillance of TDR should be immediately repeated in the same geographical area to confirm the results and expanded to additional areas.

A moderate transmitted HIVDR classification alerts programme planners to transmission of significant levels of HIVDR. National HIV programmes should react to by reviewing potential sources of HIVDR transmission in the area surveyed, through assessment of: clinic factors favoring HIVDR emergence (EWIs); rates of VL suppression 12 months after ART initiation at representative ART sites in the area of survey (WHO surveys of acquired HIVDR); performance of HIV prevention programmes to minimize HIVDR transmission; coverage of HIV testing services (high coverage of HIV testing increases awareness of HIV status and reduce the risk of unintended HIVDR transmission).

Before changing ART policy based on TDR survey results, policy-makers should conduct representative surveillance of HIVDR in populations initiating ART. The prevalence of HIVDR in population initiating ART will inform policy-makers when to (if they should consider): 1) change first-line regimens (from NNRTI-based to PI-based regimen) at the population level, 2) introduce baseline genotyping test at individual level to guide therapy (where feasible); or 3) intensifying VL monitoring (e.g. during first 12 months following ART initiation).

If a survey conducted among pregnant women at antenatal care (ANC) sites in a specific geographic area shows moderate level of TDR, and results are confirmed by repeated surveys, policy-makers should consider implementing full scale national surveillance in a representative sample of all HIV-infected pregnant women. Policy implications include: switching from NNRTI-based to PI-based PMTCT or performing resistance genotyping in all HIV-infected pregnant women to guide the decision on which PMTCT regimen will be most effective. Since policy decisions should take into account cost-effectiveness analysis, WHO is exploring the possibility to develop specific guidance to help HIV/ART policy makers to interpret survey results using an economic lens. When full-scale surveillance of HIVDR in HIV-infected pregnant women is not feasible, sub-analyses of HIVDR prevalence in women initiating ART may be considered.

7. LIMITATIONS

1. Surveillance for transmitted drug resistance is ideally conducted among recently infected individuals from longitudinal studies with estimated dates of infection. In this study laboratory evidence of seroconversion was not employed apart from using other proxy criteria like opportunistic infection and age which have been suggested to agree poorly with laboratory-based methods to detect recent infection. Hence our criteria may not have completely eliminated chronically infected individuals.
2. Although the study specifically selected newly diagnosed, antiretroviral-naive individuals, it cannot be completely ruled out that some participants had unknown or undisclosed prior exposure to antiretroviral therapy and/or prophylaxis as the study relied on participant self-report and could not objectively verify self-knowledge of participant HIV status or prior treatment history to accurately classify resistance observed as transmitted or selected.
3. The genotypic test used in this study is population sequencing. This technique fails to identify drug-resistant minority variants that are present in <20% of the virus population infecting a patient. This detection limitation is a concern, both because transmitted minority variants might persist at low frequencies and most newly diagnosed HIV infections are in persons who have been infected for several months to years, providing time for drug resistant viruses with reduced viral fitness to decay to levels that conventional testing is not able to detect. Studies have shown that some minority resistant variants have clinical implications; in particular, NNRTI-resistant variants in minority proportions may impact virological response to an NNRTI-based regimen. Therefore, the level of resistance might be underestimated using the population sequencing assay. Sensitive methods of detection of minority resistant variants like, allele-specific PCR, single genome sequencing, pyro-sequencing (ultradeep sequencing), can be used future surveillance of transmitted HIV drug resistance.

8. REFERENCE

1. UNAIDS report on the global AIDS epidemic 2013
2. FHAPCO 2014 Country progress report on the HIV response, 2014
3. Palella F.J., Delaney K.M., Moorman A.C., Loveless M.O., Fuhrer J., Satten G.A., Aschman D.J., Holmberg S.D., and The HIV Outpatient Study Investigators. 1998. Declining morbidity and mortality among patients with advanced human immunodeficiency virus infection. N Engl J Med, 338(13): 853-860.
4. Braitstein P, Brinkhof MW, Dabis F, Schechter M, Boulle A, Miotti P et al. Mortality of HIV-1-infected patients in the first year of antiretroviral therapy: comparison between lowincome and high-income countries. Lancet 2006;367(9513):817-824.
5. Bartlett JA, De Masi R, Quinn J, Moxham C et al. Overview of the effectiveness of triple combination therapy in antiretroviral-naïve HIV-1 infected adults *AIDS*. 2001; 15: 1369 - 1377
6. Xia, Q., Radzio, J., Anderson, K.S., Sluis-Cremer, N. (2007). Probing nonnucleoside inhibitor-induced active-site distortion in HIV-1 reverse transcriptase by transient kinetic analyses. *Protein Sci.***16**: 1728–1737
7. Quinn TC, Wawer MJ, Sewankambo N, et al. Viral load and heterosexual transmission of human immunodeficiency virus type 1. Rakai Project Study Group. N Engl J Med. 2000; 342(13):921-929.
8. Cohen MS, Chen YQ, McCauley M, et al. Prevention of HIV-1 infection with early antiretroviral therapy. N Engl J Med. 2011;365 (6):493-505.
9. Diane E Bennett, Silvia Bertagnolio, Donald Sutherland, and Charles F Gilks. World Health Organization's global strategy for prevention and assessment of HIV drug resistance.2008. *AntivirTher.Suppl* 2: 1 - 13
10. The Gap Report Joint United Nations Program on HIV/AIDS (UNAIDS), 2014
11. Ministry of Health (MOH). Guidelines for implementation of antiretroviral therapy in Ethiopia. January 2005. Addis Ababa, Ethiopia.

12. Federal HIV/AIDS Prevention and Control Office. Strategic Plan to Intensify the Multisectoral Response for HIV/AIDS in Ethiopia, 2010/11-2014/5. Addis Ababa, 2011.
13. Federal Ministry of Health. AIDS in Ethiopia. Sixth Report. Addis Ababa: FMOH; 2006.
14. FHAPCO 2014 Country progress report on the HIV response, 2014
15. Jahn A, Floyd S, Crampin AC, et al. Population-level effect of HIV on adult mortality and early evidence of reversal after introduction of antiretroviral therapy in Malawi. Lancet 2008; 371: 1603–11.
16. Vella, S. and Palmisano, L. (2005). The global status of resistance to antiretroviral drugs. ClinInfeDise. 41: S239-S246.
17. Hamers RL, Wallis CL, Kityo C, et al. HIV-1 drug resistance in antiretroviral- naive individuals in sub–Saharan Africa after rollout of antiretroviral therapy: a multicentre observational study. Lancet Infect Dis 2011; 11:750–9.
18. Wittkop L, Gunthard HF, de Wolf F, et al. Effect of transmitted drug resistance on virological and immunological response to initial combination antiretroviral therapy for HIV (EuroCoord-CHAIN joint project): a European multicohort study. Lancet Infect Dis 2011; 11: 363–71
19. Little SJ, Holte S, Routy JP, Daar ES, Markowitz M, Collier AC, Koup RA, Mellors JW, Connick E, Conway B, Kilby M, Wang L, Whitcomb JM, Hellmann NS, Richman DD. 2002. Antiretroviraldrug resistance among patients recently infected with HIV. N Eng J Med 347:385–394
20. Gupta RK, Jordan MR, Sultan BJ, Hill A, Davis DHJ, Gregson J, et al. Global trends in antiretroviral resistance in treatment-naive individuals with HIV after rollout of antiretroviral treatment in resource-limited settings: a global collaborative study and meta-regression analysis. Lancet [Internet]. Elsevier Ltd; 2012 Oct 6
21. Hecht, F.M., Grant, R.M., Petropoulos, C.J., Dillon, B., Chesney, M.A., Tian, H., Hellmann, N.S., Bandrapalli, N.I., Digilio, L., Branson, B., and Kahn, J.O. (1998). Sexual transmission of an HIV-1 variant resistant to multiple reverse-transcriptase and protease inhibitors. N. Engl. J. Med. 339, 307–311.
22. Franzetti M, Violin M, Antinori A, De Luca A, Ceccherini-Silberstein F, Gianotti N, et al. Trends and correlates of HIV-1 resistance among subjects failing an antiretroviral

treatment over the 2003-2012 decade in Italy. BMC Infect Dis [Internet]. 2014 Jan [cited 2014 Dec 11];14(1):398.

23. WHO (2012) HIV Drug resistance report
24. Manasa J, Katzenstein D, Cassol S, Newell M, de Oliveira T. Primary drug resistance in South Africa: data from 10 years of surveys. AIDS Res Hum Retroviruses. 2012;28:558-65.
25. Hunt G, Ledwaba J, Basson A, et al. Surveillance of transmitted HIV-1 drug resistance in Gauteng and KwaZulu-Natal Provinces, South Africa, 2005-2009. Clin Infect Dis. 2012;54(Suppl 4):S334-8.
26. Abegaz W E, Grossman Z, Wolday D et al. threshold survey evaluating transmitted HIV drug resistance among public antenatal clinic clients in Addis Ababa, Ethiopia. *AntivirTher* 2008; *Suppl* 2: 89 – 94
27. Afework kassu, Masayuki Funjino, Masakazu Matsuda, Masako Nishizawa, Fusao Ota and WataruSugiura. (2007). Molecular Epidemiology of HIV type 1 in treatment-naïve patients in North Ethiopia. AIDS Res Hum Ret Vir. 23 (4) 564 – 568
28. Mulu A, Lange T, Liebert UG, Maier M. Clade homogeneity and Pol gene polymorphisms in chronically HIV-1 infected antiretroviral treatment naive patients after the roll out of ART in Ethiopia. BMC Infect Dis [Internet]. 2014 Jan;14:158.
29. Abdissa A, Yilma D, Fonager J, Audelin AM, Christensen LH, Olsen MF, et al. Drug resistance in HIV patients with virological failure or slow virological response to antiretroviral therapy in Ethiopia. BMC Infect Dis [Internet]. BMC Infectious Diseases; 2014
30. Bennett DE, Camacho RJ, Otelea D, Kuritzkes DR, Fleury H, Kiuchi M, Heneine W, Kantor R, Jordan MR, Schapiro JM, Vandamme AM, Sandstrom P, Boucher CA, van de Vijver D, Rhee Sy, Liu TF, Pillay D, Shafer RW 2009. Drug resistance mutations for surveillance of transmitted HIV-1 drug-resistance: 2009 update. PLoS ONE 4: e4724
31. Ndembi N, Hamers RL, Sigaloff KC, et al. Transmitted antiretroviral drug resistance among newly HIV-1 diagnosed young individuals in Kampala. AIDS 2011; 25: 905–10.
32. Hamers RL, Siwale M, Wallis CL, et al. HIV-1 drug resistance mutations are present in six percent of persons initiating antiretroviral therapy in Lusaka, Zambia. J Acquir Immune Defi c Syndr 2010; 55: 95–101.

33. Aghokeng AF, Kouanfack C, Laurent C, et al. Scale-up of antiretroviral treatment in sub-Saharan Africa is accompanied by increasing HIV-1 drug resistance mutations in drug-naive patients. AIDS 2011; 25: 2183–88.
34. Price MA, Wallis CL, Lakhi S, et al.: Transmitted HIV type 1 drug resistance among individuals with recent HIV infection in East and Southern Africa. AIDS Res Hum Retroviruses 2010;27(1):5–12.
35. Hamers RL, Wallis CL, Kityo C, et al. HIV-1 drug resistance in antiretroviral naive individuals in sub-Saharan Africa after rollout of antiretroviral therapy: a multi centre observational study. Lancet Infect Dis 2011; 11:750–759.

ANNEXES

Annex I: participant information sheet

Hello, my name is (nurse's name), and I am a nurse working at Gondar university Hospital HIV Counseling and Testing center.

Purpose of the survey:

We would like to invite you to participate in a survey which looks at the drug resistance of Human Immunodeficiency Virus (HIV). You are being asked to be part of this survey because you are about to get HIV diagnosis for the first time at this particular clinic. We are doing this survey to determine whether there is transmission of HIV drug resistance to the available HIV drugs in Gondar town. If you agree to participate, we will draw 10ml of blood from your arm to do HIV drug resistance testing if you happen to be HIV positive. The results of drug resistance testing will not be returned to you or to your doctors since this is only intended to support the overall HIV treatment program at (Name of the health center) center. The MOH, WHO and CDC-Ethiopia are sponsoring and organizing this survey.

Procedures:

If you agree to participate in this survey, this is what will happen:

1. You tell your counselor that you agree to be part of the survey. In doing so, you also consent 10ml of blood (one small tube) to be drawn from your arm. From this blood you will be tested for HIV and the result of the test will be delivered to you right away after the test is complete.
2. If you happen to be HIV positive by the HIV test, your blood will be sent for 2 another extra tests. The first test will be to determine how many CD4+ T cells you have in 1ml of your blood. The result of the CD4 T cell count test will be sent to your medical chart at the follow up site you may choose to attend. The second test is HIV drug resistance test, which is to determine whether you are infected by HIV that has resisted one or more of the currently available drugs in our country. The results of your HIVDR test will not be sent to your

Doctor however because this test is only meant to do a survey and help strengthen the existing HIV treatment program at (Name of the health center) and the country at large.

3. No identifying information about you (such as your name, address, telephone numbers) will be collected for this survey. Some routine information, such as your age, sex, if you have ever been treated for HIV before, your length of residence in Gondar town, and results of your routine tests, will be collected from your interview and laboratory tests.
4. A report on the results of the extra tests for individuals taking HIV test at this VCT center may be published. No identifying information about you or any other patient will appear in the group report.

Risks/Discomforts

1. There should not be any risks or discomfort in this survey, except for a minor pain or bruise at the site of needle stick which is common with every blood draw.

Benefits:

1. Right now experts don't think that most people being treated for HIV in Ethiopia need HIV drug resistance test. Therefore, you will not personally benefit from your HIV drug resistance test and it will not be returned to your patient card for clinical use.

The result of HIV drug resistance test in this survey will help us learn more about HIV in Gondar town and how well treatment is working for patients coming to the facilities.

Your rights

Your participation in this survey is completely voluntary. You can decide not to be in the survey at all. You will still receive all the services routinely available at this clinic. You may leave this survey at any time without any impact on your treatment and care. You will be given a copy of this form to take with you. You may ask any questions about this survey or this consent form now or in the future. If you have questions about this survey, you may contact Mr. DawitAssefa at the following phone number +251911630634. You may also call this person if you have questions or concerns about your rights as a subject in this research survey.

The contact detail of the investigator is as follows:

Mr. Dawit Assefa:

Phone number: 0911-630634, Office: 0112788058

Email: dawitarm@gmail.com

National HIV Laboratory, EPHI, Addis Ababa

The contact details of the Scientific and Research Ethical Review Offices (EPHI):

Dr. Eshetu Lemma:

Phone number: 0913-864113, off: 0112771057

Protecting your privacy

Your name, telephone number and address will not be recorded in any forms or reports that come from this survey. The survey will only report group results. Your name will also not be used on either the blood collection tubes or the blood test results. A survey code number will be used instead of your name. All the information that we collect will be kept confidential and anonymous.

Annex II: Research subject informed consent form

Title of Study:

Transmitted HIV drug resistance threshold survey from voluntary counseling and testing centers in Gondar town, Ethiopia

If you agree to take part, please read this form and sign the consent sheets at the end. Please tick off every box, if you agree.

"I have read, or it was read to me, the information sheet concerning this study and I understand what will be required of me if I take part in the study."

"I am aware of the possible risk and benefits of this study."

"I know that being in this study is voluntary."

"I understand that at any time I may withdraw from this study without giving a reason and without affecting my normal care."

"My questions concerning this study have been answered by ……."

"I agree to take part in this study"

SIGNATURE _____ DATE _____

NAME (please Print) _____

Signature of Person Obtaining the Informed Consent:

SIGNATURE _____ DATE _____

NAME (Please Print)

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Annex III. Data collection form

Survey Code _____

I.	**Demographic data**			
No	**Questions**	**Answers**		**Remark**
1	**Date of birth**	**DD/MM/YY** __/__/__		**If age greater than 25 abort questionnaire** **If age less than or equal to 25, go to Q2**
2	**How long have you lived in Gondar Town?**	**Period of residence _____**		**If length of residence less than 12 months, abort questionnaire**
3	**Sex**	**M** ☐	**F** ☐	**If female continue to Q4** **If male, go to Q9**
4	**Are you pregnant?**	**YES** ☐	**NO** ☐	**If 'YES' go to question 5** **If 'NO', go to Q6**
5	**Have you ever been pregnant before**	**YES** ☐	**NO** ☐	**If 'YES', abort questionnaire** **If 'NO', go to Q9**
6	**Have you ever been pregnant?**	**YES** ☐	**NO** ☐	**If 'YES' go to Q7**

				If 'NO', go to Q9
7	**How many times have you been pregnant before?**	**Number of pregnancies (gravidity) _____**		**If gravidity greater than two abort questionnaire**
				If gravidity is less than or equal to two, go to Q8
8	**Was your first pregnancy in the past three years?**	**YES☐**	**NO ☐**	**If 'YES' go to Q9**
				If 'NO', abort questionnaire
II. HIV and ARV history				
9	**Previously HIV diagnosed at the current site?**	**YES ☐**	**NO ☐**	**If 'YES' go to Q10**
				If NO' go to Q11
10	**When did you have the diagnosis?**			**If before three years, abort**
				If less than three years, go to Q11
11	**Previously HIV diagnosed elsewhere?**	**YES☐**	**NO ☐**	**If 'YES', abort**
				If 'NO', go to Q12
12	**Have you ever been exposed to ARV drugs?**	**YES☐**	**NO ☐**	**If 'YES', abort**
				If 'NO', continue to the CONSENTING process

Annex IV. Specimen Transmittal Form

No	Code Label	Date of blood draw (2003 E.C. DD/MM	Draw time	Date plasma separated	Time plasma separated	Plasma volume (ml)	Time DBS prepared	Number of spots for DBS
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		/						
		/						

Annex V : Transmitted HIV drug resistance Threshold Survey: Sampling and Classification Plan

Location: _____ Drug class: _____ Start date: _____ End date: _____

Sample number	Lower limit	Running total of specimens with a listed mutation	Upper limit	Sample number	Lower limit	Running total of specimens with a listed mutation	Upper limit
1	ND	–	ND	25	ND	–	6
2	ND	–	ND	26	ND	–	6
3	ND	–	ND	27	ND	–	6
4	ND	–	ND	28	ND	–	6
5	ND	–	ND	29	ND	–	6
6	ND	–	ND	30	ND	–	6
7	ND	–	ND	31	ND	–	6
8	ND	–	ND	32	ND	–	6
9	ND	–	ND	33	ND	–	6
10	ND	–	ND	34	1	–	6
11	ND	–	ND	35	1	–	6
12	ND	–	ND	36	1	–	6
13	ND	–	ND	37	1	–	7
14	ND	–	5	38	1	–	7
15	ND	–	5	39	1	–	7
16	ND	–	5	40	1	–	7
17	ND	–	5	41	1	–	7
18	ND	–	5	42	1	–	7
19	ND	–	5	43	1	–	7
20	ND	–	5	44	2	–	7
21	ND	–	5	45	2	–	7
22	ND	–	5	46	2	–	8
23	ND	–	5	47	2	–	8
24	ND	–	5	STOP	STOP	STOP	STOP