

PMTCT Performance Among HIV Exposed Infants in Tanzania

by

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Coleen Cunningham

Ann Buchanan

Thesis submitted in partial fulfillment of
the requirements for the degree of Master of Science in the Duke Global Health Institute
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ABSTRACT

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Abstract

Background: In Tanzania, 68% of the estimated 86,000 HIV-infected pregnant women who deliver annually receive some intervention to prevent mother to child transmission (PMTCT) of HIV. Few data exist concerning the effectiveness of various treatment approaches in a field setting across a large geographic area. Dried blood spot (DBS) HIV DNA PCR testing of HIV-exposed infants was first rolled out in Tanzania in 2008. Using data gathered for DBS testing, we evaluated the prevalence of perinatal HIV transmission based on PMTCT regimen across three regions of Tanzania.

Methods: This was a retrospective review of all mother/infant pairs enrolled in the National PMTCT program in the Kilimanjaro, Arusha, and Tanga Regions of Tanzania from January 1, 2008 to September 30, 2010. Enrollment registries at health facilities that submit DBS PCR were reviewed to document infant date of birth, weight, feeding practice, maternal and infant PMTCT regimen, and date and result of first DBS PCR. The present analysis included mother/infant pairs for whom DBS PCR was performed at infant age ≤ 75 days. Maternal ARV regimens included: 1) none; 2) single-dose nevirapine (sdNVP); 3) sdNVP + zidovudine (combination prophylaxis); or 4) highly active antiretroviral therapy (HAART).

Results: In this field setting PMTCT is working better than hypothesized based on clinical trial results. Overall seroprevalence was 6.4% HIV transmission in the first 75 days of life. Women on HAART had the lowest transmission (2.1%), followed by those receiving combination prophylaxis (3.9%), sdNVP (8.9%), and no treatment having the highest rates (15.8%).

Conclusion: PMTCT regimens in resource-limited settings are effective and transmission rates are less than demonstrated by clinical trials data. Use of DBS for diagnosis of HIV provides an opportunity to evaluate use and effectiveness of PMTCT regimens.

Dedication

For the more than one thousand children born today with HIV: may your future be bright and may you live to see the day when there is no longer maternal to child transmission of HIV.

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1. Prevention of Maternal to Child Transmission

1.1 Burden of Disease and Current Statistics

Prevention of mother to child transmission (MTCT) of human immunodeficiency virus type 1 (HIV-1) is key to winning the global battle against AIDS. Over the last decade, great successes have reduced the burden of maternal to child transmission of HIV by an estimated 24%. [1, 2] In 2002, over half a million children became infected annually. With improved PMTCT, this number has dropped to an estimated 390,000 in 2010; though, sadly this still translates to more than 1000 children a day becoming infected with HIV, 90% of which are by perinatal route. [1-3]

Worldwide, an estimated 34 million people are infected with HIV. More than two thirds of this population resides in sub-Saharan Africa. [1] In sub-Saharan Africa, over half of those infected (59%) are women, making women 1.4 times more likely to be infected than men, most of whom are of child bearing age. [1] Not only do the women of sub-Saharan Africa bear the burden of HIV, but so then do many of their children. As of 2010, 3.4 million children are living with HIV/AIDS. [1] Of these, 90% live in sub-Saharan Africa. [1] This massive imbalance has huge implications on the need for effective and accessible PMTCT programs targeted toward this neglected population.

In Tanzania, an estimated 1.8 million individuals are HIV infected. The prevalence rate for adults age 15-49 is 7% with women 1.3 times more likely to be infected than men in a 2007 report. [4] Women ages 15-24 years old are most vulnerable just as they hit the reproductive years. [1, 4] From the UNGASS 2010 report, women ages 20-24 have a seroprevalence of approximately 6.3% as compared to 1.7% in men of the same age. The total seroprevalence in this age group is approximately 4%, reflecting

the significant burden of HIV carried by women in Tanzania of prime reproductive age.[5]

Much attention has been brought to these inequities, and the UN Millennium Development Goal (MDG) 6 targets these challenges with the goal, “To halt and begin to reverse, by 2015, the spread of HIV/AIDS.” Clearly, achieving MDG 6 requires implementation of a comprehensive PMTCT program.[2] Furthermore, in June 2011, the Global Plan towards elimination of new HIV infections among children and keeping their mothers alive was launched by the United Nations Secretary General with the goal of decreasing MTCT by 90% by 2015.[1] To achieve this goal in both developed and developing world settings, evidence-based interventions are critical to reducing and eliminating perinatal HIV transmission.[6]

1.2 Concept of Mother to Child Transmission of HIV (MTCT)

Since 1981, when the first published report of AIDS appeared in the literature [7], the realization that AIDS would become a global pandemic has brought forth one of the most profound global health campaigns to date. The need to combat the destructive physical, psychological, and social ramifications of this deadly virus have been an ongoing challenge to prevent the spread of HIV and treat the millions infected and affected by HIV every day.

The idea that AIDS was not just a disease of adult homosexual males, hemophiliacs, prostitutes, or intravenous drug users (IVDU), but was also seen in previously healthy infants was proposed in 1983.[8] What had been termed Nezelof’s syndrome, now known as a spectrum of combined immune deficiency diseases, did not explain the upsurge in pediatric cases of initially healthy babies who after a few months

of age presented with failure to thrive, fever, hepatosplenomegaly, recurrent diarrhea, pneumonia, lymphadenopathy and often succumbed to *Pneumocystis pneumonia*. [8, 9] In an eight-patient case series, Oleske wrote, "It seems, however, that the epidemiology of AIDS may now have taken an ominous new turn, with otherwise normal infants and children as additional victims." [8]

Soon thereafter, a case series was published arguing that vertical transmission of an infectious agent was the likely cause of AIDS in three half siblings born to a Caucasian prostitute/IVDU in San Francisco. [10] At that time it was known that AIDS could be transmitted by blood transfusion, but with this case series it was proposed the virus could also cross the placenta or infect the baby from cervical secretions in the birth canal. [10]

We now know that HIV is the infectious agent that causes AIDS and that vertical transmission is one of the primary ways children become infected. Without intervention in HIV-infected pregnant women, transmission is between 15-40%. [3, 11] Many trials have since taken place to develop more efficacious and cost-effective regimens to prevent mother to child transmission of HIV. [3]

1.3 AIDS Clinical Trial Group 076

The first large multicenter clinical trial evaluating treatment to prevent PMTCT was the AIDS Clinical Trials Group (ACTG) 076. This sentinel randomized controlled trial (RCT) was double blinded with a placebo control and demonstrated efficacy and safety of zidovudine (AZT) to significantly reduce MTCT. The study was conducted in the United States and France from 1991-1993 in women with no prior antiretroviral therapy, who had no clinical indication for maternal antiretroviral therapy, and CD4

>200 cells/mm³. The treatment arm was: AZT 100 mg orally five times per day, 2 mg/kg intravenously (IV) at onset of labor, followed by 1 mg/kg/hr until delivery, and AZT was administered to the newborn 2 mg/kg orally every six hours for six weeks.

The trial was stopped early when the Data Safety and Monitoring Board showed transmission was reduced from 25.5% in the placebo group to 8.3% in the treatment group, thereby demonstrating a relative reduction in HIV transmission of 67.5% and forever changing the algorithm of PMTCT.[11]

Though this pivotal trial paved the way for decreasing transmission of HIV in resource rich settings, implementing this regimen seemed insurmountable in the developing world. The prohibitive cost of AZT, administration of the drug intravenously, and limited antenatal care of women early in pregnancy were all barriers preventing the global implementation of the 076 regimen. To address this serious gap, further trials aimed at identifying a feasible and efficacious regimen in sub-Saharan Africa were begun.

1.4 HIVNET 012

The HIVNET 012 RCT took place from 1997-1999 in Uganda and was designed to test single dose nevirapine (sdNVP) compared to AZT for PMTCT among HIV-infected pregnant women. Women received 200 mg NVP orally at onset of labor and their infant received 2 mg/kg orally within 72 hours of birth. This was compared to AZT 600 mg orally administered to the mother at onset of labor, followed by 300 mg every three hours until delivery with the infant receiving 4 mg/kg orally twice daily for one week. The majority of babies in this trial were breastfed and continued to breastfeed beyond 3 months of age. This study showed that in Uganda, a resource limited setting where the

majority of HIV-exposed newborns breastfeed, the nevirapine arm fared better than short course zidovudine, lowering the risk of transmission by nearly 50% (25.1% in the zidovudine arm and 13.1% in the nevirapine arm) at 14-16 weeks of life.[12]

HIVNET 012 did not compare two-dose nevirapine to the ACTG 076 regimen and did not alter recommendations for the developed world setting where cesarean delivery, IV drug administration, and formula feeding are readily available. It did, however, demonstrate that even in women with lower CD4 counts and higher viral loads, administering one single dose of nevirapine to the mother during labor and one dose to the baby within 72 hours after delivery was a highly efficacious and a comparatively inexpensive means by which to deliver PMTCT in the developing world.

1.5 The Petra Trial

The Petra Trial sought to evaluate efficacy of short course zidovudine (AZT) + lamivudine (3TC) in a breastfeeding population of Tanzania, South Africa, and Uganda between June 1996 and January 2000.[13] The trial initially had four arms: 1) AZT + 3TC at 36 weeks gestation (antepartum) + intrapartum + 1 week treatment for both mother and baby (postpartum); 2) Intrapartum + one week postpartum dosing of AZT + 3TC for mom and baby; 3) Intrapartem only (AZT + 3TC); 4) Placebo which dropped out due to ethical concerns in February 1998.

At 6 week follow up, HIV transmission rates were found to be: 1) Antepartum/intrapartum/postpartum (5.7%); 2) Intrapartum/postpartum (8.9%); 3) Intrapartum dosing alone (14.2%); 4) Placebo (15.3%). Intrapartum dosing alone was not efficacious, mirroring placebo. The first group receiving antepartum therapy from 36 weeks through one week postpartum was found superior at 6 weeks follow up. The

18 month follow up results, however, failed to show durability of therapy and by 18 months in a predominately breastfeeding population transmission rates were 14.9%, 18.1%, 20.0%, and 22.2% respectively.[13] The Petra study concluded that further therapy was needed in the developing world to sustain the benefit of PMTCT through the breastfeeding period.[13]

1.6 SAINT Trial

The SAINT trial took place in 11 centers in South Africa from May 1999 to February 2000.[14] Building on the prior work of HIVNET 012 (sdNVP) and PETRA (AZT/3TC), this study set out to compare and confirm efficacy of short-course ARV regimens (initiating treatment at 38 weeks or greater, including those presenting in labor) to consider not only intrapartum transmission, but also the risk of early breastfeeding/postpartum transmission in the developing world. Groups were similar with the exception of viral loads at entry, which were higher in the sdNVP group (10,351 copies/mL) as compared to the AZT/3TC group (7,674 copies/mL). The average CD4 in each group was 436 cells/mm³.

Comparing the two groups, intrauterine infections utilizing infant DNA PCR within 72 hours showed transmission rate of 5.9% in the AZT/3TC group and 7.0% in the sdNVP group. Excluding these infants, those found infected between birth and 6-8 weeks of life were 3.6% in the AZT/3TC group and 5.7% in the sdNVP group. The major risk factor for intrauterine transmission included maternal viral load >50,000 copies/mL, which increased the risk of transmission by three fold. Infants born to women less than two hours after they received sdNVP as compared to those who delivered greater than two hours after the nevirapine dose also demonstrated a three

fold increased rate of transmission by four weeks of life. Duration of rupture of membranes, CD4 count, and breastfeeding also influenced rates of transmission.

This study confirmed efficacy and safety of both short-course ARV regimens, HIVNET 012 (sdNVP) and PETRA (AZT/3TC), to reduce MTCT of HIV in a developing world setting. The limitation of these studies, however, was to demonstrate sustained benefit in long-term follow-up of a breastfeeding population.

1.7 Kesho Bora Trial (“A Better Future”)

The Kesho Bora trial took place in Burkina Faso, Kenya, and South Africa at 5 sites from June 2005 to August 2008 in ARV treatment naïve women with CD4 counts between 200-500 cells/mm³. This trial compared triple-ARV drug prophylaxis regimen of Combivir (AZT + 3TC) + Kaletra (Lopinavir/ritonavir) versus standard first line prophylaxis, AZT plus sdNVP at labor.[15] Both regimens were started at 28 weeks’ gestation or later. The women in the triple-drug arm continued therapy until cessation of breastfeeding or 6.5 months postpartum; the control group received the standard AZT + 3TC one week tail, and did so only after a protocol amendment was issued midway through the study.

The rates of transmission at birth were similar in women randomized to a triple-drug regimen (1.8%) as compared to women randomized to standard antepartum AZT/sdNVP during labor (2.5%).[15] In time, however, a clear benefit to triple therapy and longer duration of prophylaxis was shown. At 6 weeks, HIV transmission was 3.3% in the triple ARV group versus 5.0% in the AZT/sdNVP group (p=0.24); by 12 months, transmission rates were 5.4% versus 9.5% with a statistically significant risk reduction of 43% (p=0.029).

This study also stratified women by CD4 <350 cells/mm³ and CD4 350-500 cells/mm³ at the time of enrollment. At 12 months, it was noted that women with CD4 <350 cells/mm³ experienced a 48% risk reduction of HIV transmission for triple therapy versus standard therapy (p=0.03). The 36% risk reduction among women with higher CD4 counts did not reach significance. This information was incorporated into the latest World Health Organization (WHO) 2010 PMTCT guidelines to start women for their own health on therapy if their CD4 count is <350 cells/mm³.

1.8 World Health Organization (WHO) Guidelines

With expanding knowledge for best PMTCT global practice, WHO recommendations have continued to evolve over time. The latest guidelines, published in 2010, focus heavily on treatment options for pregnant women based on whether they require treatment for their own health versus only prophylaxis based on pregnancy. Criteria for initiating lifelong therapy have become more aggressive. Under the latest guidelines, women with a CD4 count <350 cells/mm³ regardless of WHO stage, or women with WHO stage 3 or 4 infection regardless of CD4 count, qualify for ART for their own health. For pregnant women who do not meet the above criteria, emphasis has been placed on PMTCT not only for the antenatal and labor period, but have been revised to pay particular attention to the postnatal period and breastfeeding prevention.[6]

Current recommendations for women who do not qualify for treatment for their own health offer two options. Option A includes the combination of: 1) AZT, shifting initiation of therapy from 28 weeks gestation to now 14 weeks gestation in the 2010 guidelines; 2) intrapartum AZT plus 3TC and sdNVP; and 3) AZT plus 3TC for 7 days

postpartum. If women receive at least 4 weeks of AZT prior to delivery, intrapartem and postpartum dosing is optional, though often administered. In addition, the new guidelines offer Option B as an alternative regimen. This includes the option for triple drug therapy based on country preference and drug availability.¹ For the infant, the new guidelines recommend continuing ARV prophylaxis with daily nevirapine for a minimum of 4-6 weeks or for one week after the infant discontinues breastfeeding, if beyond the 6-week period.[6]

Implementing these guidelines has proven difficult for many sub-Saharan African countries, but the shift from sdNVP to combination therapy is now becoming a reality. As negotiations for a steady drug supply chain and a structured healthcare work force become realized, PMTCT is working to protect the next generation. In the latest 2011 Global AIDS Progress Report, data from sub-Saharan Africa in 2010 demonstrates 50% of women received combination therapy, while 10% received sdNVP alone.[1]

1.9 Tanzanian PMTCT Implementation and Guidelines

In 2000, a National PMTCT Program was started in five major hospitals across Tanzania, including Kilimanjaro Christian Medical Centre (KCMC), to determine feasibility of integrating the national program into reproductive and child health services. A national scale up plan is now under way with the goal of increasing the percentage of HIV positive pregnant women receiving PMTCT from 34% in 2007 to 80% coverage this year, 2012, and ensuring access to care for both mother and child.[5] In

¹ Option B: Triple ARV prophylaxis starting from as early as 14 weeks and continued until delivery, or one week after all infant exposure to breast milk has ended. Combivir (AZT + 3TC) and either lopinavir/ritonavir (Kaletra), efavirenz (EFV), or abacavir (ABC). A fourth option, tenofovir (TDF) + lamivudine (3TC) or emtricitabine (FTC) plus efavirenz (EFV) is another alternative proposed based on country drug availability.

2009, of the 1.2 million antenatal care attendees, 98% were tested for HIV with a seroprevalence rate of 5.8% (70,423 women).[5] The same year, 84% of those women who tested positive received some form of PMTCT (58,833 women) and about 50% of their exposed newborns received ARV prophylaxis.[5]

This data represents only those women who presented for antenatal care and were tested for HIV. The estimated number of seropositive pregnant women each year in Tanzania is 86,000 reflecting the realization that not all seropositive pregnant women are presenting for antenatal care and testing. Using the estimated number of seropositive pregnant women as compared to the number of women who actually received PMTCT, the met need of seropositive women in care fell from 84% to 68% according to a 2010 report.[5] The most recent UNAIDS Global Report of 2011, estimates approximately 86% of pregnant women in Tanzania were tested for HIV in 2010. Of those seropositive women, only 59% received PMTCT, though this number does not include women who received sdNVP only.[1]

The first Tanzanian PMTCT Guidelines were published in 2004 and have been updated as new literature became available. The 2007 Tanzanian PMTCT guidelines reflect the WHO 2006 PMTCT guidelines which defined women who qualify for treatment for their own health as those meeting WHO stage IV criteria regardless of CD4, WHO stage III criteria if CD4 <350 cells/mm³, or CD4 <200 cells/mm³ regardless of WHO stage. These women should start first line therapy HAART with Combivir (AZT + 3TC) and Nevirapine (NVP). Those women who did not require treatment for their own health should start AZT at 28 weeks or as soon as possible thereafter. If less than 4 weeks of AZT was received prior to delivery, women should receive 3TC +

sdNVP in addition to AZT during labor, followed by a one week tail of AZT + 3TC to prevent development of nevirapine resistance. If women received at least 4 weeks of AZT antenatally further maternal intervention intrapartem and postpartum was optional, but often was received.[4]

The guidelines recommended that exposed newborns receive sdNVP within 72 hours of delivery and AZT twice daily for one week if the mother was on therapy for more than 4 weeks, otherwise the infant should have continued AZT for 4 weeks if the mother did not receive prolonged antenatal AZT. The minimum PMTCT to be given was sdNVP for women during labor and an infant dose within 72 hours of delivery. Infants were recommended to exclusively breastfeed for 6 months unless replacement feeding was acceptable, feasible, affordable, sustainable, and safe (AFASS). Viral testing for diagnosis of infants was recommended at 8 weeks, but antibody test to confirm exposure was utilized when the PCR test was not available.[4]

In 2008, HIV Early Infant Diagnosis (EID) using DNA PCR was initiated in Tanzania. As part of PMTCT scale up, by June 2009, 507 sites were offering EID.[5] Though availability and scale up of EID is excellent, it has been met with challenges. Many health care workers receive little to no EID training, there appears to be lack of ownership at some sites for implementation and scale up, and when EID does occur, there is often a long wait for DNA PCR results delaying the initiation of ART for those infants found to be positive. Worse yet, occasionally results are simply never realized or documented resulting in complete loss of follow up and lack of treatment for infected infants.[5] The United Nations General Assembly Special Session Tanzanian Report of 2010 reports no data on the number of infants born to HIV positive mothers receiving a

virologic test within two months until January to June 2009 in which a reported 834 infants were tested.[5] According to the UNAIDS 2011 Global Report, only an estimated 22% of Tanzanian exposed newborns received a virologic test by 2 months of age in 2010.[1]

With varying numbers and estimates, we see a clear need to examine in a day to day setting what is happening on the ground in Tanzania's PMTCT and EID programs. Our data reflects the guidelines and information above; however in July 2011, Tanzania's third edition PMTCT guidelines were published and follow the updated 2010 WHO guidelines described in section 1.7 above. No longer is sdNVP a recommended therapy, and early combination prophylaxis starting at 14 weeks with a minimum six weeks of daily nevirapine for infants is now the standard of care.[16]

2. Case Study of PMTCT in Three Regions of Tanzania

2.1 Background

Antiretroviral medications administered to a pregnant woman and/or her infant will decrease mother to child HIV-transmission; however, few data exist on the effectiveness of various treatment approaches in a field setting across a large geographic region. Over the course of this study, PMTCT regimens were evolving as more drugs became available and a steady supply chain was formulated. Analysis of the impact of the various PMTCT regimens across the Kilimanjaro, Arusha, and Tanga regions of Tanzania are crucial to inform the Tanzanian Ministry of Health and Social Welfare (MoHSW) about true PMTCT performance on a district and regional level. It also offers insight on the effectiveness of various regimens when implemented in a day to day clinical setting in order to optimize PMTCT regimens.

In 2008, Tanzania rolled out dried blood spot (DBS) HIV DNA PCR testing nationally, thus allowing diagnosis of HIV infected infants in the first few months of life. Prior to 2008, infants often did not receive an official diagnosis until 18 months of life when maternal immunity wanes and interpretation of a positive HIV antibody could assuredly reflect infant infection. Unfortunately, many HIV infected infants would succumb to the virus before an official diagnosis or treatment could be initiated. Without therapy, 50% of HIV infected infants will die before their second birthday; therefore, current guidelines now recommend all seropositive infants less than two years of age begin ART.[16, 17]

The study presented in this thesis was designed to utilize EID results to determine the overall prevalence of perinatal HIV transmission, as well as the effectiveness of maternal and/or infant PMTCT regimen in preventing transmission across the Arusha, Tanga, and Kilimanjaro regions of Tanzania.

2.2 Hypotheses

Perinatal transmission of HIV before 2 months of life was predicted to be 25-35% among infants with no intervention; 10-15% for women receiving single dose nevirapine (sdNVP) only; 6-10% among women who receive a combination prophylactic regimen based on 2006 WHO guidelines/2007 Tanzanian guidelines; and 2% or less for women on highly active antiretroviral therapy (HAART). The combination prophylactic regimen included: 1) zidovudine (AZT) beginning at 28 weeks gestation, 2) sdNVP during labor in addition to AZT + 3TC, 3) AZT + 3TC for one week after delivery.

2.3 Methods

2.3.1 Study Design

This was a retrospective, cross-sectional HIV prevalence study including all available mother/infant pairs enrolled in the National PMTCT program in the Arusha, Kilimanjaro, and Tanga regions of Tanzania between January 1, 2008 and September 30, 2010. Regional data is comprised of 7 districts in Kilimanjaro, 7 districts in Arusha, and 8 districts in Tanga, representing 100 total health facilities performing EID in this area of Tanzania.

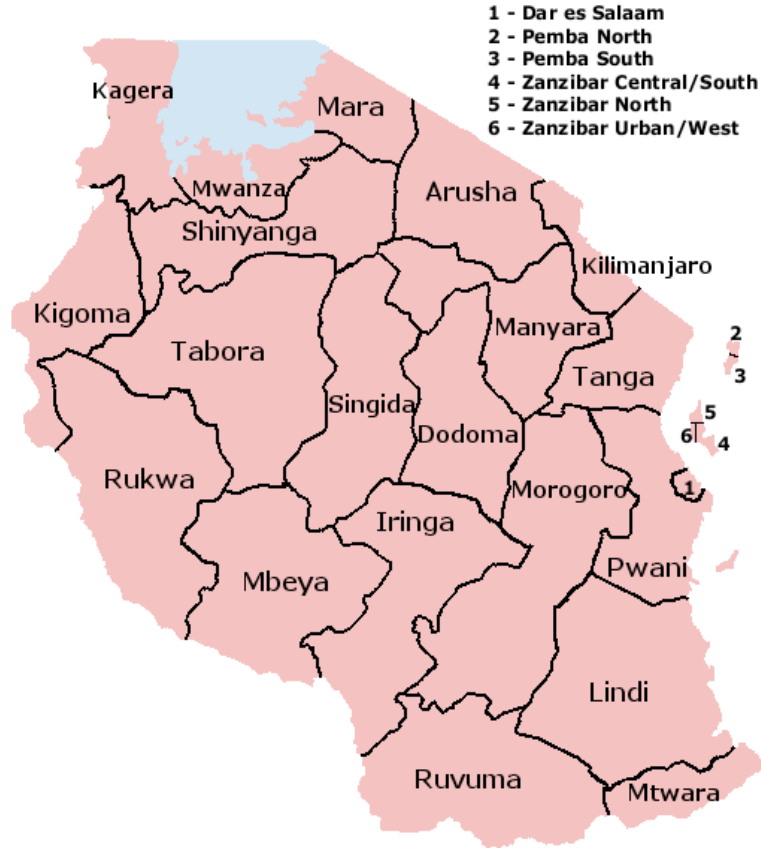


Figure 1: Regional Map of Tanzania demonstrating Arusha, Kilimanjaro and Tanga Regions. Accessed from Wikimedia Commons.

Most infants in Tanzania have their initial DBS PCR performed between 4-8 weeks of life; therefore, test results used for this study are reflective of *in utero*, perinatal, and early breastfeeding HIV transmission. We limited the analysis to children tested during this early period for two reasons. First, we wished to focus the analysis on infants born to women recognized as being HIV infected prior to delivery. If older children were enrolled, the results may be biased by inclusion of symptomatic infants who presented ill and with maternal diagnosis made retrospective of the symptomatic infant. Second, pre and intrapartum treatment will not impact late breast milk transmission; therefore, if older children were included, a significant difference in intrapartum and early breast

milk transmission might be difficult to differentiate from children with late breast milk transmission. For these reasons, we limited our study to include those infants with their first DNA PCR obtained at less than or equal to 75 days of life.

EID is performed using a dried blot spot card in which a heel stick blood sample is obtained from an exposed newborn and five separate circles are filled with the infant's blood sample on Whatman filter paper. The sample is dried and stored at room temperature and shipped to the nearest zonal laboratory (in this case, KCMC) for central repository and virologic testing of HIV DNA PCR. Quality assurance and quality control measures require 15-18% of samples and all indeterminate results to be re-run in addition to proficiency testing. Any positive DNA PCR result is confirmed by running a second as per national standards. Results are then returned to the original center and hand logged in the PMTCT registry.[16]

This study received ethical approval from Duke University, Kilimanjaro Christian Medical Centre Ethics Committee (KCMC EC), as well as the National Institute for Medical Research (NIMR) of Tanzania.

2.3.2 Patients Enrolled

Data were collected for any infant 1) born to an HIV infected mother and registered in the National PMTCT registry in one of the three participating regions, 2) age 6 months or younger at the time of the first HIV DNA PCR test, and 3) initial DNA PCR obtained between January 1, 2008 and September 30, 2010. Data were collected on all infants less than 6 months of age to ensure capture of all patients less than two months. Final inclusion criteria for the present analysis was limited to those infants confirmed to be less than or equal to 75 days of life at the time of their first DNA PCR

test. Patients whose age at the time of test was ambiguous based on their date of birth (DOB) being the same date as the date of test (DOT), or a DOT that was chronologically earlier than the DOB were excluded.

Enrollment logs at all health facilities that submit DBS PCR from the three regions were reviewed to collect information on infant date of birth, age at time of test, weight, feeding practice, maternal and infant PMTCT regimen and outcome, as well as date and result of the first DBS PCR.

2.3.3 Data Collection

Three research assistants were trained on utilization of the case report forms and data collection. The initial set of mother/infant pairs had data collected independently by all three research assistants in order to compare results and trouble shoot questions in the field. Data were then compared to identify inconsistencies and to retrain assistants so that all data were being collected in an identical manner. Data were collected with intent to link the PMTCT Mother Child Follow Up Register to the Maternity (L&D) Register and National PMTCT Care Register; however, due to incomplete and/or unavailable register log books, only data from the primary PMTCT Mother Child Follow Up Register were analyzed for this study.

One research assistant was assigned to collect data per region. Limited access was available for PMTCT data throughout much of the Tanga region, and therefore data from this region must be interpreted cautiously. A study physician provided quality assurance by independently recording case report forms from centers in all three regions at random, demonstrating <1% error rate. Data were entered manually into Microsoft Access (Microsoft Corp) with double data entry and compared using Stata 12.0 for Mac

(StataCorp LP, College Station, TX). Case report forms were reviewed to clarify any discrepancies in double data entry (<1% error) and when no further differences between the two entries were found, the master datasets from each region were merged to one dataset for final analysis.

2.3.4 Statistical Analysis

The primary analysis of this study was to evaluate HIV transmission from mother to infant by maternal/infant PMTCT regimen. We had a large sample size with statistical power for broad comparisons. For larger comparisons, chi squared analysis was appropriate; however, if groups were small (< 10), the Fisher's exact test was used. For secondary analyses, regional and district differences and the impact of time from DNA PCR rollout in each region were analyzed. Maternal ARV regimens included: 1) none; 2) sdNVP; 3) ARV combination prophylaxis including: AZT beginning at 28 weeks gestation, AZT plus 3TC and sdNVP during labor, followed by 3TC + AZT for one week after delivery; 4) HAART; or 5) Other/Not Recorded. Infant ARV regimens include: 1) none; 2) sdNVP; 3) ARV prophylaxis of twice daily AZT for one week if mother had more than four weeks of prophylaxis, otherwise for four weeks; 4) Other or Not Recorded.

2.4 Results

2.4.1 Demographics

Of the 4,701 mother/infant pairs assessed, 2,191 met inclusion criteria and had a documented DNA PCR result at ≤ 75 days of life. These were the patients included in the primary analysis (Figure 2).

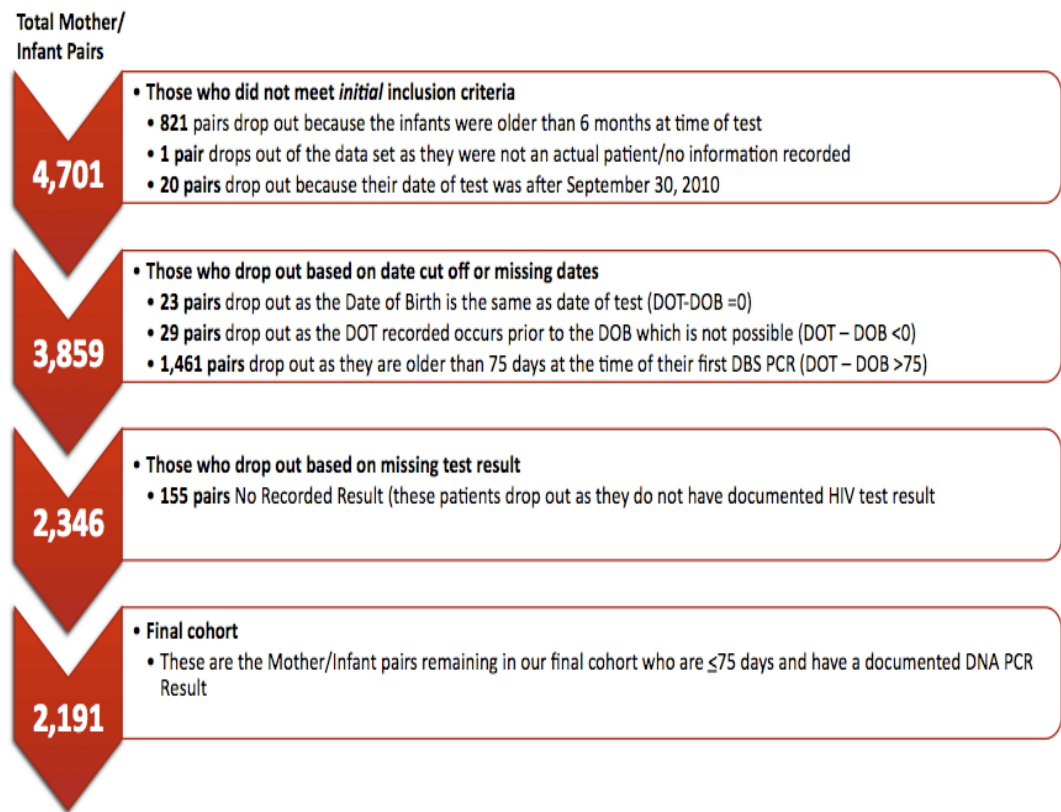


Figure 2: Flow Chart of Exclusion Criteria to Make Up Final Cohort

The 155 patients with a test result not recorded were similar to the 2,191 included in the final analysis. The age at time of first DNA PCR show those with a test result not recorded follow essentially the same distribution as the total cohort (Figure 3). The age of the child at the time of test also shows the data is skewed slightly left and not a normal distribution.

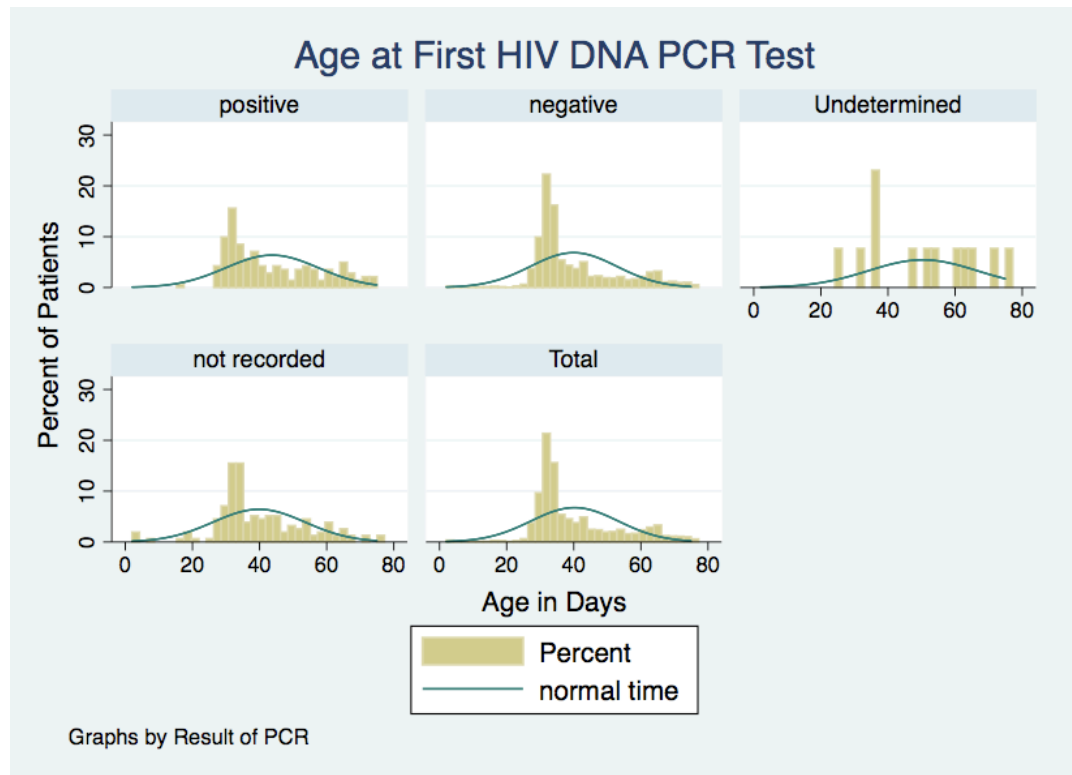


Figure 3: Age of Infant at Initial Test Categorized by Test Result

Further demographic data reveal not only is the average age at the time of first DNA PCR test the same, but so, too, are the maternal PMTCT regimens received reflective of the total cohort (Table 1). Those with test results not recorded had slight predominance from the Tanga District, but otherwise mirror the same distribution of the total cohort with respect to all other demographics; therefore, exclusion of these children does not introduce bias to our final cohort nor the final results.

Table 1: Demographic Data for Final Cohort. Note: Patients with a test result not recorded (shown in red) are demographically similar to the final cohort. Exclusion of these data, therefore, did not bias our final cohort or results.

	Total	HIV+	HIV -	Indet	Result NR
Age at test in days					
average	40	44	40	50	40
standard deviation	13	14	13	16	14
Maternal Regimen					
sdNVP	786	70	710	6	45
prophylaxis	933	36	894	3	79
HAART	240	5	235	1	19
None	164	25	136	3	9
Not Recorded/Other	66	5	63	0	3
Year of Test					
2008	318	47	254	7	10
2009	903	58	765	4	76
2010	1125	36	1018	2	69
Region					
Kilimanjaro	1,082	75	940	7	60
Arusha	1,009	57	887	5	60
Tanga	255	9	210	1	35

2.4.2 Primary Analysis

The primary analysis evaluated maternal to child transmission of HIV based on maternal ARV regimen. Overall HIV transmission rate was 6.4% (Table 2). Among HIV infected women who received no therapy, HIV transmission was 15.3%. The administration of sdNVP alone reduced transmission to 8.9%.

Table 2: Maternal ARV Regimen Based on HIV DNA PCR Test Results

Maternal Regimen (N)	HIV Infected	HIV Negative	HIV Indeterminate
None (164)	15.3%	82.9%	1.8%
sdNVP (786)	8.9%	90.3%	0.8%
Prophylaxis (933)	3.9%	95.8%	0.3%
HAART (240)	2.1%	97.5%	0.4%
Not Recorded/Other (68)	7.6%	92.4%	0%
Total (2,191)	6.4%	93.0%	0.6%

This translated to a significant overall reduction in transmission of 42% in women who received sdNVP as compared with no intervention ($p < 0.012$; CI: 0.38-0.88). Women who received combination prophylaxis had further reduction in transmission to 3.9%. This translated to a 57% overall decreased risk in transmission for women receiving combination prophylaxis versus sdNVP alone ($p = 0.000$; CI: 0.29-0.64). The highly active antiretroviral therapy (HAART) group had the lowest transmission rate of 2.1%; however, because such a comparatively small number of women were on HAART during the study, the difference between combination prophylaxis versus HAART was not statistically significant. Those on HAART did have a significant 77% decreased risk in transmission as compared to those receiving sdNVP alone ($p = 0.004$; CI: 0.10-0.57).

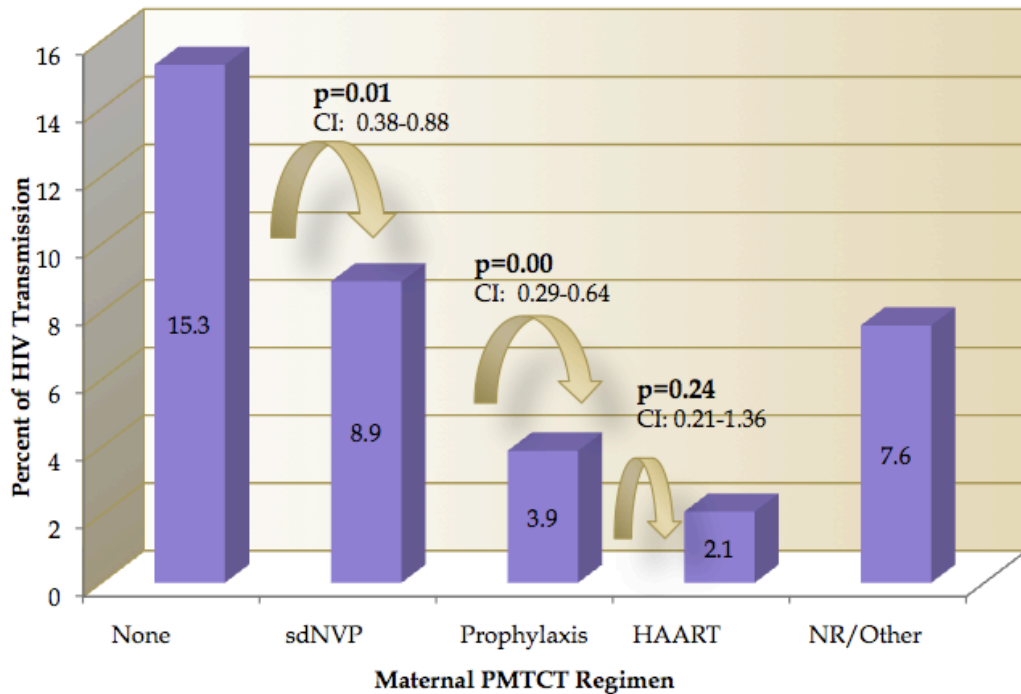


Figure 4: HIV Transmission Based on Maternal PMTCT Regimen in Kilimanjaro, Arusha, and Tanga Regions of Tanzania (01/01/2008-09/30/2010)

This study also explored the impact of perinatal transmission rates when taking into account infant ARV regimens in addition to maternal regimen categories (See Table 6 in Appendix A). Due to limited sample number in mother/infant combination groups, the power of comparisons between groups did not reach significance and was simply hypothesis generating. However, taking into account the infant regimen, if neither mother nor infant received ARV, transmission of HIV increased to 17.8% (as opposed to 15.3% in evaluating mother regimen alone). With a simple intervention of maternal sdNVP, transmission decreased to 11.5%; if the infant also received sdNVP the transmission rate was similar at 10.1% without huge added benefit of the infant dose. For infants of mother/infant pairs who received sdNVP, they were 45% less likely to acquire HIV as compared to mother/infant pairs with no intervention, and for those receiving combination prophylaxis transmission rates were even lower at 3.5%.

As predicted, maternal HAART regimen resulted in the lowest transmission rates regardless of what intervention the infant received. Of the infants born to women on HAART: 1) 22 infants did not receive prophylaxis and none were infected; 2) 54 infants received only sdNVP with one HIV infected infant, resulting in a transmission rate of 1.9%; 3) 155 infants received prophylaxis and 4 were HIV infected, a rate of 2.6%. These were not statistically significant.

2.4.3 Secondary Analysis

Further analyses were performed to describe regional and district performance (Tables in Appendix B) and to determine changes in treatment over time and with program rollout.

Kilimanjaro and Arusha had a similar representation of test results over the 2008-2010 time period. Tanga had many more tests performed in 2010 with further scale up of early infant diagnosis (Figure 5).

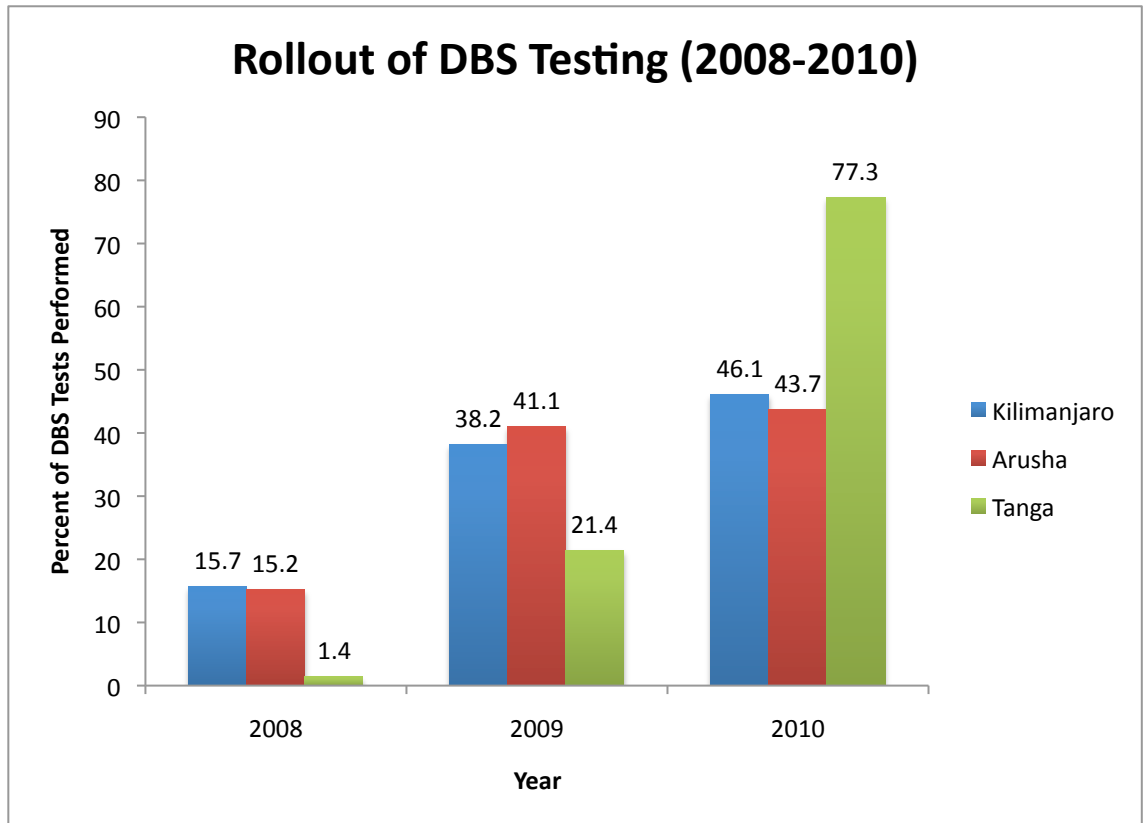


Figure 5: Percentage of Tests Performed in Each Region By Year (01/2008-09/2010)

Further differences between the regions are demonstrated in HIV transmission rates with a 4.0% transmission rate in Tanga as compared to 6.0% in Arusha and 7.3% in Kilimanjaro; however, as previously cautioned, access to PMTCT registries in the Tanga region were limited, making data interpretation of this region difficult (Table 3).

Table 3: Regional Data Based on Test Result

Region	HIV Pos	HIV Neg	HIV Indeterminate
Kilimanjaro n=1,022	75 (7.3%)	940 (92.0%)	7 (0.7%)
Arusha n=949	57 (6.0%)	887 (93.5%)	5 (0.5%)
Tanga n=220	9 (4.0%)	210 (95.5%)	1 (0.5%)
Total n=2,191	141 (6.4%)	2,037 (93.0%)	13 (0.6%)

In 2008, 71.4% of women receiving PMTCT received sdNVP. By 2010, the use of sdNVP shifted with only 15% receiving sdNVP and a dramatic increase to 61.3% of women receiving combination prophylaxis following improved access to medication and the recommended WHO 2010 guidelines. Though percentage of women on HAART trend up, still less than 15% received therapy for their own health (Table 4).

Table 4: Maternal ARV Regimen Based on DNA PCR Test Year

Maternal ARV Regimen	YEAR		
	2008	2009	2010
None (n=164)	9.4%	7.3%	7.1%
sdNVP (n=786)	71.4%	49.3%	15.0%
Combination Prophylaxis (n=933)	7.2%	31.9%	61.3%
HAART (n=240)	5.8%	8.2%	14.6%
Not Recorded/Other (n=68)	6.2%	3.3%	2.0%
Total (n=2,191)	100%	100%	100%

Concurrent with this shift, HIV transmission also decreased. The number of positive test results were twice as high in 2008 as compared to 2009 or 2010 (Table 5).

Table 5: Test Result Based on Year the Test Was Performed

Test Result	YEAR		
	2008	2009	2010
Positive n = 141 (6.4%)	47 (15.3%)	58 (7.0%)	36 (3.4%)
Negative n = 2,037 (93.0%)	254 (82.5%)	765 (92.5%)	1,018 (96.4%)
Indeterminate N=13 (0.6%)	7 (2.3%)	4 (0.5%)	2 (0.2%)
Total= 2,191	308	827	1,056

3. Discussion

3.1 Primary Analysis

The PMTCT Program in Tanzania has made tremendous progress between 2008 and 2010. In the primary analysis, transmission of HIV with no maternal intervention was 15.3%. In the subanalysis, transmission was 17.8% when neither mother nor infant received ARV. This is lower than the hypothesized 25-35% transmission with no therapy, but does fall in line with prior studies of no intervention transmission rates between 15-40%. [11]

Women who received sdNVP demonstrated a significant decrease in perinatal transmission to 8.9%. This is compared to the hypothesized 10-15%. The impact of this easily administered, cost effective intervention is clear. Nevirapine has potent antiviral activity and is rapidly absorbed and passed through the placenta. As most HIV transmission occurs during active labor, rapid intrapartum interventions can impact transmission significantly. Nevirapine has been shown to decrease HIV-RNA viral load by 1.3 logs after a single dose at 1 week postpartum. [18] In the same pharmacokinetics study, it was also shown that nevirapine concentration was maintained well above target of 100 ng/mL in infants 7 days after maternal intrapartum dose even if the infant did not receive sdNVP. [18]

For women who received sdNVP, our analysis did not demonstrate much additional benefit of delivery of an infant dose; however, this subanalysis was not powered for significance and subject only to speculative commentary. Similar infection rates in those with or without infant dosing may be secondary to nevirapine's long half-life providing the infant with therapeutic drug levels throughout the first week even

without an infant dose as described above, or it could be that those infants who received sdNVP received it beyond the recommended 72-hour window, and thus there was no added benefit.

A study in Thailand, originally intended to evaluate addition of sdNVP/sdNVP versus placebo/placebo to mother/infant pairs when the woman received AZT starting at 28 weeks and the infant received AZT for one week after birth was published in 2004. It was designed as a superiority study, but was changed to a noninferiority trial comparing sdNVP/placebo to sdNVP/sdNVP based on ethical concerns.[19] This study found high efficacy adding sdNVP to the maternal AZT PMTCT regimen, with or without a sdNVP dose in the infant.

The HIVNET 024 Ugandan study evaluated timing of sdNVP administration as a secondary analysis and found that timing of maternal and infant doses, as long as within reasonable proximity to delivery, did not affect MTCT.[20] In further literature review, no study was found that directly evaluated sdNVP only to the mother and compared sdNVP to infant within 72 hours of birth versus placebo; therefore commentary on our findings of minimal transmission reduction based on infant receiving nothing versus sdNVP is speculative.

Subanalysis of the maternal ARV regimen when considering the additional impact of infant ARV regimen demonstrates that in the maternal sdNVP group, 20% of the infants born to these mothers were given a combination prophylaxis regimen of sdNVP + AZT (Table 6 in Appendix A). It is possible the more efficacious infant regimen may have helped to drive down the significant reduction in transmission seen when analyzing maternal ARV regimen of sdNVP, 8.9% transmission, as compared to

15.3% transmission when the woman received no intervention (Table 2). Accounting for the subgroup infant regimen (Table 6 in Appendix A) offers further insight into the overall transmission rates seen in the primary maternal ARV regimen analysis.

Combination prophylaxis performed significantly better than single dose nevirapine with a 3.9% transmission risk versus that hypothesized as 6-10%. The maternal/infant analysis demonstrates that the majority of neonates received the recommended sdNVP + AZT prophylaxis if their mother was on combination prophylaxis as recommended by the WHO and Tanzanian guidelines at that time.

For those women who qualified for treatment for their own health and were on HAART, they demonstrated the lowest risk of transmission. As more women receive HAART early for their own health, the expectation is that MTCT will become a rare occurrence, even in resource-limited settings. Relatively few women, only 10.9% in our cohort, received HAART and demonstrated a predicted transmission rate of 2.1%. Though transmission was rare in infants tested at ≤ 75 days, maternal HAART may impact the ability to diagnose infection early. Without longer follow up, it is unclear if this reflects delay in diagnosis versus true protection.

Overall, the primary analysis offers a reflection of what was hypothesized, though transmission in most categories was lower than anticipated. It is unclear if women in our cohort were relatively healthier with lower viral loads as we have no data on maternal viral loads and few women in our study had documented CD4 counts. We also do not have information regarding parity of women in this study and whether they had prior exposure to sdNVP or other PMTCT regimens. These are real world realities, but still, this data offers exciting insight into what is happening in the field.

Clinical trials can be fraught with uncertainties when applied in a real world setting. Whether the intervention will be acceptable and feasible to the general population in a day-to-day setting is not known at the time of study. Strict inclusion and exclusion criteria have potential to bias results when compared to implementing therapy of all comers in a general population.

The literature was appraised to review the published experience of field studies after implementation of findings from PMTCT clinical trials. Unfortunately, few such studies were found. A recent article published by the Elizabeth Glaser Pediatric AIDS Foundation (EGPAF) demonstrates improvement in PMTCT program efficacy over a six and one-half year period, but it does not report specifically on seroprevalence of mother to child transmission and rather scale up of women receiving PMTCT services.[21] A prospective field study from Mozambique demonstrated women on HAART for 6 months postpartum showed significant reduction in HIV transmission: 1.2% at 1 month, 0.6% at 6 months, and 0.7% at 12 months for a cumulative of 2.7% transmission and significant reduction in mortality.[22] This data is supportive of having women on suppressive ART during the ante and postnatal breastfeeding period, but there is no specific RCT for which to compare outcome numbers. An adult study of a prospective Zambian cohort who started ART evaluated mortality and found data similar to that of clinical trials.[23]

A study from Coite d'Ivoire and Burkina Faso published an open label cohort of seropositive pregnant women from 1998 - 2000 who received oral short-course AZT for PMTCT and compared uptake and field effectiveness to clinical trials data of the DITRAME ANRS 049 Study Group.[24] In women, HIV counseling and testing was

significantly higher, 90.3%, as compared to 83.7% in the trial. Women who maintained compliance of the AZT intervention were 81.8% in the weeks prior to labor, 86.7% during labor, and 88.1% postnatally. In the clinical trial these numbers were 78.1% antenatal, 81.1% in labor, and 85% postnatally. Transmission rates were 19.6% at 15 months in the field setting versus 21.2% in the trial setting. Overall, the field data was found to be as good or better than the randomized clinical trial of the DITRAME ANRS 049 Study Group from the same countries.[25]

It is possible that field settings that fared worse have simply not been published; however, this experience demonstrates it is possible that a field setting can yield better results than the controlled clinical trial setting. Our study results, therefore, offer a rare glimpse at what is actually happening in a field setting and demonstrate transmission rates may be actually lower than that suggested by clinical trials data.

The DNA PCR test is another area requiring scrutiny in interpreting the field study results. DNA PCR tests from all three regions included in this study were performed at the centralized zonal laboratory at KCMC where QA/QC protocols are followed per the PMTCT guidelines.[16] A 2005 study described dried blood spot samples taken from 300 HIV exposed infants for DNA PCR at 6 weeks and demonstrated test sensitivity of 100% and specificity of 99.6% using the Roche Amplicor HIV-1 DNA test, the same machine as used by KCMC. This information demonstrates the same machine as used in this study is capable of producing accurate DNA PCR results.[26]

A meta analysis by Dunn a decade prior, in 1995, evaluated data from 271 non-breastfed infants at different time points showing DNA PCR was only 38% sensitive at

birth; however, by 14 days of life sensitivity increased to 93%, and by 28 days was 96%.[27] As the EID results from this study are from an average age of 40 days, the results are expected to be quite sensitive.

The above study found approximately 33% of infections could be attributable to intrauterine transmission, but could not comment on breastfeeding transmission. Since that study, PMTCT regimens have changed significantly. We now have more sophisticated ARV regimens with little understanding of the effect that prolonged prophylactic ARV therapy may have in delaying diagnosis of infection secondary to suppression of HIV virus. There is emerging data that AZT will delay diagnosis secondary to decreasing the viral load, but data on daily nevirapine is not yet available. It is probable, however, that a daily infant dose of nevirapine will extend the time it takes to detect intrapartem and breastfeeding infection.[28]

The primary analysis demonstrates PMTCT in these three regions of Tanzania is effective at 75 days of life; however, further data is needed to establish true seroprevalence at completion of breastfeeding exposure. As noted, with the new guidelines and longer duration of daily infant nevirapine, questions regarding the ability to diagnose and detect virus as accurately at the same time points remains to be seen. An argument for a six month DNA PCR test, even in infants who continue to breastfeed, may be an important policy change. In order to catch those infants who are infected, yet undiagnosed, a test after 4-8 weeks yet before cessation of breastfeeding may be necessary to ensure seropositive infants are started on suppressive ARV treatment as soon as possible. According to the latest 2011 UNAIDS report, only approximately 18% of Tanzanian children in need of ART are receiving HAART.[1]

3.2 Secondary Analysis

Regional data demonstrate similar transmission rates of 7.3% and 6.0% in Kilimanjaro and Arusha, respectively. Both regions had about 15% of EID testing in 2008 and with scale up, 38-46% of tests performed in 2009 and 2010 (Figure 5). Caution must be exercised when interpreting data from the Tanga region as access to the PMTCT registries were limited and may be biased. However, observations from the data available shows only 1.4% of tests from Tanga were performed in 2008 as compared to 77.3% in 2010. This may explain the lower transmission rate of 4% for the Tanga region as more efficacious regimens became available in later years.

The present study shows the trend over time is toward combination prophylaxis with a significant decreased risk of HIV transmission. The number of positive test results were twice as high in 2008 than in the following two years (Table 4). This most likely reflects implementation of Tanzanian guidelines and scale up of PMTCT programs. In 2008, sdNVP made up over 70% of maternal regimens and in this year the hypothesis of 15% transmission with a sdNVP regimen holds true. In time, however, HIV transmission dropped to 3.4% in 2010 as a shift to combination prophylaxis comprised 61.3% of PMTCT.

In the UNAIDS World Report, Tanzania reports that approximately 59% of women received combination prophylaxis in 2010.[1] In the three regions included in the present study, the shift to combination prophylaxis is reflective of this report with 61.3% of patients on combined prophylaxis and 15% receiving sdNVP. The same report states only 22% of infants in Tanzania who were born to HIV positive mothers received a virologic test within two months during 2010.[1] Our data reflects 2,191 infants ≤ 75

days received a virologic test in the Kilimanjaro, Arusha, and Tanga regions of Tanzania between January 1, 2008 and September 30, 2010 as EID was rolled out in Tanzania. The absolute number of exposed newborn births during this time period in these three regions is not known. It is clear that increase in number of EID tests have been performed with each passing year, a trend that hopefully will continue.

From this study we see a scale up in early infant diagnosis and a shift from sdNVP to more efficacious, combination prophylaxis reflected both in recommendations of the 2010 WHO guidelines and findings of the 2011 UNAIDS Global Report. Overall, only 10.9% of women in this study received HAART. The trend of women on HAART certainly increased over time to 14.6% in 2010, but this remains a very low number. Without further information about the women in this cohort, it is speculative to suggest that more than one tenth of seropositive pregnant women should qualify for HAART; however, this data should provoke urgency to re-evaluate the updated guidelines to ensure that all women who qualify for treatment for their own health receive fully suppressive HAART.

Furthermore, there is a gap between the number of women who are pregnant and HIV positive as compared to those women who receive PMTCT services. Bridging the gap between those who are aware of their HIV status, who need testing and counseling, and those who receive treatment is essential to eliminating mother to child transmission of HIV and keeping mothers alive and healthy.

4. Limitations

The length of follow up is a limitation of this study as we can only comment on the benefit of PMTCT regimen in utero, intrapartum, and early breastfeeding transmission. As is clear from clinical trials, the durability of short course ARV tends to fade in the breastfeeding population and longer follow up after breastfeeding cessation would enable this study to further address short course ARV in late breastfeeding transmission.

The database for this study was large, offering a unique glimpse of year to year progress in PMTCT in Tanzania. Limitations of the data, however, occurred in collecting data from individual site logbooks. Some sites had missing logbooks, missing results, erroneous dates recorded, and limited health workers available to assist in finding lost or missing information. The inability to collect data thoroughly at some sites had potential to create geographic and demographic bias, especially in the Tanga region. The lack of data completeness and accuracy in PMTCT data has been eloquently documented in a study from South Africa.[29] Nonetheless, this retrospective review, despite its limitations, does offer significant insight into the progress made in PMTCT in a field setting and illuminates areas that can be strengthened.

Health systems strengthening with more education on EID, the importance of complete and accurate documentation of test results, and ensuring any child with a missing or positive result is accounted for and on therapy are all crucial elements to a successful PMTCT program.

5. Conclusions

In conclusion, this field validation demonstrates PMTCT is effective in preventing mother to child transmission of HIV in the Arusha, Kilimanjaro and Tanga regions of Tanzania. Use of DBS for diagnosis of HIV provides an opportunity to evaluate the use, effectiveness, and evolution of PMTCT regimens.

The study results will be made available to the Ministry of Health of Tanzania to offer feedback on the state of PMTCT in these three regions of Tanzania. With incorporation of a steady supply chain, motivated health workers, and informed patients with testing and counseling, the goal of eliminating maternal to child transmission of HIV can become a tangible reality.

6. Next Steps

This study will continue with investigation of acquired primary HIV resistance mutations utilizing infant DBS cards, which will then be compared with maternal and infant ARV regimens. Access to the DBS cards of infants in the study will also allow for a final attempt at clarifying any missing data including dates of birth, dates of test, and non-recorded results. The cards of HIV infected infants will then be shipped to South Africa for resistance testing and mutations will be analyzed in accordance with information on maternal PMTCT regimen. This data will offer further insight on the impact of maternal and infant ARV regimens, especially with regard to the use of sdNVP and nonnucleoside reverse transcriptase inhibitor mutations.

Appendix A

Table 6: Maternal and Infant ARV Categorized by Test Result

Maternal Regimen	Infant Regimen	HIV positive	HIV negative	HIV Indeterminate
Nothing	Nothing n=73	13 (17.8%)	58 (79.5%)	2 (2.7%)
	sdNVP n=42	6 (14.3%)	36 (85.7%)	0
	sdNVP + AZT n=38	3 (7.9%)	34 (89.5%)	1 (2.6%)
	Not Recorded n=11	3 (27.3%)	8 (72.7%)	0
sdNVP	Nothing n=61	7 (11.5%)	53 (86.9%)	1 (1.6%)
	sdNVP n=524	53 (10.1%)	467 (89.1%)	4 (0.8%)
	sdNVP + AZT n=155	8 (5.2%)	146 (94.2%)	1 (0.6%)
	Not Recorded n=46	2 (4.4%)	44 (95.7%)	0
AZT + 3TC & sdNVP	Nothing n=36	2 (5.6%)	34 (94.4%)	0
	sdNVP n=125	7 (5.6%)	117 (93.6%)	1 (0.8%)
	sdNVP + AZT n=747	26 (3.5%)	719 (96.3%)	2 (0.3%)
	Not Recorded n=25	1 (4.0%)	24 (96%)	0
HAART	Nothing n=22	0	22 (100.0%)	0
	sdNVP n=54	1 (1.9%)	52 (96.3%)	1 (1.9%)
	sdNVP + AZT n=155	4 (2.6%)	151 (97.4%)	0
	Not Recorded n=9	0	9 (100.00%)	0
Not Recorded	Nothing n=7	1 (14.3%)	6 (85.7%)	0
	sdNVP n=26	3 (11.5%)	23 (88.5%)	0
	sdNVP + AZT n=19	0	19 (100%)	0
	Not Recorded n=14	1 (7.1%)	13 (92.9%)	0
Other	Other n=2	0	2 (100%)	0
Total	2,196	141 (6.4%)	2,037 (93.0%)	13 (0.6%)

Appendix B

Table 7: Kilimanjaro District level Data Based on PCR Test Result

Kilimanjaro Districts	HIV pos	HIV neg	HIV indet
Moshi Municipal n=326	26 (8.0%)	296 (90.8%)	4 (1.2%)
Moshi Rural n=156	8 (5.1%)	148 (94.9%)	0
Hai n=144	10 (6.9%)	134 (93.1%)	0
Siha n=19	1 (5.3%)	18 (94.7%)	0
Mwanga n=37	2 (5.4%)	34 (91.9%)	1 (2.7%)
Rombo n=190	24 (12.6%)	168 (86.3%)	2 (1.1%)
Same n=150	4 (2.7 %)	146 (97.3%)	0
Total n=1,022	75 (7.3%)	940 (92.0%)	7 (0.7%)

Table 8: Arusha District Data Based on PCR Test Result

Arusha Districts	HIV pos	HIV neg	HIV indet
Arusha Municipality n= 651	38 (5.8%)	609 (93.6%)	4 (0.6%)
Meru n=136	10 (7.4%)	126 (92.6%)	0
Arusha n=19	1 (5.3%)	18 (94.7%)	0
Longido n=9	0	8 (88.9%)	1 (11.1%)
Monduli n=43	3 (7.0%)	40 (93.0%)	0
Karatu n=68	4 (5.9%)	64 (94.1%)	0
Ngorongoro n= 23	1 (4.4%)	22 (95.7%)	0
Total n= 949	57 (6.0%)	887 (93.5%)	5 (0.5%)

Table 9: Tanga District Data Based on PCR Test Result

Tanga Districts	HIV pos	HIV neg	HIV indet
Tanga City n=78	4 (5.1%)	73 (93.6%)	1 (1.3%)
Muheza n=39	1 (2.4%)	38 (97.4%)	0
Mkinga n=3	0	3 (100.0%)	0
Pangani n=16	0	16 (100%)	0
Korogwe n=20	0	20 (100.0%)	0
Handeni n=43	4 (9.3%)	39 (90.7%)	0
Kilindi n=5	0	5 (100%)	0
Lushoto n=16	0	16 (100%)	0
Total n=220	9 (4.1%)	210 (95.5%)	1 (0.4%)

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