

BIRTH ORDER, DELIVERY AND CONCORDANCE OF MOTHER-TO-CHILD TRANSMISSION OF HUMAN IMMUNODEFICIENCY VIRUS IN TWIN PREGNANCIES

by

Dr Alrese Cloete

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Supervisor: Dr Gregory Petro

Department of Obstetrics and gynaecology

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Birth order, delivery route and concordance of mother to child transmission of Human Immunodeficiency Virus (HIV) in twin pregnancies

Background: Despite two decades of studies of mother to child transmission of HIV, very little data is available regarding vertical transmission in twin pregnancies. There is uncertainty whether discordance of HIV transmission exists between the first born (Twin A) and second born (Twin B) infant.

Objectives: Primary aim of the study was to examine if there is any discordance of HIV transmission in twin pregnancies when comparing Twin A to Twin B. Secondary objectives were to identify possible additional risk factors for HIV transmission in twin pregnancies. We assessed antenatal care, antiretroviral therapy, birth order, delivery route and feeding options as risk factors for mother to child transmission of HIV in twin pregnancies.

Design: Retrospective cohort study

Setting: Maternity centre in the public sector providing secondary level care

Subjects: All HIV positive twin pregnancies delivering at Mowbray Maternity Hospital from January 2010 to December 2011

Outcome measures: HIV status of first born and second born twin

Results: 49 twin pairs were included in the study. Of those there were 45 pairs with neither twin infected. 3 of the 49 pairs had either or both twins infected. Discordance of HIV transmission was found in 1 of 49 pairs of twins.

Conclusion: Twin pregnancies are not at an increased risk of HIV transmission when adequate antenatal antiretroviral treatment is given. We did not find birth order to be an important risk factor for infection in twins.

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1. INTRODUCTION

As of December 2010, approximately 3.4 million children were living with Human Immunodeficiency Virus (HIV) infection globally. 250,000 children died due to HIV-associated disease during 2010 alone. Most of these children live in the developing world, where an estimated 390,000 infants with HIV infection were born during 2010. The commonest route of HIV infection for HIV positive children less than five years is through mother to child transmission. Prevention of mother to child transmission (PMTCT) of HIV is the single most effective medical intervention to reduce the burden of HIV in communities and to achieve Millennium Development Goals 4 (reducing infant and child mortality) and 5 (reducing maternal mortality).

Medical intervention with antiretroviral (ARV) medications during pregnancy involves two separate but related goals: reduction of perinatal transmission and treatment of maternal HIV disease. It is recommended that all pregnant HIV-infected women receive a combination ARV drug regimen, regardless of CD₄ T lymphocyte count or plasma HIV RNA copy number, to prevent perinatal transmission. Post exposure prophylaxis is also administered to the infant after birth to decrease the risk of HIV acquisition. This protects the infant from virus that might have obtained access to the foetal circulation during labour or through exposure to virus during passage through the birth canal.

ARV prophylaxis is particularly important in infants born to women who initiated HIV care and management late in pregnancy. Such women may be at risk of having detectable viraemia, even if medication adherence is excellent, since it takes weeks to months for viral suppression to be attained if the patient's baseline viraemia was high. Similarly, prophylaxis is critical for the infants of women who were diagnosed with HIV at delivery and were not taking antepartum ARV drugs.

1.1 The mechanism of vertical transmission of HIV

Transmission of HIV from a mother to her infant can take place during pregnancy, labour and delivery and after birth via breastfeeding, especially mixed feeding. It is thought that the risk of transmission varies at the different stages. The risk during pregnancy ranging from 5-10%, 10-20% during labour and delivery and 10-20% through mixed feeding. In the absence of any interventions to prevent mother to child transmission, it is estimated that in about 30% of cases the virus will pass from the mother to the infant.¹ Both serological and epidemiological data show that in non-breastfeeding mothers, most vertical transmission occurs during labour or delivery. For example, about two thirds of infants who develop vertically acquired infection is seronegative by polymerase chain reaction(PCR) within the first two days of life, suggesting that a significant viraemia has not yet had time to establish.²⁻⁵

In a prospective cohort study on paediatric HIV infection, it was estimated that 65% of non-breastfeeding infants became infected at the time of birth based on the observed serial development of viral antigenaemia, culture positivity, and antibody production.⁶ Furthermore, antibodies against HIV-1-specific proteins occur at a mean of 54 days of age in almost 70% of infants. This is about the time of sero-conversion following acute infection in adults.

Preterm infants who deliver vaginally are at least as susceptible to HIV infection as those born at term. This suggests that it is labour and delivery that are associated with vertical transmission rather than the duration of the pregnancy. Perhaps, the most convincing evidence of intrapartum transmission is the observation that elective caesarean section reduces transmission by around 50%.⁷

The current HIV epidemic has a huge global impact. An unprecedented quantity of research has been applied to the problem and still little is known about the mechanisms of vertical transmission during labour and delivery. Six candidate mechanisms are as follows:

- Greater micro transfusions of blood from mother to foetus may be associated with vaginal delivery when compared to elective caesarean section. This may be related to contraction-mediated placental damage.⁸
- There may be a greater release of infected placental cells or cytoplasm into the umbilical circulation following vaginal delivery compared with elective caesarean section.⁹
- Earlier cord clamping at elective caesarean section compared with vaginal delivery could partly explain its protective efficacy. It is postulated that early clamping reduces the size of viral inoculum attributable to the first two proposed mechanisms.
- There may be a greater risk of ascending viral infection from the birth canal during the first stage of labour compared with elective caesarean section. The subsequent foetal infection may occur transcutaneous or via transmucosal routes (such as nasal, conjunctival or gastrointestinal inoculation after foetal swallowing).¹⁰
- There may be a greater acute inflammatory infiltrate of the placental membranes following vaginal delivery compared with elective caesarean section. Maternal inflammatory cells could carry infective HIV particles into the amniotic cavity and inoculate the foetus by the transmucosal route, particularly in the presence of ruptured membranes.¹¹
- There may be greater foetal exposure to infected maternal blood and body fluids during the second stage of labour compared with elective caesarean section associated for example with perineal trauma and followed by gastrointestinal or other transmucosal inoculation.¹²

1.2 Background

In 1994 the landmark Paediatric AIDS Clinical Trial Group (PACTG) 076 study found a 67% reduction in HIV transmission when pregnant women were given Zidovudine (AZT) from the second trimester onwards and intravenous AZT intrapartum. Infants received AZT for the first six weeks of life.¹³ By demonstrating that vertical transmission was preventable, these data represented the most dramatic results in HIV research at the time. Global inequities were also highlighted, as the PACTG 076 PMTCT interventions were too complex and expensive for use in resource limited settings.

Following the results of PACTG 076, short course AZT prophylaxis regimens were designed and evaluated in clinical trials in resource limited countries. This was undertaken due to accumulating data suggesting that most transmissions occurs during delivery and that in utero transmission predominantly occurs during the last two months of pregnancy. The Perinatal HIV Prevention Trial (PHPT1) was conducted in Northern Thailand. They attempted to determine the optimal duration of antenatal and postnatal infant prophylaxis in the context of a three part antepartum/intrapartum/postnatal infant regimen. The trial examined four AZT regimens that differed in the duration of maternal antenatal AZT (starting at 28 versus 36 weeks gestation) and the duration of infant AZT prophylaxis (six weeks versus three days). All infants were formula fed.

The results of PHPT1 indicated that longer AZT administration to the infant does not appear to substitute for longer duration of maternal therapy. However longer prophylaxis appeared beneficial for the infant when the antenatal component for the mother was short (for example, starting at 35 weeks of pregnancy).¹⁴ Data from the meta-analysis of individual records of African studies also suggests longer regimens are more efficacious than shorter regimens. In this analysis, a combination of AZT and Lamivudine (3TC) initiated at 36 weeks of pregnancy was significantly more efficacious than the same combination started during labour.¹⁵

In 1999 the Ugandan HIVNET 012 study, conducted in a breastfeeding population, found that just a single dose of Nevirapine (sdNVP) to the mother and a single dose to the child could reduce HIV transmission to 13%. PMTCT was now accessible in resource-limited settings.¹⁶

Over the decade after PACTG 076, evidence of the superiority of highly active antiretroviral therapy (HAART) or multidrug therapy to prevent MTCT accumulated. In 2004, the Thailand PHPT-2 study was conducted in a non-breastfeeding population. It compared short-course AZT (starting at 28 weeks gestation, given orally intrapartum and for one week to the infant) alone to short-course AZT plus single dose intrapartum/new born Nevirapine (NVP) or AZT plus sdNVP to the mother only. The study found that the use of AZT combined with NVP (dual therapy) could reduce HIV transmission to 1.9%.¹⁷ Much work has been done since 1999 and the initial drug trials to minimize, and possibly eliminate, vertical transmission of HIV.

1.3 The development of the South African PMTCT Program

A comprehensive package for a PMTCT programme was introduced in 2001, initially as a pilot programme. Later, in response to a Constitutional Court ruling, it was introduced as a full-scale national programme. This programme was expected to reduce HIV transmission by 50%.

The package included: primary HIV prevention programmes for women of child-bearing age with routine offering of voluntary HIV counselling and testing to pregnant women. A sdNVP was given to the mother and infant. There was a renewed emphasis placed on safe obstetric practices. Infant feeding was discussed and infant formula was provided to women who chose this route. Formula feeding needed to be provided safely, in an acceptable, feasible, affordable and sustainable manner. The policy was in place but significant implementation obstacles remained.

The original Departmental Plan for PMTCT was to implement 18 pilot sites in all provinces; one in a rural setting and another in an urban setting. This was done to establish the operational requirements of a nationwide programme and also to assess the effectiveness of the intervention in a real life situation. Systems were being developed to implement the pilot phase when in July 2002, lobby groups won a case against the Department of Health. The Constitutional Court ordered the Department of Health to make NVP and the provision thereof, available to all HIV positive pregnant women who wish to receive it in public health facilities.

At the inception of the program there was insufficient guidance on how to implement PMTCT, resulting in inconsistent program implementation across the country. PMTCT was mainly a vertical program that was implemented independently of maternal, neonatal and child health services. As the evidence continued to mount on the superiority of multidrug therapy, academics, clinicians and civil society in South Africa mobilized and advocated for the urgent adoption of an updated PMTCT policy incorporating dual therapy, particularly in the wake of updated World Health Organization (WHO) PMTCT guidelines in 2006.

In early 2008 the National Department of Health updated the PMTCT guidelines. Changes included a slight change in the testing strategy, calling this a routine offer of voluntary counselling and testing, the addition of AZT from 28 weeks of gestation with sdNVP at onset of labour. This antenatal PMTCT regimen was followed for all groups of women with a CD₄ cell count of more than 200. The intrapartum regimen consisted of AZT on a 3 hourly basis. The infant regimen included sdNVP with AZT for the baby for seven days. AZT was given to the baby for twenty eight days if the mother received less than four weeks of AZT or HAART antenatally or only received sdNVP. Patients with a CD₄ cell count of less than 200 or WHO stage four disease were eligible for HAART.

Regimen 1a consisted of Stavudine (d4T), Lamivudine (3TC) and Efavirenz (EFV). Pregnant women on EFV were switched to NVP in the first trimester. If they presented after the first trimester EFV was continued. The infant regimen for babies was the same. Unbooked women who presented in labour received sdNVP and AZT 3 hourly. The infant received a 28 day course of AZT and sdNVP.

There was also a renewed emphasis on getting CD₄ cell counts on all pregnant women to determine the need for initiation of HAART in pregnancy and improved guidance on infant feeding options. A greater emphasis was placed on ensuring infant diagnosis at 6 weeks of life.

1.4 2010 guidelines

The 2010 modifications included routine HIV testing and counselling for pregnant women, dual therapy to prevent MTCT of HIV. HAART was initiated for pregnant women with a CD₄ cell count of less than 350 cells/ μ l or clinical stage three or four disease. The aim was to start lifelong HAART within two weeks.

The maternal regimen consisted of Tenofovir (TDF), Lamivudine/Emtricitabine (3TC/FTC) and NVP. In patients that had a contraindication to TDF (renal pathology), AZT was used. Patients that were on HAART before pregnancy EFV was substituted with NVP during the first 12 weeks of pregnancy. Those not eligible for HAART were started on AZT from 14 weeks of pregnancy. The intrapartum regimen consisted of AZT 3 hourly in labour and sdNVP and TDF/FTC (Truvada) at the onset of labour. Unbooked patients in labour were given sdNVP and Truvada.

Infants whose mothers were on HAART were given NVP at birth and then daily for 6 weeks irrespective of infant feeding choice. If the mother was on AZT for MTCT prophylaxis the infant received NVP at birth and daily for 6 weeks if baby was formula fed. Breastfeeding infants continued as long as any breastfeeding. Where the mother did not get any antiretroviral therapy

before or during delivery the infant must receive NVP as soon as possible and daily for at least 6 weeks and continued as long as any breastfeeding.

1.5 Effectiveness of the National PMTCT Program in South Africa

Within ten years of implementing the National PMTCT program in South Africa interventions to prevent MTCT of HIV are now offered in more than 95% of public antenatal and maternity facilities country-wide. However, the 2010 South African PMTCT Evaluation (SAPMTCTE) was the first national evaluation to determine the effectiveness of the National PMTCT program. A cross-sectional facility based survey was conducted at immunization service points at public primary health care/community health centres (PHC/CHC) in all nine provinces. This methodology was chosen as immunization uptake at 6 weeks is more than 99% in South Africa.

The survey aimed to capture known and unknown HIV-exposed infants, as well as PMTCT participants and non-participants. A biomedical marker, HIV enzyme-linked Immunosorbent Assay (ELISA), tests to identify HIV antibody was used to identify HIV-exposed infants from infant dried blood spot (DBS) specimens. All DBS specimens reactive on ELISA testing were sent for DNA-based polymerase chain reaction tests (DNA PCR) to determine infant HIV infection status. Infants aged 4-8 weeks attending PHC/CHC facilities for their six week immunization were included.

The survey found that the national infants HIV exposure prevalence was 31.4% in 2010 and 32.2% in 2011. Infant HIV exposure prevalence serves as an indirect marker of maternal HIV prevalence. Perinatal mother to child HIV transmission rate measured at 4-8 weeks infant age was 3.5% in 2010 and 2.7% in 2011. Among mothers who reported being HIV negative, 4.1% had HIV-exposed infants. Therefore repeat HIV testing at 32 weeks pregnancy and couple testing is critical. Of the reported HIV-positive mothers 78.3% had a CD4 cell count done during pregnancy and 91.8% received either HAART or mother/baby ARV prophylaxis. Only 35.1% intended to access early infant diagnosis (EID) services and 89% had received infant feeding counselling.

Infant HIV testing uptake is high if offered to all infants (94%) at six-week immunization visits, indicating that EID strategies that routinely offer infant HIV testing only to known HIV exposed infants should be reviewed.¹⁸

2. HIV IN TWIN PREGNANCY

2.1 Incidence and epidemiology of twin pregnancy

Dizygotic (DZ) twins are more common than monozygotic (MZ) twins in the absence of the use of assisted reproductive techniques. The incidence of MZ twins is relatively stable worldwide at 3 to 5 per 1000 births, but there is significant ethnic variation in the incidence of DZ twinning.

The major factors influencing the incidence of DZ twins are:

- Use of fertility stimulating drugs- Artificial reproductive techniques often involves transfer of multiple embryos. DZ multiples are far more common than MZ multiples in pregnancies conceived in this way compared with naturally conceived pregnancies.¹⁹
- Maternal age - advancing age is associated with an increased prevalence of twin birth. Naturally conceived DZ twinning increase four-fold between age 15 and 35. This may be related to rising follicle stimulating hormone concentration with age. One-third of the increase in multiple births in recent decades has been attributed to increasing age at childbirth.
- Race/geographic area - The incidence of naturally conceived twins is highest among some black populations in Africa (1/30 births), lowest in Asians (less than 1/100 births), and of intermediate frequency in Caucasians (1/80 births).²⁰
- Parity - Increasing parity correlates with an increased likelihood of twin birth, even after adjustment for maternal age.
- Family history - Twinning appears to have a genetic component that is expressed in women, but can be inherited from either parent.²¹ Thus, a woman is at increased risk of having twins if she has a family history of

twin births. The family history of the biologic father appears to have little or no effect on his partner's risk of having twins.²²

- Maternal weight and height - Obese (Body mass index(BMI) more than 30kg/m²) and tall women(more than 164 cm) are at greater risk for twin birth than underweight (BMI less than 20 kg/m²) and short women (shorter than 155 cm).²³

2.2 HIV transmission in twin pregnancy

Despite two decades of research on MTCT little study of risks in multiple pregnancies in women with HIV infection has been done. Twin pregnancies are associated with an increased risk of preterm labour and premature rupture of membranes. These are known risk factors for MTCT.²⁴ The literature is conflicting whether there is an increased risk of MTCT in twin compared to singleton pregnancies and if there is discordance of transmission between the first and second delivered baby.

In a multicentre study on perinatal HIV infection including 1493 children born from 1471 pregnancies to 1415 infected mothers, 22 twin pairs and 56 sibships (115 children) were recorded. In 18 twin pairs with a known infection status 9 of the 36 children (25%) were infected. Discordance in infection status was present in only one (5.5%) dizygous pair. A high relative risk of infection (23.1) in a twin was observed when the other was infected. Infection was unrelated to gestational age, mode of delivery, or birth weight. Infection status was defined in 41 sibships (84 children including one first born twin pair and one third born child). When the first born was infected, 11/26 (42.3%) second born children were also infected, whereas this happened in only 2/16 (12.5%) second or third born children when the first born was uninfected. Two out of nine first born (22.2%) and 5/21 (23.8%) second born children prospectively followed up from birth acquired the infection. Results of this study demonstrate that neither twin nor second pregnancies are at increased risk of mother to child HIV transmission.²⁵

HIV-infected mothers were identified on a New York State Medicaid to examine factors associated with perinatal HIV transmission. In 35 twin pairs, transmission was 20.5%. The risk of transmission was increased significantly for advanced maternal HIV infection. The study found no association of birth order with twin HIV status. These data suggest that maternal stage of disease plays a greater role in vertical HIV transmission than birth order. They concluded that to prevent MTCT, reducing maternal viral load is likely to have a greater impact than modifying delivery factors.²⁶

In another study data was evaluated from 115 prospectively identified twin pairs born to HIV-infected women. Infection with HIV occurred in 35% of vaginally delivered and 16% of caesarean-delivered first born twins. 15% of vaginally delivered second born twins and 8% of caesarean-delivered second born twins were infected with HIV. Comparing vaginally delivered first born twins (infants most exposed to vaginal mucus and blood) to caesarean delivered second born twins (infants least exposed), 76% of the transmission risk was related to vaginal exposure. They concluded that these results indicate that HIV infection of twin B occurs predominantly in utero, whereas infection of twin A (and, by implication, singletons) occurs predominantly intrapartum. They proposed that intrapartum transmission is responsible for the majority of paediatric HIV infections and that reducing exposure to HIV in the birth canal may reduce transmission of the virus from mother to infant.²⁷

The mechanisms of intrapartum HIV infection in twin pregnancies remain unclear. Three hypotheses can be suggested. First, infection might occur with mixing of maternal and foetal blood during the contractions. This hypothesis would suggest that twin B is at higher risk than twin A because they are exposed to uterine contractions for a longer period. Second, breaks in the skin could become contaminated by infected maternal blood or secretions. Third, the infant might be infected through mucous membranes or by swallowing maternal blood or secretions before or during passage through the birth canal. Cell-free and cell-associated HIV can be detected in the

genital secretions of 12% to 50% of HIV-infected women even in the absence of gross blood.²⁸

One study suggested that genital shedding of HIV is significantly higher in pregnant women than in non-pregnant women.²⁹ Vaginally delivered first born twins have the greatest exposure to birth-canal products. They pass through infectious mucus and blood, and they are in the birth canal longer than second delivered twins. This could possibly explain that first born twins have an increased risk of infection. In a caesarean delivery, twin A usually is the one presenting at the cervical os and thus has ready exposure to ascending infections.³⁰ The birth experiences of twin B differ from those of twin A.

Although vaginally delivered second born twins are exposed to the birth canal, the duration of exposure usually is relatively short. In addition, the first born twin will have dilated and, to some extent, mechanically cleansed the canal.

This finding was, however, not confirmed in a study from Malawi. They examined birth order and delivery route as risk factors for MTCT of HIV in 315 twin pairs born in Malawi during 1994-1998. No ARV drugs were administered to these subjects. Infections were detected by PCR and were stratified as having occurred either in utero, perinatally, or postnatally. Risk of in utero infection for 630 infants (39 infections) did not differ by birth order (first born, 6.3%; second born, 6.0%). Similarly, in 260 vaginally delivered infants evaluated for perinatal infection (45 infections), risk did not differ by birth order (first born, 15.9%; second born, 18.7%); risk of perinatal infection was significantly lower in caesarean-delivered infants. There was no effect on postnatal transmission rates. Thus, in contrast to the authors of earlier studies, they did not find birth order to be an important risk factor for infection in twins.³¹

The French Perinatal HIV Cohort investigated whether twin pregnancies were at an increased risk of MTCT in comparison with singletons. They studied the association between twin deliveries and MTCT rates according to three time periods (ARV therapy not routinely available, prophylaxis with AZT

monotherapy and combination therapy) and the effect of birth order. The greatest difference in pregnancy outcome was the high rate of preterm delivery in twins versus singletons. Over one half of the twins were delivered before 37 weeks and severe prematurity (less than 33 weeks) in twins was fourfold that in singletons.

The other important differences were a higher rate of emergent caesarean section in twins without a difference in elective caesarean section rate, and a trend towards more premature rupture of membranes. Twins had a shorter median duration of ARV therapy, which reflected the higher incidence of preterm labour. The MTCT rate in this study was significantly higher in twin pregnancies than in singleton pregnancies. It is unlikely that the higher transmission rate in twin pregnancies could be accounted for simply by the fact that twice as many children are exposed. Considering the first born child, the transmission rate remained significantly higher in multiple pregnancies than singleton pregnancies. They found that transmission of HIV occurred more frequently to the first born twin than the second twin. The greater risk of transmission to the first twin is indirect evidence for infection via an ascending route or birth canal exposure.

Other indirect evidence is the higher risk of infection for the first born twin in case of premature rupture of membranes, as well as the protective effect of elective caesarean section. But the increased risk of transmission in twin pregnancy was only evident in the period before any ARV prevention was available, as well as in the period of AZT monotherapy. In the HAART era they found that twins were no longer at a significantly increased risk of HIV transmission compared to singletons. This study's findings indicate that twins as well as singletons, are at low risk of MTCT of HIV when the mother has an undetectable viral load with HAART.³²

The risks of postpartum transmission have been well described.³³ Extended breastfeeding is responsible for 14% of cases of HIV in chronically infected mothers, and MTCT may be as high as 29% if the acute phase of maternal infection occurs during breastfeeding.³⁴ When analysing MTCT over time a recent study found a drastic reduction in rates from 91% to 2% associated with the adoption of prevention measures, which included not breastfeeding and using ARV.³⁵ Where breastfeeding is common, it is particularly important to include sdNVP as a component of prophylactic regimens to prevent MTCT. The long half-life of NVP prevents early postnatal transmission through breast milk which is a significant component of postnatal transmission. This effect is not observed with AZT prophylaxis.³⁶

2.3 Summary

The issue of mother to child transmission of HIV in twin pregnancy is important because of increasing incidence of multiple pregnancies. This is due to advancing maternal age at first pregnancy and the use of assisted reproduction techniques such as artificial insemination and in vitro-fertilization. These women require updated information on the risks involved in the case of twin pregnancy.

Patients frequently seek antenatal care very late in pregnancy. This can lead to inadequate antenatal ARV prophylaxis. Twin pregnancies frequently result in preterm labour and preterm rupture of membranes. This puts twin pregnancies at an increased risk of mother to child transmission of HIV.

To date we are unaware of any study to investigate the risk of HIV transmission in twin pregnancy in South Africa. This was confirmed by doing a Medline search.

3. MATERIALS AND METHODS

3.1 Study design

A historical (retrospective) cohort study was conducted, comparing MTCT rates in twin pregnancies. The study period was twenty four months. It was carried out at a secondary level referral obstetric unit. The primary aim of the study was to determine if there is any discordance of transmission of HIV in twin pairs. The secondary objective was to identify and describe possible additional risk factors for HIV transmission in twin pregnancies. We assessed birth order, antenatal care, intrapartum care, mode of delivery and feeding methods.

SELECTION CRITERIA

INCLUSION CRITERIA	HIV positive twin pregnancies PCR results of both twins available
EXCLUSION CRITERIA	Intra uterine foetal demise Assisted deliveries Delivery before 28 weeks Unbooked patients

3.2 Subject identification and selection

Patients were identified on the PMTCT register and folders were reviewed for additional information. All HIV positive twin pregnancies that delivered between 01 January 2010 to 31 December 2011 were included. Since the PMTCT program changed in 2010, our study population included patients already on HAART, patients starting ARV therapy at 28 and 14 weeks and patients starting HAART during pregnancy. The type of ARV therapy was defined by the last treatment received before delivery and classified as dual therapy (AZT antenatally and intrapartum plus sdNVP and Truvada in

labour), or HAART (combinations of three or more drugs). We described demographic characteristics, obstetric care and HIV management of twins. We compared duration of ARV therapy, mode of delivery, CD4 cell count, infant feeding method used and infant HIV PCR results of twin A (first born twin) and twin B (second-born twin).

3.3 Data collection

The study was conducted at Mowbray Maternity Hospital (MMH). There are 9000 deliveries at MMH per annum. It is a secondary level care facility and provides antenatal care to pregnant patients of the local area as well as Khayelitsha, Gugulethu and Mitchell's Plain areas (during the study period). Patients were identified on the PMTCT register and additional data was extracted from folders. The PCR results of infants were traced through the National Health Laboratory Services (NHLS).

3.4 Data management and statistical analysis

All data was captured without confidential patient details. Study numbers were used. Only the principal investigator was aware of the coded study number allocation, in order to verify data at a later stage. All data was entered into an Excel spread sheet on a password protected computer. The data was accessible only to the principal Investigator. Descriptive data is presented in graphs and tables. The tables include 95% Confidence Intervals, where appropriate. Statistical analysis was performed using Stata statistical software, Version 10. Continuous data was analysed using Student-t and Mann-Whitney tests. Categorical data was analysed using Chi-square and Fisher Exact tests for the differences between proportions. Data is presented with 95% Confidence Intervals and p-values are included, where appropriate. A p-value of <0.05 was used to indicate statistical significance.

3.5 Ethical Considerations

As this was a retrospective case review there was no impact on the clinical management of patients enrolled in this study. Patient confidentiality was ensured. Only the principal investigator had access to the patient folders. The folders were reviewed at the hospital and no folders were removed from the premises. The scientific rigour of the study was assessed by the Departmental Research Committee of the Department of Obstetrics and Gynaecology of the University of Cape Town. Ethical approval to conduct the study was obtained from the Human Research Ethics Committee of the University of Cape Town (HREC REF: 493/2011). Initially the intention was to also include singleton HIV positive mothers and their infants into the study as a control group. Due to difficulties in finding patient folders and time constraints the study aim was amended and only twin pregnancies were included. (Amendment letter dated 26/08/2012)

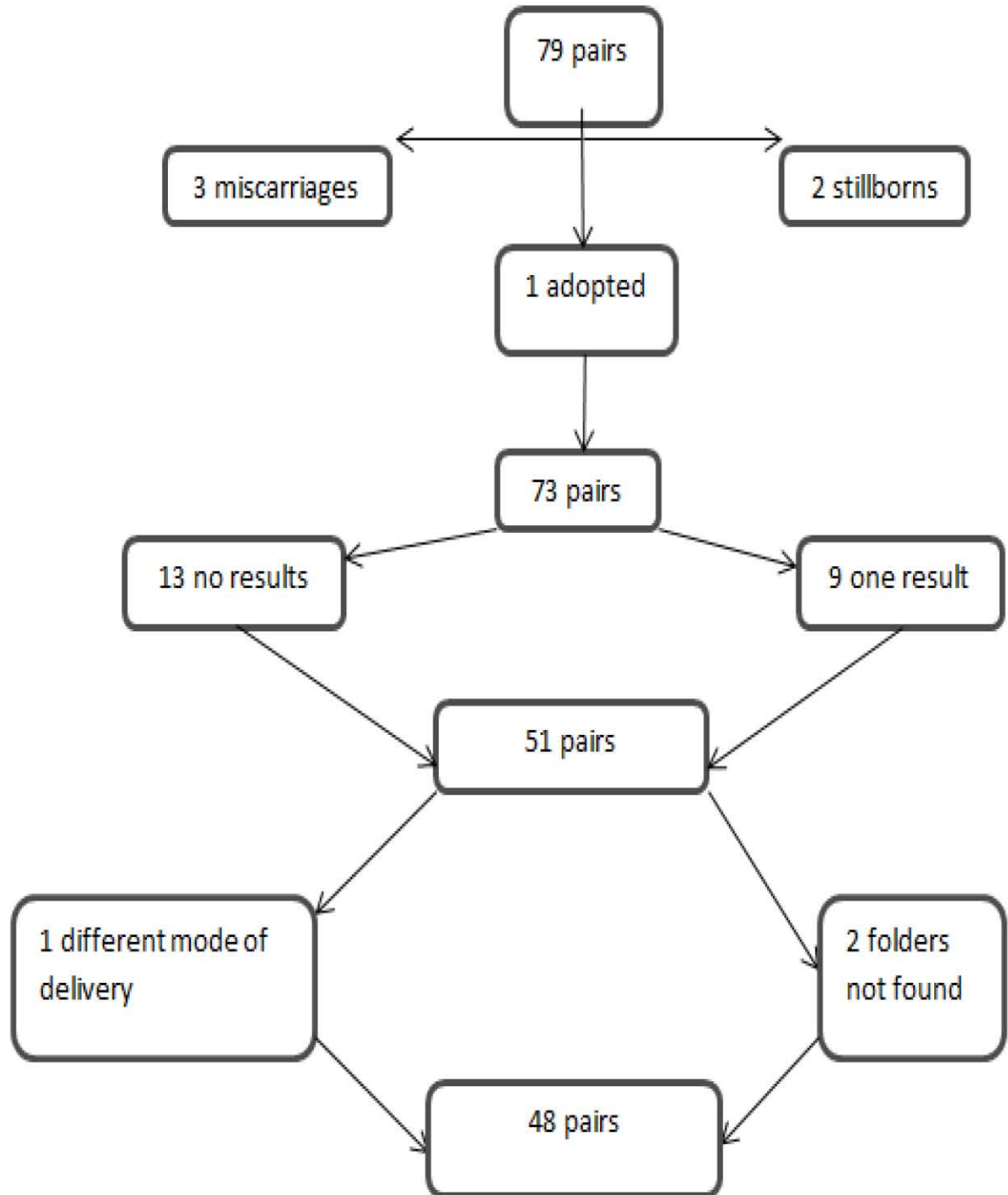
4. RESULTS

4.1 Study population

We assessed all HIV positive mothers that delivered multiple pregnancies at Mowbray Maternity Hospital between 01 January 2010 and 31 December 2011. During this time period seventy nine patients with multiple pregnancies were seen. One triplet pregnancy with a miscarriage at 25 weeks and two twin pregnancies with second trimester miscarriages were excluded. Two twin pairs where either babies or one of the pair were stillborn also had to be excluded from analysis. One twin pair was adopted and PCR results could therefore not be traced. With those six twin pairs excluded there were 73 twin pairs left for analysis. In nine pairs only one of the babies PCR results was available.

Due to the limitations of the PMTCT register and difficulty in obtaining infant folders, we are uncertain if one of the babies in these pairs demised. This would be the logical conclusion since it is unlikely that their mothers would

only bring one infant for PCR testing. Thirteen patients had no PCR results available. We obtained PCR results for 51 twin pairs. One twin pair was excluded from analysis because the delivery route for twin A and twin B differed. Two twin pairs had to be excluded because their folders could not be found. We had full data on 48 twin pairs.



4.2 Demographic details of study population

4.2.1 Maternal characteristics

The oldest patient recruited was 40 years old and the youngest 18 years old. 62% of the patients were older than 30 years. The median maternal age was 31 years old. Surprisingly there were twenty one primiparous patients which is not consistent with the literature that twin pregnancy increases with increasing parity.

	mean	minimum	maximum	Standard deviation
Maternal Age	30.396	18.000	40.000	5.1312
Gravidity	2.604	1.000	6.000	1.0865
Parity	1.583	0.000	5.000	1.1077

4.2.2 Antenatal care

Twenty seven patients were on HAART, twenty patients had received AZT for more than four weeks and one patient for less than four weeks.

Contingency table: CD₄ count and antenatal ARV therapy

	CD ₄ <200	CD ₄ 200-350	CD ₄ >350	Row
HAART	8	11	8	27
AZT>4wks	1	3	16	20
AZT<4wks	0	1	0	1
Totals	9	15	24	48

The above contingency table illustrates that the majority of the patients were receiving adequate antenatal ARV therapy based on their CD₄ cell counts. Nine patients had a CD₄ cell count of less than 200 cells/ μ l, 15 patients between 200-350 cells/ μ l and 24 patients had a CD₄ count of more than 350 cells/ μ l.

For most sets, zygosity was assessed by clinical criteria including gender and placentation where early ultrasounds were not done.

DCDA	28
MCDA	10
MCMA	1
Unknown	9

4.2.3 Intrapartum

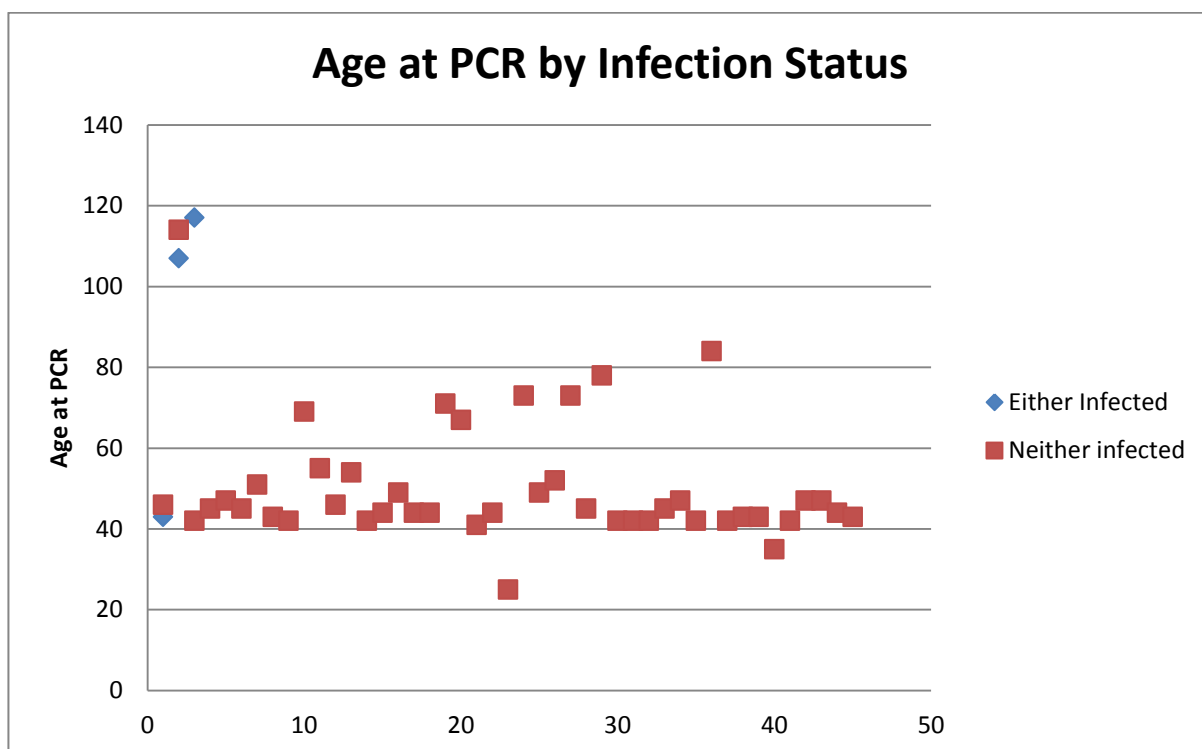
Twenty patients were in labour and six patients had rupture of membranes for more than four hours. Ten patients had an elective caesarean section, twenty seven patients had emergency caesarean sections and eleven patients had vaginal deliveries.

Of the patients receiving antenatal AZT one patient had no AZT 3 hourly in labour and no NVP/Truvada post delivery. This patient had a vaginal delivery. Another patient had no NVP/Truvada and had an emergency caesarean section. Overall the intrapartum care in patients was consistent with the PMTCT protocol.

4.2.4 Infants

The average birth weight was 2254 kg.

GENDER	
FEMALE	42
MALE	48
FEEDING	
BREAST	9
FORMULA	36



The average age when PCR was done was 52 days.

4.3 Results of primary aim

Of the 48 pairs included in the study there were 45 pairs with neither twin infected. Three twin pairs had either or both twins infected. Only one pair had discordance of infection. The mother of this pair was on HAART and had CD₄ cell count of 335 cells/μl. She was not in labour and did not have rupture of membranes for more than 4 hours. An emergency caesarean section was done and the time difference between delivery of twin A and twin B was 2 minutes. Thus the antenatal or intrapartum care cannot explain the discordance. The birth weight of twin A was 1360 gram and twin B weighed 1120 gram. The PCR of these babies were only done at the age of 117 days. They were also breastfed. In this pair it is not possible to predict the timing or mechanism of infection.

	PCR results positive	PCR results negative	Row
Twin A	2	46	48
Twin B	3	45	48
Totals	5	91	96

p=0.64599

Contingency table for HIV transmission by twin succession (not twin sets)

The Pearson's chi-square test is not significant and cannot prove that the variables are contingent on one another. Thus, there is no statistically significant discordance in HIV transmission in twin pregnancy.

4.4 Results of secondary objectives

Of the 2 pairs where both babies were infected, in one pair the mother had defaulted antenatal care and had not been taking AZT for at least 4 weeks. She was in labour but did not have prolonged rupture of membranes and had adequate intrapartum care (NVP/Truvada one dose and AZT 3 hourly). The infants also did not receive 28 days of AZT (2008 guidelines) and they were formula fed.

Contingency table of antenatal ARV therapy

	HAART	AZT > 4wks	AZT < 4wks	Row
Neither infected	26	19	0	45
Either infected	1	1	1	3
Totals	27	20	1	48

p=.00046

The contingency table shows a significant correlation between patients on HAART and AZT for more than 4 weeks and their infection status. This illustrates the significant role appropriate antepartum antiretroviral treatment plays in prevention of mother-to-child transmission.

The other twin pair where both babies PCR were positive had adequate antenatal ARV prophylaxis and mother had CD₄ cell count of 252 cells/μl. An elective caesarean section was done at 38 weeks gestation, the mother was

not in labour and there was no prolonged rupture of membranes. The PCR was however done at 107 days. It was indicated on the PMTCT register that the mother was planning to formula feed these babies. We are unable to say whether transmission of HIV occurred in utero, intrapartum or postnatally.

Contingency table of mode of delivery

	Vaginal delivery	Emergency caesarean section	Elective caesarean section	Row
Neither infected	10	26	9	45
Either infected	1	1	1	3
Totals	11	27	10	48

p=0.70812

All of the other 9 patients (that we only had one of the twin pair's results) had negative PCR results. Thus in this sample of 105 infants (48 twin pairs plus 9 infants) only 5 patients had positive PCR results. The question if age at PCR is significantly different between the Neither infected group and the Either infected group is difficult to answer because of the small sample of either infected.

Infection status	Sample Size	Mean	Standard deviations
Neither infected	45	50.44444444	15.28153306
Either infected	3	89	40.1497198

t-test
p value=0.18

5. DISCUSSION

There were very few patients in the infected group and because of the small sample size drawing conclusions from some of the contingency tables had to be done with caution. Our study population had a low background risk for vertical transmission of HIV. Acutely infected women are likely to be at a higher risk of transmitting HIV to their children than chronically infected women. Partly because the high levels of HIV viral load that occur during acute infection are associated with increased risk of perinatal transmission^{37;38} and transmission through breastfeeding,³⁹ and partly because the maternal immune response during acute infection may not be sufficiently mature to allow significant transfer of protective immunity to the child.⁴⁰

Our findings indicate that twins are at low risk of MTCT of HIV when the mother is on HAART or has taken effective ARV treatment (dual therapy) in the antenatal period. And that perinatal HIV transmission does not involve birth-canal exposure as an important contributor to risk of HIV infection, although some infants possibly could become infected via this route. In general, the risk of perinatal transmission declines with low levels of maternal HIV RNA (e.g., <1000 copies/mL) although there is still some element of risk.⁴¹

In a prospective cohort study, HIV RNA levels were serially measured in 1542 HIV-infected women who gave birth from 1990 to 2000 to assess the relationship between plasma viraemia, the type of therapeutic intervention, and the risk of HIV perinatal transmission.⁴² Overall, the number of infant infections declined with increasing complexity of the HIV treatment regimen. HIV transmission rates were:

- 20 % among 396 women who did not receive ARV drugs
- 10% for 710 women taking AZT alone
- 4 % for 186 women receiving dual ARV drug regimens
- 1 % for those taking three-drug combination ARV drug regimens

Similarly, the risk of HIV transmission declined with lower levels of maternal viraemia at delivery. HIV transmission rates ranged from 1 % for patients with a non-detectable viral load (<400 copies/mL) compared with 23 % for those with a viral load more than 30,000 copies/mL. These data support the use of combination ARV drug regimens with the goal of full viral suppression to decrease the risk of perinatal HIV transmission.⁴³

These data also highlight the fact that women with a non-detectable viral load can still transmit HIV to their infants, although the risk is low. This residual risk may be related to the presence of detectable HIV in the genital secretions. In a recent South African study of 730 pregnant women with advanced immune suppression that started ARV drug therapy during pregnancy, each additional week of treatment reduced the odds of perinatal transmission by 8 %.⁴⁴

In the one pair with discordance of HIV transmission in our study, there was a more than 10% discordance of birth weight. One study found that infected B twins tended to be smaller than uninfected B twins. This suggests that some growth retardation with in utero infection may occur but would be difficult to detect in A twins and singletons, among whom most infections probably occur in the intrapartum period. Caesarean section-delivered B twins never enter the birth canal and have only remote exposure to blood and mucus that ascends into the uterus. Except for their exposure to blood during caesarean delivery, B twins have a relatively minimal exposure that might be close to the baseline rate of HIV-1 infection in utero.

In our study the average gestational age at delivery was 35 weeks. This is also surprising since we expected more preterm deliveries in twin pregnancies. It might be explained by the fact that ten patients had elective caesarean sections, which are mostly done at or near term. We suggest that twin pregnancies represent high-risk obstetric conditions in which caesarean deliveries are more often elective and more likely to occur before or sooner after the rupture of membranes than for singletons, thus reducing the risk of ascending infections.

Because ours was a retrospective study, we were unable to separate in utero infections from perinatal infections. There is scope for a future prospective study where umbilical cord blood samples are taken as well as a postnatal sample (4-26 weeks after birth). When the umbilical cord blood samples are positive by PCR, infants are then considered to have been infected in utero. If the umbilical cord blood samples are negative by PCR but the first postnatal sample is positive by PCR, infants are then considered to have been infected perinatally. Such infections are assumed to have occurred around the time of delivery. When a postnatal sample (more than 4 weeks after birth) is negative by PCR and a later sample is positive by PCR, infants are considered to have been infected postnatally, with age at infection assumed to be the midpoint of this interval

From our study it is also evident that universal screening of HIV for all infants at 6 week immunization visit should be part of the South African guidelines. This will identify patients who seroconvert after antenatal screening, in late pregnancy or while breastfeeding.

Since completion of this study there have been dramatic changes in the PMTCT guidelines. These guidelines have been implemented since April 2013. Under the revised guidelines, the first choice ARV regimen for prophylaxis and lifelong treatment are the same (TDF+3TC/FTC+EFV, ideally as an Fixed Dose Combination(FDC)).As a result, unless there is a contraindication to TDF+3TC/FTC+EFV, all women can start this regimen as an FDC, at the first antenatal clinic visit.

The following eligibility criteria apply for pregnant mothers:

Women with a CD₄ cell count of more than 350 cells/mm³ and WHO stage 1 and 2 disease should receive ARV prophylaxis with TDF+3TC/FTC+EFV throughout pregnancy until 1 week after cessation of breastfeeding to reduce mtct. In women for whom TDF+3TC/FTC+EFV is contraindicated, AZT during

pregnancy and intrapartum is the alternative antiretroviral regimen with extended daily infant NVP until breastfeeding cessation.

Women with a CD₄ cell count of 350 cells/mm³ or less and WHO stage 3 or 4 should receive lifelong ARV treatment, both for their own health and to reduce the likelihood of mtct. As per adult guidelines, TDF+3TC/FTC+EFV is the preferred regimen for lifelong ARV therapy unless contraindicated due to psychiatric illness or renal disease. Eligibility for lifelong ARV treatment may be determined at the first antenatal visit (based on clinical staging) or at a later visit when CD₄ cell count results are reviewed.

Women on lifelong ARV therapy who become pregnant continue with treatment as per adult ARV guidelines (including those on second line regimens).

Women who initially test negative and subsequently test positive during pregnancy should be initiated onto TDF+3TC/FTC+EFV as an FDC immediately. A CD₄ cell count and serum creatinine should be taken, clinical staging and TB screening done. As for other women starting FDC, serum creatinine should be reviewed within one week to determine the safety of long-term TDF use, and CD₄ cell count should be reviewed to determine the need for prophylaxis versus lifelong ARV therapy.⁴⁵

In the era of universal ARV therapy for all pregnant women the transmission rate may be so low that it is debatable if the issue of birth order and discordance of HIV positivity may be of any relevance.

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University of Cape Town