

# MATERNAL VIRAEMIA AND MOTHER TO CHILD TRANSMISSION OF HIV

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## Abstract

**Background:** Mother-to-child transmission (MTCT) of HIV can occur perinatally and postnatally. Mechanisms of antepartum and intrapartum transmission of HIV include transfer of the virus via maternal blood and transplacental exposure. Understanding the mechanisms of transfer can lead to identification of risk factors in vertical transmission

**Study Design and Methods:** This study aims to demonstrate the relationship between the viral load and CD4+ cell counts of HIV-1 infected mothers and their babies at birth. Peripheral blood samples were obtained from fifty five mothers at delivery and their babies within the first 48 hours of birth. HIV viral load using Roche Amplicor Version 1.5, Germany and CD4+ cell counts were performed on plasma and blood samples obtained from all mother- baby pairs in the study.

**Results:** The pooled rate of transmission from HIV-1 infected mothers to their babies was 0.27 (95% CI 0.15-0.39). Maternal viraemia was significantly associated with transmission of infection to babies ( $p=0.047$ ). The odds ratio indicated that for every 1 log increase in maternal viral load the babies were 3.1 times more likely to acquire the infection (Exp (B) = 3.137 (95% CI, 1.015-9.696)). No significant association was observed between the viral load of mothers and their CD4+ cell counts ( $p=0.134$ ).

**Conclusion:** The findings suggested that maternal HIV-1 RNA is a strong predictor of risk in untreated HIV-1 positive mothers and is a valuable prognostic marker for mother-to-child transmission.

**Keywords:** viral load, CD4+ cells, vertical transmission, *in utero*, maternal viraemia

## Introduction

As human immunodeficiency virus (HIV) infection spreads amongst the adult population, the incidence of perinatally acquired HIV infection continues to be an area of concern. Mother-to-child transmission (MTCT) of human immunodeficiency virus type 1 (HIV-1) accounts for more than 95% of the cases of paediatric AIDS<sup>[1]</sup>. Accumulating evidence indicated that about one third of these infected infants will develop severe symptoms of the disease coupled with severe immunodepression by the first year of life<sup>[1]</sup>. Some of the postulations which explain the observed differences to disease progression are the timing of transmission of the virus from mother to baby and the host's capability to control the growth of the viral population<sup>[2]</sup>. Although the precise timing of viral transmission to the child cannot be pinpointed it has been proposed that detection of the virus in the infant's plasma at birth might reflect *in utero* infection<sup>[3]</sup>. Some of the risk factors associated with viral transmission from mother to child include low CD4+ cell counts during pregnancy, maternal viraemia, mode of delivery, disruption of the placenta and feeding practices<sup>[3-6]</sup>. Maternal viral load has a direct effect on intrauterine, intrapartum and breast feeding transmission, the higher the viral load the more likely is transmission<sup>[8]</sup>. To obtain better clarity on MTCT, the relationship between HIV replication, CD4+ counts and haemoglobin concentration was studied in blood samples from pregnant HIV-1 infected women and their babies at birth.

## Patients and Methods

### Patient Population, Recruitment and Selection

The study was carried out at Stanger Hospital situated in KwaZulu-Natal, South Africa. The hospital services both the local urban and surrounding rural areas. Approval for the study was obtained from the Ethics Committee, University of KwaZulu-Natal as well as Hospital Management.

Patients attending the antenatal clinic (ANC) for general monthly antenatal examination were recruited into this study. The HIV-status of the patients were established in a routine diagnostic-laboratory (Abbott, USA). Samples of peripheral blood were obtained from the women at 28 weeks of pregnancy by the attending gynaecologist. Prior to testing, pre- counselling was performed by an HIV counsellor based at the hospital's antenatal clinic. All patients who tested HIV positive at the initial screening were post-counselled and signed informed consent was obtained before inclusion into the study. Eligible participants were women aged 18 years and older and who had confirmed HIV-1 infection. Altogether 100 patients were initially recruited into the study. A total of 30 patients were lost to follow up.

The prevention of mother-to-child-transmission (PMTCT) program was in

the early stages of implementation in KwaZulu-Natal. Therefore, nevirapine was not available to the study population at this point of the study. Subsequently, during the sample collection process nevirapine was made available to HIV-1 positive pregnant women before delivery. In order to have a sample population who were antiretroviral (ARV) naive, sample collection ceased. At this point in the study a total of 70 samples were collected from mother-baby pairs for laboratory analysis. However, some samples were not suitable for laboratory analysis. Therefore, the final analysis was undertaken using complete data from 55 mother-baby pairs.

Patients were divided into two groups post delivery:

- HIV positive mothers whose babies had a viral load of >400 copies at birth
- HIV positive mothers whose babies had a viral load of <400 copies at birth

For the purpose of this study all babies who had a viral load of >400 copies/ml at birth were considered infected *in utero*<sup>[9]</sup>. Those babies with viral RNA copies < 400 copies/ml were considered uninfected pending further viral load investigations. The rate of MTCT was based on viral RNA copies of infants at birth.

### Collection and Transport of Blood Samples

Blood samples were collected in 2 EDTA vacutainer tubes. Approximately 2-5 mls of blood was collected from the mothers at delivery and  $\pm 1$ ml from babies immediately after birth. The specimens were processed for CD4+ counts within 48 hours of collection. Samples were transported from the site of collection at room temperature (20-25°C) to the laboratory for testing. Adequate transportation safety measures were taken during transport of samples. On arrival at the laboratory, specimens for viral loads were immediately centrifuged, plasma removed into sterile, labelled test tubes and frozen at -70°C for batch testing.

## Laboratory Methods

### CD 4+ Cell Counts

CD4+ cell counts were performed on unlysed peripheral whole blood samples obtained from all mothers and their babies immediately after delivery. The automated FACS Count System instrument and reagent kit (Becton Dickinson, USA) was used to calculate the absolute cell counts. Since the babies in the study were neonates the Centers for Disease Control (CDC) reference range for less than 1 year was used (CDC, 1994). The normal ranges for CD4+ cell

counts were taken as:

Male/Female: CD4+ 600 - 1500 cells/mm<sup>3</sup>  
Babies (<1year): CD4+ ≥ 1500 cells/mm<sup>3</sup>

### Viral Load

All samples were tested for number of viral RNA copies. Viral load was performed according to manufacturer's protocol (Roché Amplicor Version 1.5, Germany). Additional factors which were taken into consideration were cost of assay, limit detection and volume of sample required for analysis. The volume of sample required for the assay was crucial in this study because the study population included neonates. Only small volumes of blood were available from the babies at birth. A sample volume of 0.2 ml was used for the initial assay. A sub-sample of uninfected babies was tested using 0.5 ml of plasma for the ultrasensitive technique (Roché Amplicor Version 1.5- usCA MONITOR 1.5). The sub-samples had undetectable levels of viral RNA copies (<50 copies/ml). By extrapolation any sample <400 copies/ml was considered negative.

### Statistical Methods

SPSS statistical software was used for all descriptive analyses in the study. Some variables were unequally distributed therefore; non parametric tests were used. Correlations were assessed using Pearson's correlation test. The Chi Square test was used for assessing probabilities between variables and categories.

### Results

#### Maternal Data

Information on all mothers participating in the study was obtained by the attending gynaecologist from the antenatal and labour records which included antenatal history, past obstetric history, age of mothers, parity, method of delivery, gestational period, marital status, number of sexual partners and HIV status of partners.

The median age of the mothers was 26 years with an interquartile range (IQR) of 23 to 28 years. There were 49 (89.1%) vaginal deliveries and 6 (10.9 %) unplanned caesarian sections. Pregnancies ended in full term deliveries in 52 instances (94.5%). Only 3 (5.5 %) deliveries occurred before 36 weeks of gestation. No deliveries required assistance through instrumentation. According to patient records a positive syphilis serology was found in 4 (7.3%) patients at their initial antenatal clinic attendance. These patients were treated in the first trimester of their pregnancy. Thirty four (61.8%) women had haemoglobin concentrations ≥10g/l and 21(38.2%) with < 10g/l. At the time of delivery no maternal deaths occurred. The mothers' clinical condition was reported as satisfactory upon discharge.

Evaluation of patients' antenatal and obstetric history revealed that the median parity was 1 (IQR 1 to 2) and the median gravidity 2 (IQR 2 to 3). Only 1 (1.8%) participant was married. A total of 54 (98.2%) women attested to being single mothers. Thirty seven (67.3%) had one lifetime sexual partner and 18 (32.7%) had two or more sexual partners. Partners HIV status were known by 12 (21.8%) participants and unknown by 43 (78.2%).

**Neonatal Data:** All neonates were clinically examined at birth by the attending paediatrician. The following parameters were measured and recorded: Apgar scores at 1 minute and 5 minutes, weight at birth, gender, occipito-frontal circumference, length and a full physical examination.

The median Apgar score was 9 (IQR 1 to 9) and 10 (IQR 1 to 10) at 1 minute and 5 minutes respectively. No statistical significance was observed in Apgar scores between babies considered infected and uninfected ( $p = 0.540$ ). The median weight of babies at birth was 3 kg (range 2.0 to 4.0 kg). There was no significant difference in the median birth weight between infected and uninfected babies ( $p = 0.457$ ). Altogether, there were 38 (69.1%) males and 17 (30.9%) females born to 55 mothers in the study.

#### Maternal Viraemia and CD4+ Cell Values

Among the patients studied, association between viral load categories and CD4+ cell categories were performed to define the immune status of the patients and gauge the level of cellular response (Table 1). No significant association was observed between the mother's viral loads and CD4+ cell

counts ( $p = 0.134$ ). Despite the lack of statistical significance a trend was noted. Mothers (33.3%) with low CD4+ cell counts (<200 cells/mm<sup>3</sup>) had high viral loads (5 to 5.99 logs) compared with 7.7% with low CD4+ count and low viral load (3 to 3.99 logs). Conversely, mothers with high CD4+ counts had low viral loads (53.8% in the 3 to 3.99 log category). Twenty three (41.8%) had high CD4+ counts (>600 cells/mm<sup>3</sup>) and viral loads (3 to 5 logs) which, may indicate recent infections. Mothers with CD4+ <200 cells/mm<sup>3</sup> had a mean log viral load of 2.2 whilst those mothers with > 200 cell/mm<sup>3</sup> had a mean log viral count of 1.86.

#### Maternal Viraemia, CD4+ Counts and Haemoglobin Values in HIV-1 Infected Pregnant Women

Mothers (66.7%) with low haemoglobin (Hb) values (<10g/l) reflected high viral loads (log 5 to 5.99). Conversely, 84.6% of mothers with Hb ≥10g/l had lower viral loads (log 3 to 3.99). The association between maternal viral loads and maternal Hb values was statistically significant ( $p = 0.050$ ). It was noted that mothers with high viral loads had low Hb values and vice versa (Fig.1).

Using the CDC criteria (1993) 10 mothers with < 200 CD4+ cells/mm<sup>3</sup> were classified as having AIDS. Of these 6 (60%) had haemoglobin (Hb) values <10g/l. Fifteen (33.3%) infected mothers with CD4+ counts ≥ 200 cells/mm<sup>3</sup> had Hb values ≥10g/l. In mothers with Hb values <10g/l there was a difference of 26.7% between those with CD4+ counts <200 cells/mm<sup>3</sup> and those with CD4+ counts ≥200 cells/mm<sup>3</sup>. Although not statistically significant ( $p = 0.156$ ) there was a trend for mothers with low CD4+ counts to have low Hb values.

#### CD4+ Cell Counts of Infected and Uninfected Infants

A comparison of CD4+ counts of infected and uninfected babies was undertaken. A median difference of 500 CD4+ cells was noted between the median values of those babies infected (2000 cells/mm<sup>3</sup>) and uninfected (2541 cells/mm<sup>3</sup>). An overlap in the interquartile range between the CD4+ cell counts of babies considered infected *in utero* (IQR 928 to 2696) and those uninfected at birth (IQR 1554 to 2979) indicates no significance difference ( $p = 0.237$ )

#### Viral Load and CD4+ Cell Counts of Infected Infants

Using the CDC classification (1994) the infected babies were categorized as no evidence of immune suppression (>1500 cells/mm<sup>3</sup>), evidence of moderate immune suppression (750 to 1499 cells/mm<sup>3</sup>) and evidence of severe immune suppression (<750cells/mm<sup>3</sup>). Of 15 babies considered infected *in utero*, 6 (40.0%) had log viral loads between 3 to 4 logs of which 1(16.7%) had a CD4+ count <750 cells/mm<sup>3</sup> whilst 5 (83.3%) had >1500 CD4+ counts. The remaining 9 (60.0%) infected babies had viral loads of >4 logs. Among

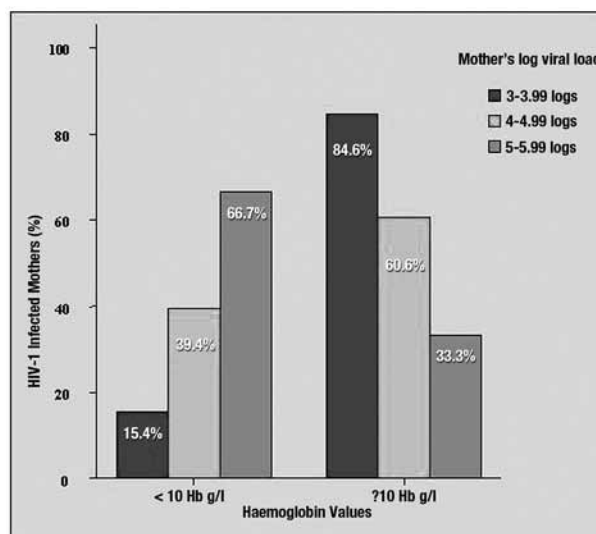


Figure 1: Haemoglobin values compared to viral loads of HIV-1 infected women

the babies with high log viral loads, low CD4+ counts were observed in 2 (22.2%). Two (22.2%) babies were found to have moderate immune suppression and 5 (55.6%) with high CD4+ counts showed no immune suppression. Overall, 66.7% of infected babies demonstrated a well preserved immune system (Table 2). The relationship between infected babies CD4+ counts and log viral loads was negatively correlated ( $r = -0.437$ ) with no observation of a statistical significance ( $p = 0.103$ ).

**Comparison between Maternal CD4+ Counts, Viral Loads and Babies Viral Load**

An evaluation between HIV-1 infected mothers with  $<200$  CD4+ cells/mm<sup>3</sup> (AIDS) and those with  $>200$  CD4+ cells/mm<sup>3</sup> and the viral loads of their babies revealed a significant association between mothers with AIDS and the viral loads of their babies at birth ( $p = 0.018$ ). Sixty percent of infected babies were born to mothers with low CD4+ counts ( $<200$  cells/mm<sup>3</sup>) as compared with 20% born to mothers with  $>200$  CD4+ cell counts (Table 3). If the mothers had AIDS the infants were more likely to be infected. The mean log viral loads of babies born to mothers with  $<200$  cells/mm<sup>3</sup> and  $>200$  cells/mm<sup>3</sup> were 3.6 (SD = 0.9) and 2.9 (SD = 0.6) respectively.

Variables such as mothers log viral load, CD4+ cell counts, age and gestational period and sex of babies were entered into a backwards selection procedure. Only two variables, log viral load of mothers and sex of babies were accepted as significant (Table 4). The log viral load of mothers was significantly associated with transmission of infection to babies ( $p = 0.047$ ). The odds ratio (Exp (B) = 3.137 (95% CI, 1.015-9.696) indicated that for every 1 log increase in viral load the risk of babies acquiring the infection increased by 3.1 times.

The predictive value of gender of babies and risk of infection (95% CI, 0.819-11.809) reflected that 7 (41.2%) of 17 female babies were infected as compared to 8 (21.1%) of 38 male babies born to HIV-1 infected mothers in the study population. Although there was no significant association between gender of babies and mothers viral load ( $p = 0.096$ ) more female babies were infected *in utero* than males.

**Discussion**

This study evaluated maternal viraemia as a risk factor in *in utero* infections. Analysis of viral loads and CD4+ cell counts indicated that there was no significant association between the viral load of mothers and their CD4+ cell counts. However, mothers with high viral RNA copies demonstrated a tendency to have low CD4+ cell counts whilst, mothers with high CD4+ counts had low viral loads. Some authors have found that there is an inverse, but variable correlation between plasma viral RNA levels and the level of CD4+ lymphocytes<sup>[10-12]</sup>.

Based on comparison with previous data, low CD4+ counts are indicators of a waning immune system<sup>[13-14]</sup>. In this study HIV-1 infected individuals with CD4+ levels below 200 cells/mm<sup>3</sup> were classified as having AIDS<sup>[13]</sup>. Ninety percent of the women with AIDS had viral loads greater than 4 logs thereby, increasing the risk for transmission to the baby<sup>[14]</sup>.

Other factors are also important for MTCT. The known risk factors are vaginal deliveries and low haemoglobin levels during pregnancy<sup>[15]</sup>. Vaginal deliveries increase the ingestion by the newborn of infected maternal blood, plasma and other secretions<sup>[6]</sup>. Studies conducted by the European Mode of Delivery Collaboration (1999) have demonstrated the efficacy of caesarian section before delivery for the prevention of MTCT<sup>[15]</sup>. Caesarian sections are also emphasized to a greater extent by The British HIV Association and the European Consensus Panel. However, in resource poor settings caesarian sections as prevention mechanism for MTCT is generally not feasible because of the lack of skilled attendants during labour<sup>[16]</sup>. The findings that caesarian sections were less likely to transmit the virus cannot be commented on in this study because only 10.9% of the total number of women in the study had the procedure performed. The numbers are too small for an accurate evaluation. As a general comment viral loads in all six babies delivered by caesarian section were

**Table 1: Maternal Viraemia and CD4+ Cell Values. N=55**

Mothers Viral Load (log)	Mothers CD4+ T Cell Counts (mm <sup>3</sup> ) Number (%)			
	<200	200-600	>600-1500	Total
3-3.99	1 (7.7)	5 (38.3)	7 (53.8)	13 (100)
4-4.99	6 (18.2)	15 (45.5)	12 (36.4)	33 (100)
5-5.99	3 (33.3)	2 (22.2)	4 (44.4)	9 (100)
Total	10 (18.2)	22 (40)	23 (41.8)	55 (100)

**Table 2: Comparison of Infected Babies Viral Loads and CD4+ Counts**

Babies Viral Load	Babies CD4+ cells/mm <sup>3</sup> (%)			
	No suppression Category 1 $\geq 1500$	Moderate suppression Category 2 750-1499	Severe suppression Category 3 $<750$	Total
3-4 logs	5 (83.3)	0 (0)	1 (16.7)	6 (100)
>4-5 logs	5 (55.6)	2 (22.2)	2 (22.2)	9 (100)
Total	10 (66.7)	2 (13.3)	3 (20.0)	15 (100)

**Table 3: Comparison between Mother's CD4+ Counts and Babies Viral Load. N=55**

Mother's CD4+ cells/mm <sup>3</sup>	Babies		
	No. Uninfected (%)	No. Infected (%)	Total (%)
<200	4 (40)	6 (60)	10 (100)
$\geq 200$	36 (80)	9 (20)	45 (100)
Total (%)	40 (72.7)	15 (27.3)	55 (100)

**Table 4: Logistic Regression to Determine the Predictive Value of Maternal Viraemia and Babies Viral Load. N=55**

	Wald	df	Sig.	Exp (B) Odds Ratio	95.0% C.I. for EXP(B)	
					Lower	Upper
Log Viral Load of Mothers	3.944	1	0.047	3.137	1.015	9.696
Gender of Babies	2.777	1	0.096	3.110	0.819	11.809
Constant	5.745	1	0.017	0.001	-	-

*Variables entered are log viral load of mothers, CD4+ cells of mothers, Pregnancy weeks, Gender of babies and age. Only viral load and gender was accepted as variables for evaluation.*

at undetectable levels at birth. It has been suggested that most infants with perinatally acquired infection, who test negative at birth, will have a positive PCR test results by 2 weeks of age<sup>[17]</sup>. The recommendations are that testing should be performed within 48 hours of birth, at 1 to 2 months of age and again at 4 to 6 months of age<sup>[17-18]</sup>. The babies in this study were not retested due to default in follow up visits.

The implication of low haemoglobin concentrations in HIV-1 infected women and the risk of vertical transmission were evaluated. Sixty percent of the women classified as having AIDS, had low haemoglobin values with a corresponding low CD4+ count. Those with haemoglobin values greater than 10g/l had CD4+ cell counts > 200 cells/mm<sup>3</sup>. Although not statistically significant in this study, there was a trend for mothers with low CD4+ cell counts to have low haemoglobin values. Parasitic infections and nutritional deficiencies could also contribute to low Hb values. However, screening for parasites or nutritional deficiencies was not done in this study because patients were discharged soon after delivery.

A statistically significant association between maternal viraemia and maternal haemoglobin was observed. Mothers with haemoglobin values less than 10g/l reflected high viral loads. Conversely, mothers with haemoglobin values greater than 10g/l had lower viral loads. A study conducted in Zaire reported an association between risk of transmission and low haemoglobin concentrations in HIV-1 infected women<sup>[19]</sup>. Some studies found that HIV-1 infected women whose haemoglobin values were <10g/l during pregnancy were at an increased risk of transmitting the virus to their babies<sup>[20]</sup>.

Detection of HIV infection in the neonatal period is important so as to provide appropriate and managed health care to the patient. In this cross sectional analysis human immunodeficiency virus in *in utero* infected and uninfected babies were compared in relation to their birth weight and Apgar scores. As in previous studies the Apgar scores and anthropometric index of weight for age showed no significant differences in both groups of babies<sup>[20-23]</sup>. However, the findings in a Rwanda study indicated differently that infected newborns were more likely to have lower birth weights and lengths<sup>[24]</sup>. Although, these studies were longitudinal studies, the initial paediatric clinical screening procedures for all neonates remain relatively constant to allow for comparisons.

Prematurity was also reported as a risk factor to vertical transmission<sup>[25]</sup>. In the European Collaborative Study (1992) there was a strong correlation with prematurity and increased risk of transmission<sup>[26]</sup>. In this study only 3 babies were born before 36 weeks. The numbers are too small to comment on its statistical significance.

The absolute risk of transmission is estimated to be 5% to 6%<sup>[26]</sup>. The mathematical modelling suggests that much of this is *in utero* transmission which occurs relatively late in gestation<sup>[14]</sup>. In addition it has been proposed that viral detection at less than 48 hours demonstrates *in utero* infection<sup>[9]</sup>. We applied this definition to the babies' viral loads in this study and found a transmission rate of 27.3%. Most studies have found that infants which fit this '*in utero*' definition have a shorter median time to onset of symptoms and death<sup>[27-29]</sup>. This pattern of decline was not demonstrated in our study because longitudinal investigations are required to identify disease progression. Mothers in this study population did not comply with scheduled follow up visits after delivery. Others studies have reported transmission rates in the range of 25%-30%<sup>[29]</sup>. In resource poor settings the values rose to as high as 42%<sup>[30]</sup>. The amount of exposure of the foetus to maternal virus is one of many factors facilitating MTCT.

According to the CDC HIV Pediatric Classification (1994), three immunological categories were established to categorise children by the severity of immunosuppression attributable to HIV-1 infection<sup>[13]</sup>. Age specific CD4+ cell count was used to analyse immunologic response in all babies in the study<sup>[30]</sup>. Overall, 20% of all infected babies were categorised as having evidence of severe immune suppression. In the same category 6.7% of babies were born to mothers with a diagnosis of AIDS. It has been reported that infants born to women with advanced disease and higher viral loads also tend to be rapid progressors<sup>[31-33]</sup>.

Altogether, 66.7% of infected babies who were classified as no evidence of immune suppression demonstrated a better preserved immune system. The mothers CD4+ counts of 7 (46.7%) infected babies in this category were > 200 cells/mm<sup>3</sup>. An observation that babies born to HIV-1 infected

mothers with immune deterioration had lower CD4+ counts than babies born to mothers with high CD4+ counts. Finally, it cannot be ascertained whether the babies with no evidence of immune suppression will be able to sustain viral suppression because again, the study population was unavailable for retesting.

Studies have indicated that without ARV treatment, transmission rates ranged from 5% to 15%. If the viral load was over 2 logs the transmission rate rose to 37%<sup>[34]</sup>. These statistics are in line with our findings. Antiretroviral treatment was not available to our study population during pregnancy. The infected babies in our study had between 3 to >5 log viral loads with a transmission rate of 27.3%. The possibility of a higher transmission rate cannot be excluded because 72.7% of the babies in our study, with an initial undetectable viral load, were not retested.

Although the relationship between infected babies CD4+ counts and log viral loads was negatively correlated, there was a trend for babies with lower viral loads to demonstrate higher CD4+ counts. An indication perhaps that babies with a greater CD4+ cell response at birth may be more capable of mounting a CD4+ cell mediated immune response. Previous studies have elucidated that in the first few months of life the course of disease appears to correlate with patterns of plasma viral dynamics<sup>[35]</sup>. In contrast, one study found low plasma RNA levels at birth for those with early infection. However, the RNA copies rapidly increased over the next two months to very high levels before slowly declining over the years<sup>[36]</sup>.

## Conclusion

The predictive value of maternal viral load and risk of transmission revealed that maternal viraemia was significantly associated with transmission of infection to babies ( $p = 0.047$ ). The odds ratio indicated that for every 1 log increase in maternal viral load the babies were 3.1 times more likely to acquire the infection. Furthermore, the study found that a higher number of female babies were infected than males. Although not statistically significant the odds ratio indicated that female babies were 3.1 times more likely to become infected than males.

The findings in this report are consistent with several paediatric studies which have shown that plasma viral RNA levels can independently predict clinical outcomes<sup>[37-38]</sup>. Infants infected by vertical transmission tend to progress more rapidly than those who acquire infections by blood products<sup>[39-41]</sup>. The clinical outcomes on babies in this study cannot be commented on due to the challenges presented in maintaining follow up visits. Some of the challenges were that patients accessed other outpatient clinics or primary health clinics for treatment when required. Most patients were from low socioeconomic backgrounds, especially from rural areas and could not afford the cost of frequent transport to the hospital. Specific outreach teams were not available to trace mothers and babies who did not adhere to follow up visits. Finally, the observations seen in this study are within a small sample population. Extrapolations of these findings to other population groups and in other geographical settings cannot be made.

## Limitations of the Study

The data are incomplete in certain important aspects such as follow up visits and continued HIV testing and monitoring of the babies. Clinical outcomes and clinical staging of babies were incomplete because mothers and babies were not available for follow up visits. The data presented here, although with limitations are from a study population in which ARV's were not administered. Sample size restricted comments to statistical significance in many areas of analyses. However, data presented in this study creates a platform for further investigations and future studies. Larger studies are required to eliminate some of the unanswered questions.

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