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ABSTRACT

Addressing Human Immunodeficiency Virus / Acquired Immunodeficiency Disease Syndrome in children is a significant global challenge and Kenya is not an exemption. Thus, Human Immunodeficiency Virus (HIV) status of infants should be determined soon after birth due to the fact that infants with HIV often develop immunodeficiency symptoms rapidly and can acquire life threatening opportunistic infections early in life. Besides this, the mortality rate of HIV infected infants is higher as compared to non-infected children. It is therefore important to quickly identify HIV infected infants to begin HIV care and management including anti-retroviral therapy and opportunistic infection prophylaxis. This will ultimately improve the infant’s quality of life, prolong life span and decrease infant mortality rate. To address this challenge in Nairobi County of Kenya, children born to HIV infected mothers were recruited from Prevention of Mother to Children Transmission clinics within Nairobi, their demographic factors recorded, blood sample collected on Dried Blood Spot and transported to Kenya Medical Research Institute –HIV laboratory for diagnosis using Roche Amplicor HIV-1 Deoxy Ribonucleic Acid Test. The generated data was stored in MS Excel, presented in graphs and charts, and analyzed using SPSS software. The pediatric HIV prevalence in Nairobi which has been lacking was determined to be 10.6%. Gender was significantly (p value= 0.05) associated with vertical transmission. Determination of HIV status provided useful information for care of infants born to HIV infected mothers. Those diagnosed positive were considered for initiation of anti-retroviral therapy upon meeting the Ministry of Health criteria. The prevalence data in association with demographic factors helped in necessitating and implementing policies that strengthens the prevention of mother to child transmissions.

Keywords: DNA-PCR, Gender, HIV, Prevalence, Pediatric, Roche Amplicor.

INTRODUCTION

Worldwide, approximately 34.0 million [31.4 million–35.9 million] people are living with Human Immunodeficiency Virus (HIV) of which an estimated 0.8% aged 15-49 years are infected adults (UNAIDS, 2012). Approximately 10000 children acquire HIV infection everyday worldwide, constituting 13% of all new HIV/ AIDS infections (Moshi et al., 2011). In 2011, 2.5 million HIV infections are estimated to have occurred in children as a result of mother to child transmission (MTCT) during pregnancy, at birth or from breastfeeding (UNAIDS, 2012). In Kenya alone about 12,894 and 12,940 were infected during 2012 and 2013 respectively (KAIS, 2014). The risk of MTCT in resource limited countries ranges from 15% to 40% much higher than the 2% experienced in high income countries (Taha et al., 2004, Kilewo et al., 2009). Where care and treatment are not available, studies suggest that 35% of infected children die in their first year of life and 75% by their first birthday (Newell et al., 2006). In kenya, 10390 deaths of children less than one year were as result of AIDS related complications (KAIS, 2014).
Almost all HIV-infected children acquire the virus from their mothers before or during birth (Covadia et al., 2004; Kuhn et al., 2007a) or through breastfeeding (UNAIDS, 2012). Some adults and children get infected through contaminated blood or blood products with HIV. A small number of children also get infected through sexual or physical abuse by HIV-infected adults. Most Mother to Child Transmission, estimated to cause more than 90 percent of infections worldwide in infants and children, probably occurs late in pregnancy or during birth. Although the precise mechanisms are unknown, scientists think HIV may be transmitted when maternal blood enters the fetal circulation or by mucosal exposure to virus during labor and delivery (Forbes et al., 2012).

The risk of MTCT is significantly increased if the mother has advanced HIV disease, high viral load and low CD4+ cells (Taha et al., 2007). Other factors that may include; maternal drug use, low maternal Hgb, low birth weight, female gender, severe inflammation of fetal membranes, or a prolonged period between membrane rupture and delivery (Temmerman et al., 2003). HIV-1 infected women who give birth more than 4 hours after the rupture of the fetal membranes are nearly twice as likely to transmit HIV to their infants, as compared to women who delivered within 4 hours of membrane rupture (Sperling et al., 2006).

The standard HIV serological tests; Enzyme linked immunosorbent assay (ELISA) and Western Blot immunoassay are not useful in the diagnosis of HIV infection during infancy. This is because of the confounding presence in infant’s blood of transparently derived maternal HIV antibodies that may persist for 18 months or longer in some cases (Temmerman et al., 2003). Thus, in the developed countries and some low income countries, the HIV Deoxy ribonucleic Acid Polymerase Chain Reaction (PCR) assay is used most widely for diagnosis of HIV infection during infancy (Zijenah et al., 2005). Testing generally should be performed in the immediate newborn period, at 1-2 months of age and at 3-6 months of age (Taha et al., 2004). Early diagnosis of vertically acquired HIV infection has important implications in decisions making on initiation of prophylactic and therapeutic medications, medical follow-up and management of intercurrent illnesses and prevention of mother to child transmission (MTCT) (Lorey et al., 2008). In light with this, a study was performed to estimate prevalence of pediatric HIV infection in Nairobi County and determine if gender is a determinant of preferred infection in infants.

**METHODOLOGY**

**Study materials, design and group**

This was a cross-sectional study involving 360 infants born to HIV positive Mothers attending PMCT Clinics within randomly selected clinics in Nairobi County. All the women were given nevirapine at the onset of labour as a measure to prevent vertical infection and the infants had to reside within Nairobi.

**Dried blood spot (DBS) collection**

Specially formulated absorbent filter papers (Schleicher and Schuell, 903 or Whatman BFC 180) were used to collect heels blood for molecular testing. The DBS were labeled and let to air dry, packaged and transported to HIV laboratory at Centre for Virus Research, Kenya Medical Research Institute for analysis.
HIV DNA extraction and PCR detection

Viral DNA extraction was done as described by Khamadi et al (2008). Briefly, one spot from DBS paper sample was cut into micro centrifuge tube under sterile condition to avoid contamination. The spot washed thrice in 1 ml of phosphate-buffered saline-0.1 % Tween for 10 minutes at room temperature and shaken. Elution was carried out for 30 minutes at 600c, followed by boiling at 1000c for 30 minutes. The samples were then quick centrifuged at 10 minutes for 2000 rpm to collect the chlex at the bottom of the tube and the supernatant used for PCR detection according to the manufacturers protocol (Roche Diagnostics). Using specific primers SK 145 and SKCC1B, a 50 µl of DNA template was used to amplify a specific conserved HIV-1 gag gene region, that is about 1500 nucleotides in length and located at approximately positions 789-2290 in HIV genome in 50 µl readily prepared master mix. The amplifications conditions were hold program for 2 minutes at 500 C, cycle program 1: (5 cycles for 10sec at 950C, 10 sec at 520C, 10 sec 920C cycle program 2 (35 cycles for 10 sec at 900C, 10 sec at 550C 10 sec 720C and another hold Program for 15 min at 720C. After amplifications, the amplicons were added to 100 µl of denaturation solution then mixed up and down 5 times and the contents detected by colometric effect using a 450nm filter. Samples were considered positive if they had an optical density (OD) of ≥0.8 and negative if they had OD of ≤0.2 according to Roche Amplicor manufacturers’ instructions.

Data storage and statistical analysis

The generated data was recorded in MS Excel and analysis done using SPSS software. Basic data findings are presented in bar graphs and pie charts. The effects of sex/gender on infection was determined using Chi–square test.

Ethical consideration

Consent for HIV testing was sought from expectant mothers. Written informed consent for participating in the study was sought and recorded from each infant’s parent or guardian before enrollment into the study. Early Infant Diagnosis in KEMRI occurs in an operations research environment, and has been approved by the KEMRI Ethical Review Committee.

RESULTS
Prevalence of HIV and AIDS in Nairobi

Of the total 360 infants sampled, 50.84% (183) were male while 49.16% (177) were female. These infants were aged between 1-18 months with mean of five months. From the overall analysis, 10.6 % of them were HIV positive (Table 1).

<table>
<thead>
<tr>
<th>HIV STATUS</th>
<th>POSITIVE</th>
<th>NEGATIVE</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>MALE</td>
<td>11 [6%]</td>
<td>172 [94%]</td>
<td>183[100%]</td>
</tr>
<tr>
<td>FEMALE</td>
<td>27 [15%]</td>
<td>150 [85%]</td>
<td>177[100%]</td>
</tr>
<tr>
<td>TOTAL</td>
<td>38 [10.6%]</td>
<td>322 [89.4%]</td>
<td>360</td>
</tr>
</tbody>
</table>

Prevalence of vertical transmission by gender

Of the total infants sampled, 183 were male and 177 female. 6% (n = 11) of the total number of males were positive while 94 % (n=172) were negative. When the females infants were
considered alone, 15% (n = 27) were HIV positive while 84.7 % (n=150) turned negative. Of the total 38 infants who tested positive, 71% (n=27) and 29 % (n=11) were female and male respectively (Figure 1). Further analysis showed that there was a significant association between the sex of the infants and the HIV prevalence ($\chi^2 = 8.142$, df = 1, $P < 0.002$). This implies that vertical HIV infection is more prevalent in females than males and gender may be a risk factor for HIV vertical transmission.

![Figure 1: Distribution of HIV positive cases by gender, showing female infants has higher risk than males](image)

**DISCUSSION**

**Prevalence of pediatric HIV and AIDS in Nairobi**

Currently, an estimated 2.5 million children are living with HIV/AIDS, 10,000 becoming infected daily and 260,000 deaths of children under 15 occur due to AIDS related illnesses (Moshi et al., 2011). More than 90% of all HIV infections in children occur in sub Saharan Africa, with 75% of these children dying of AIDS before their fifth birthday (UNAIDS, 2012). According to De cock et al (2002) in absence of any intervention, between 15 and 30% of infants of HIV infected mothers are infected before or during delivery. If all HIV infected mother’s breastfeed, another 10 to 20 percent of their infants will be infected through breast feeding. This implies that in general about a third of all children born to HIV infected mothers will become infected (Becquet et al., 2008). The report of WHO postulates the HIV vertical transmission to be at 36% in resource limited settings as opposed to less than 2% experienced in developed nations (Forbes et al., 2012). In kenya an annual average of 12900 (9300-17000) was estimated in 2013 study (KAIS , 2014).

In this study the HIV vertical transmission was determined from infants less than 18 months of age using the Amplicar DNA - PCR, conventional HIV ELISA could not be used due to confounding effect of maternal IgG transfer through the placenta (Shearer et al., 2005). The prevalence of 10.6% in Nairobi County as revealed by the study is consistent with the pediatric HIV national tally and other studies that more than a third of the infants born to HIV positive mothers don’t acquire the virus from the mothers (Taha et al 2007). The relatively lower prevalence as compared to data from other developing countries is owed to high awareness and good Prevention of Mother to Child transmission (PMCT) programme in
the County. However, all the routes of vertical transmission are assumed to be concerned, calling for a more strengthening of all PMCT programmes based on WHO recommendations through the Ministry of Health of the Kenyan government. Screening of all infants at immunization clinics using Dried Blood Spot sampling and further diagnosis using PCR method is effective and feasible for monitoring the overall impact of PMCT programmes.

**Gender is a risk factor in HIV vertical transmission**

This study show that female infants are at more risk of HIV vertical transmission than male infants. The finding is consistent with other studies carried out in Africa which reported that the risk of vertical HIV transmission was higher among girl than boy infants (Taha et al., 2004). Two explanations are possible for the observed results. It could be that HIV infection in preferentially target female offspring. The factors involved may be genetic, epigenetic, immunologic or hormonal. Preferential targeting of one gender over the other has been postulated for other adverse reproductive outcomes (Kilewo et al., 2004). For example, couples’ smoking around the time of conception were suspected to result in female offspring, presumably because the sperm carrying the male Y chromosome might be more susceptible than the sperm with the female X chromosome to the effects of tobacco and thus less likely to fertilize the egg (Fukuda et al., 2002). Whooping cough and measles diseases have also shown differential susceptibility preference in female than in boys with the consequence of increased mortality and morbidity among female infants (Garenne et al., 2002). Furthermore, this difference has been associated with T helper 1 (IL-2, IFN-gamma) and T helper 2 (IL-4, IL-5, IL-6 and IL-10) cytokine production, and a balance between these two responses could determine susceptibility, resistance, survival, or death (Garenne et al., 2002). It suggest that male infants may have early utero development of Th 1 responses associated with β chemokines that could mediate lytic mechanism of infection during HIV vertical transmission (Garenne et al., 2002).

The female and male infants are equally susceptible to HIV infection but infected boys are more likely to die before birth than are infected girls (Taha et al., 2005). Although male infants have higher mortality from most causes of death, the sex differential varies by cause (Taha et al., 2005). The decline in deaths from infection is likely to affect males and females differently. Because females have more vigorous immune responses and greater resistance to infection, female infants have lower mortality from infections and respiratory ailments. The male disadvantage begins in-utero when gonadal steroid production already differs strongly by sex (Chahnazarian et al., 2008). Males are more likely to be born prematurely and to suffer from respiratory conditions in the peri-natal period. Thus, an increase in survival among premature infants may affect the sex balance of mortality (Taha et al., 2005).

The male mortality rates in-utero among HIV infected infants are disproportionately higher, resulting in more HIV-infected female infants than male infants being born. We are aware that in Kenya HIV prevalence amongst women adults of child bearing age (19-49) is high (65%) and this could be a bearing to increased female infants infection (KAIS, 2008). Other than this we are not aware of any major recent adverse demographic or environmental factors that could affect the gender ratio at birth in this population. This finding of increased HIV infection among female infants in Nairobi is in agreement with the results of the European Collaborative Study (ECS, 2004).
Implications for pediatric HIV prevalence in prevention of HIV vertical transmission

This research finding have got implications for prevention of mother to child transmission and the data derived may help in the intervention to avoid vertical transmission. In the absence of intervention, vertical HIV transmission is estimated to be 14 to 25 % in developed countries, but in predominantly breastfed populations, it is estimated to range from 25 to 48 percent. In resource limited countries, a pregnant mother who is HIV infected is 30 to 40% likely to transmit HIV to her newborn child (UNAIDS, 2012). The baby may become infected during pregnancy, during labour, or through breast feeding (Myron et al., 2011). Determination of route of infection is important to enact and strengthen the policies for intervention and these findings may inform public health policymakers in development and implementation of comprehensive HIV prevention programs.

There are three interventions known to be efficacious in the prevention of mother-to-child-HIV-1 transmission (MTCT); anti-retroviral therapy in the mother and newborn, cesarean section before labor and ruptured membranes and complete avoidance of breastfeeding (Covadia et al., 2007). With respect to the results obtained, a large number of HIV negative infants (89.4%) could be due to a large number of expecting mothers attending clinics during gestation. Alternatively, this may be due to a large number of women in the sampling site who have undergone education on HIV testing, the use and effects of taking ARVs during the expecting period and alternative feeding regimen for their babies. However, the small percentage of the HIV positive infants observed in this study (10.6%) may be due to a small number of expecting mothers who neglect or are ignorant or due to lack of funds to enable them attend antenatal clinics and above all unwillingness to learn more about ARVs and certain specific measures undertaken in order to prevent infants from getting infected with viruses incase the parents were HIV positive.

The prevalence in Nairobi of slightly more than 10% of HIV vertical transmission among infants indicates that the epidemic setting had not yet been put under adequate control. This finding implies that some infants get HIV infection during pregnancy and delivery. To prevent this scenario administration of ARVs such as Nevirapine at the onset of labour and to exposed babies within three days after birth (Nolan et al., 2002), ministering of azydothymidine (AZT) at the last weeks of pregnancy or during labour (Cannor et al., 1994,) are recommended. A cohort study done in Argentina to access the risk of mother to child transmission, observed the transmission rate of 9.5% in the vaginal delivered group, 6.8% in elective surgery group and 17.2 % in the non elective cesarean group (Andriana et al., 2006). Thus in settings where resources are available elective cesarean should be the choice of delivery (Denese et al., 2007). Although peri-partum anti-retroviral therapy prophylaxis has been shown to significantly decrease the risk of mother-to-child transmission (MTCT) around the time of delivery, this intervention approach does not provide protection from breastfeeding transmission (Leroy et al., 2002). The postnatal transmission through breastfeeding is associated with clinical, immunologic, and virologic factors (Temmerman et al., 2003). Increased maternal RNA viral load in plasma and breast milk is strongly associated with increased risk of transmission through breastfeeding (Temmerman et al., 2003). High levels of virus in plasma, and probably also in breast milk, increases the post natal vertical transmission to nearly 100% (Dunn et al., 1992.). In a study in Kenya, the relative risk of MTCT was increased about 6-fold during primary infection of the mother (Nduati et al., 2000). Breast milk HIV-1 levels correlate with systemic viral load and are likely to be associated with risk of breast milk HIV-1 transmission (Cohen et al., 2010).
Four key actions are recommended to reduce the number of children acquiring HIV infection: (1) strengthen primary HIV prevention services to ensure that reproductive-age women and their partners avoid HIV infection, (2) take steps (such as providing contraceptives and counselling) to meet the unmet need for family planning among women living with HIV, (3) provide HIV testing, counselling and antiretroviral medicines in a timely manner to pregnant women living with HIV to prevent transmission to their children and (4) ensure proper and timely HIV care, treatment and support for women living with HIV, children living with HIV and their families.

CONCLUSIONS

The study revealed that the Pediatric HIV prevalence in Nairobi is 10.6 % and female infants are more at risk.

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