

False negative HIV antibody test in HIV infected children who receive early antiretroviral treatment in a resource-limited setting

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Abstract

With the implementation of 2010 World Health Organization guidelines, the number of infants from developing countries who will initiate antiretroviral therapy (ART) will increase considerably. In this study we describe the HIV antibody tests of 14 HIV infected children who initiated ART at age less than one year in a rural setting of India. The HIV rapid test was negative in seven and indeterminate in two cases, whereas the HIV enzyme-linked immunosorbent assay (ELISA) antibody test was negative in three and indeterminate in one case. In one child who had both negative HIV rapid test and ELISA initially, HIV serology turned positive after having a virological failure to ART, suggesting the possibility of utilizing HIV serology for monitoring ART effectiveness in children who experience HIV seroreversion. In conclusion, HIV seroreversion of children with early initiation of ART is common and should be considered for avoiding misdiagnosis of HIV infection.

Introduction

Current European and North-American guidelines recommend initiating antiretroviral therapy (ART) to all children younger than one year.^{1,2} With the implementation of the 2010 World Health Organization (WHO) guidelines which recommend initiation of ART in all children younger than two years,³ the number of infants from resource-limited countries who will initiate ART will increase dramatically in the near future.

Adults who are treated with ART soon after their primary HIV infection may not develop antibodies against HIV.⁴ In children, case reports from developed countries have also shown that early initiation of ART can pro-

duce a seroreversion of HIV.⁵⁻⁹ These children can be misclassified as HIV negative when they are requested an HIV antibody test for confirmation of the HIV infection. If the child is considered as non HIV infected and ART is stopped, the parents will feel falsely relieved. The child may not be followed up and may present in the future with opportunistic infections or other HIV related diseases.

The aim of this study is to describe the HIV serology of a cohort of infants who were initiated on ART at age less than one year in a rural setting of India.

Materials and Methods

The study was performed in Anantapur district, Andhra Pradesh, India. India has the largest burden of people living with HIV in Asia and is the third country of the world in terms of HIV infected people.¹⁰ Andhra Pradesh is the state with highest population of HIV infected people in India.¹¹ Rural Development Trust (RDT) is a nongovernmental organization that has three hospitals in the district of Anantapur. In these hospitals, medical care of HIV infected people is given free of cost, including medicines and consultation or admission charges.

The Vicente Ferrer HIV Cohort Study (VFHCS) is an open cohort study of all HIV infected patients who have attended RDT hospitals since June 2006. The study was approved by the ethical committee of the RDT Institutional Review Board.

For this study, we searched in the VFHCS database for children who started ART before one year of age and who had an HIV antibody test after 18 months. Because of the presence of maternal antibodies, diagnosis of HIV infection in children younger than 18 months requires the use of molecular assays that detect the nucleic acids of HIV in the blood of the child.³ In this study, diagnosis of HIV infection was performed using a real time HIV polymerase chain reaction assay (Roche COBAS TAQMAM 48, Roche-Diagnostics, Germany). Technical details of the HIV serological tests have been described elsewhere.¹²

Results

Seventeen children from the VFHCS were started on ART at age less than one year. In three children, HIV serology was not performed, so fourteen children were included in the study. All children had at least an HIV rapid test and an HIV enzyme-linked immunosorbent assay (ELISA) at age more than 18 months (Table 1). The proportion of

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children with negative HIV rapid test was higher than the proportion of children with negative HIV ELISA antibody test. Half of the children had a negative HIV rapid antibody test (Wilson 95% confidence interval 27%-73%) and in two cases the HIV rapid test was indeterminate. HIV ELISA antibody test was negative in three cases (21%, Wilson 95% confidence interval 8-48%) and indeterminate in one. Six children were born by caesarean section and eight children received breastfeeding. Four out of six children who started ART before six months of age had a negative HIV rapid antibody test, whereas only three out of eight children who started ART after six months of age had a negative HIV rapid antibody test. Eight children were started on syrup formulation of zidovudine, lamivudine and nevirapine and six children were started on fixed dose combinations of stavudine, lamivudine and nevirapine.

Discussion

The results of this study indicate that a sizeable proportion of children who initiate ART at age less than one year will not develop antibodies against HIV and, therefore, HIV antibody tests may not be reactive when per-

Table 1. Characteristics of 14 children who initiated antiretroviral therapy at age less than one year.

Case #	Age at ART initiation (months)	Caesarean section	Breastfed	HIV rapid test	HIV ELISA	ART regimen
1	3.1	Yes	Yes	+	+	AZT+3TC+NVP
2	3.5	No	No	-	-	AZT+3TC+NVP
3	3.6	Yes	No	-	+	AZT+3TC+NVP
4	3.7	No	Yes	-	-	d4T+3TC+NVP
5	4.3	No	No	+	+	d4T+3TC+NVP
6	5.5	No	Yes	-	-	AZT+3TC+NVP
7	6.6	Yes	No	-	+/-	AZT+3TC+NVP
8	7.9	Yes	No	-	+	d4T+3TC+NVP
9	7.9	Yes	No	+/-	+	AZT+3TC+NVP
10	8.2	No	Yes	+/-	+	d4T+3TC+NVP
11	10.2	No	Yes	+	+	d4T+3TC+NVP
12	10.8	Yes	Yes	-	+	AZT+3TC+NVP
13	11.7	No	Yes	+	+	d4T+3TC+NVP
14	11.8	No	Yes	+	+	AZT+3TC+NVP

ART, antiretroviral treatment; ELISA, enzyme-linked immunosorbent assay; +, reactive; +/-, indeterminate; -, non reactive; AZT, zidovudine; 3TC, lamivudine; NVP, nevirapine; d4T, stavudine.

formed for confirmation of HIV infection. These HIV infected children may be misclassified as non HIV infected. In our experience, one child who had both negative HIV rapid test and HIV ELISA stopped ART and follow up to our hospital after the parents attended other health care facility where they were told that the child was not infected. Guidelines for starting ART in children should inform about the possibility of HIV seroreversion in infants who initiate ART, especially if ART is initiated in children younger than six months.

In this study, HIV rapid test had lower sensitivity than ELISA for diagnosing HIV infection in children with early initiation of ART. WHO recommends the use of rapid antibody tests in developing countries because they do not require sophisticated laboratory service, running water or electricity, and results can be obtained within shorter time than for traditional ELISA.¹³ WHO and UNAIDS also recommend the use of serial testing in most setting, so if the first HIV rapid test is non reactive, no additional HIV test is performed.¹³ However, according to the results of this study, this strategy may not be appropriate for diagnosing HIV infection in children who initiate ART at age less than one year.

Case number 4 was particularly interesting. HIV serology became positive after having a virological failure to ART confirmed with high HIV viral load levels. In a previous study, the absence of HIV antibodies was maintained if the suppression of viral replication was maintained.⁷ Our case suggests that, in resource-limited settings where viral load is not available, HIV serology could be utilized for monitoring the ART response in children with early initiation of ART who do not develop HIV antibodies.

The study has some limitations. Children may acquire HIV while in uterus, during delivery or during breastfeeding.¹⁴ Delivery by elective caesarean section reduces significantly the chances of acquiring HIV infection during delivery.¹⁴ In this study, we did not perform serial HIV viral loads to detect the moment of HIV transmission from the mother to the child. Seroreversion of HIV probably occurs in those children who are initiated on ART soon after the acquisition of HIV infection, but we were not able to identify the exact point when the children were infected.

In conclusion, the results of this study show that HIV seroreversion of children with early initiation of ART is a common phenomenon that should be considered for avoiding misdiagnosis of HIV infected children.

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