

Role Of Dried Blood Spot In Early Infant Diagnosis Of HIV Infection

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Abstract: Background: Routinely the test that we use in HIV screening are HIV rapid test or Enzyme Immuno Assay (EIA), these methods can't accurately diagnose HIV infection in infants up to the age of 18 months. Only way to confirm diagnosis is detection of HIV DNA from the children by nuclear techniques like polymerase chain reaction (PCR), which is not available at the resource limited laboratories. The technique of Dried Blood Spot (DBS) is a simple effective method of sample collection & transport for HIV testing of children below 18 months, especially at resource limited laboratories, which is the part of PPTCT programme. Method : The study group consists of 87 infants born to HIV reactive mothers under the PPTCT programme, at tertiary care center in Ahmedabad, Central Gujarat, India over a period of one year, from May-2010 to April 2011. Earliest DBS collection is recommended at the age of 6 week. Results: Total 87 children were included in the study. Total prevalence of HIV infection in children below 18 month of age is 8%. 2(12.5%) children born to mothers who had not received Nevirapine were tested positive for HIV by DNA PCR. 3 (20%) children became infected with HIV who had not received syrup Nevirapine. Rate of HIV infection in children who were breast fed is 4 (12.5%). 2 (20 %) mothers with CD4+ count less than 200/mm³ transmitted HIV infection to their babies. Conclusion: Ninety percent of the children living with HIV were infected through mother-to-child transmission during pregnancy, around the time of birth or through breast feeding. The technique of Dried Blood Spot (DBS) especially at resource limited laboratories & detection of HIV infection through the most sensitive and specific method PCR in diagnosis of HIV at earliest age, six weeks. Different interventions can be taken to prevent mother to child transmission during pre, intra and postnatal periods. [Dharsandia M et al NJIRM 2012; 3(1) : 85-89]

Key words: Dried Blood Spot (DBS), HIV, DNA, Infants

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Introduction: Globally the number of children younger than 15 years living with HIV has increased from 1.6 million in 2001 to 2 million in 2007, though the newly infected children have been decreasing since 2003 due to increasing services of Prevention of Parent to Child Transmission (PPTCT) ¹. Routinely the tests that we use in HIV screening at various centers in India -Integrated Counseling & Testing Center (ICTC) and under PPTCT programme are HIV rapid test or Enzyme Immuno Assay (EIA). Major limitations of these tests are that they can't accurately diagnose HIV infection in infants up to the age of 18 months, because the antibody detected by these tests might be due to transplacental transfer from mother. Only way to confirm diagnosis is detection of HIV DNA from the children by nuclear techniques like polymerase chain reaction (PCR), which is not available at the resource limited laboratories. HIV infected infants have high mortality, because of delay in diagnosis especially in developing countries like India and

more in rural and developing areas. The challenge in resource limited laboratories is to diagnose HIV infected children till 18 months and to provide them early access to life saving medicines ². This study specially emphasizes on role of Dried Blood Spot(DBS) in diagnosis of HIV at earliest age, six weeks, in infants through the most sensitive and specific method PCR and different interventional parameters which can prevent mother to child transmission during pre, intra and postnatal periods ^{1,2,3,4}.

The technique of DBS is a simple effective method of sample collection & transport for HIV testing of children below 18 months, especially at resource limited laboratories. This is the part of PPTCT programme.

Materials and Methods The study group consists of 87 infants born to HIV reactive mothers under the PPTCT programme, at tertiary care center in Ahmedabad, Central Gujarat, India over a period of

one year, from May-2010 to April 2011. These women were followed throughout their antenatal period and various related parameters were noted and analyzed. Whether she was taking Anti Retroviral Therapy (ART), her CD4+ cell count, mode of delivery (Normal delivery/Cesarean section), she had received Inj. Nevirapine or not, whether baby received syrup Nevirapine, babies were breast fed or were on skimmed milk were noted and during their first visit after delivery, infants blood samples were collected on DBS card after 6 weeks of birth (Fig 1).

Figure 1: Medical personnel collecting sample on DBS card, infant with leaning position for free flow of blood directly on to the card



Parents were counseled before each test to ensure their understanding for the need of early diagnosis of HIV and the treatment options available for them if child's tests turn out to be positive. Also parents were counseled for possibility of negative test result. Other important features were also covered during counseling like possibility of HIV transmission through breast feeding.^{1,3}

Strategies of DBS collection are:^{1,3} Earliest DBS is recommended at the age of 6 week.

Children below 6 month: At the time of first visit, DBS is collected and sent for testing. If HIV DNA is not detected, again the infant is tested at 6 months.

Children from 6- 18 months: DBS collection & testing of these children is not recommended;

hence they were tested first with rapid screening test which is available at the Integrated Testing & Counseling Centre (ICTC). If rapid test is reactive, then DBS collection is done & then sent for HIV DNA PCR testing.

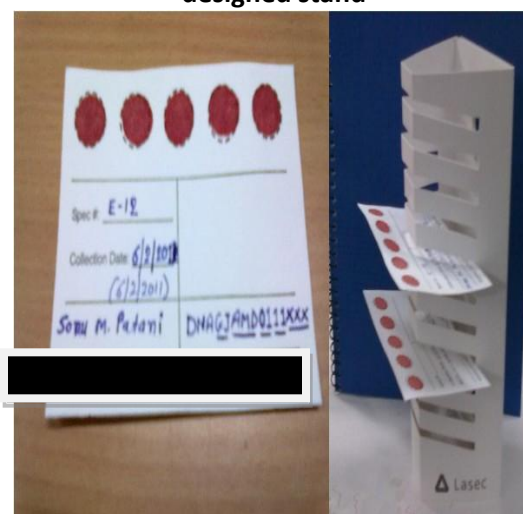
The next step in children in whom HIV genome is detected: Whole blood samples of these children are sent for HIV DNA PCR testing at reference level laboratory for confirmation.

DBS collection procedure^{1, 2, 3} Steps included in sample collection are: First baby's foot warmed (or hand, if older than 10 months or larger than 10 kg) to facilitate blood flow.

Baby is positioned with its foot down; foot is cleaned with appropriate antiseptic agent. Wearing the powder free gloves, baby's foot is pricked with a lancet and allows the blood to flow. First drop of blood is wiped away with gauge or cotton wool. Large drop of blood, enough to cover circled area on DBS card is collected. Blood is collected at least on two circles, completely filled on DBS card. The foot then cleaned with antiseptic.

Samples were put for drying horizontally, preventing from direct sunlight for at-least four hours³ (Fig 2).

Figure 2 : Collected sample on DBS card & left at room temperature for drying on specially designed stand



After drying samples were stored in sealable plastic bags with desiccant packets and a humidity card

and transport to the reference laboratory where they were tested by HIV DNA PCR³.

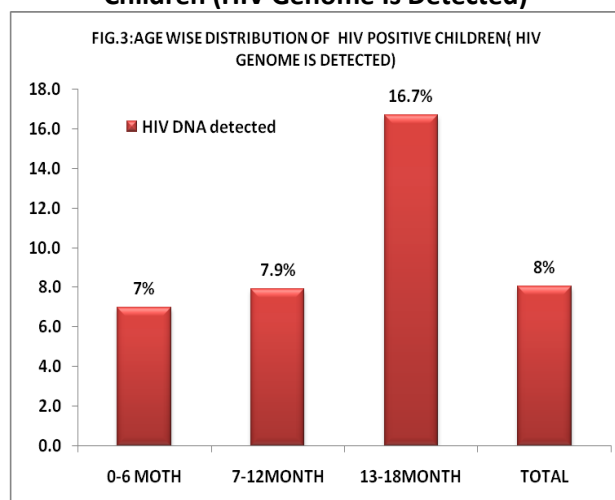
Results: Total 87 children were included in the study, 45 children were male and 42 were females, this shows almost equal number of male and female children tested. In 7(8.04%) children HIV DNA was detected. They were divided into three age groups shown in (Table 1).

Table 1: Age and gender wise distribution of children tested with DBS

Age Group (Month)	Total No. Of Infant	Sex		HIV DNA detected by DBS
		MALE	FEMALE	
0-6	43	23 (53.5%)	20 (46.5%)	3
7-12	38	19 (50%)	19 (50%)	3
13-18	6	3 (50%)	3 (50%)	1
TOTAL	87	45 (51.7%)	42 (48.3%)	7 (8.04%)

There were 1 (16.7%) children in whom HIV DNA is detected in the age group 13-18 months, and in the age group 0-6 month and 7-12 month 3(7%) and 3(7.9%) were found positive by DBS respectively. Total prevalence of HIV in children below 18 month of age is 8%.Age wise positive cases shown in the (Fig-3).

Fig.3: Age wise distribution of HIV positive Children (HIV Genome Is Detected)



71 mothers who were having HIV infection were given Tab. Nevirapine during labor and 16 women

were not able to receive this medicine. Only 3(4.23%) children born to these mothers who had received Nevirapine were tested positive for HIV by DNA PCR compared to 2(12.5%) children born to mothers who had not received Nevirapine were tested positive for HIV by DNA PCR (Table 2).

Only 4 (5.56%) children who had received Nevirapine syrup became HIV infected, compared to 3 (20%) children became infected with HIV who had not received syrup Nevirapine (Table 2).

Table 2: Results of HIV testing on 87 infants along with different interventional parameters

Parameters	Numbers (n)	Children Having HIV	Children Not having HIV
Tab. Nevirapine at the time of delivery	Given(n=71)	3(4.23%)	42
	Not Given(n=16)	2(12.5%)	14
Nevirapine syrup to baby	Given(n=72)	4(5.56%)	68
	Not Given(n=15)	3(20%)	12
Mode of Delivery	Normal delivery(n=67)	6(8.96%)	61
	Cesarean section(n=20)	1(5%)	19
Mother taking ART	Yes(n=24)	2(8.33%)	22
	No(n=63)	5(7.89%)	58
Type of Feeding	Breast fed(n=32)	4(12.5%)	28
	Non Breast Fed(n=55)	3(5.54%)	52

Six (8.96 %) children born by normal delivery were tested positive for HIV by DNA PCR and 1 (5%) children born by cesarean section were tested positive for HIV by DNA PCR (Table 2).

Two (8.33 %) children born to mother on ART were tested positive for HIV by DNA PCR while 5 (7.89 %) children born to mother not on ART were tested positive for HIV by DNA PCR (Table 2).

Rate of HIV infection in children who were breast fed is 4 (12.5%) while only 3 (5.54%) children were infected by HIV, who was not breast fed (Table 3).

We followed the CD4+ count of HIV infected mothers. None of mothers with CD4+ count $>500/\text{mm}^3$ transmitted HIV infection to their babies. 5 (12.20%) HIV infected mothers with CD4+ count between $200-500/\text{mm}^3$ transmitted HIV infections to their babies while 2 (20 %) mothers with CD4+ count less than $200/\text{mm}^3$ transmitted HIV infection to their babies. Unfortunately there were 9 mothers whose CD4+ count, we were not able to follow, due to transfer of patient to other ART centre.

Table 3: Association of CD4+ count of mother

CD4+ Count	Total	Children Having HIV Infection	Not Having HIV Infection
$<200/\text{MM}^3$	10	2(20%)	8
200- $500/\text{MM}^3$	41	5(12.5%)	36
$>500/\text{MM}^3$	27	0	27

Discussion: Ninety percent of the children living with HIV were infected through mother-to-child transmission during pregnancy, around the time of birth or through breast feeding. In year 2007 alone 3,70,000 children under the age of 15 were newly infected, that is around 1,000 per day; and 2,70,000 died, the majority under the age of five^{1, 2, 3}.

Our study shows higher HIV infection rate among children born to those mothers, who did not receive Nevirapine at the time of delivery. Our Study is comparable with the study by Laura Johnes who has shown 25% chances of HIV infection to children born to mother having HIV if she had not received nevirapine^{5, 6}.

Present study shows there are 4 times more chances of HIV infection in children who did not receive Nevirapine syrup at the time of birth. Study shows that even a regimen as simple as one dose of nevirapine given to the baby within the first 72 h of birth (but as close to birth as possible) was associated with a 41% relative reduction in the risk of HIV transmission to baby⁶. There are more chances of getting HIV infection, if the child is born through normal delivery rather than Cesarean section. Research indicates that the majority of babies who pick up HIV infection from their mothers probably acquire the virus during the birth

process⁵. The risk of transmission is reduced if the baby is delivered by planned cesarean section, rather than by vaginal delivery⁷. There is negligible difference in rate of HIV infection among children whose mother is on ART in antenatal period and who are not on ART before birth of children. Our study is comparable with the study of Laura Johns⁵.

The study shows there are 2 to 3 times higher chances of HIV infection in children who are breast fed. Because there are documented cases showing that HIV can be transmitted from mother to infant through breastfeeding, HIV positive women are counselled to avoid breastfeeding if safe alternatives to breastfeeding exists^{5, 7}. In a breast fed population, the added risk of postnatal transmission from breastfeeding adds 5%-20% to the baseline risk, such that the total risk increases to as much as 50% (average range, 20%-50%)⁶. Duration of breast feeding also increases chances of transmission by 10 %⁶. These percentages are averages of transmission rates; an individual patient could have much higher or lower risk depending on the particular clinical scenario^{5, 6}.

Study shows that low CD4+ count increases chances of acquisition of HIV infection among children from mother. Mother whose CD4+ count was $<200/\text{mm}^3$ transmitted HIV infection to their children in 20% cases⁶.

Factors that increase the risk of acquiring HIV from mother to children⁷:

A child is more likely to contract HIV from its mother if:

- She has advanced HIV infection or AIDS;
- She has high viral load or a low CD4 count;
- Her waters break at least four hours before delivery;
- She has a vaginal delivery ;
- The labor is difficult, requiring episiotomy or forceps;
- She has a genital infection (e.g. a sexually transmitted infection, such as Chlamydia);
- She gives breast feeding.

Advantages of DBS collection for HIV diagnosis:

- For DBS whole blood sample in large amount is not required, instead few drops of whole blood

collected on pre designed filter paper are only required³.

- It is easy to collect blood for DBS card. No special collection training is required^{1, 2,3,8,9}.
- No need to store the DBS filter paper at specific temperature, while, whole blood or serum sample is requires 2-8°C for storage. DBS can be left at room temperature for drying. In refrigerator (2-8°C) it can be stored for at least 6 months under low humidity condition^{3,8}.
- DBS poses little biohazard risk^{2,3}.
- It is easy to collect & transport to testing facilities without any specific requirements for temperature during transportation. Whole blood requires 2-8°C temperature during transportation^{9,10}.
- Infant can be tested as early as 6 week of age. While antibody detection only helpful after 18 month of age.
- Also DBS card can be used for testing of other metabolic and genetic disorders such as phenylketonuria, galactosemia, hemoglobinopathies and hypothyroidism^{2,4,11}.
- Testing DBS collected card by PCR is as effective as to test whole blood sample by PCR with sensitivity 100% and specificity 99.6%^{2,4}.

Conclusions: Ninety percent of the children living with HIV were infected through mother-to-child transmission during pregnancy, around the time of birth or through breast feeding. The technique of Dried Blood Spot (DBS) especially at resource limited laboratories & detection of HIV infection through the most sensitive and specific method PCR in diagnosis of HIV at earliest age, six weeks. Different interventions can be taken to prevent mother to child transmission during pre, intra and postnatal periods.

Acknowledgment: We are thankful to Gujarat AIDS Control Society & National AIDS Control Organisation to include our centre for this project under PPTCT programme.

References:

1. Guideline for care of HIV exposed infant and children less than 18 month. National AIDS Control Organization (NACO); January 2010.
2. Newell, M.L. et al. Mortality of infected and uninfected infants born to HIV-infected

mothers in Africa: a pooled analysis. The Lancet.2004 Oct; 364 (9441): 1236-43.

3. Guidelines for HIV testing. National AIDS Control Organization(NACO); March 2007.
4. Sharon C, Teresa S, Max A, Peter N, Martin TS, Michael O. Use of Dried Blood Spot Specimens in the Detection of Human Immunodeficiency Virus Type 1 by the Polymerase Chain Reaction. J Clin Microbiol. 1991 April; 29 (4): 667-71.
5. Sharon C, Teresa S, M. John G, Michael M, James R, Cheikh TS, et.al. Stability of Dried Blood Spot Specimens for Detection of Human Immunodeficiency Virus DNA by Polymerase Chain Reaction. J Clin Microbiol 1992 Dec; 30(12):3039-42.
6. Ada C, Gayle GS, Annette HS, Consuelo B, Susan AF. Diagnosis of Human Immunodeficiency Virus Type 1 Infection in Infants by Use of Dried Blood Spots and an ultrasensitive p24Antigen Assay. J Clin Microbiol 2009 Feb; 47(2):459-62.
7. Sharon C, M. John G, Richard P, Michelle C, Robert FV, Brian W, et.al. Quantification of Human Immunodeficiency Virus Type 1 RNA from Dried Plasma Spots Collected on Filter Paper. J Clin Microbiol 1997 Nov; 35(11): 2795-801.
8. Sharon C, Ann B, Sharon K, Joanne S, Tidiane SY, Normand L, et.al .Rapid Screening for Early Detection of Mother-to-Child Transmission of Human Immunodeficiency Virus Type 1. J Clin Microbiol 1994 Nov; 32(11):2641-645.
9. Antonio CT, Jose NJ, Renata MS, Arminda LS, Bernardo FM, Erica LF.et al. Dried blood spots as a practical and inexpensive source for human immunodeficiency virus and hepatitis C virus surveillance. Mem Inst Oswaldo Cruz. 2005 July; 100 (4):365-70.
10. Laura Johnes, Perinatal HIV Transmission and Birth Options for HIV positive Mothers .[Internet].2003. Available from: http://www.tpan.com/publications/positively_aware/sep_oct_03/treatment_series_perinatal.html
11. Michael Carter, Mother-to-baby transmission.[Internet].2011. Available from: <http://www.aidsmap.com/Mother-to-baby-transmission/page/1044918/>