



## Research Article

### STUDY OF EARLY INFANT DIAGNOSIS(EID) AT THE PPTCT CENTRE IN CIVIL HOSPITAL AHMEDABAD

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**Abstract-** Early infant diagnosis (EID) project was implemented in 18 districts of Gujarat through 33 PPTCT (Prevention of Parent to Child Transmission) centres, one of them being Civil Hospital, Ahmedabad, from 1<sup>st</sup> April 2010 to diagnose HIV (Human Immunodeficiency Virus) in infants below 18 months. The method used for this purpose was Total Nucleic Acid-PCR (Polymerase Chain Reaction). This study was done on 97 infants whose mothers were enrolled under the PPTCT program at Civil Hospital, Ahmedabad in Gujarat, India from January 2016 to December 2017. The study determined the infant positivity rate and evaluated the associated factors. PPTCT is a component of the National AIDS Control Program (NACP) which was upgraded to NACP-IV in 2012. Therefore, the obtained results were also compared with the data from 2012-2015 from the PPTCT centre record. The results indicated that six infants out of 97 were found to be HIV positive (6.18%).

**Keywords-** HIV, PCR, PPTCT, Infants, DBS

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#### Introduction

HIV caused its first outbreak in the 1980s. One of the several consequences of the generation of enormous amount of information about HIV was discovery of AZT (Zidovudine) and successively other effective anti-retroviral drugs. UNAIDS has set an ambitious treatment target of '90-90-90 by 2020' for the world and India is committed to work towards it. The PPTCT intervention under the NACP was started in 2002, targeting special population-Pregnant women and their children [1-4]. It provides access to HIV testing services to all pregnant women enrolled in ANC (Ante-natal Care) along with ARV (Antiretroviral) prophylaxis to the mothers and their neonates. Neonates and infants are the most vulnerable of all the HIV infected patients, as the virus replicates at a higher rate in the first year of life as compared to adults, which leads to higher viral load and rapid disease progression [5]. The neonate may acquire the infection anytime during pregnancy, during delivery or during lactation; and the risk of transmission ranges from 15-35% [6]. The treatment policy of NACP to reduce this risk underwent a transition from SD-NVP (Single Drug Nevirapine) to MD-ARV (Multi-drug Antiretroviral) prophylaxis in 2012.[7] Now, lifelong ART is given to all pregnant and lactating women living with HIV / AIDS, regardless of the CD<sub>4</sub> count, WHO clinical stage or duration of pregnancy. The aforementioned initiatives are aimed at eliminating new HIV infection in infants. EID is one of the provisions of CST package of NACP, which stands for Care, Support and Treatment [7]. It is necessary because early diagnosis will lead to early linkage of the infant to CST services and early ART initiation will delay the negative effects of HIV. Determination of infant positivity shall quantify the efficacy of the prophylactic measures and let us know the current status of HIV in that geographical area. By tracing the infants and their mothers, we can find out the relationship between its vertical transmission and the associated risk factors.

#### Method

To determine the above-mentioned variables, we studied 97 infants borne by HIV positive mothers enrolled under the PPTCT program at Civil Hospital, Ahmedabad in Gujarat, India from January 2016 to December 2017. These women and their infants were followed throughout till the confirmation of HIV status of infants, *i.e.*

from six weeks or the age of first visit at PPTCT Centre to up to the age of 18 months. The women also underwent a pre-test counselling prior to the collection of the first sample of the infant. Diagnosis of HIV in infants less than 18 months cannot be relied upon by the antibody-based tests, because of the transfer of maternal HIV antibody. Therefore, detection of the virus itself has diagnostic significance for which the method of choice is Qualitative Nucleic Acid Amplification but this test is not available at the counselling and testing Centre. The sample for DBS was collected according to National AIDS Control Organization (NACO) guidelines and sent to Kasturba Hospital, Mumbai which is one of the premier EID laboratories in India [2]. The NACO EID laboratories have switched over from conventional PCR based on pro-viral DNA hybridization to detection of Total Nucleic Acid by PCR which detects pro-viral DNA and RNA. This RT-PCR (Real Time – PCR) is pre-qualified for testing DBS samples as it is more sensitive [2]. For infants more than six months, whole blood was collected, and the baseline status of the infant was determined by three rapid tests based on antibody prior to the dispatch of the sample for DBS PCR in accordance with the testing algorithm of EID guideline. A second DBS sample was collected if HIV was detected in the first instance and the baby was said to have HIV infection only if both the test results were concordant. In case of a discordant result, a repeat DBS was taken to establish the diagnosis. Chi-square test was used to determine the statistical significance of the obtained results.

#### Result

A majority of the infants *i.e.* 85 out of 97 were enrolled for EID before six months of age (87.6%). The positivity rate was 5.88% amongst the infants of less than 6 months and 8.33% in those more than six months. 81 out of 97 infants (83.5%) were on exclusive breastfeeding (EBF) while 16.49% infants were on exclusive formula feeding (EFF). All but one pregnant woman did not receive any kind of prophylaxis during gestation and during delivery. Her infant also did not receive any prophylaxis and was enrolled at EID Centre at nine months of age where he was diagnosed to be positive for HIV. Positivity rate was found to be higher among the male infants in comparison to the female infants.

Table-1 Results and their Significant Values

Parameter	Total	Positive	Rate	$\chi^2$	Degrees of freedom	Significance (P value at 0.05)
Age						
<6 months	85	5	5.88%	0.1089	1	P = 0.74144
6-12 months	12	1	8.33%			
Nutrition						
EBF	81	5	6.17%	0.0001	1	P = 0.99065
EFF	16	1	6.25%			
ART						
Received	96	5	5.20%	-	-	-
Not received	1	1	100%			
Mode of birth						
Normal	70	4	5.71%	0.0963	1	P = 0.75637
LSCS	27	2	7.40%			
Sex						
Male	63	5	7.93%	0.3298	1	P = 0.9496
Female	34	1	2.94%			

Table-2 Positivity rates among exclusively breastfed infants according to the maternal CD<sub>4</sub> count at the time of pregnancy

CD <sub>4</sub> count of mothers providing EBF	Total infants on EBF	Infants on EBF found to be HIV positive	$\chi^2$	Degrees of freedom	Significance (P value at 0.05)
<200 /mm <sup>3</sup>	6	2 (33.3%)	8.5865	2	0.013661
200-500 /mm <sup>3</sup>	35	2 (5.71%)			
>500 /mm <sup>3</sup>	40	1 (2.5%)			

Table-3 The infant positivity rate according to the maternal CD<sub>4</sub> count

CD <sub>4</sub> Count of mothers	Total infants	Positive infants	$\chi^2$	Degree of freedom	Significance (P value at 0.05)
<200 /mm <sup>3</sup>	6	2 (33.3%)	9.1372	2	0.01037
200-500 /mm <sup>3</sup>	42	3 (7.1%)			
>500 /mm <sup>3</sup>	49	1 (2.0%)			

## Discussion

### Fall in infant HIV

As per NACO annual report 2015-16, 3.7% babies born to PLHA mothers were found to be HIV positive by DBS DNA PCR [7]. The positivity rate declined to 1.73% in the year 2016-17. There has been a national decline in the prevalence of HIV infection among pregnant women from 0.40% in 2010-11 to 0.29% in 2014-15 [7]. We studied 97 infants and their mothers who were enrolled with PPTCT Centre at Civil Hospital, Ahmedabad, out of which six were found to be HIV positive (Rate = 6.18%). [Fig-4] illustrates the rate of positivity in infants and pregnant women who had visited the Centre for ANC. The upgrade of NACP-III to NACP-IV happened in 2012 when 0.95% of women visiting the Centre for ANC were positive for HIV and infant positivity rate was 12.32%. As far as Southern Gujarat is concerned, infant positivity rate was 11.4% in 2010 [1]. After the introduction of new guidelines as per NACP-IV, there has been an uneven but noticeable decrease in both HIV positivity and infant positivity rates as illustrated in [Fig-4]. This coincides with a general decreasing trend of HIV prevalence in the country [8]. However, the HIV positivity rate among pregnant women enrolled for PPTCT at Civil Hospital was higher than the national prevalence rate. This could be due to the large number of migrating populations in this state which is a high-risk group. In 2016-17, 2,34,000 immigrants were covered under the program out of which 1,00,000 were in Ahmedabad [7].

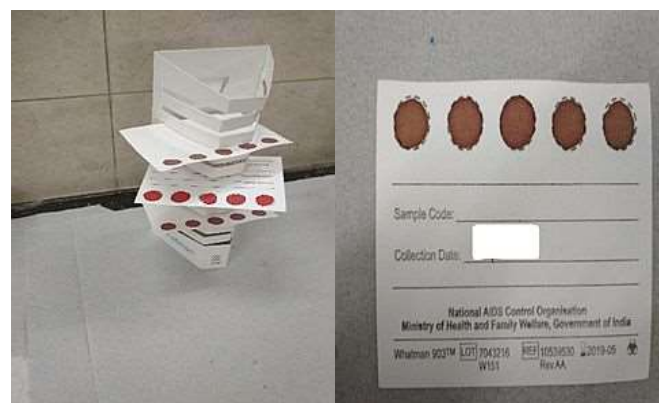


Fig-2 DBS are air dried prior to dispatch Fig-3 DBS ready for dispatch

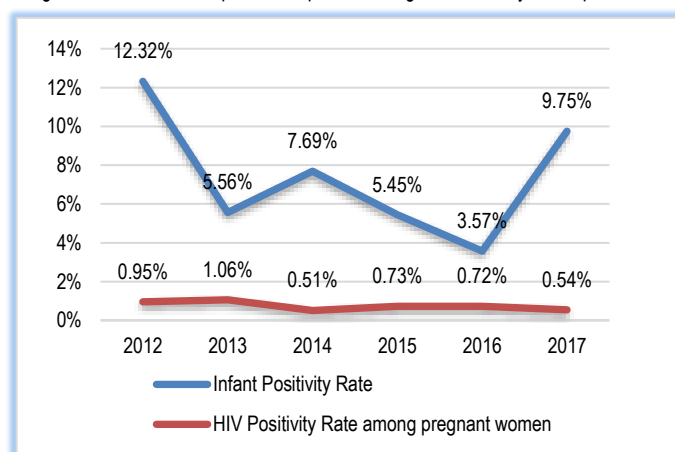


Fig-4 Infant Positivity Rate and HIV Positivity Rate among pregnant women (2012–17)

### Prophylactic Nevirapine and ART are vital

We found that 96 out of 97 mothers were on ART and were given intra-partum prophylactic NVP. The one infant, whose mother missed the prophylaxis and ART, was diagnosed with HIV by DBS and antibody detection.



Fig-1 DBS obtained from heel prick of a 6-week-old infant on a DBS card

In a study conducted in Southern Gujarat in 2010, infants who received prophylactic ARV were eight times less likely to acquire HIV infection from their mothers [1]. According to Dharsandhia *et al*, in 2010-11 which was before the transition to MD-ARV from SD-NVP, 16 out of 87 pregnant PLHAs and their babies had no prophylactic Nevirapine [9]. Moreover, only 24 women were on ART and the infant positivity among them was 8.33% while it was 7.89% in those who were not on ART[9]. This contradiction could be due to the high viral load and low CD4 count which was the initial criteria for administration of ART. As illustrated in [Fig-5], the MD-ARV coverage for pregnant women has increased from 54.34% in 2012 to almost 100% in 2016-17.

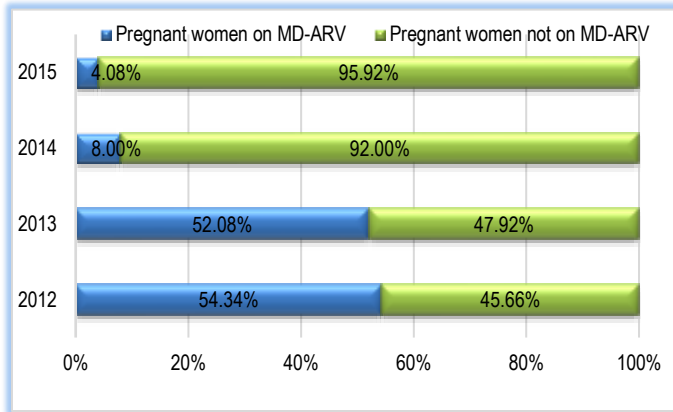


Fig-5 Percentage of pregnant women on MD-ARV after upgrade to NACP-IV (2012-15)

#### The significance of 'Early' in EID

85 (87.6%) infants were enrolled for EID before six months of age with the median age being 1.7 months as shown in [Fig-6]. The oldest infant was 9-month-old at the time of first visit to the PPTCP Centre and was found to be HIV positive.

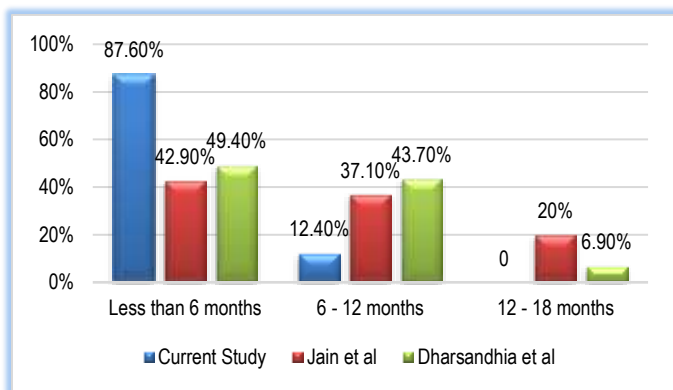


Fig-6 Percentage of infants enrolled for EID according to age

A general higher rate of positivity was observed in infants who had delayed first visit to the PPTCT Centre in the current study [Table-1] and previous studies [1,9]. This finding is not statistically significant. However, the 'Early' in EID cannot be undervalued. The optimum age for HIV detection by PCR is 6 weeks and it should not be delayed beyond 18 months. The infant is referred to the ART for initiation for ART as soon as HIV infection is detected because early ART will aid in palliation of negative effect of HIV on development of the infant. It may also promote immune recovery and delay immunosuppression caused by HIV.

#### The choice between breastfeeding and formula feeding

The risk of transmission from breast milk is 12-20% [6]. Thus, formula feeding is recommended as a preventive measure to theoretically eliminate the risk associated with exposure to breast milk. But for the developing and under-developed nations, the cost and risk associated with exclusive formula feeding outweighs the benefits of exclusive breastfeeding necessitating recommendation of the latter. However, it is important that the nursing mothers are on ART during the lactation period. To ensure that there was no transmission during lactation, the

infant is tested once again for HIV six weeks after the last breast milk feeding or at 12 months, whichever is earlier. In this study, 81 infants were given exclusive breastfeeding (EBF) out of which five infants were detected positive for HIV (6.17%). The positivity rate amongst the infants on exclusive formula feeding (EFF) was 6.25% but the difference was not found to be statistically significant. In the study by Jain *et al*, infant positivity was significantly higher in breastfed infants [1]. Dharsandhia *et al* found infant positivity to be 12.5% in breastfed infants and 5.54% in formula-fed infants. This could be due to the lack of universal MD-ARV coverage for pregnant women in that time period, since it was not recommended. On studying the distribution of HIV positivity amongst the EBF infants on the basis of maternal CD4 count, it was found that the low maternal CD4 count increases the risk of transmission of HIV to the infants from breast milk [Table-2].

#### The role of mode of delivery

Caesarian section is not recommended for prevention of MTCT and it is done only when there is an obstetric indication [10]. Safe delivery techniques are to be practiced to minimize the risk of transmission. The majority of infants were delivered by normal vaginal delivery (NVD) and the positivity rate was 5.71% in them which was 7.40% in the infants delivered by CS. It is contradictory to the previous studies in which the positivity rate was found to be a higher in infants delivered by NVD [1,9]. This could have been due to high viral load, low CD4 count or increased intra-partum exposure. The probable cause cannot be commented upon since we were unable to find the obstetric indication for the same. However, it was not found to be statistically significant since the P-value was less than 0.05.

#### Maternal CD4 count during pregnancy is important

Mother's CD4 count is a major determining factor for vertical transmission of HIV to the infant. It is an indicator of the viral load, status of the immune system, general well-being of the mother and successful ART. A high CD4 count ensures a delayed development of AIDS, decreased susceptibility to opportunistic infections which usually occur when the count falls below 200/mm<sup>3</sup> and consequently delayed death, that are desirable outcomes. It was found in this study that the CD4 count of a majority of women was more than 500/mm<sup>3</sup> which is conducive for bearing a healthy infant as shown in [Table-3] while in 2010 the majority of HIV positive pregnant women had CD4 count between 200-500/mm<sup>3</sup>.

#### Conclusion

Infant HIV is not only a part of new HIV infection but also an indicator of several other HIV-associated factors such as maternal HIV infection and efficacy of HIV infection control measures. It was found in this study that there was an overall decline in the rate of HIV positivity in infants which is an indicator of efficacy of PPTCT measures. Not only the numerator but the denominator has also decreased implying a general decrease in HIV prevalence among pregnant women. Prophylactic Nevirapine and MD-ARV prophylaxis at pregnancy improve the outcome and lower the risk of transmission. This also coincides with the finding that women with CD4 count of less than 200 are more likely to transmit HIV in comparison to the women with count of more than 500. MD-ARV prophylaxis helps in maintaining the CD4 levels and keeping a check on the viral load, thereby decreasing the transmission risk. Planned pregnancy and lactation within the purview of CD4 count and viral load will further help in control and subsequent elimination of infant HIV. As long as the nutrition is adequate enough to boost the immune system, there is no mixed-feeding and the nursing mothers are on ART and have healthy CD4 count (>500/mm<sup>3</sup>), there is not much harm in exclusive breastfeeding considering the associated risks in the developing and under-developed world. Thus, exclusive breastfeeding is recommended up to six months and practiced in these countries which also have the maximum HIV patient load [11]. Formula feeding can be given if it is deemed acceptable, feasible, affordable, sustainable and safe (AFASS) [10]. Early diagnosis, prompt treatment, timely prophylaxis and regular monitoring are vital for prevention of transmission. In the year 2016 NACP-IV was given a Mid-Term Appraisal (MTA) [12]. The success of the program upgrade and MTA should be evaluated in terms of target achievement, program implementation and monitoring indicators at national and regional levels.

This should be followed by formulation of more effective strategy, expansion of coverage, effectual implementation and customization as per the regional requirements.

**Application of research:** This study evaluated the regional transmission rate and the trend of variables which could be used to formulate the mitigation strategies for this area. It will also help in determining its position nationally as well as globally in terms of disease burden and control.

**Research Category:** Medical Microbiology

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**Sample Collection:** Civil Hospital, Ahmedabad

**Ethical approval:** This article does not contain any studies with human participants or animals performed by any of the authors.

Ethical Committee Approval Number: Nil

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