EARLY DIAGNOSIS OF HIV IN INFANTS USING DRIED BLOOD SPOT- A RETROSPECTIVE STUDY FROM 2010 TO 2016

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ABSTRACT

BACKGROUND

Mother-to-child transmission of HIV is a major cause of HIV in children. Detection of virus by DNA PCR is of utmost importance in <6 months of age as antibody detection is not helpful. Dried blood spot (DBS) method is a cheap, less invasive, easy and feasible method as compared to whole blood collection. Followup of babies up to 18 months is needed before declaring them negative. The study was undertaken to know the HIV transmission rates in babies up to 18 months.

MATERIALS AND METHODS

Sample collection and processing was done strictly according to National AIDS Control Organisation (NACO) guidelines.

RESULTS

55.36% HIV positive Antenatal care (ANC) females were in 20-24 years age group. 48.21% HIV Positive ANC females presented for the first time in 2nd trimester. 53.57% females were primigravida. In 55.36% cases, even spouse was positive for HIV. In 78.57% there was normal delivery. In year 2010, samples collected at 6 weeks, 6 months, 12 months, 18 months were 33, 21, 21 and 20 respectively; and in year 2011 were 32, 18, 9, 29; in 2012 were 30, 28, 23, 16; in 2013 were 33, 30, 25, 14; in 2014 were 30, 14, 19, 23; in 2015 were 27, 21, 20, 14; in 2016 were 14, 17, 15, 12 respectively. The positivity rate at 6 weeks, 6 months, 12 months, 18 months in 2010 were 1, 0, 1, 2; in 2011 were 4, 0, 0, 1; in 2012 were 2, 0, 0, 0; in 2013 were 1, 1, 0, 0; in 2014, there were no positive cases; in 2015 were 0, 1, 0, 0; and in 2016 were 0, 1, 0, 0 respectively.

CONCLUSION

Decrease in positivity of samples in years 2014-2016 may be due to effective Prevention of Parent-to-child transmission (PPTCT) programme, increase in awareness amongst people, and early initiation of Anti-Retroviral Therapy (ART). The number of samples also reduced significantly in the year 2016. No deaths were recorded after 2011.

KEYWORDS

ART, DBS, DNA-PCR, NACO, PPTCT, 2010-2016.

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BACKGROUND

In 2012, approximately, 2.3 million new HIV infections occurred worldwide and 1.6 million of these infections were in sub-Saharan Africa. Everyday around 6300 adults are infected worldwide of which 34% are women aged 15 - 24 years.¹ There are 30% chances of mother-to-child transmission of HIV infection (transplacental, at birth, after birth, pregnancy).² According to studies, almost 2000 infants in developing countries are infected everyday with HIV through mother-to-child transmission.³

Most of the PPTCT programmes have successfully achieved high rates of initial HIV testing in pregnant women. The tests of HIV are repeated if the mother is negative.

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But the partner involvement and testing in mother and child health services is mostly limited to requests in ANC women.⁴ Under PPTCT programme, the exposed children of the seropositive mothers are benefitted.⁵

As maternal antibodies can be present in infants after delivery, antibody testing is not useful to achieve the diagnosis till 18 months. So, detection of virus is of utmost importance. DNA PCR that detects HIV-1 proviral form integrated with human genome has been implemented across world since 2010.⁵

Whole blood needs to be stored in the refrigerator. Hence, DBS is of much importance. DBS sampling is microvolume sampling technique that involves quantification of analytes from whole blood spotted on filter paper. This technique is discovered in 1960 and since been used for screening newborn errors of metabolism.⁶ Need of less amount of blood and that the sample can be collected and stored without refrigeration with low biological hazard are the advantages of DBS.⁷ Also, a prick is sufficient so use of needles and vacutainer can be avoided. Blood coated on filter paper lyses the cells and binds the DNA. This facility is mainly used in resource limited nations.⁸ The HIV-1 PCR test is 99% sensitive and 98% specific which means that a confirmatory test is critical to eliminate the rare cases of false positives.

As prophylactic antibiotic and antiretroviral therapy can increase the longevity and promotes healthy survival, early diagnosis of HIV should be done. This study was undertaken to assess the detection rate of HIV in infants using DBS. We took the data from year 2010-2016.

MATERIALS AND METHODS

The present study was carried out retrospectively at Shri Bhausaheb Hire Government Medical College, Dhule from 2010-2016.

All the babies whose mothers were positive for HIV or who were suspected cases of HIV as referred by the clinicians were included in the study. Our study also included babies with unknown identities.

An informed consent from parent or guardian was taken before pre-test counselling and collecting the sample. The sample collection was done according to NACO guidelines.⁹

DBS was collected from heel prick in children aged 6 weeks to 4 months, from big toe in children aged >4 months to 10 months and from 3rd and 4th finger prick in children >10 months to 18 months.

DBS card was labelled and we filled test requisition and result form (TRRF) just before taking the specimen. DBS samples were stored at 2-8°C until transported and packed appropriately. We ensured packaging appropriate specimens after verifying the TRRF and delivery checklist. Transportation of sample in person or through courier was done every Tuesday of the month.

Algorithm for Diagnosis of HIV Infection at <6 Months of Age

At ICTC Level

We have collected and sent DBS of babies between 6 weeks to 6 months of age for HIV-1 PCR test to National AIDS Research Institute (NARI) PCR testing laboratory. Antibody testing was not recommended in babies <6 months. If baby is <6 weeks old, then PCR test is not recommended; 6 weeks and above is the optimal age for the routine first PCR test.

If Result is HIV-1 Detected

Infant is probably HIV-1 infected. NARI lab asks for another DBS sample. If the sample is positive, baby is HIV-1 infected. We refer the baby to Anti-Retroviral Therapy Centre (ARTC) for treatment. If sample is negative, then lab asks for another DBS sample and will rely on the result of this sample for establishing diagnosis.

If HIV-1 is not detected

Baby is asked to visit at 6 months of age again or when the baby develops sign and symptoms of HIV (whichever is earlier).

If DBS test is positive, then another sample collected at different time (not 2 samples spotted at same time) were sent for confirming diagnosis.

Algorithm for Diagnosis of HIV Infection at >6 Months of Age

At ICTC Level

Blood was collected and tested for HIV antibodies using all the 3 serological tests (Triline, Trispot and ImmunoComb). Also we prepared a DBS for HIV-1 PCR test simultaneously.

If all the 3 or any 2 or any 1 of the Serological Tests is Positive

We send DBS of child for HIV-1 PCR test and follow the Early Infant Diagnosis (EID) testing algorithm. [Serologically unreliable in infants (maternal antibodies cross placenta; can

give a positive HIV antibody test in baby), Testing of EID is by qualitative nucleic acid tests (NAT)].

If all the 3 rapid serological tests are negative, baby does not need HIV-1 PCR test at this point. We asked the baby to come back for testing at 6 weeks after the last breastfeeding or at 12 months or if baby develops symptoms of HIV whichever is earlier.

RESULTS

The current study was conducted at Shri Bhausaheb Hire Government Medical College from year 2010-2016.

The age distribution of ANC females presenting to our department from 2010 to 2016 is as follows.

According to Table I, total 112 ANC females positive for HIV were enrolled in our hospital. Maximum number of females were in 20-24 years age group (55.36%) and minimum number was recorded in >35 years age group (1.79%).

As per Figure I, 48.21% females presented for the first time in 2nd trimester of pregnancy, 32.14% in the third trimester and 19.64% in the first trimester.

53.57% HIV Positive ANC females were gravida 1, 32.14% and 14.29% were gravida 2 and gravida 3 respectively. (Table II).

According to Figure II, in 55.36% cases spouse was having history of HIV while in 31.25% cases the spouse was negative for HIV. In 13.39% cases, the history could not be traced.

As per Figure III, 78.57% HIV Positive ANC females delivered by normal delivery while 21.43% delivered by caesarean section.

In year 2010, samples collected at 6 weeks, 6 months, 12 months, 18 months were 33, 21, 21 and 20 respectively; and in year 2011 were 32, 18, 9, 29; in 2012 were 30, 28, 23, 16; in 2013 were 33, 30, 25, 14; in 2014 were 30, 14, 19, 23; in 2015 were 27, 21, 20, 14; in 2016 were 14, 17, 15, 12 respectively. The positivity rate at 6 weeks, 6 months, 12 months, 18 months in 2010 were 1, 0, 1, 2; in 2011 were 4, 0, 0, 1; in 2012 were 2, 0, 0, 0; in 2013 were 1, 1, 0, 0; in 2014, there were no positive cases; in 2015 were 0, 1, 0, 0; and in 2016 were 0, 1, 0, 0 respectively. (Table III).

According to Table IV, 2 babies died in 2010, 1 baby in 2011 and 1 baby migrated in 2016.

Year	15-19 Years	20-24 Years	25-34 Years	>35 Years	TOTAL
2010	1	11	8	1	21 (18.75%)
2011	1	9	7	0	17 (15.18%)
2012	2	10	7	0	19 (16.96%)
2013	2	7	7	0	16 (14.29%)
2014	1	10	5	0	16 (14.29%)
2015	1	9	2	1	13 (11.61%)
2016	0	6	4	0	10 (8.92%)
Total	8 (7.14%)	62 (55.36%)	40 (35.71%)	2 (1.79%)	112

Table I. Age-wise Distribution of ANC Females Positive for HIV

Figure I. Trimester of ANC Patients Positive for HIV

Year	Gravida 1	Gravida 2	Gravida 3	Total
2010	10	7	4	21
2011	7	8	2	17
2012	9	7	3	19
2013	8	5	3	16
2014	10	4	2	16
2015	9	3	1	13
2016	7	2	1	10
Total	60 (53.57%)	36 (32.14%)	16 (14.29%)	112

Table II. Distribution of HIV Positive ANC Females According to Gravid Status

Figure II. Status of Spouse Positive, Negative or having Unknown History of HIV

Figure III. Mode of Delivery in HIV Positive ANC Females

Sl. No.	Year	6 weeks Test	Positive	6 months Test	Positive	12 months Test	Positive	18 months Test	Positive
1	2010	33	1	21	0	21	1	20	2
2	2011	32	4	18	0	9	0	29	1
3	2012	30	2	28	0	23	0	16	0
4	2013	33	1	30	1	25	0	14	0
5	2014	30	0	14	0	19	0	23	0
6	2015	27	0	21	1	20	0	14	0
7	2016	14	0	17	1	15	1	12	0
Total	2010-2016	119	8 (6.72%)	149	3 (2.01%)	132	2 (1.52%)	128	3 (2.34%)

Table III. Number of Tests Conducted and Positive for HIV at 6 Weeks, 6 Months, 12 Months and 18 Months Respectively

Year	Fate of the Baby
2010	2 babies died
2011	1 baby died
2012	No deaths
2013	No deaths
2014	No deaths
2015	No deaths
2016	1 baby migrated
2010-2016	3 deaths, 1 migration

Table IV. Fate of the HIV Positive Babies

DISCUSSION

In India, it is estimated that currently about 115,000 children are living with HIV, and approximately 40,000 children below 15 years are provided with antiretroviral therapy (ART) by the national program. While 35% children infected with HIV do not see their first birthday, 53% do not celebrate their second birthday.¹⁰ Hence, early diagnosis and testing is of utmost importance.

In our study, total 112 ANC females who were tested as HIV positive were included. 20-24 years age group was the most commonly affected. 55.36% females were in this age group followed by 25-34 years (35.71%), 15-19 years (7.14%) and >35 years (1.79%). (Table I). This correlates with the study of Sumitra Yadav et al in which 60% of the females were in 20-25 years age group.¹¹ This also correlates

with the study of Maia et al in which <30 years was the most commonly affected age group.¹² In the study of Berhan et al, 25-34 years age group was most affected (59.9%) with HIV in ANC patients.¹³ The age variation depends upon age at the time of marriage and infectivity of the partner.

The reason for less number of visits in first trimester may be ignorance, lack of awareness regarding pregnancy, lack of hospital facilities especially in rural areas and late diagnosis of pregnancy.

According to Table II, 53.57% HIV infected females were primigravida, 32.14% were gravida 2 and 14.29% were gravida 3. This is in contrast to the study of Sumitra Yadav et al in which 70% of affected ANC females were gravida 2 and 10% each of G1, G3 and G4.¹¹ This result also varies individually and depends on age at the time of exposure and infectivity of partner, in case of home deliveries HIV status may be unknown.

According to Figure II, 55.36% spouses were also reactive for HIV, 31.25% were non-reactive for HIV and in 13.39% cases the history of HIV was unknown in the spouses. The reason for not knowing HIV status in 13.39% cases was partner not willing to come for HIV testing, social stigma of HIV, lack of awareness and ignorance about the disease. Single motherhood, rural residence, low maternal education, and higher paternal age were associated with unknown HIV status. In the study of Maia et al, 23.65% cases had their partners infected with HIV.¹²

In our study, as per Figure III, 78.57% females delivered by normal delivery and 21.43% females delivered by Caesarean section. There are more chances of transmission of HIV through normal delivery as there is more exposure of mucosal surface of baby in normal delivery as compared to Caesarean section. This finding correlates with the study of Hondegla et al, Kaizad et al, Berhan et al and Sumitra Yadav et al.^{11,13,14,15}

As mother-to-child transmission rates have been reported in USA and Western European untreated populations as 15-20%; in developing countries, rates were as high as 40%.¹⁴

In our study, according to Table III, the infectivity rate in infants of HIV positive mothers was 3.03%. In the study of Hondegla et al, the infectivity rate was 5.6%.¹⁵ They conducted a 2-year retrospective study. In the study of Oladeinde et al, the HIV prevalence was 10.2%.¹⁶ Decrease in mother-to-child transmission rate may be due to increase in awareness, proper counselling, availability of drugs free of cost which decreases mother-to-child transmission.

So, as per Table III, samples positive at 6 weeks were 6.72%, 6 months were 2.01%, 12 months were 1.52%, 18 months were 2.34%. In the study of Berhan et al, samples tested at 6 weeks, 6 weeks to 6 months and after 6 months were 120, 255 and 69 respectively and samples positive at 6 weeks, 6 weeks to 6 months were 6 (5%), 14(5.49%), 24 (34.78%).¹⁴ In the study of Olana et al, 4.2% were positive at 6-8 weeks.¹⁷ In the study of Sumitra Yadav et al, the HIV positive infants at 6 weeks were 4.27%, 6 months were 0.61% and 18 months were 1.21%.¹¹

According to Table IV, 2 babies died in 2010, 1 baby in 2011 and 1 baby migrated in 2016 i.e. there were 18.75% deaths and 6.25% migrations. In the study of Sumitra Yadav et al, 20% of babies died and 20% lost to followup which correlates with our study.¹¹ The reason for death may be premature labour, missed dose of nevirapine at the time of labour and low birth weight.

CONCLUSION

HIV remains the important cause of mortality and morbidity amongst children. Diagnosis of HIV in mother during ANC is important. As serological tests are not useful during first 6 months of age, diagnosis by DNA PCR should be done. DBS is an easy, cheap, readily available test requiring very less amount of sample. In our study, there was much decrease in positivity of samples in year 2014-2016. This may be due to effective PPTCT programme, increase in awareness amongst people, free testing of HIV everywhere and early initiation of ART therapy. The number of samples also reduced significantly in the year 2016. No deaths were recorded after 2011.

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