Summary and Conclusion
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The evaluation of molecular methods used in HIV infant diagnosis as well as abnormalities of liver marker enzymes, hematological and immunological findings have been reported in western countries but very limited studies have been done in India. HIV infection and its treatment have been associated with a wide range of liver marker enzymes abnormalities, hematological and immunological disturbances, the quantification of viral loads and the determination of drug resistance.

For the present study, the subjects were recruited from the Namakkal district, Tamilnadu, South India. The total number of subjects included in this study was 397; the number of HIV exposed study infants was 125 and their HIV positive mothers (n=125), and the number of control children was 47 (27 HIV positive and 20 HIV negative), the number of HIV non exposed infants was 50 and their HIV negative mothers (n=50). All HIV positive mothers received nevirapine prophylaxis at the time of delivery and nevirapine syrup provided by the Indian government was given to their infants. Details of socio-demographic data and breast feeding practices were recorded. The control mothers were also recruited from similar socio-economic background as that of study mothers. Mothers were counseled to ensure the understanding of the procedure and written informed consent was obtained from eligible infants’ mothers and for the children it was obtained from parents or guardians.
The main objective of the study was to identify the early diagnosis of HIV-1 infection in infants using molecular methods. Blood samples were collected from the infants and were spotted onto three different filter papers to identify HIV-1 infection in infants born to HIV positive mothers. The sensitivity, the specificity, negative predictive and positive predictive values were calculated for each filter paper. Traditionally, the test required a whole blood sample, which if taken in a rural area and transported to a testing facility, needed to be kept refrigerated. But according to the new technique developed for HIV-1 DNA PCR was tested on a small blood spot on filter papers. The blood spotted on filter papers was easy to prepare in a resource-limited setting and could be stored and transported to testing centers without refrigeration.

In this research, three different filter papers such as S& S isocodestix, protein saver card 903 and FTA card were used for the detection of HIV-1 infection in infants; nested PCR was performed with gag, env and pol genes of HIV. It was observed from the present study that protein saver card 903 and FTA card filter papers could be used for blood collection from the infants with gag gene which was found to be more conserved than env and pol genes for early diagnosis of HIV-1 infection in infants. The sensitivity, the specificity, NPV and PPV were 100 % of HIV-1 DNA PCR using whole blood spotted on protein saver card 903 and FTA card filter papers with gag gene. These two filter papers were suitable for blood collection and could be easily transported from resource limited settings to the testing laboratories for early infant diagnosis of HIV-1 infection.
This study further examined the side effects of nevirapine prophylaxis which was received by HIV positive mothers at labour and to their infants after birth. The abnormalities of liver marker enzyme activities in the infants and their mothers were carried out and compared with their respective controls. Toxicity grading tables were used to check the severity of the disease, the activity of ALT was increased in HIV exposed infants rather than controls and it was observed in grade 1 level and elevated levels of both AST and ALT enzyme activities were observed in HIV positive mothers.

Hematological and immunological findings were also performed for the infants and their mothers and the severity of anemia was studied based on the toxicity table. The prevalence of anemia was found in HIV exposed infants and their mothers. Positive correlation between total lymphocyte count and CD4 count was observed in the present study. HIV positive mothers were divided into 2 groups on the basis of their CD4 levels which was < and > 350 cells/mm³, hemoglobin levels were found to be lower in mothers who had CD4 < 350 cells/mm³.

The quantification of viral load was performed for HIV positive infants and their mothers and a strong inverse correlation was found between CD4 cell counts and viral load. Oligo nucleotide ligation assay was performed in HIV positive mothers and their HIV positive infants for the evaluation of primary mutations associated with nevirapine resistant, the drug which was given at labour for the mother and the infant after birth and mutations were observed in 4% of the
mothers while none of their infants had developed any mutations. These observations were relevant for future treatment for the mothers and their infected infants.

**Conclusions**

Identification of HIV-1 infection in the infants should be definitively diagnosed at an early age of their life. This present study provides a unique opportunity to determine the HIV-1 status of the infants born to HIV positive mothers in a rural population in Southern India where the resources are limited. The findings of present study revealed that Protein saver card 903 and FTA card had good sensitivity, specificity, positive predictive and negative predictive value using *gag* gene when compared with whole blood methods. From this study it was observed that these two filter papers were suitable for collecting the blood from the infants for earlier diagnosis of HIV-1 infection in infants. It can be suggested that blood may be spotted directly on filter papers by heel prick, and this method is found to be more convenient for health care workers.

**Advantages of using filter papers in the infant diagnosis**

Whole blood collected on filter paper, offers a simple, sensitive, and specific test appropriate for the diagnosis of HIV-1 infection among infants.

- The small amount of blood required, the ease of collection, storage, and transport of samples, and the low cost of the test make this assay ideal for HIV-1 testing of infants where resources are limited.
Few droplets of blood samples are required and can be directly coated on filter paper from heel prick rather than the blood drawn from venipuncture, thus avoiding the use of syringes and vacutainer tubes. Drawing the blood from the infant is very difficult and minimal training is sufficient for the health care workers to collect the blood on filter papers.

Storage of filter papers is easy since they can be kept at room temperature and DNA has a good stability in blood spotted on filter papers.

It can be transported easily from the resource limited regions to the testing laboratories without any cold chain.

These data demonstrated that it could be possible to extract proviral DNA form the blood spotted filter papers using both Chelex resin for protein saver card 903 and FTA purification reagent for FTA card with comparable efficiency to that of standardized DNA extraction kit using whole blood.

For these reasons, filter papers could be used for specimen collection in HIV-1 diagnosis in infants and it could be concluded that this simple DNA extraction and PCR amplification methods gave a reliable molecular diagnosis of HIV-1 infection in infants born to HIV positive mothers.

Regarding the results liver marker enzymes, mild elevations were observed in activity of ALT levels and no significant differences in AST, ALP and total bilirubin compared with the control infants. Mild anemia was observed in HIV exposed infants rather than the controls and no significant association were seen.
according to gender. It was observed that correlation between TLC and CD4 counts was good in this study and the testing of total lymphocyte count is always cheaper, simpler and easily available, if CD4 is not available, total lymphocyte counts can be used as an alternate to find out the immune status. In conclusion, a considerable proportion of HIV positive infants required antiretroviral treatment at presentation and age-specific CD4 cells remain the most appropriate immunological criterion to determine when to initiate therapy in HIV positive children. It is generally recognized that total lymphocyte count is a useful predictor of mortality in HIV infected adults and children. Also more convenient and less expensive technologies are needed as alternatives to currently available CD4 cell assays in resource-limited settings.

It was observed from this study that the liver marker enzyme activities were higher in HIV positive mothers than controls and anemia was more common in HIV positive mothers. The side effects of nevirapine such as skin rash and abnormalities of liver marker enzymes were observed between 23-60 days after administration of a single dose nevirapine. In conclusion the liver marker enzyme activities and hematological levels should be monitored periodically before starting any antiretroviral drugs.

An inverse correlation between viral load and CD4 counts was observed in HIV positive mothers. It was not possible to do the correlation for the infants due to the presence of undetectable viral load results in 11/13 infants. Mutations were
observed in 4% of HIV positive mothers and no mutations were found in HIV-1 positive infants. In conclusion, the findings of the nevirapine mutations can cause resistance to that particular combination of antiretroviral drugs; if the mutations are detected, the combination of the drug may be stopped and a new combination of the drug should be started. It is observed from the present study that the exclusive breastfeeding is safer than mixed feeding. Exclusive breastfeeding will reduce the mother to child transmission and WHO recommends that all HIV positive mothers can avoid mixed feeding and also to take antiretroviral drug throughout the breastfeeding period.

**Recommendations**

1. All HIV-exposed infants should be assessed (by review of test results or recent testing of the mother or by testing the infant) for exposure to other infections that may be acquired in utero or at delivery.

2. Counseling and education of the parents or care givers about HIV infection should be provided by pediatricians caring for HIV-exposed infants.

3. All HIV-exposed infants should undergo testing for HIV DNAPCR to determine infection status. If the PCR is positive for infection, the test should be repeated for confirmation. If PCR is negative, the infant should have serologic testing to document disappearance of HIV antibody at 18 months of age.
4. Liver marker enzymes, hematologic and immunologic levels should be monitored periodically in all HIV-exposed infants while infection status is being determined.

**Future Prospects**

In future all the mother and infant pair should be tested for Human Leukocyte Antigen (HLA) B5701 to check for possible hypersensitivity reaction to the drug abacavir. If the HLA B5701 test is positive, then the child should not receive abacavir combination of any antiretroviral drug.

To identify the effect of highly active anti-retroviral therapy on prevention of mother to child transmission of HIV and on infant growth and development. The biochemical and hematological findings will be monitored periodically to find out whether these drugs will influence the baby's growth and development.

In future these studies will help to follow the HIV infected mother and infant pair for monitoring their immune system and the growth of the infant before initiation of antiretroviral treatment.