PREVALENCE OF HIV INFECTION IN HIV EXPOSED INFANTS IN CHENNAI

DISSERTATION SUBMITTED FOR
M.D DEGREE (PEDIATRICS)
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CHENNAI

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CERTIFICATE

Certified that this dissertation entitled **"PREVALENCE OF HIV INFECTION IN HIV EXPOSED INANTS IN CHENNAI"** is a bonafide work done by **DR.S.SEENIVASAN,** post graduate student of Paediatric Medicine, Govt. Kilpauk Medical College Hospital, Chennai – 10, during the academic year 2008-2011.

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DECLARATION

I, **Dr.S.SEENIVASAN** declare that this dissertation entitled **"PREVALENCE OF HIV INFECTION IN HIV EXPOSED INFANTS IN CHENNAI"** has been conducted by me at Govt. Kilpauk Medical College Hospital. It is submitted in part of fulfillment of the award of the degree of M.D., (Paediatrics) for the April 2011 examination to be held under the Tamil Nadu DR.M.G.R Medical University, Chennai. This has not been submitted previously by me for the award of any degree or diploma from any other university.

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CONTENTS

INTRODUCTION

Children of today are youth of tomorrow. Any disease affecting them will have a major impact on future of the nation. HIV is one of the important infectious diseases causing major morbidity and mortality in children. In India with 27 million pregnancies, 189,000 HIV infected women deliver every year with transmission rate of 15 to 25 % [1.] With breast feeding, the overall transmission rises to 30 – 45 % [1].The infected infants appear healthy at birth without any signs or symptoms. Most of these infected innocent infants die without being diagnosed because of the non availability of proper diagnostic techniques in the developing countries [2]. This study aims to find the prevalence of HIV infection among HIV exposed infants in Chennai by early detection (HIV DNA PCR).

Definition of HIV exposure [1]

Infants and children born to mothers living with HIV, until HIV infection in the infant or child is reliably excluded and the infant or child is no longer exposed through breastfeeding.

HIV INFECTION IN CHILDREN

ETIOLOGY

AIDS is caused by Human Immunodeficiency Virus 1&2 of the Retro viral family and belongs to the genus Lentivirus. The HIV-1 genome contains two copies of single stranded RNA. The genome includes 3 major sections.

- GAG region - encodes viral core proteins P24, P17, P9, P6.
- POL region-encodes viral enzymes reverse transcriptase, protease and integrase.
- ENV region-encodes the viral envelope proteins gp120 & gp41.

Reverse transcriptase is the enzyme useful for the HIV RNA virus to form DNA templates that get integrated into the host DNA.

Gp 120 is a complex molecule that includes the highly variable region V 3 loop. This region is immunodominant for neutralizing antibodies. The heterogeneity of gp 120 presents major obstacles in establishing an effective vaccine. The gp 120 glycoprotein also carries binding site for the CD 4 molecules. The gp 41 molecules is highly immunogenic and is used in diagnostic assays.

The chemokine CXCR- 4 acts as a co receptor for HIV attachment to lymphocyte while CCR- 5 facilitates HIV entry into macrophages.

HIV-2 is known to cause infection in several monkey species .It is a rare cause of infection in children. The diagnosis of HIV 2 infection is more difficult because standard diagnostic tests give indeterminate results. But third generation ELISA can capture both HIV 1 and HIV 2.

EPIDEMIOLOGY

HIV infection is prevalent all over the world. Worldwide there are 2.1 million children< 15 years, infected with HIV constituting 6 % of all HIV infection. But 18% of HIV deaths occur in children.430, 000 new HIV infections occur in children every year.

In India, 189,000 HIV exposed infants are delivered every year of which 56,700 are infected [1]. There were 202,000 HIV infected children in 2004 in India [6]. In Oct 2006, NACO [1] admitted that half of the HIV infected children die before their second birthday without being diagnosed. With current coverage of HIV testing of pregnant women, status of 90 % of the HIV exposed infants is unknown. Even if the HIV status of the mother is known, only 6 % of the infants are followed up to 8 weeks. Tamil Nadu had HIV prevalence of 0.50 % in antenatal mothers in 2003, which declined to 0.25 % in 2007 and attained 0 % growth in HIV infection in the same year. This was mainly due to the successful organization of PPTCT (Prevention of Parent To Child Transmission of HIV) Programme.

TRANSMISSION

The modes of transmission in children are

1) Vertical transmission-Primary route
2) Transfusion of infected blood products
3) Sexual transmission-rare in children but common in adolescents.
4) IV drug abuse in adolescents.

Of the four routes of transmission, vertical transmission is the most important mode of transmission in children as it constitutes more than 93 % of infections. Vertical transmission may be during pregnancy (5 - 10 %), child birth (10 – 15 %) or through breast feeding (5- 10 %) [1] .Safer transfusions after proper screening had made blood products a less common mode of transmission. Transmission through sex and IV drug abuse is rare in children less than 15 years of age.

RISK FACTORS FOR MOTHER TO CHILD TRANSMISSION [1,2,31]

1) Preterm delivery
2) Low birth weight
3) Rupture of membrane more than 4 hours.

4) Vaginal delivery

5) Maternofetal blood transfusion or contact of infant skin with maternal blood or vaginal secretions.

6) Breast feeding

7) High risk factors in mother like smoking, illicit drug abuse, multiple partners and chorioamnionitis.

8) Maternal CD 4 count < 200/mm^3

9) Advanced maternal disease.

RISK OF VERTICAL TRANSMISSION-NACO [1]

- During pregnancy-5-10%
- During Labour-10-15%
- Breast feeding-5-10%
- Overall without breast feeding-15-25%
- Overall with breast feeding up to 6 months – 20 – 35 %
- Overall with breast feeding up to 24 months-30-45%
- Mixed feeding doubles the postnatal risk of transmission.

PATHOGENESIS

When the mucosa serves as the portal of entry for the HIV the first cells to be infected are the dentritic cells. They transport the virus to the lymphoid tissue. HIV selectively binds to CD4 (T-Helper) cells.Chemokines, CCR5 and CXCR4 are necessary for HIV infection and entry in to the cell. HIV preferentially infects the CD4 cells, the very cells respond to it. This accounts for the progressive loss of these cells' response and the subsequent loss of control of HIV replication. When HIV replication reaches a threshold (3-6 weeks), a transient plasma viremia occur and the disease progresses.

3 distinct patterns of disease can be described in children[3, 7].

RAPID PROGRESSORS

They are infected in utero. Symptoms occur during first few months of time and if untreated death occurs within 6-9 months. In resource poor countries, majority of the HIV infected newborn will have rapid disease progression.

SLOW PROGRESSORS

They are infected intrapartum. The viral load peaks at 2 – 3 months of age and slowly declines over 24 months.

LONG TERM SURVIVORS

They are infected perinatally, constituting only < 5 % of the cases.They have minimal or no disease progression and relatively normal CD 4 counts. The viral load is low for longer than 8 years of age.

CLINICAL STAGING OF HIV/AIDS [2]

WHO categorized clinical stages as 1 through 4, progressing from primary HIV infection to advanced HIV/AIDS.

Clinical stage 1

- Asymptomatic
- Persistent generalized lymphadenopathy

Clinical stage 2

- Unexplained persistent hepatosplenomegaly
- Papular pruritic eruptions
- Extensive wart virus infection
- Extensive molluscum contagiosum
- Fungal nail infections
- Recurrent oral ulcerations
- Unexplained persistent parotid enlargement
- Lineal gingival erythema
- Herpes zoster
- Recurrent or chronic upper respiratory tract infections

Clinical stage 3

- Unexplained moderate malnutrition not adequately responding to standard therapy
- Unexplained persistent diarrhoea(14 days or more)
- Unexplained persistent fever
- Persistent oral candidiasis(after 6 -8 weeks of life)
- Oral hairy leukoplakia
- Acute necrotizing ulcerative gingivitis or periodontitis
- Lymph node tuberculosis
- Pulmonary tuberculosis
- Severe recurrent bacterial pneumonia
- Symptomatic lymphoid interstitial pneumonitis
- Chronic HIV associated lung disease including bronchiectasis
- Unexplained anemia (8g/dl), neutropenia (500/mm^3), thrombocytopenia (<50,0000/mm^3)

Clinical stage 4

- Unexplained severe wasting, stunting or severe malnutrition not responding to standard therapy
- Pneumocystitis pneumonia
- Recurrent severe bacterial infections

- Chronic herpes simplex infection
- Extra pulmonary tuberculosis
- Kaposi sarcoma
- Esophageal candidiasis
- CNS toxoplasmosis
- Cytomegalovirus infection
- Extra pulmonary cryptococcosis
- Disseminated non tuberculous mycobacterial infection
- Cerebral or B cell non Hodgkin lymphoma
- Progressive multifocal leukoencephalopathy
- Symptomatic HIV associated nephropathy or HIV cardiomyopathy

DIAGNOSIS [3, 32]

❖ HIV ELISA antibody qualitative assay is used for children more than 18 months of age. For children < 18 months, maternal antibodies may interfere with the test and is not recommended.

❖ For children < 18 months of age the following tests are used.

1) HIV DNA PCR – highly sensitive and specific.
2) HIV p 24 Ag - less sensitive.
3) HIV culture – not easily available, requires at least 4 weeks.
4) HIV RNA PCR – not recommended for routine testing.

Among the above ,HIV DNA PCR with a sensitivity of 96 % and a specificity of 100 % [7,9,10] is currently recommended by WHO & NACO for detection of HIV infection in children < 18 months of age.

TREATMENT

Anti Retroviral Therapy once started not only has to be taken lifelong, but also has to be taken strictly as per schedule [1]. Besides, it requires regular monitoring. All these factors demand careful patient selection and thorough counselling to ensure adherence.

All HIV positive children are evaluated with detailed history and clinical examination [18]. They are classified into 4 clinical stages 1 to 4 as per WHO. Clinical staging and CD 4 counts are necessary for starting ART.

CD 4 counts are higher in children when compared to adults. Hence CD 4 % is used in children < 5 years rather than absolute CD 4 counts.

Normal CD 4 counts/ % in children are [1]

Age	**CD 4 count/ mm^3**	**CD 4 %**
< 12month	>1500	>= 25 %
1 – 5 years	>1000	>= 25 %
>6 years	>500	-----

Guidelines to start ART based on CD 4:-

Age	CD 4 count/mm^3	CD 4 %
< 11 month	< 1500	<25 %
12 – 35 month	< 750	<20 %
36 – 59 month	< 350	15 %
>5 years	< 350	------

Clinical and Immunological Criteria for starting ART [1]

WHO Pediatric staging	Availability of CD 4	Age specific < 12 month	Recommendation >12 month
4	CD 4	Treat all	Treat all
	N o CD 4		
3	CD 4	Treat all	Treat all, for TB,LIP,OHL CD 4 guided
	No CD 4		Treat all
2	CD 4	CD 4 guided	CD 4 guided
	No CD 4	Don't treat	Don't treat
1	CD 4	CD 4 guided	CD 4 guided
	No CD 4	Don't treat	Don't treat

Treatment is based on AIDS staging and CD 4 counts. NACO recommends a combination of Nucleoside Reverse Transcriptase

Inhibitors (NRTI) and Non Nucleoside Reverse Transcriptase Inhibitors (NNRTI) [1,3,2,6].

- AZT + 3 TC + NVP or EFV
- D4T + 3TC + NVP or EFV

AZT – Zidovudine, NVP – Nevirapine, EFZ - Efavirenz

3TC – Lamivudine, D4T - Stavudine

EFV is not used in children < 3 years or < 10 kg.

PREVENTION

Till now no vaccine is found for HIV.

Vertical transmission is controlled by

1) Prevention of disease progression in mother by CD 4 count monitoring and starting ART as needed.
2) National AIDS Control Organisation, NACO recommends single dose Nevirapine to mother (200 mg) at labor and to baby (2 mg/kg) within 72 hours of birth.
3) ACOG recommends Elective Caesarean Delivery if maternal viral load > 1000 copies/ml.

Safe sexual practices in adolescents, avoiding needle sharing among IV drug abusers and proper screening of blood products are other methods of prevention.

POLYMERASE CHAIN REACTION

PCR is used to amplify a specific region of a DNA strand (the DNA target). Most PCR methods typically amplify DNA fragments of up to ~10(kb), although some techniques allow for amplification of fragments up to 40 kb in size. It was developed by Kary Mullis in 1983.

A basic PCR set up requires several components and reagent. These components include:

- ***DNA template*** that contains the DNA region (target) to be amplified.
- Two ***primers*** that are complementary to the 3' (three prime) ends of each of the sense and anti-sense strand of the DNA target.
- ***Taq polymerase*** or another DNA polymerase with a temperature optimum at around 70 °C.
- ***Deoxynucleoside triphosphates*** (dNTPs; also very commonly and erroneously called deoxynucleotide triphosphates), the building blocks from which the DNA polymerases synthesizes a new DNA strand.

- ***Buffer solution,*** providing a suitable chemical environment for optimum activity and stability of the DNA polymerase.

- ***Divalent cations****,* magnesium or manganese ions; generally Mg^{2+} is used, but Mn^{2+} can be utilized for PCR-mediated DNA mutagenesis, as higher Mn^{2+} concentration increases the error rate during DNA synthesis

- ***Monovalent cation*** potassium ions.

Procedure : Typically, PCR consists of a series of 20-40 repeated temperature changes, called cycles, with each cycle commonly consisting of 2-3 discrete temperature steps, usually three (Fig. 2). The cycling is often preceded by a single temperature step (called *hold*) at a high temperature.

- ***Initialization step*****:** This step consists of heating the reaction to a temperature of 94–96 °C. It is only required for DNA polymerases that require heat activation by hot-start PCR.

- ***Denaturation step*****:** This step consists of heating the reaction to 94–98 °C for 20–30 seconds. It causes DNA melting of the DNA template by disrupting the hydrogen bonds between complementary bases, yielding single-stranded DNA molecules.

- ***Annealing step*:** The reaction temperature is lowered to 50–65 °C for 20–40 seconds allowing annealing of the primers to the single-stranded DNA templates.

- ***Extension/elongation step*:** The temperature at this step depends on the DNA polymerase used; Taq polymerase has its optimum activity temperature at 72 °C. At this step the DNA polymerase synthesizes a new DNA strand complementary to the DNA template strand by adding dNTPs that are complementary to the template in 5' to 3' direction.

- ***Final elongation*:** This single step is occasionally performed at a temperature of 70–74 °C for 5–15 minutes after the last PCR cycle to ensure that any remaining single-stranded DNA is fully extended.

- ***Final hold*:** This step at 4–15 °C for an indefinite time may be employed for short-term storage of the reaction.

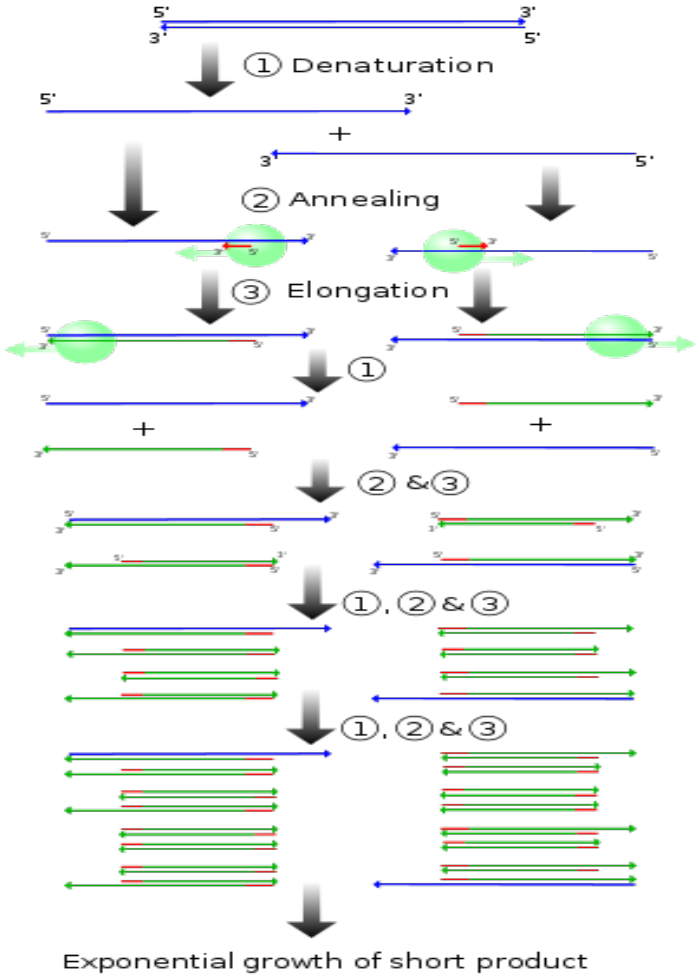


Figure 1 : Schematic drawing of the PCR cycle. **(1) Denaturing at 94–96 °C. (2) Annealing at ~65 °C (3) Elongation at 72 °C**. Four cycles are shown here. The blue lines represent the DNA template to which primers (red arrows) anneal that are extended by the DNA polymerase (light green circles), to give shorter DNA products (green lines), which themselves are used as templates as PCR progresses.

Figure 2 : Ethidium bromide-stained PCR products after gel electrophoresis

PCR stages

The PCR process can be divided into three stages:

1) ***Exponential amplification*:** At every cycle, the amount of product is doubled (assuming 100% reaction efficiency). The reaction is very sensitive: only minute quantities of DNA need to be present.

2) ***Levelling off stage*:** The reaction slows as the DNA polymerase loses activity and as consumption of reagents such as dNTPs and primers causes them to become limiting.

3) ***Plateau*:** No more products accumulate due to exhaustion of reagents and enzyme.

REVIEW OF LITERATURE

1. Gbadegesin, et al in Lagos conducted a study in 45 HIV exposed infants. All the mothers and infants were given single dose Nevirapine. 5 of the 45 infants were HIV DNA PCR positive with a prevalence of 11 % [9].

2. Ugochukwo EF, et al from Nnewi, Nigeria did a similar study in 4 groups of infants based on prophylaxis with Anti Retro Viral drugs(PARV).The results were as follows [10]:

   i. PARV for both mother & baby: 7/192 (3.6 %)

   ii. PARV for mother & not for baby 23/284 (8.7 %)

   iii. PARV for baby and not for mother 2/16 (12.5 %)

   iv. No PARV for mother & baby: 39/73 (53.4 %).

   Transmission among breastfeeding, formula feeding and mixed feeding were 18.5 %, 4.8 % and 68 % respectively.

3. Jacob SM, et al from Chennai reported an 8.7 % transmission (n= 19) from a sample of 359 HIV exposed infants at 2 months of age [11].

4. Shah. I, et al from Mumbai documented a high transmission rate of 41.8 % from a group of 52 infants from 1.5 to 7 months of age. [12]

STUDY JUSTIFICATION

In India, among 27 million pregnant mothers a year with HIV infection of 0.7 %, 189,000 HIV exposed infants are delivered every year of which 56,700 are infected [1]. In India, there were 202,000 HIV infected children in 2004 [6]. With current coverage of HIV testing of pregnant women, the status of 90 % of the HIV exposed infants is unknown. In Oct 2006, NACO admitted that half of the HIV infected children die before their second birthday without being diagnosed. This is because the readily available HIV ELISA assay is not useful in children < 18 months [1, 2, 3]. On the other hand, the useful tests like DNA PCR, HIV culture, p 24 assay are not readily available. In addition, even if the HIV status of the mother is known, only 6 % of the infants are followed up to 8 weeks and their further course is unknown [1].

The prevalence of HIV infection in HIV exposed infants is unknown with current PPTCT measures. Few studies are available in India that did DNA PCR to the infants. Some of these studies had taken symptomatic infants who do not represent real prevalence of HIV infection born to HIV positive mothers. NACO did a pilot study and found a prevalence of 10 to 20 %, with single dose Nevirapine to mother and the baby. It recommends further studies to know how the preventive measures work in reality. Hence, we decided to follow all HIV positive mothers delivered in all 4 Obstetrics Institutes in Chennai to do DNA PCR for their babies to find out the prevalence of infection and the risk factors associated with transmission.

AIM

To study the prevalence of HIV infection among HIV exposed infants in Chennai by HIV DNA PCR.

SUBJECTS AND METHODS

1)METHODOLOGY

STUDY DESIGN : Prospective study

PLACE OF STUDY :

1) Kilpauk Medical College Hospital, Chennai 600 010
2) Institute of Obstetrics & Gynecology, Chennai 600 008
3) Kasturba Gandhi Hospital, Chennai 600 005
4) Govt. Raja Sir Ramaswamy Mudhaliyar (RSRM) Hospital, Chennai 600 013

PERIOD OF STUDY : Jan 2009 to Oct 2010

STUDY POPULATION : Babies born to HIV positive mothers.

INCLUSION CRITERIA : All infants born to HIV positive mothers registered in the ART/ICTC centers of the above 4 Obstetric institutes.

EXCLUSION CRITERIA :

1. Infants with suspected inborn errors of metabolism, serious medical illness like complex congenital heart diseases.
2. Mothers who wish to deliver & follow up in private hospitals.

SAMPLE SIZE : 100

2) MANOEUVER

This study evaluated the antenatal period, birth and postnatal period up to 18 months of age of all children born to HIV positive mother registered in ART centers of above 4 maternity hospitals in Chennai.

All mothers registered in the above hospitals were counseled for HIV screening by qualitative HIV ELISA Antibody testing. If mother was found to be positive, the spouse was called for HIV ELISA testing. Any history of blood transfusion, IV drug abuse etc. was noted. WHO AIDS clinical staging was done for them [1]. Both the mother and the father were started on Anti Retroviral Therapy (ART) based on CD 4 counts and AIDS staging, as per NACO guidelines. They were educated about the risk of parent to child transmission of HIV [1,2,24]. The parents were regularly followed. Counseling regarding breast feeding and artificial feeding along with their risks and benefits was done [1, 2, 25, 27, 28, 29]. The choice of feeding was left to the mothers. We advised them to follow the principle of AFASS (Acceptable, Feasible, Affordable, Sustainable and Safe), if the baby was artificially fed.

For confinement, we did not recommend Elective Cesarean Delivery (ECD). Cesarean delivery was done on Obstetric indications only. Single dose Nevirapine was given to both mother (200 mg) and the

baby (2 mg/kg) [1,2 20,23,26,29,30]. Risk factors like bleeding PV, prolonged rupture of membrane > 4 hours etc. were documented. APGAR score was noted. The baby was assessed clinically and anthropometry measured. Feeding options were reemphasized. BCG was given if not symptomatic of HIV infection [1]. Mothers were asked to bring the baby for HIV DNA PCR testing at 6 weeks of age [1, 2, 29, and 30].

At 6 weeks of age[1], baby were assessed clinically along with nutritional(anthropometric) assessment (Length, Weight, Head circumference & Chest circumference). Consent for blood sampling was obtained. Blood sample(2 ml) was taken in Lavender colored vacutainers containing EDTA for Whole blood HIV – 1 DNA[17,19,21,22].We did not do dried blood spot DNA PCR for whole blood DNA PCR is the gold standard [16]. If the baby is on breastfeeds, a second sample was advised 6 weeks after stopping breast feeds [1]. All the babies were started on Cotrimoxazole prophylaxis (5 mg/kg) at 6 weeks of age [1,2,4,5].

The samples were taken immediately to Tuberculosis Research Center, an ICMR Institute in Chennai where the samples were analyzed. It is a reference laboratory for Tuberculosis and AIDS approved by WHO and NACO. We used Roche Amplicor HIV 1 DNA PCR machine

(Test Version 1.5) for testing the samples. This is more sensitive and specific than HIV RNA PCR and LTR Real Time PCR [14, 3]. We simultaneously did CD 4 count and complete blood count. CD 4 count was done by BD FACS Calibur machine, BD Biosciences, California, USA. The results were available within a month. If any child was DNA PCR positive, the child was referred to Pediatric ART center at the Institute of Child Health. ART was started according to NACO guidelines. Routine follow up was done with CD 4 count monitoring.

For those babies on breastfeeds, a second sample for DNA PCR was taken 6 weeks after stopping breastfeeding [1,2]. They were followed upas described earlier.

3) STATISTICAL ANALYSIS

Proportions and mean with SD were arrived as applicable.

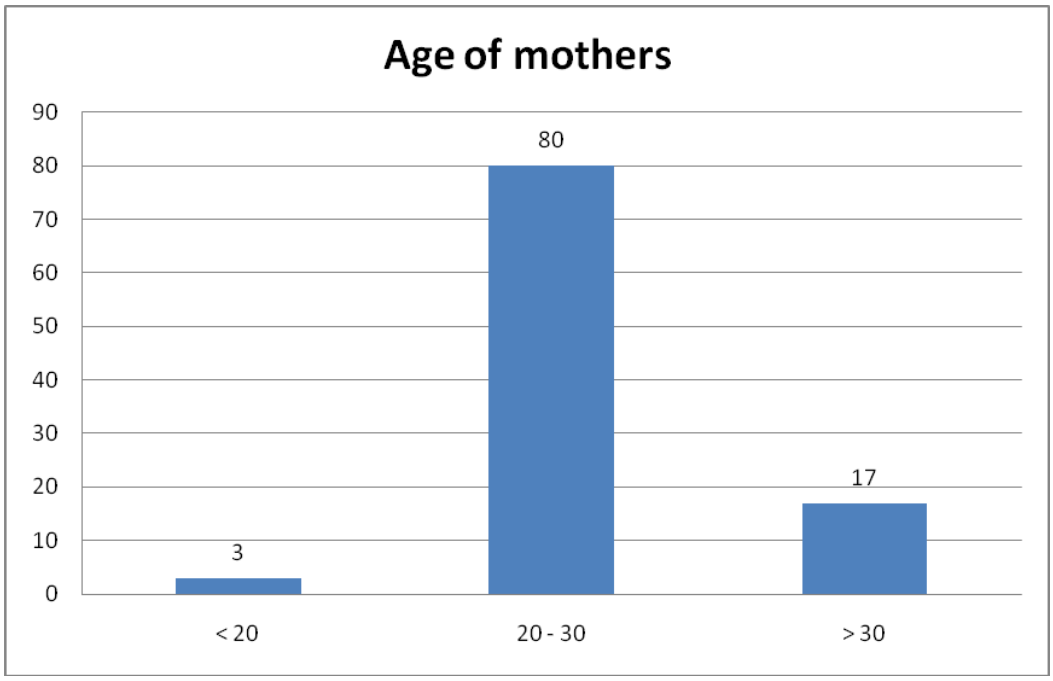
OBSERVATIONS

In our study period of 22 months from Jan 2009 to Oct 2010, there were 1,23,786 delieveries in Chennai [44] of which 79,268 deliveries were conducted in the 4 Obstetrics Institutes in Chennai.

Sl. No	Hospital	Deliveries	HIV positive Mothers
1)	KMC	12,122	25
2)	IOG	28,025	96
3)	RSRM	22,360	38
4)	KGH	16,761	17
	Total	79,268	176

The total number of HIV positive mothers delievered in the four above hospitals was 176 (prevalence = 0.22%). Out of 176, we followed up 100 mothers. The results of our study were discussed in the following pages.

AGE OF THE MOTHERS



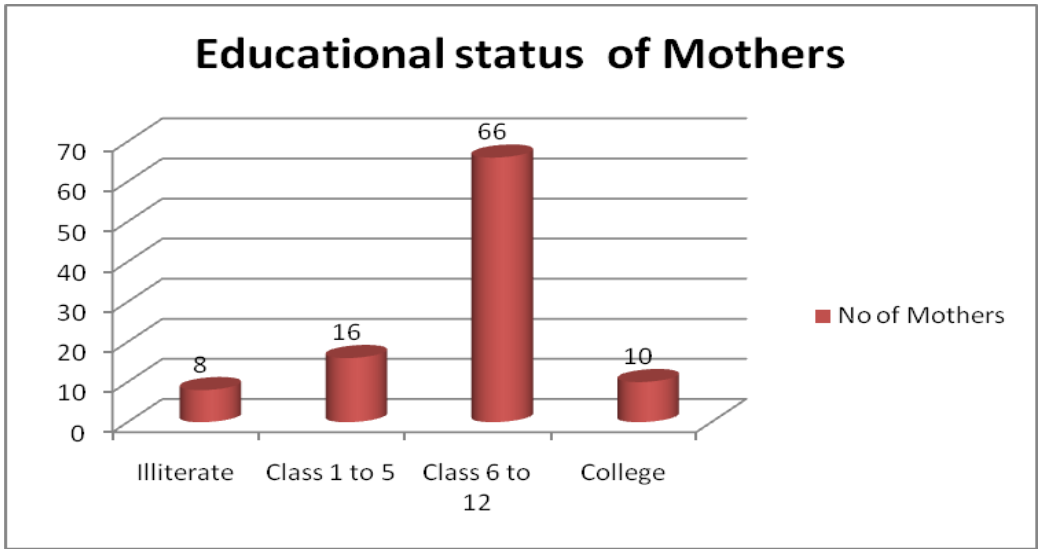
X axis: Age in years

Y axis: No. of mothers

Most of the mothers (97%) are above 20 years of age and only 3% had teenage pregnancy.

EDUCATION STATUS OF MOTHERS

The female literacy rate of Chennai was 75.32% in 2008 [43] . In our study the literacy rate of the mothers was 92 %, only 8 mothers were illiterate. All of them knew that they are HIV positive and the infection can be transmitted to the baby during pregnancy, child birth and through breast feeding.



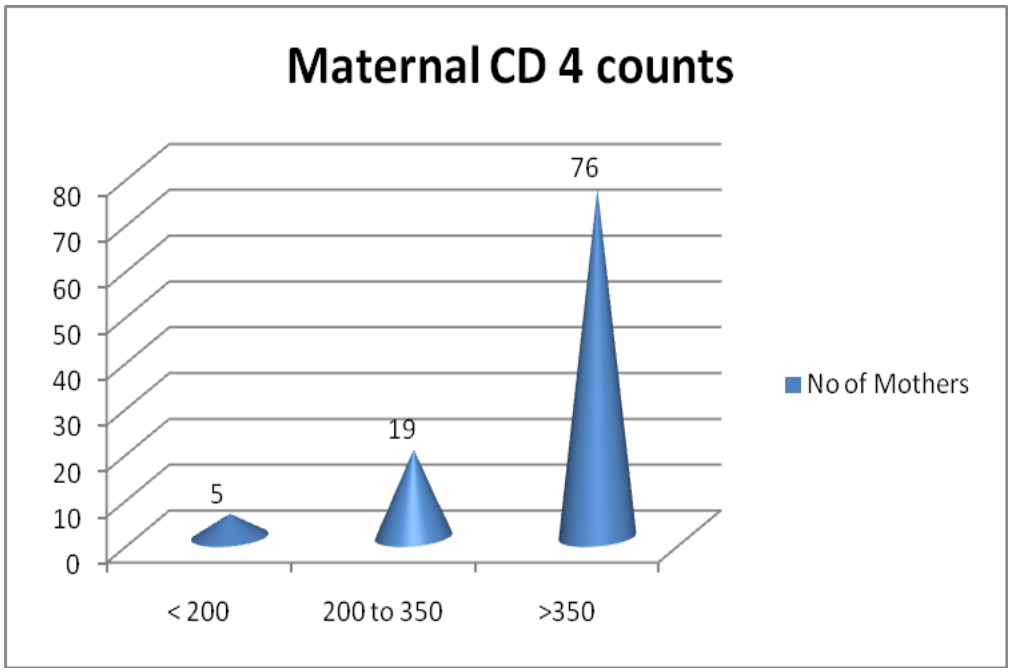
Time since diagnosis

The approximate time of diagnosis in the mother before they presented to us is as follows. The longest duration since diagnosis for one mother is 10 years.

Time since Diagnosis	No. of mothers
< 1 year	55
>1 year	45

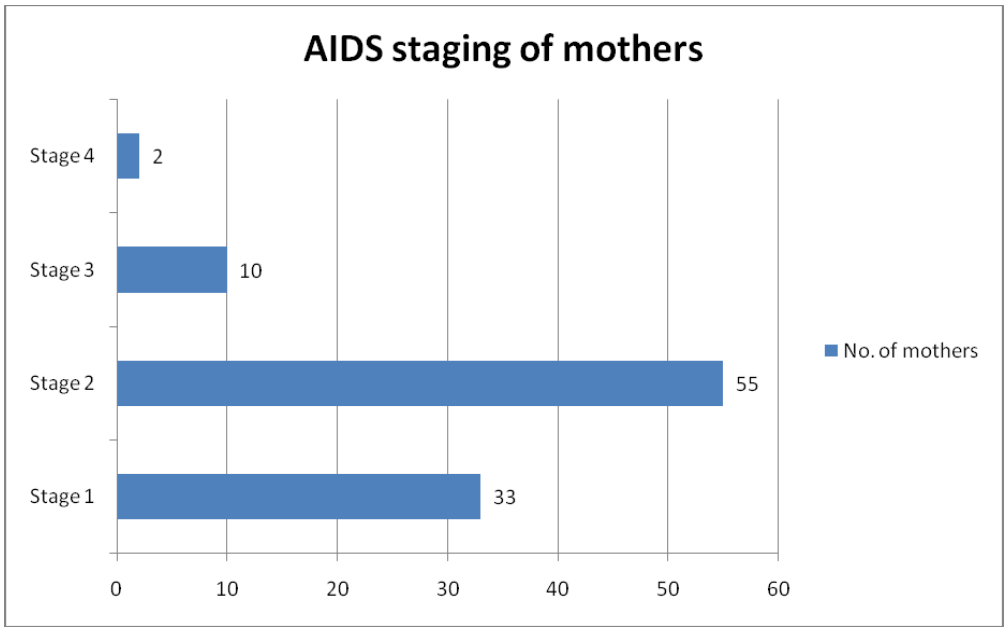
MATERNAL CD 4 COUNTS BEFORE DELIVERY

Maternal CD 4 count was done at the time of diagnosis and once in 6 months or whenever necessary. The CD 4 counts of 100 mothers, prior to delivery were as follows. The highest and lowest counts were 1177 and 65 respectively. We started Anti Retroviral Therapy if CD 4 count < 200 irrespective of clinical stage, considered in stage 1 and 2 if CD 4 between 200 and 350, and for all patients in stage 3 and 4 irrespective of CD 4 counts[1]. We divided the mothers into 3 groups based on CD 4 counts as per WHO guidelines [2].



AIDS STAGING OF MOTHERS

All the mothers were classified into 4 clinical stages as per WHO, as soon as they were diagnosed. Most of them (n= 88) were either in stage 1 or 2 and only a few were in advanced stages (stage 3 = 10, stage 4 = 2).

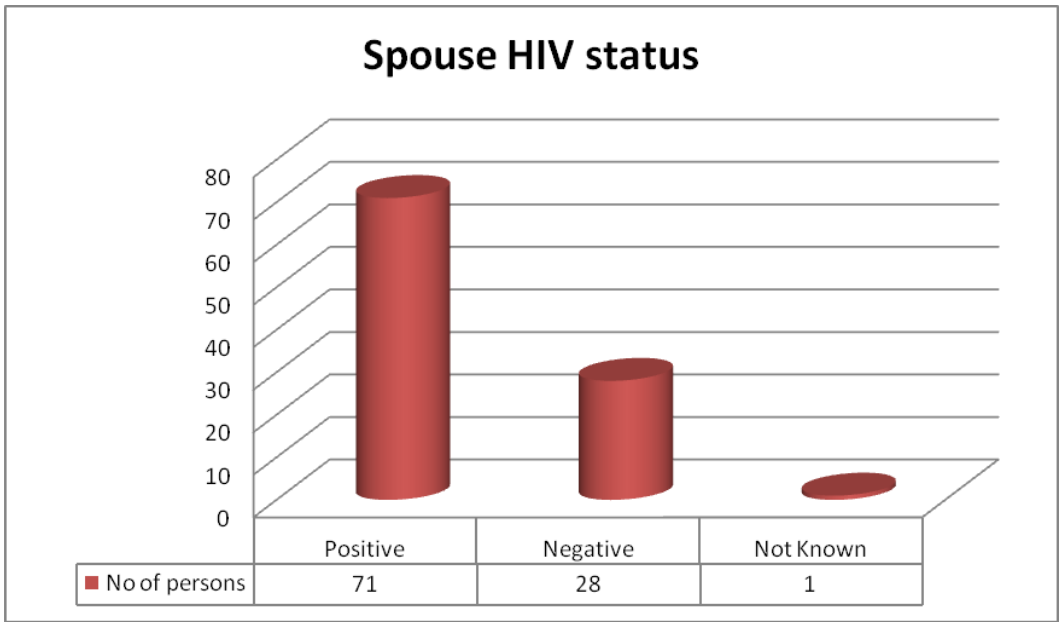


MOTHERS ON ANTI RETROVIRAL THERAPY

All the mothers in Clinical stage 3 or 4 were started on ART.

For mothers in stage 1 or 2, ART were started, if CD 4 < 200. 37 % mothers were on ART and 63% were not on ART.

SPOUSE'S HIV STATUS

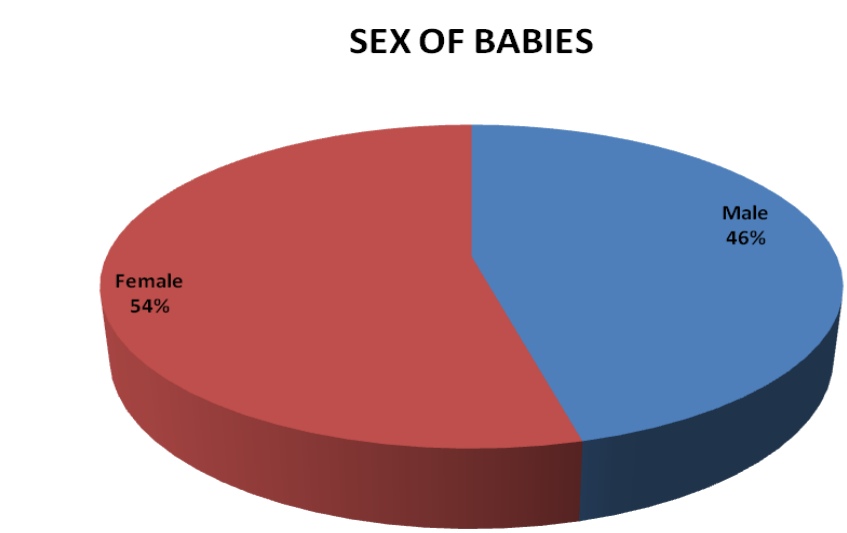


99 out of 100 spouses agreed to HIV testing when their wives are positive.71 of them were positive and 28 were negative. One husband refused for HIV testing. Among the 28 spouse negative pairs, 17 of them were second marriages. Out of 17, 13 mothers got transmitted through their HIV positive first husband and 4 mothers had their first husband expired without knowing their HIV status. In the remaining 11 pairs, the mothers alone were positive and the husband negative.

SIBLING'S HIV STATUS

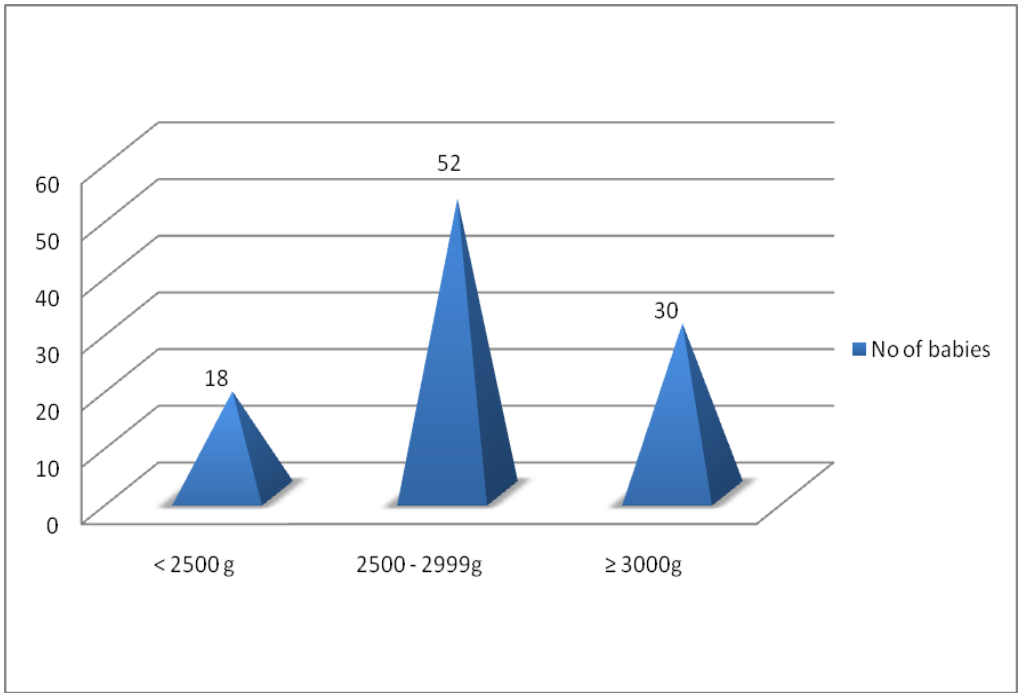
53% babies were first born. 47% babies had siblings. Among these, 10 were HIV positive.

SEX OF BABIES



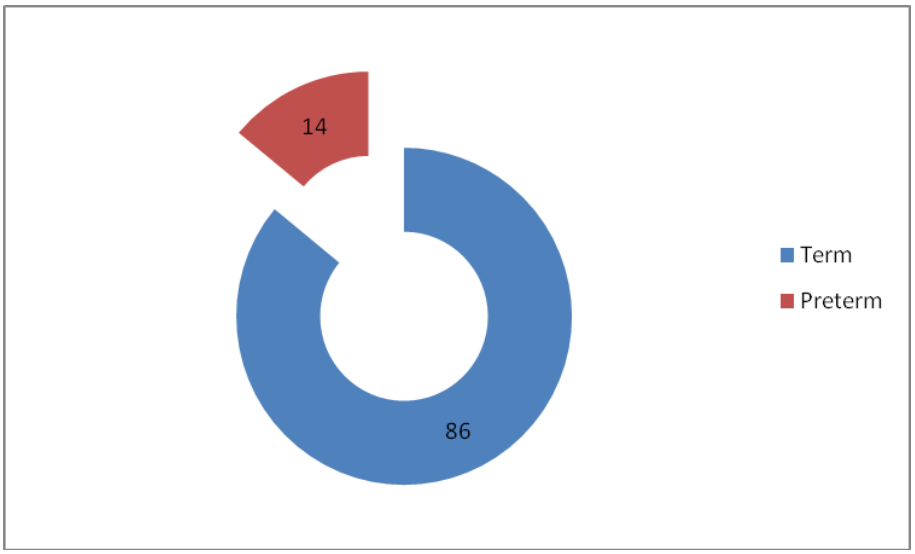
- There is a slight female predominance in our study.

BIRTH WEIGHT



Only 18% babies were low birth weight among which 14% were preterm and 4% were term SGA babies. One of the 4 PCR positive babies was a term SGA baby (Birth weight 2250g).

GESTATIONAL ASSESSMENT AT BIRTH



Our study showed that 14 % of babies were preterm. All the 4 PCR positive babies were term, 3 were appropriate for gestational age and one small for gestational age. No preterm baby turned out to be PCR positive.

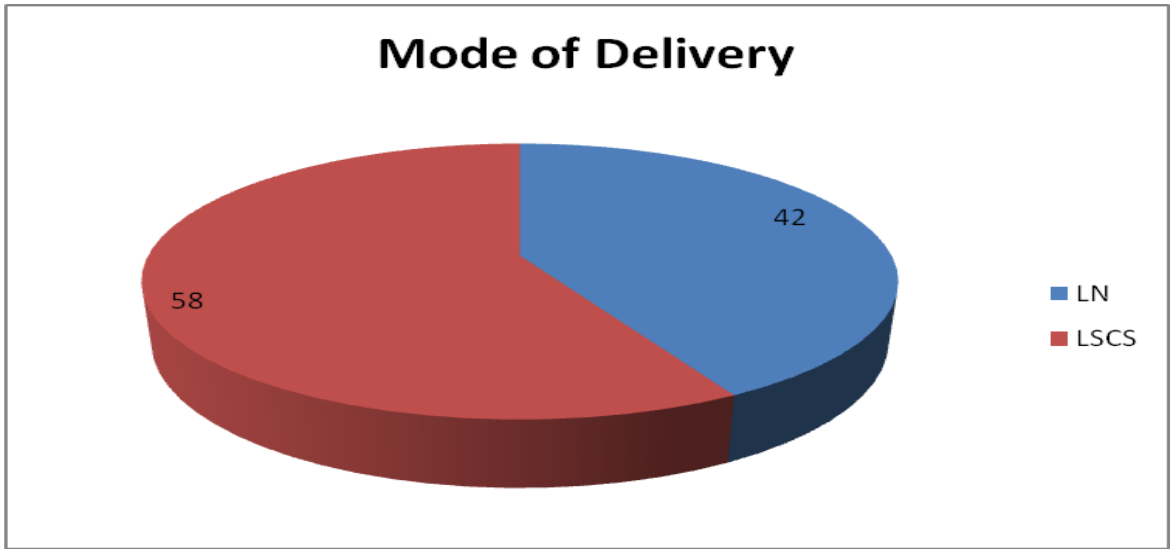
MODE OF DELIVERY

98 deliveries were institutional.2 deliveries were conducted at home by trained dais. The mode of delivery is as follows:

Labor Natural - 42

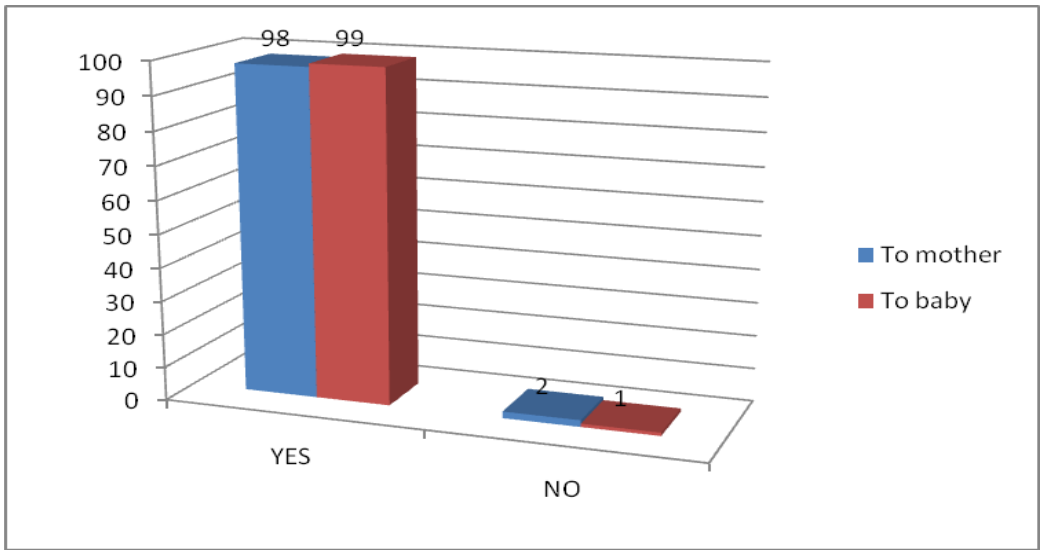
Caesarean delivery – 58

Caesarean delivery (LSCS) was done for Obstetric indications only.



(LN – Labour Natural, LSCS- Lower Segmental Caesarean Section)

NEVIRAPINE PROPHYLAXIS



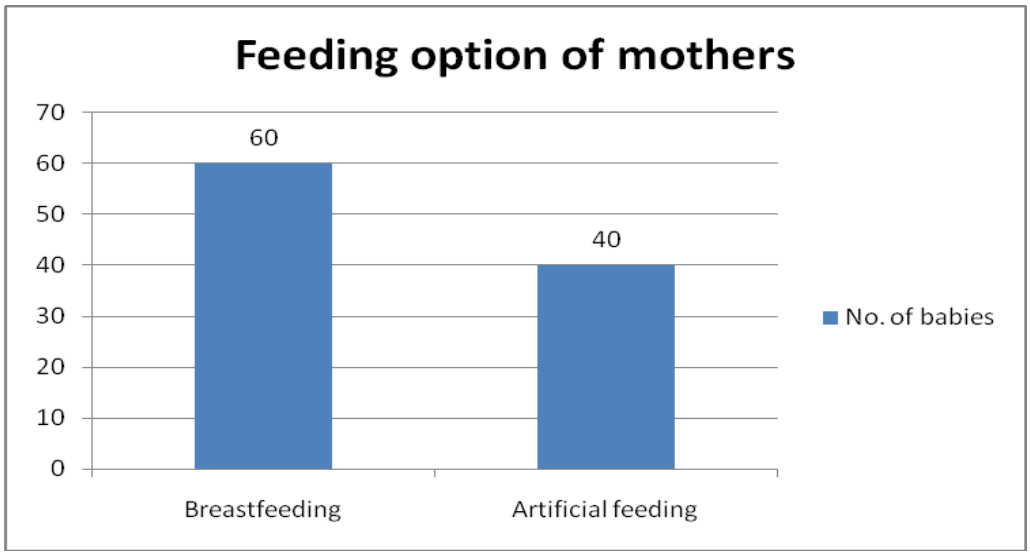
We followed single dose Nevirapine regimen to mother(600 mg) and baby (2 mg/kg).Nevirapine was given in our study as below.

To mother – 98

To baby – 99

One baby was delivered at home and so Nevirapine was not given to mother but the baby was given Nevirapine the next day. Another baby was also delivered at home in their native place but Nevirapine was given to neither to mother nor to baby, for the baby was not brought within 72 hours after delivery.

FEEDING PRACTICE



Breastfed – 60

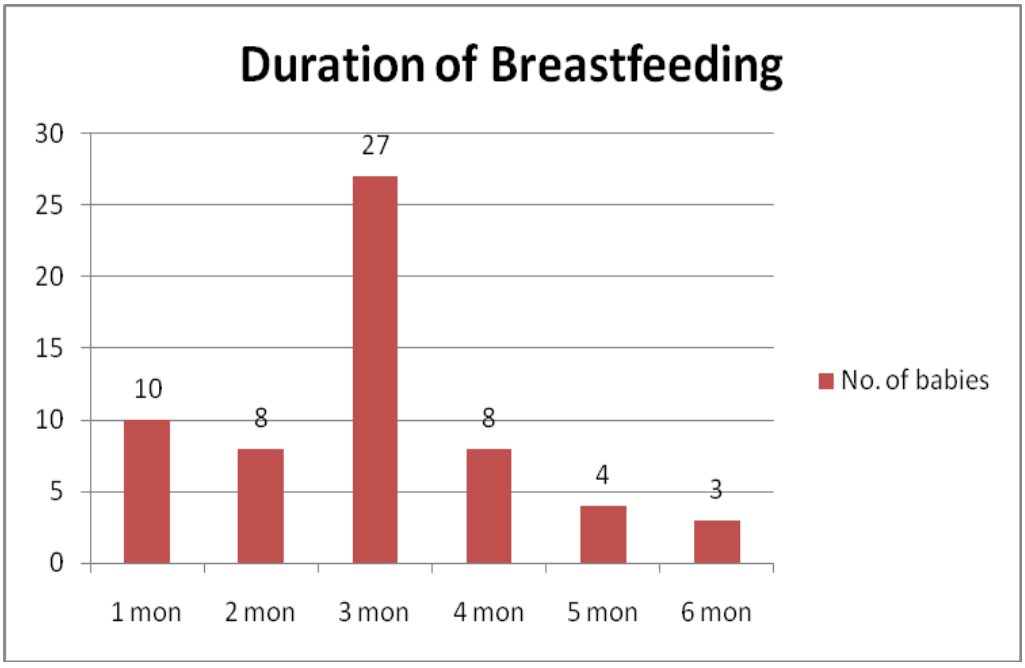
Artificial feeding (Not breastfed) – 40

40% babies were not at all breast fed. They were given cow's milk or formula feed from day 1.The mothers were advised to strictly follow proper dilution. Mixed feeding was not advised.

The babies on artificial feeds did have 1 or 2 episodes of diarrhoea and respiratory infections but none of the episodes was severe enough for admission. This was similar to that in the breastfeeding group.

Infection rate among breastfeeding and artificial feeding were 6.7% and 0% respectively.

DURATION OF BREASTFEEDING



10 mothers breastfed their babies for < 1 month and went for replacement feeds.3 mothers exclusively breastfed for 6 months. The others breast fed for a varied period from 1 month to 6 month as shown in the above bar chart. All the mothers stopped breastfeeding their babies after 6 months of age.

Nutritional status among breastfed and non breastfed babies

Feeding	Normal	Undernourished	p value
Breastfeeding	67 %	33 %	>0.1, not significant
Artificial feeding	64 %	36 %	

We assessed nutritional status of the infants at each visit. The nutritional status of breastfed and non breastfed infants were as shown in table above. We do not find any statistically significant difference (p value is > 0.1) in nutritional status of breastfed and non breastfed babies.

HIV DNA PCR positive babies

Out of 100 babies, 4 babies were DNA PCR positive .All the four were on breastfeeds. Among the four, 3 babies were positive at 6 weeks of age. The fourth one was PCR negative at 6 weeks, breastfed up to 4 months but was positive 6 weeks after stopping breastfeeds.

Prevalence = 4 %

Prevalence among artificial feeds =0 / 40 = 0 %

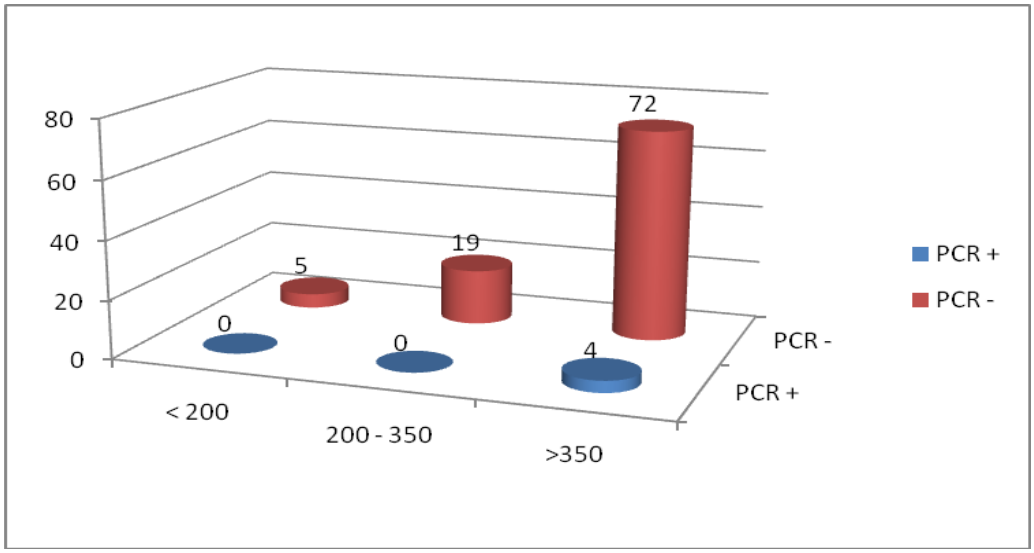
Prevalence among breastfed babies = 4 / 60 = 6.7 %

The details of the PCR Positive babies are given the following table:

HIV DNA PCR positive Babies

	A	B	C	D
Mothers age	23	31	30	27
Mother's wt.	45 kg	66 kg	57 kg	56 kg
Gravida	Primi	Primi	G4P3L3AO	G2P1L1A0
Education	Class 6	Class 5	Illiterate	Class 4
Mother'sCD4	467	834	890	491
HIV stage of mother	1	1	2	2
ART	NO	NO	NO	NO
Spouse HIV status	Positive	Positive	Positive	Positive
Sex of baby	Male	Female	Male	Female
Birth wt.(g)	2500	2900	2250	3000
Mode of deli.	LSCS	LSCS	LN	LN
NVP	YES	YES	YES	YES
PROM>4hrs	YES	YES	NO	YES
Bleeding PV	NO	NO	NO	NO
Feeding	Breastfed 3 month	Breastfed 4 month	Breastfed 2 month	Breastfed 4 month
Baby's HIV stage	Stage 1	Stage 1	Stage 1	Stage 1
CD 4 of baby	3411(47%)	2718(41%)	1880(27%)	1898(32%)

Comparison of CD 4 counts of mothers of positive and negative babies

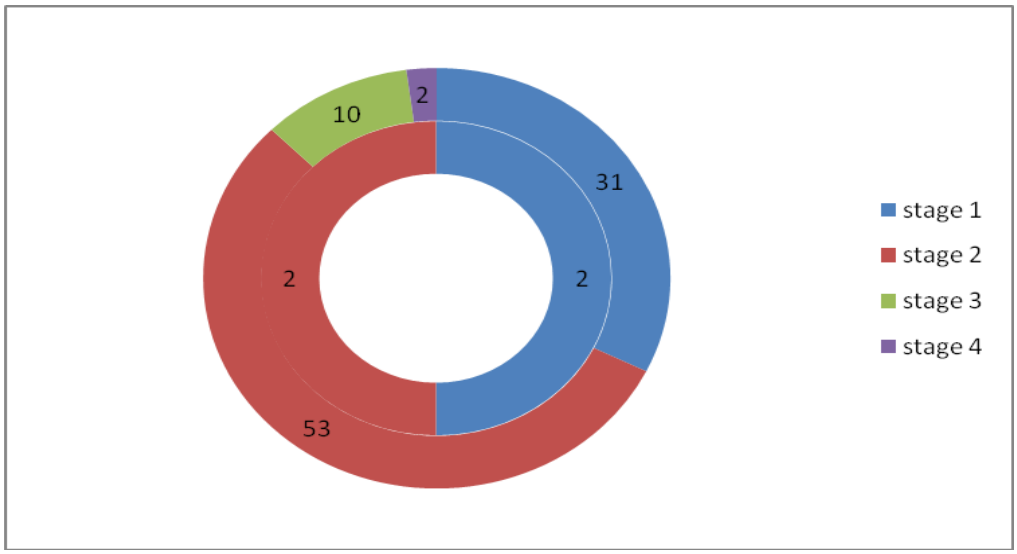


X axis – Mothers' CD 4 count

Y axis – CD 4 count/mm3

We know that CD 4 count < 200 is a risk factor for mother to child transmission. In our study, the mothers of all the four PCR positive babies had CD 4 counts more than 350. But none of these 4 mothers were on ART. On the other hand, the babies of the 5 mothers with CD 4 < 200, were all PCR negative. These 5 mothers were on ART.Thus we see in our study, that though CD 4 < 200, if the mother is on ART, it there is no risk of transmission and similarly even if CD4 > 350, if the mother is not on ART, there is risk(4/72 = 5.5 %) of mother to child to transmission.

COMPARISON OF MATERMAL HIV STAGING

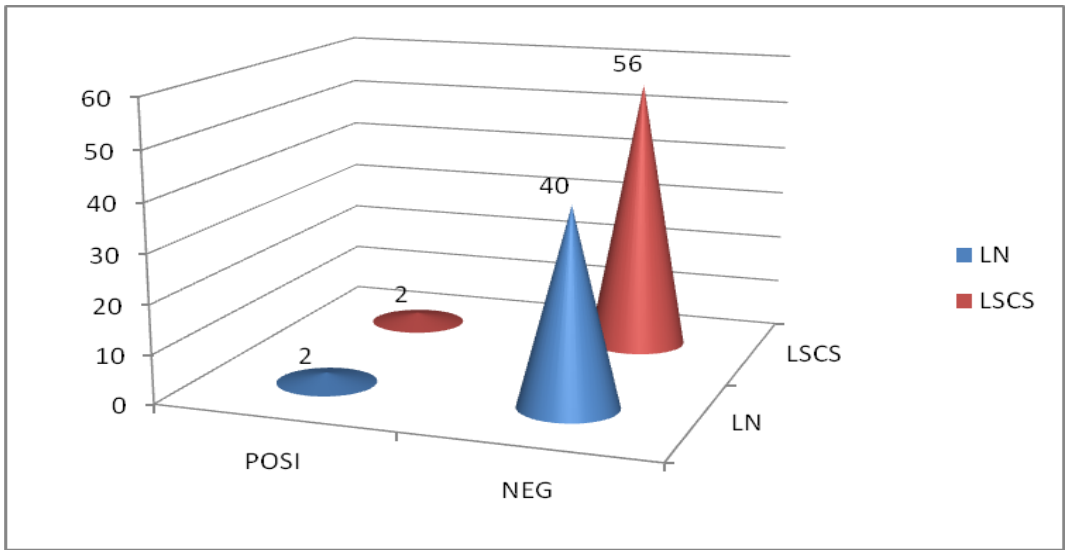


Inner circle – Mothers with PCR positive babies

Outer circle – Mothers with PCR negative babies

Among PCR positive babies, 2 mothers were in stage 1 and 2 mothers were stage 2. The mothers of PCR negative babies were in stage 1- 31, stage 2 - 53, stage 3 - 10 and stage 4 - 2. Though higher the maternal AIDS stage , higher the risk of mother to child transmission[34], those mothers with stage 3 or 4 had no PCR positive babies. This may be due to the fact that all of them were on triple drug regimen ART since diagnosis throught the pregnancy.

MODE OF DELIVERY



X axis – PCR positive and negative

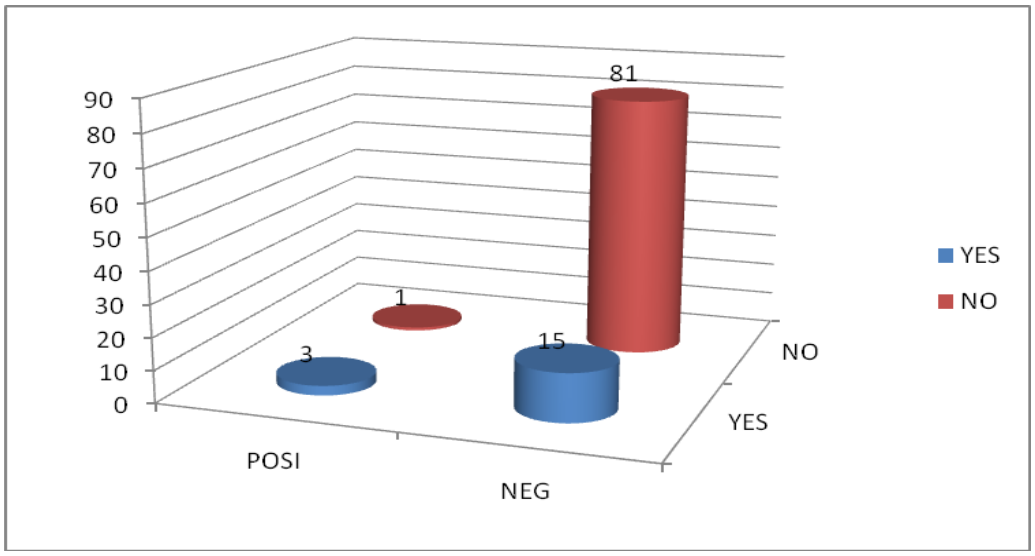
Y axis – No of deliveries

Z axis – Mode of delivery

	LN	LSCS	P value
PCR Positive	2%	2%	>0.5(not significant)
PCR Negative	40%	56%	

- We do not find any difference in the mode of delivery(p value >0.5) among PCR positive and negative babies

PROM >4 HOURS



	PROM YES	PROM NO	p value
PCR Positive	3%	1%	< 0.005 (significant)
PCR Negative	15%	81%	

X axis – PCR positive and negative

Y axis – No of mothers

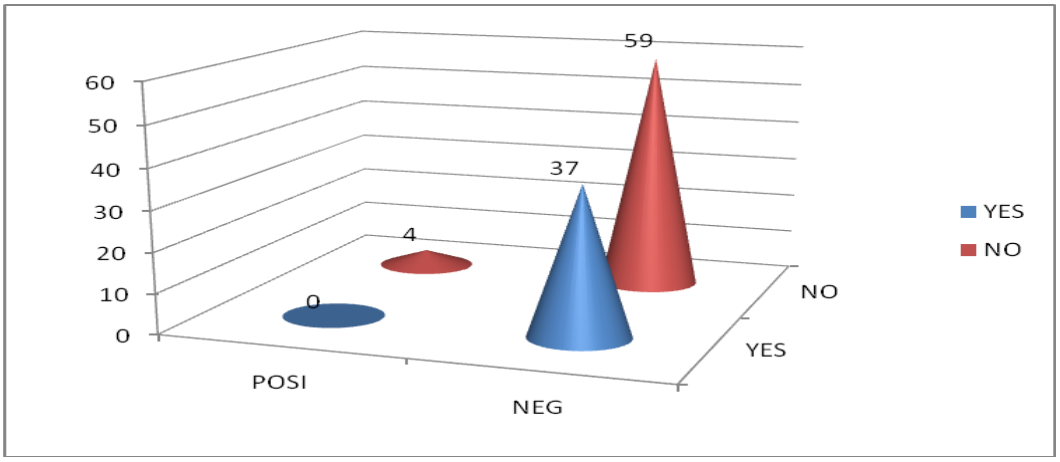
Z axis – PROM, YES/NO

PROM- Prolonged Rupture Of Membrane

Among PCR positive babies, 75% of mothers had PROM where as among the PCR negative babies, 15 % of mothers had PROM($p<0.005$)

MOTHERS ON ART

On ART	POSITIVE	NEGATIVE	p value
YES	0	37	0.000 (significant)
NO	4	59	



X axis – PCR positive and negative babies

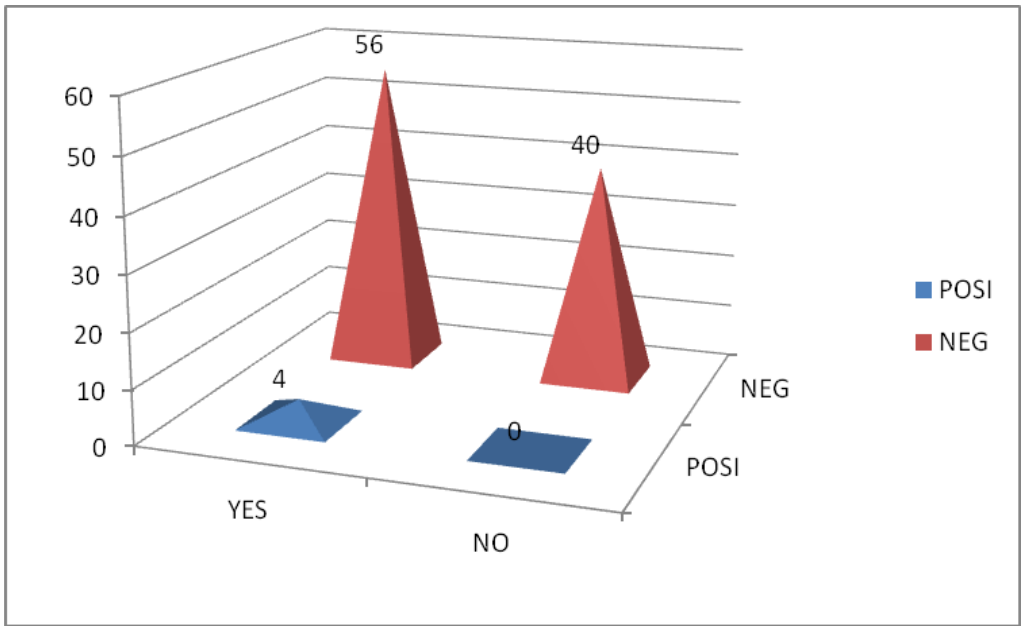
Y axis – No of Mothers

Z axis – ART for Mothers YES/NO

We know that the risk of transmission correlates with maternal AIDS staging. But none of the babies born to stage 3 and 4 were PCR positive(all mothers of stage 3 and 4 were on ART). All the 4 PCR positive babies were born to mothers with stage 1 or 2 who were not on ART. No baby was PCR positive born to mothers with stage 1 or 2 who

were on ART. p value fot the above data is 0.000, which is significant. This shows that not starting ART to the mother is a definite risk factor for mother to child transmission. Single dose Nevirapine given to these mother and babies was not effective for prevention of mother to child transmission who were not on ART. We do not say that all mothers should be started on ART but stress that mothers must be given long term prophylaxis as advised by WHO in the 2010 guidelines [5].

BREAST FEEDING



X axis – Breastfeding YES/NO

Y axis – No of babies

Z axis – PCR positive and negative babies

40 mothers chose to start replacement feeds from Day 1 and 60 mothers chose to breastfeed. Among the breastfeeding group, the duration of breast feeding varies from a few days to 6 months. None of them were on mixed feeds. 4 PCR positive babies were from breastfeeding group and none from the the other group. The first 3 babies were PCR positive at the first sample and the fouth one was PCR negative at 6 weeks and positive at the second sample taken 6 weeks after stopping breastfeeds.Though breastfeeding is a risk factor from above data(p value <0.001), we do not attibute PCR positivity to breastfeeding alone.

DISCUSSION

The objective of our study was to find the prevalence of HIV infection in HIV exposed infants in Chennai by doing HIV DNA PCR. We followed up 100 mothers registered in the ART/ICTC centers of 4 Obstetrics institutes in Chennai.All the 100 mothers knew that they were HIV positive, they also knew that it can be transmitted to their babies.They consented for the study and were willing to rule out HIV infection in their babies earlier by HIV DNA PCR. All but one spouse agreed to HIV ELISA test when their wives were found to be positive.Their weight,clinical staging, initial CD 4 counts were documented. ART was started in clinical stage 3 and 4,and considered in stage 1 & 2 if CD 4 count was $< 350/mm^3$, preferably before $<200/mm^3$. ART was not started only for prevention of mother to child transmission.The 37 mothers who were on ART, were for maternal indications only.The complainance was good.

Maternal CD4, HIV status , ART status

In our study, we found no PCR positive baby was born to mothers with HIV stage 3 and 4.This is contrary to the results of Marinda E, et al [34] who showed that risk was associated with advanced maternal disease. But in our study, the 0 % transmission rate in stage 3 and 4 may be due to the fact that they were all on ART. All the 4 positive babies were born to mothers with CD4 > 350 and HIV stage 1

or 2 and none of the 4 mothers were on ART. No PCR positive baby was born to mother with CD 4 < 200.We know that MTCT occurs when maternal CD4 < 200 [1,2].But our results are similar to the results of Marazzi, et al [33] where 50.6 % of transmission occurred in mothers with CD 4 > 350/mm^3 and which stressed that absence of maternal ART prior to delivery was associated with HIV transmission. This shows that triple drug ART regimen prevents mother to child transmission in spite of low CD4 count or advanced HIV stage

Birth history

96 deliveries were conducted at one of the 4 Obstetrics institutes, 2 at private hospitals,2 at home.The percentage of institutional deliveries(98 %) is much higher when compared to general population of the locality and also higher than that of Marazzi, et al[33].This shows that these mothers were keen about their safe confinement.42 babies were delivered through vaginal route and 58 underwent Caesarean section.Though caesarean delivery was conducted only for obstetric indications, the percentage of caesarean delivery is higher in our study when compared to general population.We did not find any difference in PCR positivity from vaginal and caesarean delivery in our study. Shah I et al [39] found no statistically significant difference in MTCT in vaginal and caesarean delivery when the mothers were on ART and babies were on artificial feeds. Similar to Shah I, et al , Legardy – Willaiams et al [41]

showed that the benefit of Elective Caesarean Delivery (ECD) in preventing MTCT of HIV is substantial, but the benefit of ECD for women with low viral loads or for women using combination antiretroviral drugs is unclear.

Bleeding PV

Eight babies were born to mothers with bleeding per vaginum before delivery. Though it is a risk factor [31], all the babies of these mothers were turned out to be PCR negative.

PROM

Prolonged rupture of membrane(PROM) more than 4 hours is known risk factor for mother to child transmission[31].We found PROM >4 hours in 15 mothers with PCR negative babies and 3 mothers in PCR positive babies with a p value of < 0.005 which is significant, showing PROM >4 hours is a definite risk factor for mother to child transmission.However Alvarez JR, et al [42] concluded that MTCT rate of HIV did not seem to be related to duration of rupture of membrane prior to delivery. But all the mothers(100%) in his study received antenatal highly active antiretroviral therapy and intrapartum Zidovudine whereas in our study only 37% were on ART.

Birth weight

The birth weight was similar to that of general population.The average birth weight was 2710 g. 18 babies were low birth weight and one baby of this group was PCR positive. 3 babies of birth weight >2500 g were PCR positive. On the other hand, all the 14 preterm babies with birth weight < 2500 g were all PCR negative.

Nevirapine prophylaxis

Nevirapine was given to 98 % of the mothers and babies. One of the 2 babies delivered at home, received Nevirapine the next day.We, as per NACO guidelines, did not use extended ART for prevention of MTCT alone when mothers did not require ART for her own health. With this we found a transmission rate of 4 %. Marazzi et al [33] showed very low MTCT rate of 2.8 % at 12 month with extended antenatal antiretroviral therapy to the mother. Namakwaya Z et al [26] demonstated a transmission rate of 11.2 % for single dose Nevirapine as against 4.9 % with triple drug ART regimen.

Feeding practice

Though counselling about breastfeeding was done before delivery most mothers were unclear whether to give breastfeeds or not soon after delivery. Only 40 % of mothers chose not to breastfeed .60 mothers decided to breastfeed. Out of 60, only 3 continued exclusive

breastfeeding upto 6 months. All others stopped breastfeeds after a variable period from a few days to 5months and switched over to replacement feeds.None of the babies were on mixed feeds.We found they need more counselling regarding the choice of feeding during antenatal visits and at the time of delivery.Among those who chose not to breastfeed from Day 1, most of them gave cow's milk and only a few babies were on formula feeds.The chilren on replacement feeds, had 1 or 2 episodes of diarrhoea and respiratory infections similar to breastfeeding group, but none of the episodes were severe enough for admission. We found no significant difference in the nutitional status of breastfed and non breastfed group. This is similar to Palombi L, et al [35] who showed a postnatal transmission rate of less than 2 % using alternatives to breastfeeding without a higher mortality rates. But Kagaayi J, et al [37] and Rollins NC, et al [38] found that alternatives to breastfeeding have shown a worrying increase in overall mortality among formula fed children, probably due to lack of access to clean water, incorrect dilution of formula and inadequate access to formula or postnatal follow up.The normal nutritional status in replacement group in our study may be due to acceess to clean water in Chennai, proper dilution techniques and high literacy rate of the mothers.None of the babies who were put on replacement feeds were PCR positive. All the 4 PCR positive babies were breastfed .Thus replacement feeds can be consideredcto offer superior protection against mother to child

transmission than breastfeeding. Our results were similar to that obtained by Ugochukwu et al [10]. But virus transmission may not be attributed to breastfeeding alone.

PCR positive babies

Since we had the adressess and phone numbers of the mothers, we reminded them to come for HIV 1 DNA PCR at 6 weeks of age during their visit for DPT immunisation.From the 100 samples taken, 3 were DNA PCR positive.The positive samples were tested twice before the results were given to parents.The negative results were given to the mothers saying that further tests are necessary if the babiy is on breastfeeds.We advised follow up for all babies irrespective of the PCR results.We taught them when to return if the baby manifests symtoms like oralthrush, multiple skin infections, respiratory distress etc.We do HIV ELISA to PCR positive and negative babies at 18 months of age.All the three PCR positive babies A,B,C were in HIV stage 1 and their CD 4 counts were 2718(41 %), 1880(27 %) and 1898(32 %) respectively.They were refered to pediatric ART center at the Institute of Child Health, Egmore.

All the babies on breastfeeds were to be brought for second sample 6 weeks after stopping breastfeeds.The mothers showed reduced interest to bring their children for second sample as the first one was negative.Yet they did after a delay of 1 or 2 months.Among those who

were on breastfeeds and DNA PCR negative at 6 weeks of age, one baby turned out to be PCR positive 6 weeks after stopping breastfeeds.This child was also in stage 1 with a CD 4 count of 3411(47 %).

One of the 4 PCR positive babies developed bronchopneumonia. He was admitted at ICH, and died of respiratiry failure. All the other three were started on ART at the pediatric ART center at ICH, as per the newer recommendations of NACO[2] which says to start ART in PCR positive infants irrespective of CD 4 %.Of the 3 babies on ART, 2 are regularly taking ART and are thriving well.The third one is on irregular treatment as her mother has psychiatric illness. Among the 96 PCR negative babies, one baby died of respiratory infection/sepsis at 6 months of age.The remaining 95 are on follow up and are doing well.

Compared to previous studies which showed a prevalence of 10 – 20 % [1], our study showed a low prevalence of 4 %. But this is high when compared to that of less than 1 % in western countries [40].Shah. I, et al [12] from Mumbai showed a high prevalence of 41.8 % but sample size was small (n= 36) and false positivity was aslo 80 %. Our results were comparable to Jacob SM, et al[11] from Chennai who showed a prevalence of 8.7 % with a sample size of 218,the samples being taken in second month of life .But this study did not analyse the risk factors of MTCT and no mention had been about the HIV stage, CD 4count, ART status of the mother.. The reasons for our low prevalence may be high

literacy rate of 92 % among the 100 mothers, good health awareness in urban area like Chennai city, 100 % follow up in the antenatal period, 100 % adherence to ART , 98 % institutional deliveries, higher rates of caesarean delivery, 98 % coverage of Nevirapine prophylaxis, 40 % received replacement feeds, shorter duration of breast feeding(< 6 months) and no mixed feeds.

In resource limited countries[3], most of the positive babies are rapid progressors dying at 6 – 9 months of age.A study conducted in Madurai, Tamilnadu in 2007, by Poorna Ganga Devi et al supports this statement [7].On the contrary, our study shows 75 % of them thriving well. The one baby died of bronchopneumonia was not because of immunosuppression as the CD 4 count was 1880(27 %), normal values being >1500(>25 %) .This shows rapid progressors are of smaller proportion in our study. We can not come to a definite conclusion as our sample of positive babies is small(n = 4).We recommend further studies in this regard. Marazzi, et al [33] showed a cummulative HIV – 1 free survial of 91 % at 12 months of like ours.

The limitations of our study were : a small number of PCR positive babies (n = 4) available for comparison and non availability of facilities to do viral load for all the mothers.

As a whole, our study found that the PPTCT program works well and awareness and care of HIV/AIDS have improved in Chennai.

SUMMARY

1) The prevalence of HIV infection among HIV exposed infants in Chennai is 4 %.The prevance was 0 % among those babies on artificial feeds.The prevalence among breastfed infants was 6.7%.

2) The literacy rate of the mothers was 92 % and all the 100 mothers were aware that they were HIV positive and there was a risk of mother to child transmission.

3) None of the babies born to mothers on ART was PCR positive eventhough the CD 4 count was < 200. The 4 PCR positive babies were born to mothers with CD 4 >350 and not on ART.

4) Most of the mothers(n=88), were in HIV clinical stage 1 or 2 and all the 4 positive babies were from this group.All those in stage 3 or 4 and on ART had no PCR positive baby.The prevalence of infection among babies born to mothers on ART irrespective of CD 4 count and HIV stage was 0%.

5) 71 % of spouses were HIV positive.13 mothers whose spouses were negative acquired the infection from HIV positive first husband who had expired.

6) 98 % of the deliveries were institutional and all the institutional deliveries were given Nevirapine prophylaxis.

7) We did not find bleeding per vaginum as a risk factor for virus transmission in our study. However, prolonged rupture of membrane > 4 hours is a risk factor in our study.

8) We found the mothers need more counselling regarding the choice of feeding during antenatal visits and at delivery. 40 % of mothers chose for replacement feeds from day 1 and none of baby was PCR positive from this group but positivity in breastfeeding group may not be attributed to breastfeeding alone. We couldnot see any major difference in the nutritional status or incidence of diarrhoea and respiratory infections among breastfed babies and those on replacement feeds.

CONCLUSION

1) The prevalence of HIV infection among HIV exposed infants in Chennai is 4 % with single dose nevirapine prophylaxis to mother and baby.

2) Maternal CD 4 count < 200/ mm^3 or advanced HIV staging of mothers were not risk factors for MTCT if the mothers were on combination antiretroviral drugs. Similarly, transmission do occur from mothers of stage 1 or 2 and CD 4 >350 when they were not on ART.

3) PROM > 4 hours is a risk factor for MTCT of HIV.

4) Breastfeeding is a definite risk factor for MTCT. There was no significant difference in the nutritional status of babies on breastfeeds and replacement feeds.

RECOMMENDATIONS

1) Replacement feeds may be advised in all HIV exposed babies from day 1 of life, in urban population like Chennai where literacy, economy and hygiene are better, after adquate counseling regarding artificial feeding (AFASS)

2) Prolonged rupture of membrane more than 4 hours is found to be a definite risk factor for MTCT and hence baby must be delivered soon after rupture of membrane.

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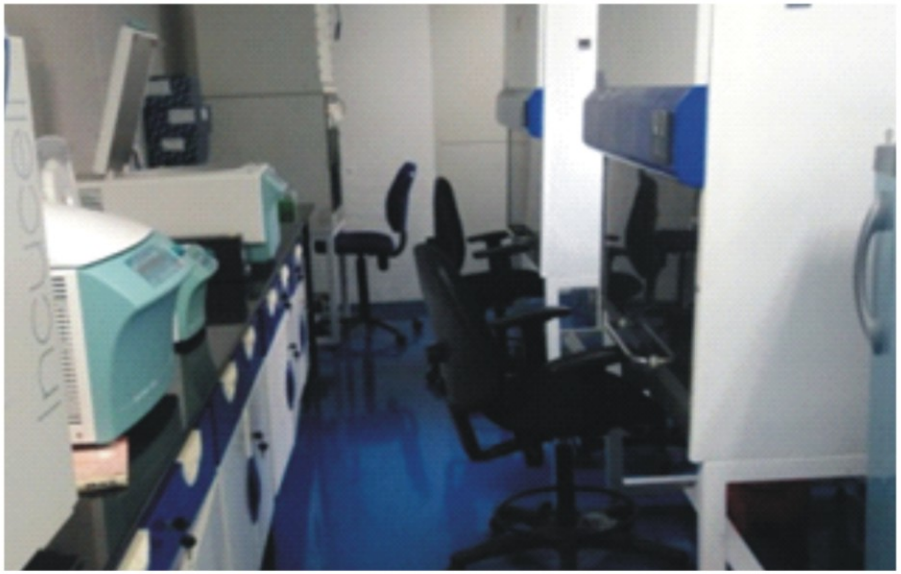
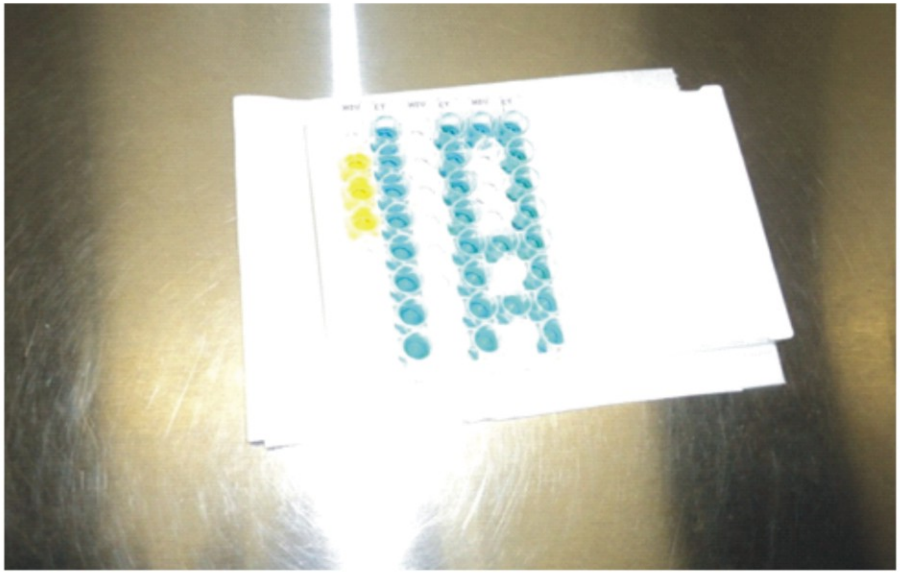


Part of Our Lab at TRC

DNA PCR during process



DNA PCR during process

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