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Antiretroviral treatment, viral load of mothers & perinatal HIV transmission in Mumbai, India

Swati P. Ahir, V. Chavan, S. Kerkar, P. Samant-Mavani[*], R. Nanavati[**], P.R. Mehta[+] & J. Mania-Pramanik

Department of Infectious Diseases Biology, National Institute for Research in Reproductive Health (ICMR), [*]Departments of Obstetrics & Gynaecology, [**]Neonatology & [+]Microbiology, Seth G.S. Medical College & K.E.M. Hospital, Mumbai, India

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***Background & objectives*: Mother-to-child transmission (MTCT) is the most significant route of HIV transmission in children below the age of 15 yr. In India, perinatal HIV transmission, even after treatment, accounts for 5.4 per cent of HIV cases. The present study was conducted to evaluate the efficacy of anti-retro viral therapy (ART) or prophylactic treatment (PT) to control maternal viral load in HIV positive women, and its effect on vertical HIV transmission to their infants.**

***Methods*: A total of 58 HIV positive women were enrolled at the time of delivery and their plasma samples were obtained within 24 h of delivery for estimation of viral load. Viral load analysis was completed in 38 women. Infants received single dose nevirapine within 2 h of birth and zidovudine for 6 wk. At the end of 18 month follow up, HIV positive or negative status was available in 28 infants.**

***Results*: Results revealed undetectable levels of viral load in 58.3 per cent of women with ART compared to 30.7 per cent of women with PT. No women on ART had viral load more than 10,000 copies/ml, whereas seven (26.9%, *P*=0.07) women receiving PT had this viral load. Median CD4 count of women on PT (483 cells/µl) was high compared to the women on ART (289 cells/ µl). At the end of 18 months follow up, only two children were HIV positive, whose mothers were on PT. One had *in utero* transmission; infection detected within 48 h of delivery, while the other child was infected post partum as HIV was detected at six months follow up.**

***Interpretation & conclusions*: Women who received a single dose of nevirapine during delivery had higher levels of viral load than women on ART. Combination drug therapy for pregnant women is now a standard of care in most of the western countries; use of nevirapine monotherapy at the time of delivery in our settings is not effective in controlling viral load. This highlights initiation of ART in pregnant women to control their viral load and thus to inhibit mother to child HIV transmission.**

Key words ART - MTCT - perinatal HIV transmission - prophylactic treatment - viral load

Human immunodeficiency virus (HIV) may be transmitted from mother to infant during the antepartum, intrapartum, or postpartum period. More than 3,70,000 children were newly infected with HIV in 2009, predominantly through mother-to-child transmission (MTCT) and an estimated 42,000-60,000 pregnant women died because of HIV[1]. Among the infected children nine out of ten children were infected

either during pregnancy, labour and delivery or during breastfeeding. Without treatment, 15-30 per cent children become infected during pregnancy and delivery[2]. India is the third largest population with HIV/AIDS with 2.39 million people living with HIV (adult HIV prevalence 0.31%). Parent-to-child transmission accounts for 5.4 per cent of the newly infected cases[3]. This population needs constant monitoring since treatment is not only life saving for women but also control their viral load that plays a direct role in reducing HIV transmission to the child.

Taking this into consideration the Prevention of Parent to Child Transmission (PPTCT) programme was launched with the findings of PACTG 076 trial published in 1994[4]. Zidovudine was used antenatally, during intrapartum period and postnatally in newborns and 67 per cent reduction in HIV transmission was demonstrated[4]. The subsequent trial (CDC-Thai study) showed almost similar efficacy despite using shorter regimen of zidovudine and ultra-short regime using nevirapine[5]. World Health Organization (WHO) recommended several prophylactic treatment (PT) regimens to prevent MTCT which are in use since 2000 with a latest revision in 2010[6]. Prophylactic treatment reduces the risk of MTCT by decreasing viral replication in the pregnant women, thus reducing viral load and through post-exposure prophylaxis of the neonate during and after exposure to the virus.

Besides viral load other factors implicated in MTCT of HIV are advanced maternal AIDS-related illness, breastfeeding, route of delivery, other infectious and obstetric conditions and low CD4 cell counts during pregnancy[7,8]. Further, viral subtype[9], viral concentration in maternal genital fluids[10] and genetic factors[11] have also been identified. Short duration of antiretroviral therapy (ART) during the course of pregnancy, problems regarding adhesion to treatment[12] and co-infections such as hepatitis C virus[13], genital herpes[14], cytomegalovirus[15], syphilis[16] and toxoplasmosis[17] further increase the risk of MTCT. However, in practical situation, particularly in resource-poor settings a few of these factors such as viral subtype, viral concentration in genital fluids, genetic factors or use of recreational drug abuse are not taken into considerations while initiating preventive steps in MTCT of HIV.

In India, in pregnant women free ART is initiated only when the CD4 count is below 350 cells/µl or if the woman is at WHO stage III or IV of infection. As of now protease inhibitors (PIs) are not included in HIV treatment as per the National Guidelines led down by NACO[18,19]. If the pregnant woman is not on ART, the current prophylactic treatment uses two drugs monotherapy: zidovudine (ZDV) and nevirapine (NVP). Single dose nevirapine (Sd-NVP) to the women at the time of delivery followed by a single dose to the infant immediately after birth is the current strategy for PPTCT. This is followed by zidovudine therapy to the infant for 6 wk[18,19]. The efficiency of the drugs, currently being used as treatment options, in reducing the viral load is not widely studied. Maternal plasma HIV RNA level is the strongest individual predictor of risk of MTCT of HIV infection[20]. Seroconversion during pregnancy was associated with viral loads and a major determinant of HIV transmission[21]. No reports are available from India on the efficacy of PT and ART on the maternal viral load and their role in MTCT. Considering these factors, this study was undertaken to evaluate the efficacy of current treatment regimen during pregnancy or at the time of delivery to HIV positive women for controlling their viral load and restricting HIV transmission to their infants.

Material & Methods

Subjects: This study was designed to enrol HIV-1 infected pregnant women and follow them up to delivery as well as HIV-1 infected pregnant women at the time of delivery to include their newborn for evaluation of effect of treatment *i.e.* ART or PT on mother's viral load and its association with HIV transmission to their newborn. The enrolment of the study population was done at the PPTCT centre of the Department of Obstetrics & Gynaecology and Integrated Counselling and Testing Centre (ICTC) of the Department of Microbiology, Seth G.S. Medical College and K.E.M. Hospital, Mumbai, India. Ethics committees of the participating institutions had approved the study protocol. The study period was between January 2010 and December 2011.

HIV positive pregnant women were informed about the study and those who were ready to give consent for enrolment along with their newborn infants were included. Each parent was counselled on the benefit of follow up of their child at different time intervals for HIV screening. For infant enrolment the consent was taken from both the parents. If one of them refused to consent, the mother-child pair was excluded. At birth, infants who were very sick and kept under Neonatal Intensive Care Unit (NICU) were excluded from the study. Infants who did not come for follow up were also excluded along with their mothers.

Maternal characteristics, treatment regimens and delivery option: A detailed questionnaire was prepared to obtain the maternal data during delivery or during the enrolment. This included information on other illness or any opportunistic infections that the women might be infected with during gestation or delivery. Other factors such as maternal age, time of HIV detection (during or before pregnancy), route of HIV infection, recent CD4 count and onset of ART were also noted. ART was initiated free of charge if the CD4 count of the woman fell below 350 cells/µl or WHO stage III or IV. ART included a combination of three reverse transcriptase inhibitors such as stavudine (30 mg) + lamivudine (150 mg) + nevirapine (200 mg) (SLN combination) which was the initial treatment and was changed if an allergic reaction or hypersensitivity reaction was observed. The PT regimen included the single dose of nevirapine (200 mg) at the onset of labour for mothers who were not on ART. PT prophylaxis given to the infant included nevirapine syrup (2 mg/kg) single dose immediately or within 2 h of delivery followed by zidovudine syrup (4 mg/kg) twice daily for 6 wk as per the policy of the local neonatology department. Mode of delivery was classified as FTVD- full term vaginal delivery, LSCS- Low segment caesarean section, ID - Instrumental delivery and PD-preterm delivery.

Feeding options: All the mothers were counselled for feeding option of their babies. All advantages and disadvantages of top feeding and breast feeding were explained[22] and the final decision of the feeding option was left to the parents. For mothers who could not afford top feeding, exclusive breast feeding up to four months and rapid weaning in order to restrict transmission was recommended. Change in the feeding options, if made, was noted during the follow up visits.

Determination of maternal viral load: The HIV-1 viral load in the blood plasma was estimated in mothers whose samples were obtained within 24 h of delivery. All the women goes through huge metabolic changes during delivery, it was decided to estimate the viral load after delivery to see its impact on transmission and to maintain a balance on time after delivery with viral load. Before delivery it was difficult to measure the effect of PT as the time of delivery in each individual varied. The viral load analysis was done with isolation of total nucleic acid using the MagNa pure Compact Nucleic Acid Automated System (Roche Diagnostic, Germany) and spectrophotometrically checked for the purity at 260/280 nm. Subsequently, the viral load was measured by Cobas Taqman Real time PCR (Roche Molecular Systems, USA) according to the manufacturer's instructions. To assess the quality of the viral load results, randomly selected samples (n=16), such as those with undetected values, high viral load values and with intermediate values were reassessed.

Testing of the infants: Whole blood samples (1-2 ml) of the infants collected in EDTA vacutainer, within 48 h of birth, were used to detect *in utero* infection using an in-house standardised Proviral DNA assay[23]. Briefly, two sets of primers were used for this nested PCR which amplified a specific part of the gp41 region of the *env* gene. The primers used were synthesized by Bangalore Genei (Custom Oligo Synthesis Division, Merck Specialitis Private Limited); 1st round: JH38 (5'CAG CAG GAA GCA CTA TGG G 3') and JH41 (5'GGT GAG TAT CCC TGC CTA AC3'), 2nd round: Menv27 (5'AAG CCT CCT ACT ATC ATT ATG A3') and Env19 (5'CTG GTA TAG TGC AAC AGC A3'). All infants were followed up at six weeks and again between 4-6 months dried blood spot (DBS) were collected for DNA PCR. In some cases where the status was indeterminate, whole blood was collected and PCR was repeated. All the follow up tests up to 18 months for confirmation of the child's status were performed at the collaborative centre (ICTC).

A child was confirmed as being negative if two consecutive DNA PCR results were negative or a negative serology (ELISA) result after 18 months of age was obtained on follow up visits. Any child tested positive by DBS was again tested for PCR using whole blood sample collected in EDTA vacutainer. If again the child was tested positive, that child was confirmed as being perinatally infected and referred to the respective department for further treatment.

Statistical analysis: StatCalc program (Epi Info version 6.0.4. CDC Atlanta, USA) was used for estimation of the Fisher's exact two tailed, *P*-values. Odds ratios (OR) of treatment profile were calculated at 95% confidence intervals. Z test was used as the test of significance for proportions. Student t test was used to compare between two mean values; this was performed using the SPSS software version 19 (SPSS, Chicago, IL, USA).

Results

During January 2010 to December 2011, 81 seropositive women who gave birth to live infants at the collaborative centre were counselled for participation in the study. Among them 58 (71.6%) women were enrolled with informed consent. They belonged to

diverse ethnic races, *i.e.* Maharashtrian Hindu (n=21), North Indian Hindu (n=19), Muslim (n=10), South Indian Hindu (n=3) and others (n=5). Heterosexual transmission (n=38, 65.5%, husbands were HIV positive) was the major cause of infection, while 31.0 per cent (n=18) did not know how they were infected, only two (3.4%) revealed on needle abuse. Among the enrolled women, 33 (56.9%) were diagnosed as being HIV positive after conception.

Among the 58 enrolled women, 18 women during pregnancy were eligible for initiation of ART (CD4 count < 350 cells/µl) and were given a combination of SLN as a first line of therapy (Fig. 1). The median age of the women on ART was 25.5 yr, and 27.5 yr for women not on ART. The women on ART were not given single dose nevirapine at labour. The remaining 40 women were given single dose nevirapine (200 mg) prophylaxis at the onset of labour. All infants were given anti-retroviral prophylaxis.

Sixty infants were born to the enrolled 58 mothers (2 had twins). Twelve deliveries (20.7%) were done by emergency low section caesarean section (LSCS), for obstetric indication and the remaining 46 (79.3%) were vaginal deliveries. Of the 60 infants, 58 were born at full term and two were preterm. At the end of 18 month follow up, decisive HIV status was available for 28 infants (Fig. 1). The rate of vertical transmission among children born during the study period was 7.1 per cent (two of 28 determined cases with known HIV status positive, of whom 6 were breastfed). Among the two HIV positive male children, one was breastfed. Data on infection during ante- and intrapartum were available for 25 infants, 24 were found to be negative and one was positive by DNA PCR (Fig. 2). In other three infants, the neonatologist on duty could not collect the blood within 48 h of delivery due to collapsed veins and their blood sample was obtained at 6 wk follow up. The initial PCR result of the breastfed baby was negative at 48 h as well as up to 6 wk of delivery, but

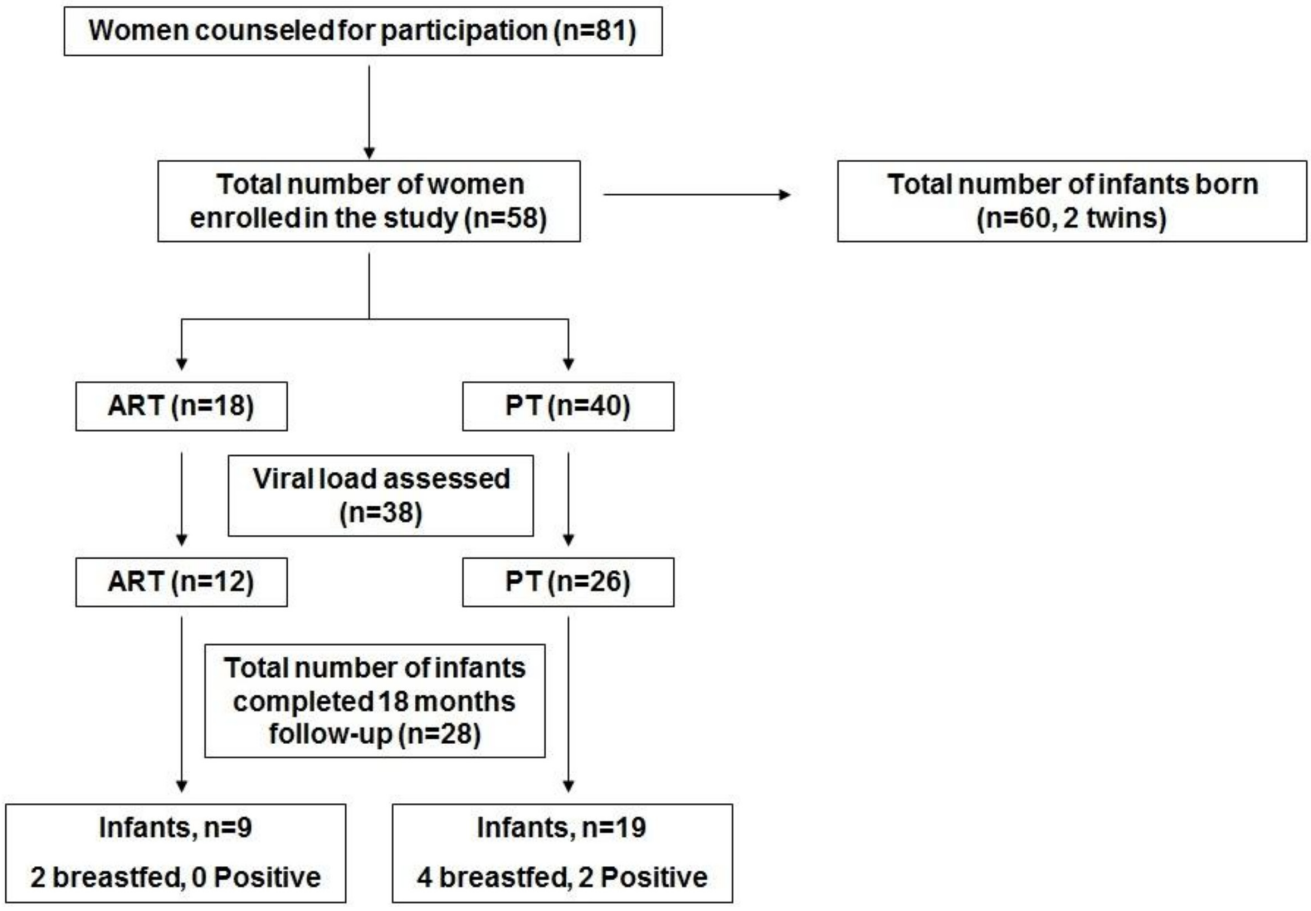

Fig. 1. Enrolment of HIV positive mothers and follow-up of their infants. ART, anti retroviral prophylaxis; PT, prophylactic treatment.

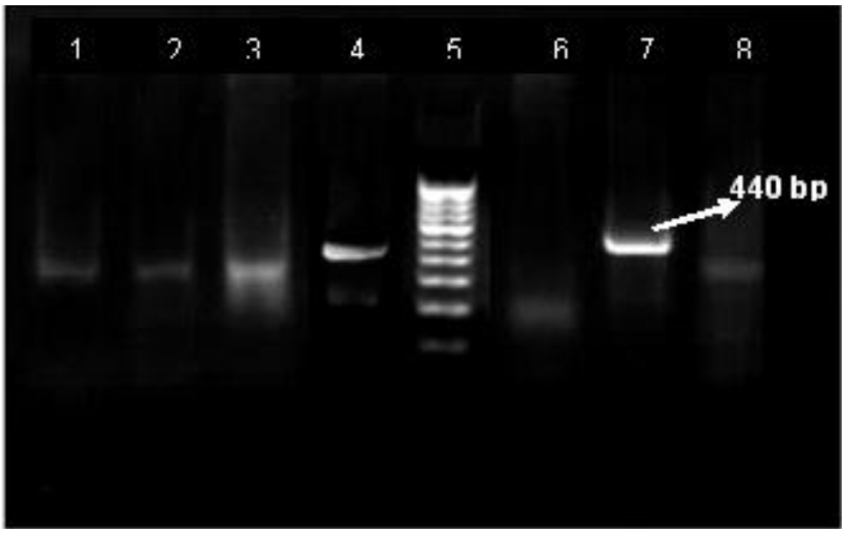

Fig. 2. Proviral DNA product for detection of infant's HIV status. Lane 1-Control negative; Lane 2-negative infant sample; Lane 3- negative infant sample; Lane 4-positive infant sample (440 bp); Lane 5 - 100 bp Ladder; Lane 6- negative infant sample; Lane 7- control positive; Lane 8- PCR negative control.

was found to be positive at six months follow up. The PCR result of the top fed baby was positive at birth and also at 6 wk whose mother's viral load was 47 copies/ml but with HCV co-infection.

Viral load was done in 38 samples of the enrolled women, within 24 h of delivery. Twelve of these were on ART, among them five had started ART during pregnancy, three had been on ART for more than a year and four were on ART for more than two years. Reassessment of viral load results in 16 samples revealed association with the first viral load assay, indicating the acceptability of the test used. Results revealed non significant difference in the median viral load among women receiving ART when compared to women on PT (2058 copies/ml in women on ART vs. 4234.5 copies/ml in women on PT, *P*=0.879). A subset of women (n=7) who were on PT showed a very high median viral load of 52323 copies/ml (viral load >10,000 copies/ml), when compared to the women on ART, *P*=0.001. The median CD4 count in women on PT was slightly higher than those on ART (483 vs. 289 cells/µl, *P*=0.183) (Table I).

When an overall comparison was made between the ART and the PT groups, higher viral load was found in women on PT (Table II). Viral load was in control (*i.e.* below detectable limits) in women receiving ART, none had a viral load >10,000 copies/ml, whereas seven women on PT had >10,000 copies/ml (0 vs.26.7%,

Table I. Characteristics of enrolled women in the study

Variables		PT (n= 26)	ART (n=12)
Median age in years		27.5	25.5
Median CD4 count: cells/µl		483	289
Median viral load: copies/ml		52323[*+] 4234.5[#]	2058[+]
Infections	Tuberculosis	1	1
	Hepatitis C virus	1[@]	0
	VDRL (syphilis)	1	0
	Hepatitis B virus surface antigen	1	0
	Others (renal disorder)	1	0
Full term vaginal delivery	Normal	21[*]	9
	Instrument delivery	1	0
Low segment caesarean section		4	3
Feeding options	Formula feeding	19	8
	Breast feeding	7	4

PT, prophylactic treatment; ART, antiretroviral treatment
[*]Women on PT having viral load >10,000 copies/ml (n=7); [#]Women on PT having viral load <10,000 copies/ml (n=19); [+]*P*=0.001(unpaired t-test) compared to women on ART; [@]infant positive; [*]One was HIV positive

P=0.07). Undetectable viral load was seen in 8 of 26 women (30.7%) on PT. This was significantly less (*P*<0.001) when compared with the remaining women (69.2%, 18 of 26) on PT with detectable viral load (Table II).

Infants of all the mothers who were on ART were negative. Two of 19 infants (10.5%), were positive whose mothers were given PT prophylaxis (Table III). Infant birth weight (Median ± SD) was lower (2.18 ± 0.51 kg) in positive infants as compared to the negative ones (2.792 ± 0.65 kg). Fathers of both the infected children were HIV negative. Among the remaining 26, five children had negative fathers, 20 had positive fathers and one father's status was not known (Table IV).

Discussion

The rate of MTCT obtained in the current study was 7.1 per cent, which was more as compared to NACO's report (5.4%)[3]. This difference might be due to the use of specific and sensitive DNA PCR (started in April 2010) for early infant diagnosis (EID) at the collaborating ICTC. It was evident from our study that single dose nevirapine (*i.e.* prophylactic treatment given to the HIV seropositive mothers in our settings) was not efficient in reducing the maternal viral load and consequently the transmission rate, indicating importance of ART use for treatment and prophylaxis to decline viral loads. Current treatment option helped only, 39.5 per cent (15 of 38) of these infected women to control their viral load, while another 18.5 per cent had high rates of virologic failure. This highlighted the need to implement long-term treatment options with ART for HIV infected women to control the viral load.

Viral load is the strongest predictor of HIV transmission during unprotected sex or transmission from infected mother to her child and low maternal plasma viral load is the key factor for preventing MTCT[24]. The transmission in the present study happened only in one of five infants born to mothers on PT with very high viral load. The viral load (85314 copies/ml) of the mother of this newborn was very high as well as the infant was breastfed. However, the mother of the other HIV positive infant had low level of viral load (47 copies/ml) and also the infant was

Table II. Effect of treatment regimens on viral load in mothers with anti retroviral (ART) vs prophylactic treatment (PT)

Viral load copies/ml	On ART		On PT		Odds ratio	Fisher's exact *P* value (two-tailed)
	n=12	%	n=26	%		
Undetectable*	7	58.33	8	30.7	3.5	0.104
<1000	2	16.66	4	15.38	1.0	1.0
>1000	3	25	7	26.9	0.9	1.0
>10000	0	0	7	26.9	0.0	0.07
Mean viral load ± SD	2464.40±2665.2 (n=5)#		24854.00±41656.8 (n=18)#		Levene's test for equality of variances	
					F	*P*
					4.413	0.048

*Undetectable viral load: Indicates a viral load less than 40 copies/ml, which was the detection limit of the kit; #Mothers with detectable viral loads

Table III. Maternal viral load and HIV status of infants

Mothers (n=28)	HIV status of infants	Viral load (copies/ml)				N
		Undetectable	<1000	<10000	>10000	
PT (n=19)	Negative	6	3	4	4	17
	Positive	0	1*	0	1@	2
ART (n=9)	Negative	5	1	3	0	9
	Positive	0	0	0	0	0
n (%)		11 (39.28)	5 (17.85)	7 (25)	5 (17.85)	28

Viral load: *47 copies/ml; @85314 copies /ml

Table IV. Maternal treatment regimen, father's HIV status and infant birth weight

Treatment of mothers	Fathers HIV status	Infant HIV status	Infant birth weight (kg)		
			Median	Standard deviation	Average
PT (n=19)	Negative (n=2)	Positive (n=2)	2.18	± 0.51	2.18
	Negative (n=3) Positive (n=13) Unknown (n=1)	Negative (n=17)	2.792	± 0.65	2.81
ART (n=9)	Negative (n=2) Positive (n=7)	Negative (n=9)	2.406	± 0.47	2.49

not breastfed, and was delivered at full-term indicating *in utero* transmission. This supports the earlier report of about 1 per cent perinatal HIV-1 transmission even with the maternal viral load <1000 copies/ml[25], though other studies have indicated that high viral load and premature deliveries are major risk factors of MTCT in non-breast feeding populations[20,26]. Several studies have reported the beneficial role of early and combination drug therapy to lower the rate of MTCT[27,28]. Current recommendations of the United States for pregnant HIV-1 infected women suggest early and sustained control of HIV viral replication, which favours initiation of combination drugs early in pregnancy[29]. Our result on mothers on ART who did not transmit HIV to their newborns supports this recommendation. Though the time period for which they were on ART was different, but their viral load was in control and hence no transmission was observed. It was also observed these infants did not have a significantly low birth weight.

In conclusion, women on ART had lower levels of viral load compared to women who received a single dose of nevirapine during delivery, showing that nevirapine monotherapy at the time of delivery was not effective in controlling viral load in our settings. The results of the present study also highlighted the need for an initiation of combination drug therapy such as use of three drugs antiretroviral regimens for treatment and/or prophylaxis as early as possible during pregnancy so that the risk of perinatal transmission is reduced. In addition, if possible necessary resources may be allocated to provide formula feeding for children at risk of vertical transmission (MTCT) of HIV. The findings of our study need to be shared for the day-to-day clinical practice and to improve the patient care in any Indian settings to make it clear that less you treat the more MTCT will occur.

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Reprint requests: Dr Jayanti Mania-Pramanik, National Institute for Research in Reproductive Health (ICMR), J.M. Street, Parel, Mumbai 400 012, India
e-mail: jayantimania@rediffmail.com