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Paternal Transmission of HIV to the Child of A Seronegative Mother and A Seropositive Father

KEYWORDS: HIV paternal transmission, seronegative mother, seropositive father

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ABSTRACT *Mother to Child Transmission (MTCT) of HIV is considered during perinatal period. However possible risk of paternal transmission of HIV is still not understood. A family of HIV infected father, mother and child was recruited for the study. The mother during first pregnancy was tested HIV seronegative and found seropositive during subsequent pregnancy. The seronegative wife of HIV infected husband delivered first male child who was tested at the age of four and a half due to pulmonary tuberculosis and found HIV positive. Translated amino acid sequence of C2-V3 region of env gene of HIV1 in PBMCs of father and son showed 86% homology and that with mother and son showed 48% homology. These HIV variants showed the sequence identity with Chinese isolates. The study suggests the paternal transmission of HIV to the son of HIV negative mother and need for parental diagnosis of HIV during perinatal period.*

Introduction

The risk of HIV transmission from an infected Mother to child in perinatal period has been reported to be in the range of about 9·1 to 55 % in the absence of any interventions such as antiretroviral therapy (ART) (1, 2). Studies of Paediatric AIDS Clinical Trials Group (PACTG) 076 and PACTG 185 (3) demonstrated that the administration of zidovudine to HIV infected pregnant women and their infants reduced the rate of transmission by two-thirds, from 22·6% to 7·6% (4-7). The Indian public health programme of Prevention of Parent to Child Transmission (PPTCT) recommended the administration of single dose Navirapine prophylaxis at the onset of labour and to new born infant within 72 hours of birth. MTCT of HIV may be during pregnancy (*in-utero*), during delivery or after birth during breast feeding (8,9). However the possibility and mechanism of HIV transmission from father to infant is not fully understood. Presence of cell free virus as well as proviral DNA has been detected in the sperm (10-12). Bagasra et al demonstrated the localization of HIV DNA in the ejaculated spermatozoa of infected individuals. HIV proviral DNA has also been detected in germ cells at all stages of spermatogenesis, of HIV infected individuals. Additionally our studies on genotypic and phenotypic characterization of HIV variants also demonstrated the presence of distinct variants in spermatozoa of HIV infected individuals. Translated amino acid sequence of C2-V3 region of env gene of sperm proviral DNA of infected males showed the homology with HIV1 C isolates (13).

Studies have been reported that HIV-infected spermatozoa have the ability to fertilize oocytes and transfer the virus into the resulting embryo, but cell-free virus is not able to bind or penetrate the oocyte in vitro (14) suggesting the possible risk of sperm associated virus in paternal transmission of HIV to infants at the time of fertilization (15-17).

Present study demonstrates the characterization of translated amino acid sequence of C2-V3 region of *env* gene of HIV1 in PBMCs of the infected family (Father, mother and child) and possible association of sperm associated HIV in parental transmission of HIV to infant.

Materials and methods

Participants

A HIV infected Family of father, mother and children registered at Center of Excellence for HIV Care, Sir J J Group of Hospitals, Mumbai was investigated for parental transmission of HIV to their children. 27 years old father (DF) with unexplained fever for more than three months was detected to be HIV infected and referred for treatment at HIV Care Center in September 2007. Family history revealed that his wife (DM) during her first pregnancy was tested at the time of labor and found to be HIV negative in February 2006. She delivered a male child (D) which was breast fed till the age of one year. In 2007 DF was counseled for testing of DM for HIV however she declined. In 2009 DM was detected to be positive during her second pregnancy and administered ART as per National guidelines. The second female child born was tested by DNA PCR method and found HIV negative. The first child D had Herpez Zoster and pulmonary TB in July 2010 and following testing by ELISA for HIV was found seropositive. The child was started on ART. Testing for HIV for D, DF and DM was done as per the Indian National Guidelines.

Blood sample of HIV infected DF, DM and D was collected in EDTA coated vacutainers in March 2011 after Informed consent. The family was evaluated for CD4 count, viral load estimation and sequencing of C2-V3 region of *env* gene of HIV1 in PBMCs as described below.

Viral load

The HIV-1 viral load in the blood was estimated in total nucleic acid which was isolated using the MagNa pure Compact Nucleic Acid Automated System (Roche) and the viral load was estimated using Cobas TaqMan Real time PCR (Roche Diagnostic System) according to Manufacturer's instruction.

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Blood CD4 count was estimated by flow cytometry using cocktail of antibodies to CD4 and CD3 from Guava technologies and analyzed using Gauva Cytosoft software and measured the CD4 Count.

Genotypic characterization of viral variants in PBMCs
PBMCs were isolated by layering of blood-diluted 1:1 with RPMI 1640 (Sigma) onto Ficoll-paque plus (GE Health Technologies). A white buffy coat layer thus separated was washed twice with RPMI-1640 to obtain PBMCs and DNA from was extracted using Qiagen DNA mini kit and quantified spectrophotometrically.

C2-V3 region of HIV1 *env* gene from purified DNA in PBMCs was amplified by nested PCR according to the method described (18,19)using two sets of primers as mentioned below. The DNA sequence of the second round product thus obtained was determined by automated DNA sequencing using second round set of primers.

Primer set for first round PCR amplification of C2-V5 *env* gene

ED5 5′ ATGGGATCAAAGCCTAAAGCCATGTG 3′ (6556-6581)

ED 12 5′ AGTGCTTCCTGCTGCTCCCAACCCAA 3′ (7822-7792)

Primer set for second round PCR amplification of C2-V3 *env* gene

ED31 5′ CCTCGCCATTACACAGGCCTGTCCAAAG 3′ (6816-6844)

ED33 5′ TTACAGTAGAAAAATTCCCCTC 3′ (7359-7380)

0·5 – 1µg of DNA template from PBMCs was PCR amplified using first round 20 pmole each of ED5 and ED12 primer set in the presence of 1·25 mM MgCl, 2·5 mM dNTPs (Bangalore Genei) and 2·5 units of Taq polymerase (Banglore genei) in a total volume of 25 µl. PCR conditions were 94°C 15 min; 3 cycles of 1min each at 94° C, 50°C and 72°C; 35 cycles at 94°C for 15 sec, 55°C for 45 sec, 72°C for1 min and final extension at 72°C for 5 min. Subsequently C2-V3 region from 5µl of first round of product was similarly amplified by second round PCR using ED31 and ED33 set of primers.

Results
The family was responding to ZDV+LAM+NVP as evident from the CD4 count of 790, 560 and 710 cells/mm^3 of DF, DM and D respectively while their blood viral load was less than 47copies/ml.

Genotypic characterization of C2-V3 region of env gene of HIV1 in PBMCs
C2-V3 region of *env* gene of HIV1 isolated from PBMCs was amplified by nested PCR and sequenced. The translated amino acid sequence of C2-V3 region of HIV1 env gene demonstrated 91 and 95 % identity of the variants from PBMCs of son and father respectively with HIV 1 Chinese isolate CRF07_BC China (Fig 1a) while HIV isolates in mother's PBMCs showed 87% identity with CRF07_BC18 of Chinese isolate (Fig 1b). The comparative identity of HIV1 variants in PBMCs of Son and Father was found to be 86% and that of son and mother was 48% while Father and Mother was 45% (Fig 1a, b and c, The bold letters represents constant region while remaining are the variable regions.

D: Son, DF: Father and DM: Mother).

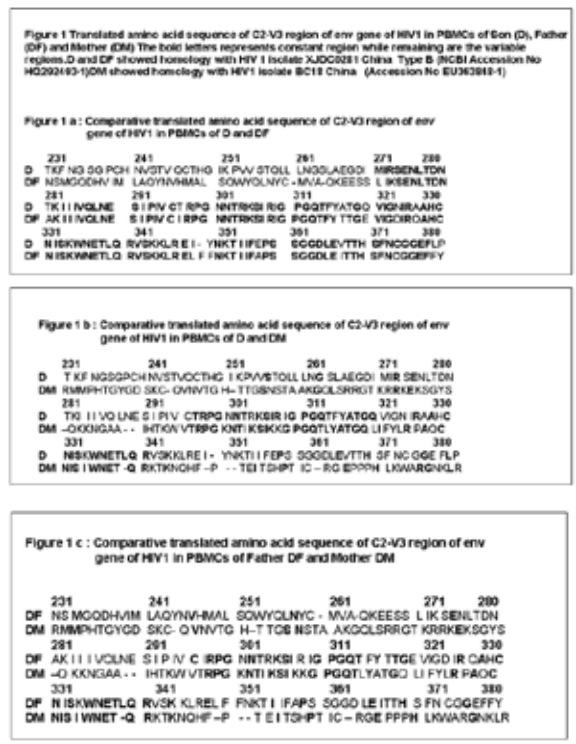
Figure 1 Translated amino acid sequence of C2-V3 region of *env* gene of HIV1 in PBMCs of Son (D), Father (DF) and Mother (DM) The bold letters represents constant region while remaining are the variable regions.D and DF showed homology with HIV 1 isolate XJDC0251 I China Type B (NCBI Accession No HQ292493-1)DM showed homology with HIV1 isolate BC18 China (Accession No EU363818-1)

Figure 1 a : Comparative translated amino acid sequence of C2-V3 region of *env* gene of HIV1 in PBMCs of D and DF

	231	241	251	261	271	280
D	TKF NG SG PCH	NVSTV OCTHG	IK PVV STGLL	LNGSLAEGD	MIRSENLTDN	
DF	NSMGGDHV IM	LAQYNVHMAL	SQWYQLNYC -	MVA-QKEESS	L IKSENLTDN	
	281	291	301	311	321	330
D	TKI I I VQLNE	S I PIV C I RPG	NNTRKSI RIG	PGQTFYATGQ	VIGNIRAAHC	
DF	AK II I VQLNE	S I PIV C I RPG	NNTRKSI RIG	PGQTFY TTGE	VIGDIRQAHC	
	331	341	351	361	371	380
D	N ISKWNETLQ	RVSKKLRE I -	YNKT I IFEPS	SGGDLEVTTH	SFNCGGEFLP	
DF	N ISKWNETLQ	RVSKKLR EL F	FNKT I IFAPS	SGGDLE ITTH	SFNCGGEFFY	

Figure 1 b : Comparative translated amino acid sequence of C2-V3 region of env gene of HIV1 in PBMCs of D and DM

	231	241	251	261	271	280
D	T KF NGSGPCH	NVSTVOCTHG	I KPVVSTGLL	LNG SLAEGDI	MIR SENLTDN	
DM	RMMPHTGYGD	SKC- QVNVTG	H-TTGSNSTA	AKGDLSRRGT	KRRKEKSGYS	
	281	291	301	311	321	330
D	TKI I I VQ LNE	S I PIV CTRPG	NNTRKSIR IG	PGQTFYATGQ	VIGN IRAAHC	
DM	-QKKNGAA - -	IHTKW VTRPG	KNTI KSIKKG	PGQTLYATGQ	LI FYLR FAQC	
	331	341	351	361	371	380
D	NISKWNETLQ	RVSKKLRE I -	YNKTI I FEPS	SGGDLEVTTH	SF NCGGE FLP	
DM	NIS I WNET -Q	RKTKNQHF -P	- - TEI TSHPT	IC – RGE PPPH	LKWARGNKLR	

Figure 1 c : Comparative translated amino acid sequence of C2-V3 region of env gene of HIV1 in PBMCs of Father DF and Mother DM

	231	241	251	261	271	280
DF	NS MGGDHVIM	LAQYNVHMAL	SQWYQLNYC -	MVA-QKEESS	L IK SENLTDN	
DM	RMMPHTGYGD	SKC- QVNVTG	H-T TGS NSTA	AKGDLSRRGT	KRRKEKSGYS	
	281	291	301	311	321	330
DF	AK I I I VQLNE	S I P IV C IRPG	NNTRKSI R IG	PGQT FY TTGE	VIGD IR QAHC	
DM	-Q KKNGAA - -	IHTKW VTRPG	KNTI KSI KKG	PGQTLYATGQ	LI FYLR FAQC	
	331	341	351	361	371	380
DF	N ISKWNETLQ	RVSK KLREL F	FNKT I IFAPS	SGGD LE ITTH	S FN CGGEFFY	
DM	NIS I WNET -Q	RKTKNQHF –P	- - T E I TSHPT	IC – RGE PPPH	LKWARGNKLR	

Discussion
MTCT of HIV is known to be during pregnancy, delivery and/or breast feeding. The possible risk of paternal transmission of HIV was not fully understood. Present study showed that the child born to HIV seronegative mother was found seropositive when tested at the age of 4½ years. This suggests the possibility of HIV transmission from father to the fetus. The HIV variants in PBMCs of father showed 86% homology with that of the son. But HIV variants in PBMCs of mother and son showed 48% sequence homology. Interestingly the viral isolates in PBMCs of son and father showed homology with Chinese isolate CRF07_BC while the isolate of PBMCs from mother showed homology with BC 18 Chinese isolate. Additionally during pregnancy and at the time of delivery mother was tested to be seronegative and obviously not administered ART to mother or the child. No evidence of unhygienic conditions at the hospital environment at the time of delivery, sexual assault or transfusion was recorded for the child. Moreover, due to close viral sequence homology between son and father suggests the possibility of HIV transmission from father. The paternal transmission of HIV could be at the time of fertilization as evident from in-vitro studies which showed that the sperm associated virus but not free virus enters into oocyte and the infected sperm can successfully fertilize oocyte (13).

The study results also supported by our earlier observation of presence of proviral DNA in spermatozoa and in some cases the viral load in blood was undetectable but showed the presence of proviral DNA in the sperm as well as detectable viral load in seminal plasma (20). It has also been reported that due to acidic pH in vagina the cell free virus in seminal plasma may have limited survival and may not be possibly responsible for transmission of HIV to fetus at the time of fertilization. However the sperm associated

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virus may likely transmit the virus either to female sexual partner or to the fetus at the time of fertilization.

The father of this child was tested positive by DNA PCR and Rapid Test and found seropositive while mother was detected to be seronegative using 4th Generation ELISA, DNA PCR and Rapid tests. The HIV infectivity of the mother was also tested twice by rapid test after delivery. The author's ruled out the possibility of HIV transmission due to unhygienic health care environment, blood transfusion, sexual abuse and accidental switching of babies and therefore concluded the possibility of HIV transmission from father. Further Struik et al., 2008 in UK also reported the five cases of HIV positive children of HIV negative mothers and one out of two children died within five months after birth. However the HIV testing of their father has not been reported.

Present study as per the guidelines of PPTCT programme only the mother was tested during pregnancy and found negative while the father's sero status remained unknown at the time of birth of the child. The results of the present study along with the earlier reports suggest the HIV transmission from father possibly at the time of fertilization. The study also suggests that the sperm washing may not always be the safe procedure. The results also suggest the need for appropriate perinatal testing of couples and post natal testing of their infants born. The study also suggests the need for revised strategies for prevention of paternal transmission of HIV.

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Conflict of Interest:
All the authors declare that they have no conflicts of interest.

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