

## 11. Studies on HIV/AIDS

### 11.1 Molecular Characterization of HIV-1 strains circulating in West Bengal

Investigator: Sekhar Chakrabarti

One of the most important routes of HIV-1 transmission worldwide is through sharing of needles and syringes among injecting drug users (IDUs) and ten percent of the HIV/AIDS cases globally, have been attributed to IDUs. As far it is reported, the number of IDUs estimated all over the world, is 13.2 million (0.3% of the estimated 4 billion adult population), the majority of which (10.3 million) live in the developing and transitional countries. HIV-1 subtype C has been found to be prevalent among IDUs of Manipur, one of the northeastern states of India. The presence of dual subtype infection as well as intersubtype recombination between subtype C and ThaiB have also been reported. IDU population from central Chennai (southern India) revealed 30% HIV prevalence and studies on the drug addict population from Orissa revealed a prevalence of 7% HIV infection. Majority of HIV-1 infection in West Bengal has been detected among the female sex workers and subtype C was found to be prevalent. Only 1 to 2% of HIV-1 infection among the IDU population had so far been detected from Calcutta, the capital city of West Bengal. Recent epidemiological study revealed, an occurrence of 11.8% (95% CI: 7.9-16.7) HIV-1 seropositivity among a group of Injecting Drug Users (IDUs) in Darjeeling, a hilly district in northern West Bengal. Most of the IDUs (75.3%) were found to use Brown Sugar followed by morphine and dextro-propoxyphen. Route of drug intake was predominantly intravenous or intramuscular route. In the present communication the molecular characterization of HIV-1 among the injecting drug users of West Bengal with respect to *env*, *gag* & *tat* genes has been studied.

Darjeeling is situated in the northern hilly region of West Bengal surrounded by Nepal, Bhutan and Bangladesh. Except Siliguri, rest of the district is situated on hilly terrain of mountain Himalaya. Siliguri, the only subdivision located on plain, serves as the gateway for Nepal, Bhutan, and Bangladesh as well as all north-eastern states and Sikkim. Therefore it is the gateway for drug trafficking in India through the various international boundaries. The IDU samples from Darjeeling were screened for HIV-1 seropositivity by an unlinked anonymous method. HIV was tested by ELISA (Immunogenetics, Belgium) followed by tridot assay as per policy of country's National AIDS Control Programme. 14 out of 118 samples (11.8%) were tested positive for HIV-1. Blood samples were collected in Na-citrate solution and peripheral blood mononuclear cells (PBMCs) were separated from whole blood by Ficoll-Hypaque gradient centrifugation. DNA was extracted by using the QIAamp DNA Blood Mini Kit 250 (QIAGEN, Germany) according to the manufacturer's protocol. Primer pairs used for *env* C2-V3 amplification were ED3, ED14 (1<sup>st</sup> round) and ED31, ED33 (2<sup>nd</sup> round) and that for *gag* p24-p7 amplification were H1G777, H1P202 (1<sup>st</sup> round) and H1gag1584, g17 (2<sup>nd</sup> round).

5 $\mu$ l of the amplicon of the unknown sample and 5 $\mu$ l of the reference amplicon in presence of 1.1 $\mu$ l of 10X annealing buffer (1M NaCl, 100Mm Tris-HCl [pH 7.8], 20Mm EDTA) for *env* gene. Similarly 4.5 $\mu$ l of the unknown and 4.5 $\mu$ l of each reference amplicon for the *gag* gene were mixed in presence of 1 $\mu$ l of 10X annealing buffer. It was then denatured at 94°C for 2 minutes followed by snap freezing in ice for renaturation to form heteroduplex molecules.

The fragments were then run on a 5% Polyacrylamide Gel (5% polyacrylamide for *env*-HMA; 5% Polyacrylamide / 20% Urea for *gag*-HMA) in 1X TBE buffer at 250V for about 2h 30 minutes. Based on the relative mobility of the heteroduplex molecules towards homoduplexes, subtypes were assigned accordingly. Heteroduplex molecules formed between the unknown sample and the most closely related subtype exhibited the fastest mobility. HMA analysis of Darjeeling IDUs showed that all the 14 samples belonged to subtype C for both *env* & *gag* genes.

Results of the HMA analysis were further confirmed by sequencing and phylogenetic analysis. Amplicons of the *env* & *gag* gene segments were purified by a QIA quick PCR purification kit (QIAGEN, Germany) and were subjected to cycle sequencing reactions using fluorescent dye-labeled dideoxy nucleotides in an ABI PRISM 3100 automated sequencer following the manufacturer's protocol. The sequences were edited using BIOEDIT sequence alignment editor program (version 5.0.6; Department of Microbiology, North Carolina State University) and were subsequently aligned with the reference sequences from different geographic regions available in the database (<http://www.hiv.lanl.gov/content/index>). All the nucleotide sequences obtained in this present study were screened using the BASIC BLAST program ([http://www.hiv.lanl.gov/basic\\_blast.html](http://www.hiv.lanl.gov/basic_blast.html)) to search for sequence similarities for previously reported sequences in the database, and to rule out potential laboratory errors. Each sequence was divergent from the previously published sequences in the database by more than 5%, suggesting an absence of sample mix-ups. Alignment was done using CLUSTAL W (version 1.4) multiple sequence alignment program. Evolutionary distances were measured by a Kimura two-parameter distance matrix method and phylogenetic tree was constructed by Neighbor-Joining (NJ) method using the Interior Branch Test of Phylogeny with 1000 bootstrap replicates, in MEGA version 2.1. Finally the tree was viewed and edited by the Tree Explorer in MEGA 2.1.

BLAST search and alignment study of the *env* and *gag* gene sequences of IDUs with respective reference sequences of different subtypes (A-K) of HIV-1 showed that the sequences belonged to subtype C of Indian origin. Further analyses were then done mainly with the reference IDU sequences from India (Manipur), neighbouring countries like Nepal (for *env* C2-V3 only, as found in the database), China, Myanmar belonging to subtype C or BC recombinant and also with outgroup reference IDU sequences of other subtypes from different regions of the world including sequences from Vietnam (AE recombinant), Thailand (subtype B and AE recombinant), Russia (subtype A and AB recombinant) and Spain (subtype G and BG recombinant) with the corresponding genomic regions. A few prototype sequences of heterosexual mode of transmission from India (subtype C) and Africa (subtype C and A) were also included in the analyses.

Phylogenetic analysis based on *env* C2-V3 region showed two main groups, one comprising of the subtype C strains from Darjeeling along with the C strains from Manipur, Myanmar, China and Nepal while the other included all outgroup (A, AE, B, AB, G and BG) subtype / subtype-recombinant reference sequences. IDU sequences from Darjeeling showed a better relatedness to the C strains from Manipur and formed a strong cluster with the Manipur IDU sequences (along with the Myanmar and Chinese IDUs) and were different from the Nepalese IDUs cluster. The mean interpopulational diversity calculated between the Darjeeling and Manipur IDU sequences was 1%; that between Darjeeling IDUs and Nepalese IDUs was 2% for the corresponding genomic region. Similarly, the *gag* p24-p7 phylogenetic analysis revealed that Darjeeling IDU sequences forms a strong cluster with the Manipur IDU strains. Outgroup IDU sequences from Russia, Thailand and Spain formed separate cluster from the

subtype C cluster as in *env*. The mean interpopulational diversity calculated between the Darjeeling and Manipur IDUs was 1% for the corresponding genomic region.

After assigning of the subtypes with respect to *env* and *gag* genes and their analysis, polymorphic variation of the *tat* regulatory gene was further studied among these Darjeeling IDU samples. The *tat* exon-1 (~216bp) was amplified by nested polymerase chain reaction (PCR) in a thermal cycler (Geneamp PCR system, 2700, Perkin Elmer) using primers TAT OF1: 5'ACAGGAGTCGAAGCT ATAATAAG 3' and TAT OR1: 5' TTCTATATATACTATGGTCCACACAATTAT 3' for the 1<sup>st</sup> round and TAT INF1 : 5' GACTACTGCAACAACACTACTGTTTAT 3', TAT INR1 : 5' ATT AATGCTACTACTATCAATGCTCCTACTCC 3' for the 2<sup>nd</sup> round. Conditions followed in the first round PCR was: 94°C for 15 min, 35 cycles consisting of 94°C - 30 sec, 52°C - 30 sec and 72°C for 1min, with a final extension at 72°C for 7 min. 1µl of the first round PCR product was used as a template for the second round amplification and the condition followed in the second round was: 94°C for 15 min, 35 cycles consisting of 94°C - 30 sec, 55°C - 30 sec, 72°C for 1 min; and a final extension at 72°C for 7min. Amplicons of the *tat* fragments were purified similarly using QIA quick PCR purification kit (QIAGEN, Germany) and were subjected to sequencing and phylogenetic analysis.

Alignment and phylogenetic analysis of the *tat* exon-1 region of Darjeeling IDUs with subtype C and other reference strains like subtype A, Thai-B, US-B, D along with the above mentioned IDU outgroup reference sequences available in the database (<http://www.hiv.lanl.gov/content/index>) clearly showed that samples belonged to subtype C. HIV-1 *tat* sequences of seropositive IDUs from India was not available in the database and hence, the reference panel of subtype C *tat* sequences included the strains from heterosexually transmitted population and general population in India. Darjeeling IDU *tat* sequences formed characteristic cluster among themselves and is close to the Myanmar and China IDU sequences along with the prototypic Indian and African C sequences. IDU outgroup sequences from Thailand, Russia, Spain formed separate groups from the C subtype cluster as shown previously. It would be interesting to continue further studies on other genomic regions of HIV-1 in this population in order to monitor possible emerging routes of HIV-1 transmission in India and to see whether any new strain is entering in this part of the country.

## **11.2 A study on biological markers of HIV-1 resistance conferring polymorphisms and their distribution in injecting drug users population of north-eastern states of India**

Investigator: K. Sarkar

This study was initiated with the following objectives:

- To find out the existence of mutant genes responsible for resistance to HIV-1 among IDUs and its distribution in north-eastern states of India
- To find out any association with these factors and HIV infection in IDUs
- To attempt to make an IDU cohort based on presence of mutant genes for future follow up if possible

The project was funded by ICMR as an extra-mural project of two-year duration. The project was just initiated by recruitment of staff, training of staff, standardization of laboratory

procedures. As per the proposal, samples would be collected from IDUs of Manipur, Meghalaya, Mizoram and Nagaland for studying the association between HIV and mutant genes in IDUs. A questionnaire for studying risk-behaviour and risk perception of IDUs has been developed and field-tested for using it in the field prior to sample collection. Till date a total 200 IDU blood samples are collected from IDUs of different districts of Manipur and samples are under process.

### 11.3 Sex trafficking, violence, negotiating skill and HIV infection in brothel-based sex workers of eastern India, adjoining Nepal, Bhutan & Bangladesh

Investigators: Kamalesh Sarkar, Baishali Bal and Sekhar Chakraborty

A study was conducted among brothel-based sex workers of West Bengal, eastern India. The objective was to understand the sex trafficking, violence, negotiating skill and HIV infection in them. It was a community-based cross-sectional study. A total of 580 sex workers from four districts' brothels participated in this. A pre-tested questionnaire was introduced to study their socio-demography, sex-trafficking, violence and negotiating skill. About 4–5 ml. blood sample was collected from each of them using unlinked anonymous method to study their HIV status. Data was edited and entered in computer using Epi-Info software. Both univariate as well as multivariate analysis were done to find out any association between HIV and relevant risk factors.

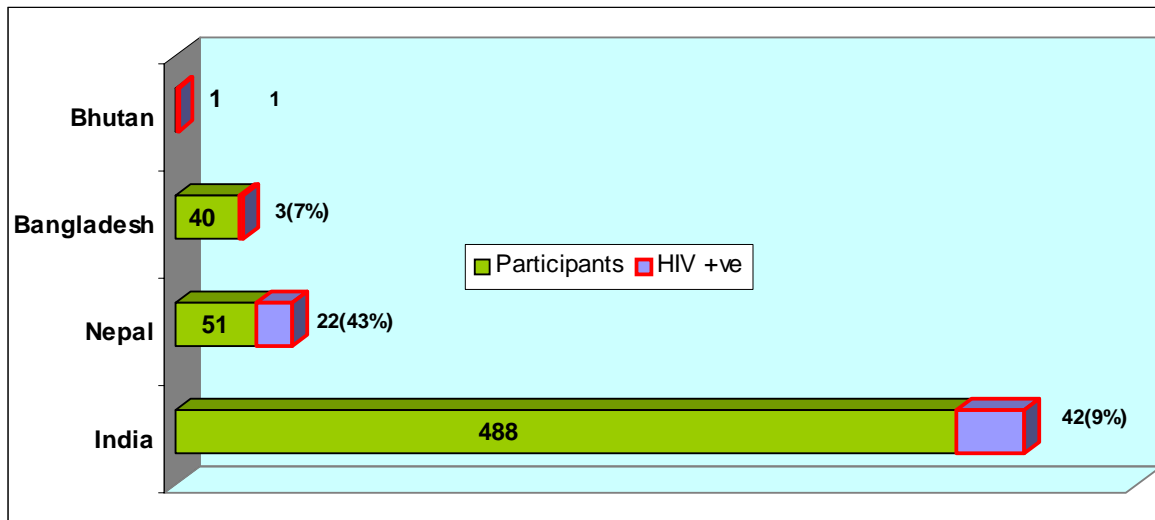
**Fig.11.3.1: The studied districts of West Bengal with its location in India (inset)**



**Results:**

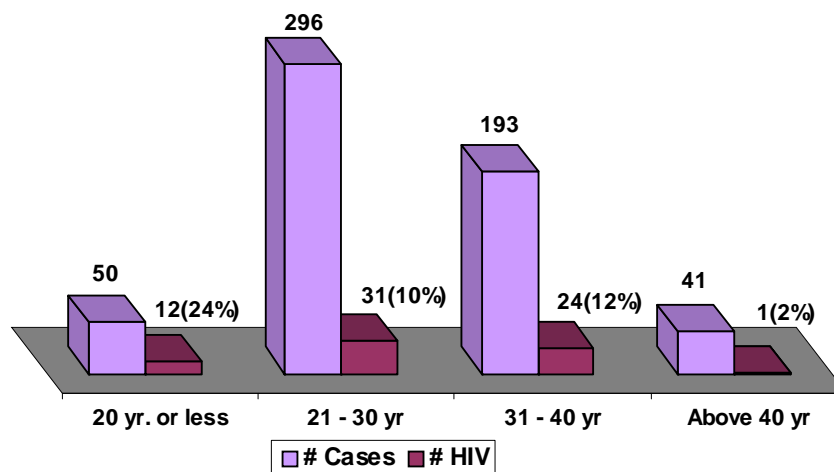
A total of 580 sex workers from 4 different district brothels of West Bengal participated in this study. Of them, 488 were from India, 51 (9%) from Nepal, 40 (7%) from Bangladesh and 1 participant was from Bhutan as their country of origin. Overall HIV sero-prevalence rate was found to be 12% (n=68) among the study participants. Figure-2 shows the study participants with their country of origin and corresponding HIV sero-prevalence in them.

**Fig.10.3.2: Country-wise distribution of studied sex workers with HIV status [N=580]**



From above figure, it is evident that sex workers coming from Nepal had HIV sero-prevalence rate of 43%. Sex workers of Indian origin had a HIV sero-prevalence of 9% and that of Bangladesh had the same of 7%. Only one sex worker from Bhutan participated in this study and was found to be infected with HIV.

**Fig.10.3.4: Age distribution of studied sex workers with corresponding HIV status [N=580]**



The study revealed that a sizable number of study participants were from Nepal (9%) and Bangladesh (7%). HIV sero-prevalence was strikingly higher among Nepalese (43%) compared to Bangladeshis (7%) and Indians (9%). Almost 1 in every 4-sex workers (24%) was found to have joined this profession by being trafficked. Violence at the beginning of this profession was found to be more among the trafficked victims including those sold by their family members (57%) compared to those joined voluntarily (15%). Overall condom negotiation rate with most recent 2 clients was found to be 38%. HIV was found to be significantly associated with sexual violence by multivariate analysis (OR = 2.3; 95% CI: 1.2 – 4.5).

The study has documented that trafficked victims face violence including sexual violence to a greater magnitude and sexual violence is associated with acquiring HIV in them. There is a need for an in-depth study to understand the problem of trafficking & its consequences.

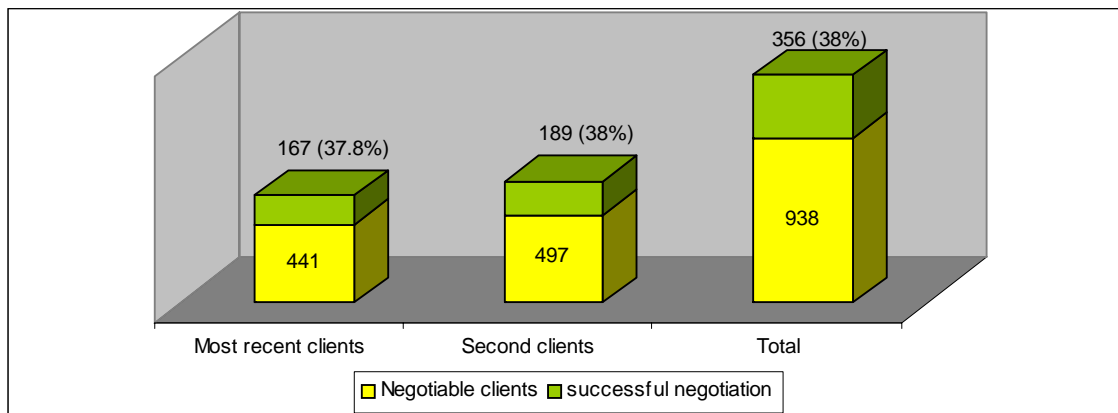
The study shows that the violence was faced more by the trafficked sex workers including those sold by their families (57%) compared to those joined voluntarily (15%). HIV sero-prevalence was found to be 13% (n=24) among sex workers who were trafficked including those sold by their families compared to those joined voluntarily (10%; n=40).

**Table-11.3.1: Violence and trafficked victims**

	<b>Violence</b>	<b>Trafficked victims including sold by family member/s</b>	<b>Joined voluntarily</b>	<b>OR with CI at 95% level</b>
1	Suffered from violence	105	61	7.4 (4.8 – 11.3)
2	Didn't suffer from violence	78	336	

Regarding assessment of negotiating skill, sex workers were asked on condom use with their most recent 2 clients. If the response was yes with any one of them, the respondent was asked again whether it was with or without negotiation as some clients were found to use condom voluntarily and did not require any negotiation. A total of 566 participants responded to this question. A total of 292 and 258 sexual acts took place with condoms with client no. 1 (most recent) and client no. 2 respectively. Out of 292 sexual acts using condoms with first clients, 125 clients used condoms voluntarily by themselves and did not require any negotiation. So, actual scope of negotiation for condom use with first clients was = 566 – 125 = 441. Out of these 441 clients, who didn't have any plan to use condoms, the sex workers negotiated successfully with 167 clients. This gives rise to a rate of 38% (167 out of 441) as their successful condom negotiation rate. In this way, the rate was 38% with their 2<sup>nd</sup> clients. So, overall successful condom negotiation rate was found to be 38% (rounded off) among the studied participants as shown in figure-4.

**Fig.11.3.4: Negotiated condom use rates with most recent two clients (n=566)**



## 12. Activities of NACO National Reference Laboratory at NICED, Kolkata

National AIDS Control Organization (NACO) of Ministry of Health and Family Welfare, Government of India funds the National HIV Reference Laboratory of the Institute since 1992. The activities of the reference center comprises of (1) Sero-surveillance for HIV infection. (2) Confirmation of serum samples received from different surveillance and zonal blood testing centers located in different states of Eastern India. (3) Training man-power (Doctors and Medical Laboratory Technologists etc.) for HIV surveillance and laboratory diagnosis of HIV infection as and when requested by Institute of Serology, Govt. of India, State Health authorities, Hospitals etc. and (4) HIV, HCV, HBV kit evaluation for West Bengal State AIDS Prevention and Control Society.

Between April 2006 and March 2007 a total of 2962 serum samples were screened by highly sensitive ELISA and positive samples were confirmed by either highly specific ELISA or Western mark.

## NACO National Reference Laboratory

Division of Virology,  
National Institute of Cholera & Enteric Diseases, Kolkata- 700 010  
Sample screened for Human Immunodeficiency Virus (HIV)  
Antibody by ELISA, Rapid and/or Confirmatory Test  
From 1<sup>st</sup> April, 2006 to 31<sup>st</sup> March, 2007.

Source of Samples	No. of Tested	No. of Positive
<b>A. WEST BENGAL: -</b>		
1. Drug Users	35	07
2. Eastern Command Hospital	04	Nil
3. Foreigners	02	Nil
4. People with High Risk Behavior	2815	326
5. Patients from Hospitals	70	19
6. Miscellaneous	08	02
<b>Sub Total</b>	<b>2934</b>	<b>354</b>
<b>B. OTHER STATES: -</b>		
1. Meghalaya	07	07
2. Jharkhand	21	17
<b>Sub Total</b>	<b>28</b>	<b>24</b>
<b>GRAND TOTAL</b>	<b>2962</b>	<b>378</b>

1) **Sentinel Surveillance:** Sentinel surveillance was organized by West Bengal State AIDS Prevention and Control Society among the commercial sex workers, STD patients and Men sex with Men etc. of West Bengal. We screened 1250 samples for HIV, VDRL from Aug 2006 to November 2006 and the results (Positive individuals were 3.92% & 3.36% for HIV & VDRL respectively) of the same were communicated to the Project Director West Bengal AIDS Prevention & Control Society, Kolkata.

2) **Kit Evaluation:** In this year, West Bengal AIDS Control & Prevention Society was entrusted by NACO to procure diagnostic kits for detection of HIV, HBsAg, HCV antibody in serum by ELISA and Rapid tests. We evaluated 15 nos. of HIV kits, 8 nos. of HBsAg kits, 13 nos. of HCV kits and 7 nos. of RPR kits for detection of antibody by ELISA and Rapid tests from time to time for West Bengal State AIDS Prevention and Control Society.

3) **NACO External Quality Assessment Scheme (EQAS) for HIV Testing:**  
The serum samples for HIV testing under EQAS Programme were received from different states viz. Assam, Meghalaya, Orissa and Andaman & Nicobar Islands and the results of the same were communicated to NACO and respective State AIDS Control Society.

This year we visited four State Reference Laboratories of Guwahati Medical College & Hospital, Assam, Pasteur Institute, Shillong, Meghalaya, G.B. Pant Hospital, Port Blair, Andaman & Nicobar Islands and Central Hospital, Red Cross Blood Bank, Bhubaneswar, Orissa. We arranged the EQAS Training Programme for Doctors and Technicians.

As per the responsibility of National AIDS Control Organization (NACO) National Reference Laboratory (NRL) at NICED We organized EQAS workshops and hands on training at the State Reference Labs (SRL) for HIV testing and panel sera preparation for the states of Andaman & Nicobar Islands, Assam, Meghalaya & Orissa

**EQAS** Workshop and panel sera preparation for evaluation of performance of the HIV testing laboratories were conducted as following States:

- 1) **Assam:**  
During 18<sup>th</sup> -19<sup>th</sup> January 2007 at Microbiology Department. Guwahati Medical College and Hospital.
- 2) **Meghalaya:**  
During 1<sup>st</sup> – 2<sup>nd</sup> February 2007 at Blood Bank, Pasteur Institute Shillong,
- 3) **Andaman and Nicobar Islands:**  
During 26<sup>th</sup> – 27<sup>th</sup> February 2007 at G B Pant Hospital, Port Blair.
- 4) **Orissa:**  
During 7<sup>th</sup> - 8<sup>th</sup> March 2007 at Capital Hospital Blood Bank, Bhubaneswar
- 5) **EQAS & Panel Sera preparation workshop for all SRLs of the states (A&N Islands, Assam, Meghalaya and Orissa)** conducted at NACO-NRL, Virology Division, NICED on 27<sup>th</sup> – 28<sup>th</sup> March 2007. Professors, Asst. Professor of Department of Microbiology of Medical Colleges of Orissa and Assam, In charge of State Reference Laboratory of Meghalaya and Technicians were trained on EQAS in HIV Testing.
- 4) **Training of Laboratory Technician:** Thirty four Laboratory Technicians were trained who attended the workshops from different hospitals from different states for laboratory detection of HIV antibody along with a lecture on principles and operation of above tests. The training programme (one in each month) was organized at the request of Institute of Serology, Govt. of India.