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Activities of National HIV Reference Centre of NICED, Kolkata

Investigator :

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National AIDS Control Organization (NACO) of Ministry of Health and Family Welfare, Government of India funds the National HIV Reference Centre 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 about) 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 2004 and March 2005 a total of 1202 serum samples were screened by highly sensitive ELISA and positive samples were confirmed by either highly specific ELISA or Western Blot.

National HIV Reference Centre

Division of Virology,

National Institute of Cholera & Enteric Diseases,
Kolkata- 700 010Sample screened for Human Immunodeficiency Virus
(HIV)

Antibody by ELISA and/or Confirmatory Test

From 1st April, 2004 to 31st March, 2005.

Source of Samples	No.of Tested	No.of Positive
A. WEST BENGAL: -		
1. Antenatal Mother	Nil	Nil
2. Blood Donor	Nil	Nil
3. Border Security Force	Nil	Nil
4. Drug Users	Nil	Nil
5. Eastern Command Hospital	127	126
6. Foreigners	Nil	Nil
7. People with High Risk Behavior	1069	285
8. Patient with Blood diseases	Nil	Nil
9. Miscellaneous	001	Nil
Sub Total	1197	411
B. OTHER STATES : -		
1. Meghalaya	02	02
2. Jharkhand	02	02
3. Orissa	01	Nil
Sub Total	05	04
GRAND TOTAL	1202	415

1) **Sentinel Surveillance:** West Bengal State AIDS Prevention and Control Society organized Sentinel Surveillance among the high risk groups such as Commercial Sex Workers (SW), Truck Drivers and Men those who had sex with Men etc. of West Bengal. We screened 2000 samples for HIV, VDRL from July 2004 to November 2004 and the results (Positive individuals were 9% & 11% for





HIV & VDRL respectively) of the same were communicated to the Director, West Bengal AIDS Prevention & Control Society, Kolkata.

2) Kit Evaluation : In recent year, West Bengal AIDS Control & Prevention Society was entrusted by NACO to procure diagnostic kits for detection of HIV, HBsAg, HCV antibody by ELISA and Spot tests. We evaluated 40 HIV kits, 16 HBsAg kits, and 10 HCV kits for detection of antibody by ELISA and Spot test from time to time for West Bengal State AIDS Prevention and Control Society.

3) EQAS Programme of NACO : The serum samples for HIV testing under EQAS Programme were received from different states of Eastern India including Andaman and Nicobar Islands and the results of the same were communicated to NACO and respective laboratory.

We also organized an EQAS training programme, which was held on 15th & 16th February 2005. Professors, Associate Professor of Department of Microbiology of seven Medical Colleges of West Bengal and In-charge of State Regional HIV Reference Centres of Assam were trained on EQAS in HIV Testing.

4) Training of Laboratory Technician : We trained 50 Laboratory Technicians from different hospitals from different states of Eastern India on 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.

Polymorphism of HIV-1 gag (p17) gene detected from female sex workers in Calcutta, India.

Investigator :

S. Chakrabarti

The human immunodeficiency virus type 1 (HIV-1) matrix (MA) protein p17 is a multifunctional protein that is pro-

duced by posttranslational modifications from the precursor Pr55 Gag. The processed matrix protein forms the outer protein core of HIV. It is involved in the early stages of viral replication and helps in transport of unspliced viral RNA to the plasma membrane. Proper assembly of the viral DNA-preintegration complex into the host nucleus involves the role played by the matrix domain. HIV-1 p17 causes increased production of TNF-alpha and IFN-gamma, which might be a mechanism for the virus for its replication. HIV-1 evolves and mutates giving rise to a wide range of subtypes, which varies in different geographic regions worldwide. A detailed genetic analysis of the p17 gag gene isolated from HIV positive female sex workers in Calcutta, India is presented in this report.

An unlinked anonymous study was carried out among female sex workers in Calcutta. The competent authorities ethically approved this study. Blood samples were collected in Na-citrate solution after obtaining the informed consent and pre- and post-test counseling. HIV-1 seropositivity was determined by rapid spot test (Immunocomb HIV-1/2 Bi-spot, Organics, Israel), followed by ELISA (Immunogenetics, Belgium) and line immunoassay (Inno-LIA, HIV-1/HIV-2). Out of 185 blood samples screened for the presence of antibodies for HIV-1, 22 (11.8 %) samples were positive and were used for further study. All of them were heterosexually transmitted female sex-workers between an age group of 20-30 and were naïve in respect to anti-retroviral therapies. Many of them were clinically asymptomatic while others showed some features like fever, diarrhoea, tuberculosis, skin rash, genital ulcer etc. Peripheral blood mononuclear cells (PBMCs) were separated from whole blood by Ficoll-Hypaque gradient centrifugation and the DNA was extracted by using the QIAamp DNA Blood Mini Kit 250 (QIAGEN, Germany) according to the manufacturer's protocol. The DNA fragment comprising of full-length p17 along with the part of p24 was amplified by nested polymerase chain reaction (PCR) in a thermal cycler (Geneamp PCR system, 2400, Perkin Elmer). PCR





amplicons were purified by a QIAGEN PCR purification kit (QIAGEN, Germany) and the purified products were subjected to cycle sequencing reactions in both directions using fluorescent dye-labeled dideoxy nucleotides in an ABI PRISM 310 automated sequencer following the manufacturer's protocol. Sequences were submitted to Genbank and the accession numbers were assigned (Fig.14.1).

The p17 gene sequences were edited by using the BioEdit sequence alignment editor program (version 5.0.6.; Department of Microbiology, North Carolina State University [<http://www.mbio.ncsu.edu/BioEdit/BioDoc.pdf>]) and were subsequently analyzed on the BASIC BLAST program (http://www.hiv.lanl.gov/basic_blast.html) which revealed a close relatedness to subtype C. About 5% divergence of each sequence with that of the other in the database was indicative of the absence of sample mix-ups or laboratory error. All the sequences were then aligned with a reference panel of reported sequences and/or related sequences of strains isolated from different geographic regions available in the HIV sequence database (<http://www.hiv.lanl.gov/content/index>) provided by the Los Alamos National Laboratory, operated by the University of California to generate the nucleotide substitution pattern among them. The reference panel included thirty-eight sequences of different global strains with respect to the same p17 region of HIV-1 consisting of all subtypes (A-K). Two to four sequences of each reference subtype (A,B,D,F,I,G,H,J,K) were taken into account while subtype C reference sequences were considered as the majority of the panel as previous data from blast analysis showed the strains from Calcutta to be more close to subtype C. The multiple alignments were done by the Clustal W program. Phylogenetic and molecular evolutionary analyses were conducted using MEGA version 2.1. Evolutionary distances were measured by a Kimura two-parameter distance matrix method. Mean genetic diversity between the strains from Calcutta and other subtype C global strains was 7.8% and among the Calcutta samples was found to be 6.4%,

ranging from 2.2% to 13.1%. A phylogenetic tree was constructed by Neighbor-joining (NJ) method using the Interior Branch Test of Phylogeny - a t-test which is computed using the bootstrap procedure. Bootstrapping was done for 1000 replicates and finally the tree was viewed and edited by the Tree Explorer in MEGA 2.1. Figure 14.1 shows the phylogenetic tree generated for all 60 strains (Calcutta and others). Confidence probability values above 50% had been given only. The sequences from Calcutta were found to be within the subtype C cluster and more often with the Indian C types. Sample cal1017 was close to the C strain from Myanmar while cal908 and calD51 were more close to the Chinese C strain. However, two samples, cal709 and cal242 were found to be residing far from the Indian and other Calcutta strains and showed a better relatedness to the non-Indian C strains from south-central and east Africa, south America, Middle East etc.

Another phylogenetic analysis was done among HIV-1 subtype C strains only, to see the distribution pattern of the Calcutta strains with those from different regions of India and also from other parts of the world (Fig.14.2). A total of thirty-two Indian C sequences and twenty-eight global C sequences other than India were taken for comparison based on the p17 gene. Majority of the non-Indian C strains were from Zambia and South Africa along with a few from Tanzania, Angola, Brazil, Israel, Bangladesh, China, Myanmar etc as were available in the database. The Indian sequences were subdivided according to their regional origin within the country. Sixteen sequences from western India, four from the southern part and twelve sequences were taken from the northern region of India. Samples from Calcutta represented eastern India. Clustal W multiple sequence alignment program was used and a phylogenetic tree was computed using the NJ method by MEGA version 2.1. The radial pattern of the tree was viewed for better observation. Two main clusters were formed. Cluster-1, as depicted in the figure, showed the gathering of non-Asian strains whereas the Indian strains





along with a few Asian countries constituted cluster-2. The Indian sequences (cluster-2) were found to belong separately from the global C strains (cluster-1), except for three sequences that were from the close neighbouring countries of India like Bangladesh (C.BD_01P1gag), Myanmar (C.MM.mIDU11) and China (C.CN98003). Three small subclusters were formed among the Indian strains and they did not follow the regional separation, rather a discrete distribution was observed. The Calcutta-strains did not form any strong monophyletic subcluster within the Indian strains but majority of them were dispersedly distributed among the strains from different regions of India. Interestingly, two samples, cal242 and cal709 were seen to be residing away from the Indian C strains but among the African strains within cluster-1.

Legends to figures :

Fig. 14.1. Phylogenetic analysis of HIV-1 p17 gene from 22 female sex workers in Calcutta, India. The construction of the tree was described in text. Samples from Calcutta are designated as 'cal'. Reference isolates of different subtypes used are - A: 98HM8ZA, BABMNT, 00ZM4750; B: mIDU1, HXB2, HCM312, AD87; C: 93IN101, 95IN21068, mIDU11, 93IN9999, IN98011, CN98003, 01AOSNS09, 96BW01B03, 96BW01, 93ZA030, 98BR004, BR97001, IS98001, IS98003, 93ZA036_he, 00ZM4719, 98IS002, 98TZ017; D: KEM389clone37, 99CM4ZA, GER; F1: 93BR020_1, BZ163B; G: SE6165, 98ZAM507ZA; H: V1557, OSA_MT; J: 01AOHDC236, SE7887; K: EQTB11C, V1325. Genbank Accession numbers of the Calcutta sequences are- AF491232 (cal126); AF491233 (cal202); AF491234 (cal242); AF491235 (cal250); AF491236 (cal352); AF491237 (cal441); AF491238



Vaccine supply system.





(cal525); AF491239 (cal554); AF491240 (cal709); AF491241 (cal759); AF491242 (cal1060); AF491243 (calD51); AF491244 (cal998); AF491245 (cal1007); AF491246 (cal1058); AF491247 (cal1017); AF491248 (cal908); AF491249 (cal1094); AF491250 (cal462); AF491251 (cal760); AF491252 (cal145); and AF491253 (cal1056).

Fig. 14.2. Phylogenetic study of p17 gene sequences of 22 HIV-1 seropositive strains from Calcutta, the eastern region of India with 32 C-strains from other regions of India and 28 non-Indian C-strains available globally. Different coloured symbols have been used before the isolate names to recognize the origin of the strains and the specification of each symbol has been given in the figure. Samples from Calcutta are designated as 'cal'. Genbank Accession numbers of the Calcutta sequences are same as given in the legend to Fig.1. Different isolates taken for the study are-Non-Asian countries: 00ZM4740, IN80496, IN50561, IN70177, IN60161, IN50950, IN50823, IN50581, IN50322, IN5081300ZM4800, 93ZA035, 93ZA201_he, 93ZA029_he, 93ZA040_he, 00ZM4781, 00ZM4795, 00ZM4796, 00ZM4739, 00ZM4783, 98IS002, 00ZM4725, 00ZM4737, 00ZM4719, 94ZA422, 93ZA036_he, 93ZA205, 98BR004, BR97001_P1, 93ZA207_ve, 98TZ017, 01AOSNS03, 00ZM4779, 00ZM4810; Other Asian Countries: mIDU11, CN98003_P1, BD_01P1gag; Northern India: IN60080, IN50437, IN49670; Western India: NARI_GAG_1, NARI_GAG_2, NARI_GAG_3, NARI_GAG_4, NARI_GAG_5, NARI_GAG_6, IN301904, IN21068, IN98014, IN301905, IN11246, IN301999, IN565.10, IN565.11, IN565.13, IN565.14; Southern India: IN97009_P1, IN98006_P1, IN97005_P1, IN98008_P1.



