12. Studies on HIV/AIDS

12.1 Study on sexually transmitted infections among female sex workers of West Bengal

The project was undertaken to study the magnitude of problem of STIs (HIV, HBV & Syphilis) among female sex workers of West Bengal and to study the factors associated with HIV infection in sex workers.

The districts of West Bengal have been categorized into high, medium & low prevalent districts based on rate sex workers per lakh population in a particular district. Two districts, each from high, medium & low prevalent category have been selected randomly for studying these sex workers. Sample size of sex workers in each selected district has been calculated using stat-calc programme of Epi-Info software, which are as follows:

1. High prevalent districts - Kolkata & Darjeeling with sample sizes of 604 & 696 respectively
2. Medium prevalent districts - 24 Parganas (N) & Midnapore (East) with sample sizes of 693 & 872 respectively
3. Low prevalent districts - Nadia & Murshidabad with sample sizes of 356 & 417 respectively

Sex workers of Kolkata & East Midnapore district were approached till date. A total of 420 and 806 blood samples were obtained from sex workers of Kolkata & Midnapore district till date. About 3 ml of blood sample was collected from each study subject by unlinked anonymous method. About 20% of the study subjects was interviewed on their risk behaviour and other factors associated with HIV infection. Samples are under process. Study is in progress.
12.2 Detection and Characterization of HIV-2 in Calcutta, India.

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AIDS pandemic is spreading unchecked in almost every parts of the world. Currently more than 34 million individuals are infected with human immunodeficiency virus (HIV). Although majority of infections are due to HIV Type-1 (HIV-1) strains, a significant number of cases with HIV Type-2 (HIV-2) infections has been reported in many countries. HIV Type-2 was first identified in 1986 in West Africa and subsequently from other places. A second epidemiological pattern of HIV-2 infection has been suggested from reports of HIV-2 in Portugal which accounts for around 10%-13% of total HIV infections and 4.5% of AIDS cases (UNAIDS/WHO, 1998) and also in Germany, Spain, Angola, Mozambique and Brazil. The prevalence of HIV-2 is a growing concern in certain parts of Europe and in Southwestern parts of India. In India 3.5 million people are HIV seropositive as estimated by National AIDS Control Organization. The presence of HIV-2 was reported from different parts of India and in most of the cases HIV-2 infection was associated with HIV-1 infection also. However, isolation and neutralisation of two HIV-2 isolates from Pune has been also reported in 1999. Since the beginning of the national serosurveillance of HIV in the eastern part of India, only HIV-1 infection has so far been reported. During a recent survey for HIV among the female sex workers and their clients, five cases with single HIV-2 infection were found. Detail genetic analysis of one of the HIV-2 cases is being reported here.

The subject was a male client of 29 years and has history of sexual relations with sex workers for 6 years. He was tested negative for Syphilis (serologically) that was undertaken as part of investigation. He was receiving
recurrent attack of loose motion for last couple of months. Then he suffered from blood dysentery associated with waist pain and was relieved of his symptoms after a course of antibiotic. Serum of the patient was tested for HIV infection by a Rapid Spot Test (ImmunocombHIV-1/2 Bi-spot, Organics; Israel), ELISA (Immunogenetics, Belgium) and Line Immunassay (Inno-LIA HIV-1/HIV-2). Further the presence of HIV-2 antibody was confirmed by Western blot analysis (Immunetics Qualicode HIV-1/2 Western Blot).

Peripheral Blood Mononuclear Cells (PBMCs) were separated from the whole blood by Ficoll-Hypaque gradient centrifugation. DNA was extracted from PBMC. Full-length envelope gene (2.5 Kb) was amplified from PBMC DNA by nested PCR and then cloned in PUC-18 vector.

Full length envelope clone was then subjected to cycle sequencing using M13 forward and reverse primer along with several HIV-2 envelope gene specific primers using fluorescent dye labeled dideoxy-nucleotides in an ABI prism 310 automated sequencer and was submitted to Gene bank (accession no AY309063).

The nucleotide sequence of the clone was aligned with all the HIV-2 reference sequences from database and a phylogenetic tree was constructed to show the evolutionary relationship of our sequence with all the global by the neighbor-joining method with 1000 bootstrap replicates (Fig. 12.2.1).

Phylogenetic tree analysis based upon full length envelope gene of CAL1HIV-2 strain (Acc. no. AY309063) and all HIV-2 isolates reported worldwide reveals that CAL1HIV-2 is closely related to HIV-2 Rod and Rodx strains, isolated from offshore Senegal. There is also a close relatedness with HIV-2/Cam sequence, isolated from Guinea Bissau, a former Portuguese colony. Phylogenetic tree analysis also included 1.5 Kb of gp120 region of env gene of HIV-2 strains (Acc.no. HIV2U07104, HIV2U07106, HIV2U07107, HIV2U07108) isolated from Mumbai. This software analysis placed CAL1HIV-2 strain in a monophyletic cluster with closest similarity to U07107. To ascertain the degree of diversity of CAL1HIV-2 with other four reference strains pair wise distance matrices were also calculated.

Relationship to previously reported Indian HIV-2 env gene sequences and the degree to which these sequences represent variants circulating in India were studied in detail through several software analysis. As there was no previously reported full-length envelope gene sequence (2.5 Kb) of Indian HIV-2 isolates in database, construction of Phylogenetic tree and Similarity plot included only 1.5 Kb region of env gene of HIV-2 strains previously reported from India by other groups.

Nucleotide sequence analysis of gp120 region of HIV-2 env gene revealed that overall genetic distance between Indian HIV-2 strains was 6.9% to 14.3% while the mean genetic distance of CAL1HIV-2 from other four reference strain was estimated to be 13.5% (range, 12.4 to 14.3%). Within this region of env gene, the mean interpatient nucleotide sequence divergence estimated from the four reference HIV-2 strains isolated from Mumbai was 8.2% (range, 6.9 to 9.1%). This mean pairwise distance within the HIV-2 strains isolated from Mumbai is small enough for the strains to consider as being derived from a common ancestor. This clearly demonstrates that percent diversity of CAL1HIV-2 sequence is more than other Indian HIV-2 strains reported previously.

Similarity plot (Fig. 12.2.2) shows that CAL1HIV2 sequence differs to a large extent from other HIV-2 sequences isolated from Calcutta, mainly in 200-500 basepair and 1200-1400 basepair region and maximum similarity of
95% is found at 190-200 basepair region. Predicted amino acid sequences of 1.5Kb region of env gene of all Indian HIV-2 isolates were subjected to IdPlot identity plotter analysis program. Examination of the corresponding amino acid sequences of Indian HIV-2 strains revealed the presence of only 7 conserved amino acids Methionine (M) at 138, Aspartic acid (D) at 149, Isoleucine (I) at 159 and 409, Asparagine (N) at 165, and Arginine (R) at 412 and 417 positions. This result shows that gp 120 region of Cal1HIV-2 envelope gene varies significantly from HIV-2 Mumbai isolates at amino acid level also.

Isolation & cross-neutralization of two HIV-2 strains from Pune with Senegal 6F and 8F sera indicated that the isolates might have some relation to Senegal strain. Genetic analysis of only 1.5 Kb region of HIV-2 envelope gene of four Mumbai strains revealed close relationship to HIV-2 Rod isolate. But the striking feature is that though Cal1HIV-2 isolated from Calcutta showed close relatedness to HIV-2 Rod, a significant divergence at 200-500 base pair and 1200-1400 base pair region has been found compared to the HIV-2 strains isolated from Mumbai. In this context, it is to be mentioned that HIV-1 strains isolated from Calcutta also differ significantly from those of other states. The sequence variations of 1.5 Kb envelope gene of CAL1HIV-2 with other Indian HIV-2 strains and corresponding changes at amino acid level reflected the possible variations in physico-chemical properties of the env protein of Cal1HIV-2 strain. Our results are in agreement with previous observation, which have suggested that highly variable domains of gp120 are responsible for pathogenesis of HIV-2. In addition to this the existence of seven amino acids in the external envelope glycoprotein which were conserved in most of the Indian HIV-2 strains may define these amino acids as sites of potential functional importance for the spread of the virus. Abundance of Proline residue in gp120 region of Cal1HIV-2 strain might have some role in folding of the protein and binding with the cellular receptors. Above all, these changes can alter the antigenic properties of the Cal1HIV-2 strain, which might have evolved as immunologically important strain. Work is in progress to express the gene and study the envelope protein to establish the hypothesis.
Fig. 12.2.1 Phylogenetic analysis of 2500 base pair total envelope gene sequence of a HIV-2 strain isolated from Calcutta. Sample from Calcutta is designated as Cal1HIV-2. Indian HIV-2 strains previously isolated from Mumbai are IND102, IND766, IND868, IND808.

Fig. 12.2.2 Plots of similarity (generated by Simplot) of a set of reference sequences to the Cal1HIV-2.