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HIV LONG TERM NON DISEASE PROGRESSORS AND HIGHLY EXPOSED PERSISTENTLY SERONEGATIVE POPULATIONS OF WARANGAL, INDIA

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In India, 4 million people have been infected with AIDS disease. Nearly 1% of the adult population in India is infected with HIV. Extraordinary burden of HIV-related morbidity and mortality lies ahead in India. In Andhra Pradesh about 4-5 Lakh people are infected so far, which accounts 20.60% in STD clinics and above 1% in general population. In Warangal district HIV/ AIDS victimize 40.4% of STD and 6.25% of general population during 2002. Every day 5-10 new HIV cases are recorded on an average in various government & private hospitals in Warangal district. This study identifies HIV highly exposed persistently seronegative and non disease progressors in the south Indian set up. Study design includes Pregnant women and their husbands, sero negative babies who were born to seropositive parents of maternity hospitals, serodiscordent couples of the STD clinic & VCTC center, full blown AIDS patients and their non disease spouses in HIV hospital were studied for their stage of infection and highly exposed but persistently seronegative individuals. During 1996 to March 2006, serodiscordant couples and seropositive patients of various hospitals who returned to collect the report of their blood test for HIV were encouraged to enroll in this prospective study. HIV infection and manifestations were recorded with the help of clinicians. HIV seropositivity was confirmed by three individual tests : (1) HIV TRIDOT, (2) COMB AIDS or EIA (Enzyme immuno assay) and (3) NEVA TEST. Finally sero states are confirmed by Western blot. This study was conducted in maternity, civil and HIV hospitals in and around Warangal, Andhra Pradesh, South India. According to World Health Organization clinical manifestations classification staging system 60 HIV long-term non-disease progressors and highly exposed persistently seronegative subjects were found out of 1021 HIV discordant couples and other positive subjects. These epidemiological results of our investigation suggesting an association between humoral and cellular responses and HLA (human leucocyte antigen) of these highly exposed persjstently seronegative subjects and non diseases progressors are very important observation for future epidemic.

Key words : General population, HEPS, serodiscordant couples, humoral, cellular responses and HLA.

INTRODUCTION

India has become epicenter of AIDS pandemic. Globally an estimated 65 million people had been infected with HIV, 25 million had died and 40 million living with HIV/AIDS. Every day 14000 people are infecting worldwide.(AIDS epidemic update December, 2004: UNAIDS/WHO). HIV/AIDS pandemic has devasted many countries reversing national development, widening the gap between rich and poor and pushing the already stigmatized group close to the margins of society. HIV was not seen in Asia and India till 1980. Now India has become epicenter of AIDS pandemic. Presently 3.82 to 4.58 millions are infected in India. In Maharashtra, Tamilnadu, Karnataka, Andhra Pradesh, Nagaland and Manipur infection rate is over 1% in antenatal clinics(National AIDS Control Organization 2003; Bulletin). In Andhra Pradesh 4 lakh people are infected with HIV. The most prevalent

district in A.P. is Warangal. According to 2002 ANC cases highest positive infection rate is 6.75%. In STD clinic 40.40% is found (Andhra Pradesh AIDS Control Society 2003. Bulletin) 3.20% of seropositivity is found in Warangal during 2003-2004 (Radhakrishna *et al.*, 2005). UNAIDS/ WHO warned that by 2010 India faces 20 million HIV positive people. Nearly 20 years after the virus was discovered, we still have no vaccine, no cure and no therapies. HIV is one of the most deadly viruses that human race has faced. In central African pigmies (Rowland-Jones, 1998), Gambian (Lin *et al.*, 1996), Kenyan sex workers (Rowland-Jones, 1998) and Caucasians (Samson *et al.*, 1996), a few individuals seem to have conquered the virus. These people are with HIV positive for the last 15 years but have not fallen sick and their blood CD4 counts are also good.

MATERIALS AND METHODS

First HIV case was recorded in Warangal in 1996 at a private hospital. Complete data on HIV positive subjects and their spouses, children from the various private hospitals before 2002 was obtained. In 2002 MTCT and VCTC centers were established in Warangal hospitals. Pregnant women from rural and urban areas visit maternity hospital for regular check up from 3rd month till delivery, when they visit, they would be screened for HIV antibody with their informed consent in the hospital settings, HIV/AIDS testing lab and counseling room were well established. Pre test counseling and post test counseling were performed. During April 2002 to January 2006 HIV positive pregnant mothers and their spouses were encouraged to enroll for prospective study. Uninfected babies of HIV positive parents are also included in the prospective study.

Patients attending the various STD clinics & VCTC center at State Government Mahatma Gandhi Memorial Hospital in Warangal, A.P. India, were screened for HIV antibody during April 2002 to January 2006. Serodiscordant couples were enrolled for prospective study with their informed consent. We recorded 40 epidemiological case studies of serodiscordant couples and their children in the prospective study. Cause of death was recorded. The first author at hospital during the year April 2002 to January 2006 and interacted with the HIV/AIDS patients with the help of clinicians. The study protocol consisted of informed consent. Questionnaire elaborating the age, educational status, high-risk behavior, occupation, marital status and clinical manifestations, cause of death and mortality observed during their visit to the hospital in frequent intervals. All the subjects were classified by the clinical manifestation following World Health Organization staging system.

4-5 ml peripheral blood is collected intravenously by needle pricks. In the given sample the seropositivity was confirmed by three individual tests as under :

HIV TRIDOT : HIV tridot is a rapid test developed and designed using gp41, C-terminal of gp120 and gp36 representing the immuno dominant region of HIV-1 and HIV-2 envelop gene structures respectively. The HIV tridot test is a visual, rapid, sensitive and accurate immuno assay for the differential detection of HIV-1 and HIV-2 antibodies in human serum (or) plasma using HIV-1 and HIV-2 antigens immobilized on an immuno filtration membrane. The test is a screening test for anti HIV-1 and HIV-2 and in vitro laboratory use (Roland *et al.*, 2003).

COMB AIDS or EIA (Enzyme Immuno Assay) **:** It is an in vitro visually read DOT immunoassay intended for the qualitative detection of Ig-G/Ig-M antibodies to the HIV type 1 & 2 in human serum (on) plasma. A Comb AIDS employs the principle of enzyme immunoassay. In the test a positive result is indicated by the presence of magenta red colored DOT on the surface of the Comb where peptides have been spotted. (Constantine *et al.*, 1994).

NEVA (Naked Eye Visible Agglutination) **:** The NEVA test kit comprises of a set of several recombinant molecules. All these molecules have RBC binding sites. Such Universal RBC-Protein-Binding sites have been selected. The molecules are so designed that each molecule has only one RBC binding molecules that contain fusion proteins, which are RBC binding molecules with different immuno dominant HIV antigenic regions fused at the other end these fusion proteins capture one arm of the anti HIV antibodies (Constantine *et al.*, 1994).

RESULTS AND DISCUSSION

The present study in maternity, civil, HIV hospital and private clinics near Warngal Andhra Pradesh, India. 60 HIV long-term non-disease progressors and highly exposed persistently seronegative subjects were found, out of 1021 HIV patients and serodiscordant couples and their non infective children. These individuals did not develop any HIV related clinical signs and symptoms for the past few years (5 years). One of the partners is HIV positive and they all had regular unprotected sex for three or more years. Few of the subjects are HIV antibody negative for the past several years, however, their spouses died early. All the clinical manifestations and clinical signs and symptoms compared with the World Health Organization classification system (Lifson *et al.*, 1995) but not based on the CD4 cell count. In various maternity hospitals setting 25 noninfective husbands identified and those wives are infected.10 HIV negative babies who born HIV positive parents also identified. In civil, HIV hospital and private clinics 25 highly exposed persistently seronegative and non-diseases progressors were identified.

It is intriguing that a few ethnic minorities of people who never become infected at all despite repeatedly having unprotected sex with HIV positive partners. They are known as exposed-uninfected. HIV positive persons who have not required treatment and continue to survive and do well despite the HIV infection are generally termed as long term non progressors (LTNP). Early HIV positive cases of this region were recorded in 1996. It was observed in immigrant labour. All these labourers came from Bombay, Kalyani, Bimandi and also from Gulf. During their stay, they visit sex workers and might have contacted the disease. Returning from the work place they have started to transmit the virus to wives and to sex workers resulting in transmission in this region. Now this region is adversely affected, with an incidence of 3-6% in antenatal clinics, above 20% in STD clinics and VCTC centers. HIV mortality is increasing, some of the individuals are committing suicide on the other side of the epidemic serodiscordant couples and HIV negative babies who are born to HIV positive parents, subjects were identified. In Kenya, Gambia and also Caucasian they are not infected by the virus and highly exposed but persistently sero negative for several years. They are called long-term non-disease progressors they have mutation in expression of CCR5 receptor a protein that normally resides on the surface of

CD4 cells (Rowland-Jones, 1998) HIV must first bind to CD4 and then to CCR5 before it can get into cell. In this CCR5 receptor 32 amino acids are missing in the middle therefore it is defective and hence the HIV will not anchor on CD4 cell. 1% of Caucasians is homozygous for CCR5 mutation, 18% heterozygous mutation (one mutation and one normal copy). The mutation of homozygous CCR5 was never observed in African and Asian populations. In other parts of the world in discordant couples the uninfected woman have HIV antibodies in the mucous membrane lining their vagina and cervix (HIV immunology database 2001). In Warangal, antenatal clinics 10 babies who are born to HIV positive parents are seronegative, where the pregnant women received 200 mg of nevirapine and 2gm syrup for the babies within 24 hours. In this study 10 babies were detected HIV antibody negative for 3 years from their birth. All the babies' breast feeded for more than 1 year. In Britain research showed that after birth 20% of the uninfected babies had CTLs that recognized HIV (Rowland-Jones, 1998). The discordant people have a special type of CTLs (Cytotoxic T Lymphocytes). In a group of African sex workers from Nairobi, the Pumwani sex workers a potent effect against HIV sero conversion seen with HLA (Human leucocyte Antigen) class II DRB *019 and in particular DRB1*01 0102 restricted CD4 cells may play a role in protecting. In Kenyan sex workers the CD4 cells have 5 MHC (Major His to Compatibility Complex) genes and helix 3 protein inhibited viral replication. (Rowland Jones, 1998).

In our recent study conducted in Warangal Andhra Pradesh South India 60 HIV long-term non-diseases progressors were found out of 1021 HIV Patients. These individuals are not developing any HIV related clinical signs and symptoms. In a hospital, setting, 25 non-infective husbands were identified were infected. In civil and HIV hospital setting 25 HIV exposed but not infected and non-disease progressors were identified. Serodiscordant subjects frequently had unprotected sex with HIV infected individuals. Some of the non disease progressors having CD8 cells blocking viral replication in CD4 cell without killing them. These subjects have HLA epitopes HLA (human leucocyte antigen) class 1 restriction with cytotoxic T lymphocyte playing a major role in the immune response to destruction of virally infected cell. The major histo compatibility complex-HLA region in humans has long been shown to be an important host genetic risk factor in infectious diseases as well as a variety of auto immune diseases and cancers association with susceptibility or resistance over 50 different diseases. HLA class I restriction with cytotoxic T lympocytes plays a major role in the immune responses to and distribution of virally infected cells. In highly exposed persistently seronegative subjects having B-57, B-14, C-8, B-27 factors present on HLA system in various geographical areas. In India highly exposed but persistently sero negative subjects B-1801, B-3520, C-1507 human leucocyte antigen is present (Shankar kumar *et al.*, 2003). In Andhra Pradesh people HLA B17 is associated in the CTL, but in non progressors and highly exposed persistently seronagative subjects HLA antigen and association of disease progression is unknown (Pitchappan, 2006)

These observations suggest that ,in some cases ,natural immunity may protect exposed individuals from HIV infection and that HLA restricted CTLS may be responsible for the protective immunity.This primary data usefull for investigating factors host-genetic make up *i.e.* (Chemokine receptors, SDF-1 Variants). Host Immune responses (Cytokine T-Cell dynamics and cellular immune responses) of these non progressive individuals, and HIV

characteristics and replication dynamics (Viral Virulence, replication fitness and Genetic diversity)of this highly exposed,persistently sero negtive subjects.

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