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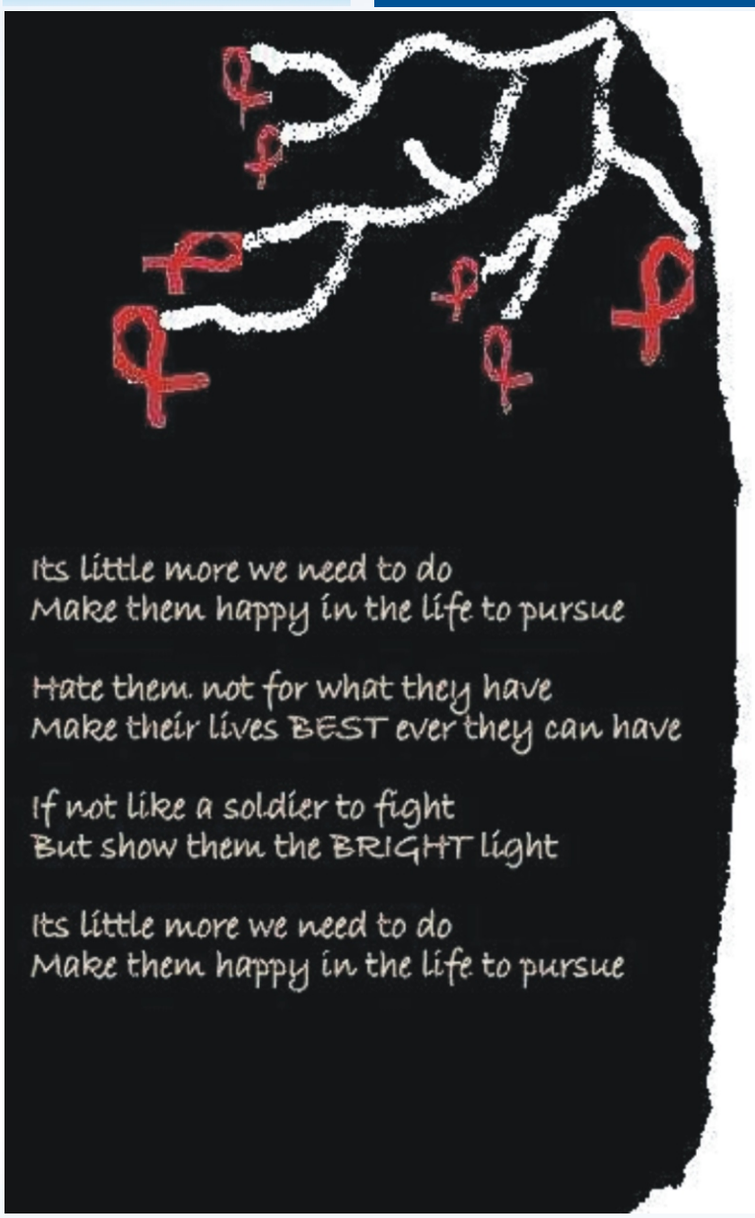

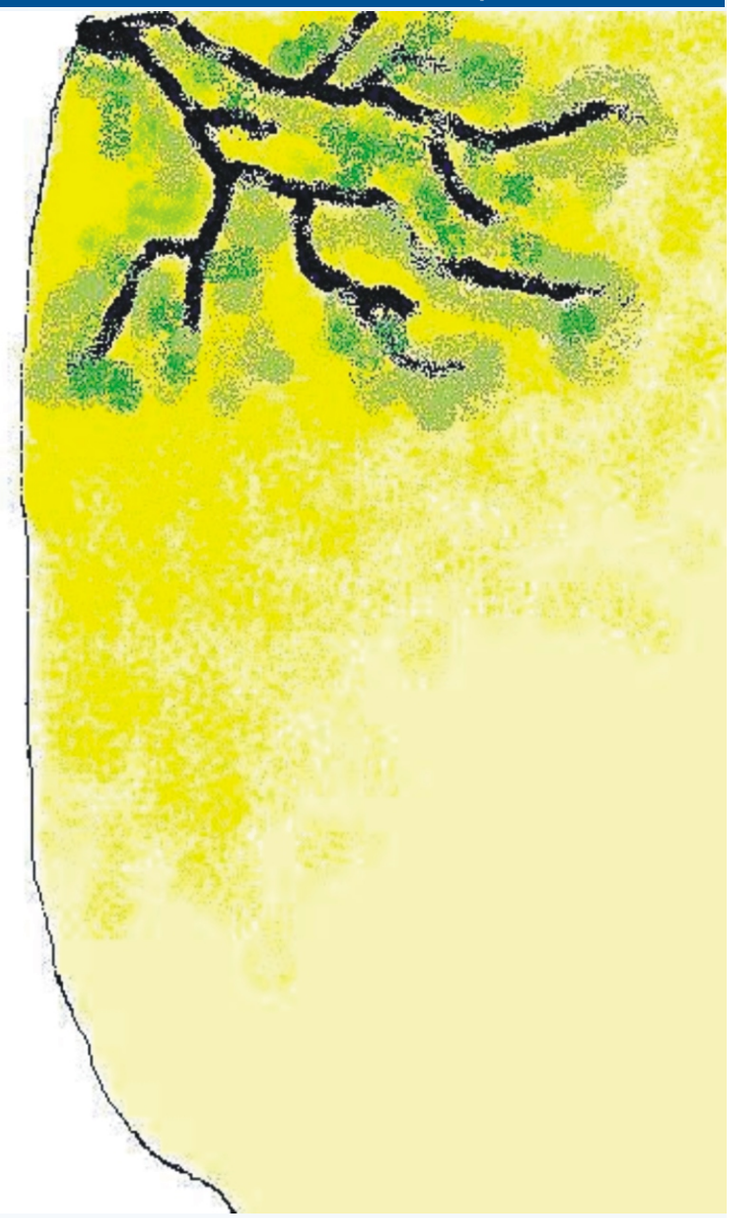

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INVITING LITERARY CONTRIBUTIONS

We invite
original research articles
or review articles in the field
of HIV/AIDS for publication
in forthcoming issues
of NARI Bulletin.

The Editorial Committee is happy to announce that NARI Bulletin has been assigned ISSN No. 2278-6694 by the Indian National Centre for ISSN, NISCAIR, New Delhi.

EDITORIAL

The year 2012 marks the completion of National AIDS Control Programme Phase III and the initiation of Phase IV. Phase III has been underlined by many successes, including decline in new infections as well as a drop in the overall prevalence of HIV, exceeding the targets of bringing PLHIV under ART programme, strengthening HIV-TB cross referral and many others. However, the challenges still exist. There is not much dent made on the epidemic among men having sex with men and intravenous drug users. The change from predominantly brothel-based sex work to street-based sex work and the use of newer communication modes to solicit, makes reaching to sex workers more difficult.

However, a second line of treatment has been introduced, and we are ready to deal with the increasing numbers that may need second line treatment as the programme becomes older. The surveillance of HIV drug resistance is still in infancy. Migration as one of the drivers of the epidemic continues. The NACP IV should be able to face these challenges in addition to operational issues. The NACP IV should also include research agenda that not only addresses the needs of today but also of the coming decade.

One of the areas of future concern is going to be living healthy with HIV. As a result of the introduction of ART, the longevity and quality of life of PLHIV has increased. However, this may bring in health concerns that involve chronic illnesses of cardiovascular system, central nervous system, metabolic disorders, malignancies, and so on.

This issue of the Bulletin carries two articles on the neurological aspect in HIV infection. National AIDS Research Institute is studying neurocognitive abnormalities in HIV infected persons with high and low CD4 counts. Initial findings indicate that though HIV associated dementia is not as common in HIV-1 subtype C infections seen in India as in HIV-1 subtype B infections, there is evidence of neurological impairment in Indian HIV infected patients.

This issue also highlights the team that is engaged in this research along with scientists from University of California, San Diego. Dr. Igor Grant, the renowned scientist who was instrumental in developing this very productive collaboration is aptly the Scientist in Focus in this issue.

In the end we are very proud to report a high number of quality publications from NARI. The Bulletin is our vehicle to share new information, thoughts of the scientists, and happenings at NARI. We strive to improve the quality. We look forward to your feedback to encourage us to do still better.

Dr. R.S.Paranjape
Director, NARI

Topics in Management of HIV in the Central Nervous System

Scott Letendre, M.D.

Antiretroviral therapy has improved survival among people living with HIV disease and, as a result, HIV disease has become a chronic illness in many individuals. Unfortunately, several other medical conditions seem to have become more common in people living with HIV than in the general population. These conditions include cardiovascular diseases, kidney diseases, bone loss, and neurocognitive impairment.

Several comprehensive studies have now confirmed that HIV-associated neurocognitive disorder, or HAND, occurs in a substantial proportion of people living with HIV and have linked the condition to antiretroviral characteristics, advancing age, worse immune suppression, co-infections, and central nervous system (CNS) conditions that can affect this population (such as psychiatric, mood, or substance abuse disorders). This article will briefly focus on the effects of antiretroviral characteristics and ageing.

All drugs circulate into tissues, including the brain, based on their characteristics, such as their molecular weight and the extent to which they bind themselves to blood proteins, such as albumin. The brain is protected by a structure named the blood-brain barrier (BBB). The BBB typically functions so well that it either completely excludes a molecule, such as, some toxins; or actively transports another, such as nutrients into the brain. The BBB allows some antiretrovirals to enter the brain and the fluid that surrounds it, the cerebrospinal fluid (CSF), but not others. For example, nevirapine reaches relatively high concentrations; while another, saquinavir, is almost completely excluded.

Research has established that when combining multiple antiretrovirals into a treatment regimen, selecting those that reach higher concentrations in CSF results in lower concentrations of HIV RNA in CSF, less inflammation, and better neurocognitive functioning. However, some of the drugs may also cause neurotoxicity. In other words, some of the drugs themselves can injure the brain. As a result, selecting the best regimen to treat HIV in the nervous system must be approached carefully. The therapy should be tailored for each individual, taking into account not only the nervous system, but also the potency of the regimen, its impact on other organs, and the person's preferences.

The challenges in understanding the impact of ageing on people with HIV include:

- Whether the effects of HIV and age are additive: Determining if age-linked complications occur more frequently in people with HIV than in general population
- Whether the effects of HIV and age are multiplicative: Determining if complications occur at a younger age

For example, Valcour et. al. (2004) identified that HAND was more common in older people with HIV but did not consistently find a multiplicative interaction between age and HIV[1]. Ances et. al. (2012) also found additive associations between older age, smaller subcortical grey matter volumes, and less cerebral blood flow[2].

In contrast, recent analyses from the CHARTER cohort

in the U.S. identified interactions between age and HIV in specific cognitive abilities (speed of information processing, learning and memory, executive functions, and motor skills).

The relationship between HIV, age, and medical complications does appear to be at least additive. The possible risk factors for this include:

- More severe past HIV disease. Several reports have confirmed that the lowest level that someone's T-cells has ever reached (also referred to as the nadir CD4 count) is one of the most reliable correlates of HAND risk. The reasons for this are not completely understood, but possible explanations include:
- Adaptation of HIV to cells in the brain
- Reduced immune control of other infections, particularly an infection that is common in the general population, Cytomegalovirus (or CMV)
- Injury of the nervous system caused by the immune system itself
- Immune senescence. The effects of HIV-mediated immune system injury could be compounded by the changes in the immune system that occur with ageing. Ageing-related immune changes include characteristics that are commonly associated with HIV disease, such as declines in naive T-cells, increases in immune activation, and declines in the CD4: CD8 ratio. Interestingly, CMV infection has been implicated as a co-factor in immune senescence in people who do not have HIV disease.
- Immune activation. Medical complications in people with well-controlled HIV disease may occur as a result of persistent immune activation. This immune activation may occur because HIV attacks the immune cells in the gut early in the disease, allowing the bacteria that typically live there to cross into blood. Evidence of these bacteria in the blood and the response of the immune system to the bacteria have been linked to HAND.
- Comorbid conditions. Other conditions occurring in ageing people with HIV, particularly vascular and metabolic disorders (e.g., diabetes), also increase the risk for neurocognitive complications.
- Drug toxicity. As people with HIV age, the number of prescribed and over-the-counter medications they use typically, increases. This can lead to polypharmacy and increased risk for drug interactions and drug toxicity.

Screening, diagnosis, and management of people with HAND can be complex. Incorporating standardized approaches for diagnosing HAND into clinical practice enables medical care providers to identify other conditions that can affect the CNS, provide social support for patients, and consider potentially beneficial therapeutic interventions. Unfortunately, ageing further complicates the screening and diagnostic process since HAND must be distinguished from conditions such as cerebrovascular disease and Alzheimer's dementia.

No single screening approach has proven consistently sensitive and specific but several groups, such as the European AIDS Clinical Society, the Mind Exchange Program, and others, have provided guidance. In 2012, Mind Exchange will publish comprehensive summaries and recommendations from experts in the field for HAND screening and management. For instance, HAND can usually be differentiated from other neurodegenerative disorders such as Alzheimer's by clinical findings. Clinical features of HAND are those of a dominant subcortical type of cognitive impairment, with the chief abnormality being psychomotor slowing. There are no cortical features such as difficulty in speaking, reading, or writing,

which are features of Alzheimer's dementia. Assessment often includes the following:

- Symptom questionnaires
- Functional assessments (i.e., activities of daily living)
- Mood assessment
- Blood tests
- Imaging
- CSF analysis and neuropsychological evaluation when feasible

In HAND, but not typically in neurodegenerative disorders, blood levels of vitamin B_{12}, red cell folate, and thyroid stimulating hormone are normal.

Recent data regarding CSF examination indicates that "viral escape" meaning that HIV is present in CSF when it is undetectable in blood can occur in CSF in more than 10% of ART-treated people. When viral escape occurs, it may respond to antiretrovirals that are both guided by drug resistance testing and reach higher concentrations in the CNS.

Ageing typically also increases the permeability of the BBB, which may then result in even higher concentrations of antiretrovirals in the CNS. Higher drug concentrations in the CNS should better control HIV but they could also increase the risk of toxicity. Therefore, changes in therapy should be closely monitored. In addition to considering antiretroviral modification, management of other conditions, such as vascular disease, diabetes, and psychiatric conditions, is also essential. Treatment of certain co-infections, such as hepatitis C, may also be important. However, the cognitive benefit of treating others, such as CMV, remains to be proven. No clear strategy for reducing persistent immune activation has been identified. Finally, new findings support that cognitive rehabilitation techniques may benefit people with HAND.

In summary, neurocognitive problems are common in people with HIV, even those on successful ART. In addition to HIV, several other common conditions can injure the brain, making diagnosis and management challenging. Successful management is worthwhile, because it can help patients lead more functional lives.

References:

1. Valcour V, Shikuma C, Shiramizu B, et al. Higher frequency of dementia in older HIV-1 individuals: the Hawaii Aging with HIV-1 Cohort. *Neurology* Sep 14 2004; 63(5): 822-827.
2. Ances BM, Ortega M, Vaida F, Heaps J, Paul R. Independent Effects of HIV, Aging, and HAART on Brain Volumetric Measures. *J Acquir Immune Defic Syndr* 15 Apr 2012; 59(5): 469-477.

Neurocognitive studies in HIV infected individuals in India

Dr. Manisha Ghate, Scientist 'D', NARI

HIV commonly enters the CNS early after infection, and although it does not directly infect neurons, it is frequently associated with structural and functional brain abnormalities[1, 2]. Neurocognitive changes occur most often in individuals with an AIDS-defining condition, but also appear in about a third of persons in the early, medically asymptomatic phases of the disease[3,4].Neurocognitive impairment in HIV infected individuals may involve cognition (thinking), motor control, and psychological state.

Symptoms may include a combination of slowing of psychomotor, poor concentration or attention, confusion, impaired memory, decreased problem-solving ability, changes in speech and language, impaired movement, decreased fine motor skills, poor coordination, mood and personality changes, and altered behavior. Specific neurological manifestations depend on which parts of the brain are affected. Impairment can range from so mild that it is not apparent without specialized testing, to so severe that it prevents independent living.

Neurocognitive assessments are important tools to know HIV effects on the CNS. Reliable and valid assessments may provide valuable estimates of functional impairment. Important information on motor skills, memory, executive functioning, language, attention, and so on can be obtained from various standardized tests and appropriate intervention can be provided in the form of medication and counseling sessions.

Studies in India have reported prevalence of dementia from late eighties. A study in one hundred patients with various neurological disorders associated with HIV infection during 1989-1996 was done at NIMHANS, Bangalore. Total twenty patients had non-opportunistic conditions and of these, two had cortical dementia[5].

An important cross sectional study was published in 2001 from Pune, India. This was the first largest study on both outpatient and inpatient HIV infected individuals that documented the prevalence of neurological complications from a large clinical cohort. 21 out of 457 (4.3%) patients had HIV associated dementia[6].

In a hospital-based study in Hyderabad, 411 patients had neurological manifestations and 8.03% patients had AIDS Dementia Complex[7]. Another study from Mumbai on non-opportunistic manifestations showed that 4 out of 67 patients (5.6%) with non-opportunistic conditions had AIDS dementia[8]. Though all these studies have reported that the prevalence of dementia is less than 10%, the methods for diagnosis of dementia are not clearly mentioned.

In 2004, Ranga et. al have reported that in clade C virus has an important natural variation in the dicysteine motif of the Tat protein (C31S). Since the Tat protein promotes viral replication directly, a functional change in the tat protein could have a significant impact on the virulence of the infection[9]. The authors have concluded that the Tat variation may in part explain the low prevalence of HAD in India.

Over the time, neurocognitive consequences of HIV gained attention, because research showed that cognitive performance affects routine activities, employment, adherence to treatment, and quality of life.

An article was published from Chennai on neurocognitive aspects in AIDS patients. 30 patients with median CD4 count 97/mm^3 with age and education matched controls were enrolled in a cross-sectional study. A battery of cognitive tasks sensitive to the effects of HIV on brain function was translated and administered in local language. Results revealed significant differences on most cognitive tests, with lower performances obtained by the HIV-positive individuals. Cognitive difficulties were present in 56% patients with advanced HIV meeting the criterion for impairment in two cognitive domains[10].

A study conducted in Bangalore compared a sample of 119 adults in India infected with HIV-1 subtype C who were not

on antiretroviral therapy, with normative data derived from an Indian sample of 540 healthy volunteers (with comparable gender distribution, age, and education) and with a matched cohort of 126 healthy, HIV-1-seronegative individuals[11]. They found a high rate (60.5%) of mild to moderate cognitive deficits in the HIV patients but no evidence of true dementia. The neuropsychological profile was characterized by deficits in fluency, working memory, and learning and memory.

A study was conducted in Pune using International HIV Dementia Scale (IHDS) to screen a well-characterized cohort of HIV-infected discordant couples. 48 HIV+ subjects with CD4 cell count <200 cells/mm^3 and 48 HIV- subjects were studied. The HIV+ subjects had significantly lower IHDS scores compared to the HIV- subjects. 35% of the HIV+ subjects and 15% of the HIV- subjects scored < 10 on the IHDS[12].

A study was carried in patients with early stages of HIV. 50 patients with early stages of HIV and 50 matched controls were compared in various neuropsychological tests along with demographic profiles. The results showed that seropositive patients had poorly performed in digit symbol substitution test, trail making test, and controlled word association test. This impairment had no association with the detection of illness[13].

To examine the neurologic effects of HIV in India, collaborators at NARI and the HIV Neurobehavioral Research Program at the University of California, San Diego, developed an NIMH-funded project with aims to the following:

a) Better characterize the neurologic complications associated with clade C HIV in India

b) Examine the effect of ART initiation on cognition

c) Determine viral and host correlates of HIV Associated Neurocognitive Disorders (HAND) and its treatment

This ongoing study has recruited HIV infected patients prior to starting the ARV treatment, and uninfected individuals as controls. All participants have received a comprehensive neuropsychological evaluation administered in Marathi, the official language of Maharashtra, as well as neuromedical assessment and laboratory evaluation. After baseline, HIV infected individuals have been started ARV treatment and follow ups are going on with them with comprehensive evaluations annually. Additionally, various biomarkers that may be associated with central nervous system injury, as well as host (participant) and viral genetic correlates will be examined.

It is hoped that by incorporating viral and host studies, as well as the tracking of comorbidities, this project may potentially identify the inter-individual differences that put one at risk for HAND, or affect CNS benefit from treatment. This would also yield insights regarding the neuropathogenesis of HAND in our population.

Challenges to detection, treatment and research in India:

Though it is reported that the prevalence of HIV Associated Dementia (HAD) is less in patients with Clade C, the studies conducted so far have shown high prevalence of mild to moderate neurocognitive impairment in HIV infected patients. With the increasing availability of HAART in the country, it is expected that the life span of HIV infected population will increase. Reports in western literature have shown increased prevalence of HAND in the HAART era, though the severity of the condition has reduced. So it would be important to conduct multicentric research studies to know the extent of HAND in the Indian population with and without HAART, and the impact of HAND on daily activities.

The prevalence of HAND could be studied by using a small screening battery in the busy programme clinics. Such small batteries could be administered by trained non-neurologist staff in a short time. Patients with high scores could be evaluated using comprehensive batteries that are standardized in our population.

A systematic review and pooled analysis of diagnostic accuracy of IHDS presented in the CROI 2012 (poster#501) has mentioned that IHDS (with cut off <10) have low diagnostic accuracy and specificity. Another interesting data was presented in the same conference (poster#499) using combination of different tests with good specificity and sensitivity for diagnosing NCI. The authors have concluded that combinations of widely accepted NP tests that can be administered in less than 10 minutes have demonstrated

adequate sensitivity and specificity. The domains of executive functioning, verbal learning, and working memory appeared to be particularly useful. If the norms are generated for such battery, it could be an excellent tool for detecting neurocognitive impairment.

For Indian population, two important issues of literacy and language should be kept in mind while combining and developing such tests. The tests for illiterate population should also be taken into consideration. The tests also need to be translated in different languages specific to the region and then standardized for the population. The classification of Asymptomatic Neurocognitive Impairment and Mild Neurocognitive Disorders depend upon the patient's activities of daily living. All the available data is based on the questionnaire that is based on western culture. So we need to develop culture-specific questionnaire that would capture the data on affection of activities of daily living. This will help in correctly differentiating and diagnosing asymptomatic and mild disorders.

There have been no published cohort treatment effect studies in India so far. Studies of patients after initiating ARVs should be conducted with a view to describing the effect of antiretrovirals on cognition and the course and progression of neuropsychological impairment. Important issues like potential antiretroviral neurotoxicity, adherence and drug resistance can be addressed in these studies.

References:

1. Langford, T. D., Everall, I. P., & Masliah, E. (2005). Current concepts in HIV neuropathogenesis: Neuronal injury, white matter disease, and neurotrophic factors. In H.E. Gendelman, I. Grant, I.P. Everall, S.A. Lipton, & S. Swindells (Eds.), *The neurology of AIDS* (2nd ed.), pp. 405-414. New York: Oxford University Press
2. Heaton, R. K., Marcotte, T. D., White, D. A., Ross, D., Meredith, K., Taylor, M. J., et al. Natural and vocational significance of neuropsychological impairment associated with HIV infection. *The Clinical Neuropsychologist* 1996;*10*, 1-14
3. Heaton RK, Grant I, Butters N, et al. The HNRC 500--neuropsychology of HIV infection at different disease stages. HIV Neurobehavioral Research Center. *J Int Neuropsychol Soc.* May 1995;1(3):231-251.
4. White DA, Heaton RK, Monsch AU. Neuropsychological studies of asymptomatic human immunodeficiency virus-type-1 infected individuals. The HNRC Group. HIV Neurobehavioral Research Center. *J Int Neuropsychol Soc.* May 1995;1(3):304-315.
5. Satishchandra P, Nalini A, Gourie-Devi M, Khanna N, Santosh V, Ravi V, Desai A, Chandramuki A, Jayakumar PN, Shankar SK. Profile of neurologic disorders associated with HIV/AIDS from Bangalore, south India (1989-96). *Indian J Med Res* Jan 2000;111:14-23.
6. Wadia RS, Pujari SN, Kothari S, Udhar M, Kulkarni S, Bhagat S, Nanivadekar A. Neurological manifestations of HIV disease. *J Assoc Physicians India* Mar 2001; 49:343-8.
7. Teja VD, Talasila SR, Vemu L.Neurologic manifestations of HIV infection: an Indian hospital-based study. *AIDS Read* Mar 2005;15(3):139-43, C3.
8. Deshpande AK, Patnaik MM. Nonopportunistic neurologic manifestations of the human immunodeficiency virus: an Indian study. *Med Gen Med* Oct 2005;7(4):2.
9. Ranga, U., Shankarappa, R., Siddappa, N., Ramakrishna, L., Nagendran, R., Mahalingam, M., Mahadevan, A., Jayasuryan, N., Satishchandra, P., Shankar, S.K., & Prasad, V.R. Tat protein of human immunodeficiency virus type 1 subtype C strains is a defective chemokine. *Virology* 2004; 78, 2586 -2590
10. Yepthomi T, Paul R, Vallabhaneni S, Kumarasamy N, Tate DF, Solomon S, Flanigan T. Neurocognitive consequences of HIV in southern India: a preliminary study of clade C virus. *J Int Neuropsychol Soc* May 2006;12(3):424-30.
11. Gupta, J. D., Satishchandra, P., Gopukumar, K., Wilkie, F., Waldrop-Valverde, D., Ellis, R., et al. Neuropsychological deficits in human immunodeficiency virus type 1 clade C-seropositive adults from South India. *Journal of Neurovirology* 2007; 13(3), 195-202.
12. Riedel D, Ghate M, Nene M, Paranjape R, Mehendale S, Bollinger R, Sacktor N, McArthur J, Nath A. Screening for human immunodeficiency virus (HIV) dementia in an HIV clade C-infected population in India. *J Neurovirol* Feb 2006; 12(1): 34-8
13. Mandal N, Singh OP, Bhattacharya S, Chatterji S, Biswas A, Sen S. Neurocognitive impairment in early HIV-positive individuals. *J Indian Med Assoc* Jul 2008;106(7):442, 447-9, 453.

Researcher in Focus

Dr. Igor Grant

Dr. Igor Grant is the Director of the HIV Neurobehavioral Research Program (HNRP) at the University of California, San Diego (UCSD). After completing medical studies at the University of British Columbia, and internship and residency at the University of Pennsylvania, Dr. Grant began his career at UCSD in 1972 as an Assistant Professor of Psychiatry, School of Medicine. Today, Dr. Grant is Distinguished Professor and Executive Vice-Chair of Psychiatry.

His academic interests focus on the effects of various diseases on the brain and behavior, with an emphasis on translational studies in HIV and drugs of abuse.

He has contributed to approximately 600 scholarly publications and is the principal investigator of several NIH studies, including the Translational Methamphetamine AIDS Research Center (TMARC), California NeuroAIDS Tissue Network (CNTN), CNS HIV Anti-Retroviral Therapy Effects Research (CHARTER), University of California Center for Medicinal Cannabis Research (CMCR), and is a co-director of the HIV Neurobehavioral Research Center (HNRC). Professor Grant founded the Journal of the International Neuropsychological Society, serving as Editor-in-Chief for 10 years. He also co-founded the journal AIDS and Behavior.

Over the past 23 years, HNRC research has grown from a limited group of research projects and core functions into an infrastructure of support mechanisms to promote numerous US-based and international HIV-related research programs now under the umbrella of the HIV Neurobehavioral Research Program (HNRP). One of the international programs involves collaboration with NARI on the comprehensive neurological and neurocognitive study of HIV infected persons in Pune.

Dr. Grant's work in HIV/AIDS research began in the late 1980's with HNRC colleagues by observing and characterizing neurocognitive and neuropsychological changes linked to HIV infection. Their studies showed that in the absence of advanced medical manifestation, subtle cognitive impairments such as memory and attention disorders contributed to the inability to hold a job, decreased adherence to antiretroviral medication, and led to more rapid disease progression and ultimately, death.

More recently, HNRC studies have shown that using a CNS penetrating anti-retroviral that drives down virus in the CSF leads to improved cognition. Current investigations from the TMARC focus on translational research combining human, animal, and in vitro studies on the combined CNS effects of HIV infection and the use of methamphetamine. Dr. Grant and the collaborations of HNRP in China, India, Brazil, Romania, and Ethiopia are exploring clade effects on neurological complications. He is also working towards creating sustainable and renewable resource programs geared towards fighting HIV.

Part of the inhumanity of the computer is that, once it is competently programmed and working smoothly, it is completely honest.~Isaac Asimov

Commentaries

Surveillance of HIV infection in suspected tuberculosis patients

Michael Pereira, Technical Assistant, Tuberculosis Laboratory, Division of Microbiology.

Testing of tuberculosis and suspected TB patients for HIV is important for early case detection and initiation of appropriate treatment. WHO has recommended provider-initiated HIV testing and counseling (PITC) of TB and suspected TB patients. However, currently in India the policy is that HIV testing is offered only to tuberculosis patients. Consequently, data on HIV prevalence in persons suspected of TB is lacking. To bridge the gap of this paucity of information Naik B et al., conducted a study to show how adopting WHO's HIV/TB testing policy would change the detection rates of HIV. The study included 1668 patients tested for tuberculosis in December 2010, all of whom were offered HIV testing. Of these, 92% accepted HIV testing, and 7% were tested positive for HIV. The authors have projected that, had there been in place a policy of HIV testing for all suspected TB patients throughout 2010, about 534 new HIV infections (51% increase) could have been identified in Mandya district.

The study demonstrates a major opportunity for improved HIV case finding through continuous surveillance in suspected TB cases nation-wide to detect the burden of HIV. Hence, more studies are required to be conducted to gather evidence of the importance of HIV testing in TB suspects, so that Indian policy makers are convinced of revising the National TB Control Program guidelines to include HIV testing for both TB patients and TB suspects. This would help in the early detection of HIV and managing patients who test positive for HIV by providing better care in the form of effective antiretroviral treatment and planning effective control strategies.

To conclude, considering the uneven distribution of the HIV epidemic in India, surveillance of HIV in TB and suspected TB patients should be conducted routinely by providing on-site testing at TB clinics for early detection and timely initiation of ART to reduce the morbidity and mortality associated with HIV infection.

References:

Naik Balaji, Kumar MV Ajay, Lal Kumaraswamy, Doddamani Sangamesh, Krishnappa Mohan, Inamdar Vikas, Satyanarayana Srinath, Gupta Devesh, Dewan Puneet Kumar. HIV prevalence among persons suspected of tuberculosis: policy implications for India. *J Acquir Immune Defic Syndr* 2012, 59(4): e72 - e76.

New weapon in the arsenal of HIV treatment: Cxcr4-Zinc-finger Nuclease modified CD4+ T cells

Ms. Sneha Sant and Ms. Nidhi Abraham, Research Assistants, Division of Serology.

HIV-1 cellular entry requires the cell surface expression of CD4, and either CCR5 or CXCR4 chemokine co-receptors on host cells. HIV-1 uses CCR5 as the primary co-receptor in naïve and infected individuals. However, it could also evolve to use CXCR4 as the alternative co-receptor post-infection. Several strategies including development of small molecules and genetic engineering have focused on the disruption of CCR5 receptor. However, mutations in envelope protein render resistance to these antagonists and individuals on Highly Active Antiretroviral Treatment harbor upto 50% dual-tropic viruses, or have mixtures of CCR5 or CXCR4-tropic viruses, thus making gene therapies less effective.

In this study, Yuan *et al.* compared the efficacy to disrupt the CXCR4 receptor using adenovirus-mediated transient delivery of an engineered zinc- finger nuclease (ZFN) to gene delivery of lentiviral vector expressing short hairpin RNAs (shRNAs) siX4-1 and siX4-2 to SupT1 T cells. ZFN permanently knocks out the targeted gene, whereas, shRNAs need continuous expression to modulate CXCR4. Post-modification, the cells were challenged with CXCR4-tropic HIV-1. Within 15 days, >85% parental, siX4-1 and siX4-1-expressing cells were infected whereas ZFN-modified cells remained <1% viral positive for upto 42 days, thus demonstrating that ZFN-mediated *cxcr4* disruption conferred CD4+ T cell to HIV-1 resistance for a longer period in cell lines than the shRNAs.

The second part of the study aimed to assess the possibility of selective advantage of cxcr4-ZFN-modified T cells over unmodified ones. The study showed that when a minor

population of cxcr4-disrupted cells were introduced in the CD4+ T cell population and subjected to viral challenge, there was a steady increase in CXCR4 negative cells early in the infection and loss of virally infected cells. The loss of infected cells and rise of cxcr4-disrupted cells thus resulted in decreasing viral load. Similar results were observed in HIV-1 infected humanized mice models. The findings also provided insights into the growth patterns of CD4+T cells with and without the loss of CXCR4 in absence of viral infection. In both tissue culture and humanized mice models, the loss of CXCR4 did not promote the expansion of CXCR4-disrupted CD4+ T cells in absence of HIV-1.

The study thus provides a rationale to consider use of cxcr4-ZFN-modified CD4+ T cells in HIV-1 infected individuals requiring a salvage therapy. Designer ZFNs targeting the CCR5 co-receptor are already in Phase-I clinical trials and have demonstrated promising results. Future studies can be conducted to evaluate the effect of knocking out both CCR5 and CXCR4 co-receptors to reduce dual-tropic HIV-1 infection, and to check if this promotes viral evolution to utilize other chemokine receptors for viral entry and infection.

References:

Yuan J, Wang J, Crain K, Fearns C, Kim KA, Hua KL, Gregory PD, Holmes MC, Torbett BE. Zinc-finger Nuclease Editing of Human cxcr4 Promotes HIV-1 CD4(+) T Cell Resistance and Enrichment. *Mol Ther*. 2012 Apr;20(4):849-59.

Research Assistant, Department of Serology

A Change in the vaccine strategies for Tuberculosis

Ms. Anjali R. Patel, JRF, Division of Immunology.

Conventional CD4+ and CD8+ T cell responses recognize mycobacterial peptide antigens presented by polymorphic MHC class I and II molecules, and are targeted by current vaccine and immunodiagnostic strategies. However the lipid-rich cell wall of M.tb is essential for pathogenicity and provides targets for unconventional T cell recognition.

Mycolic acid (MA), the predominant mycobacterial cell wall lipid is a key virulence factor in patients with active TB infection. MA protects M.tuberculosis from dehydration, exposure to drugs, and the hostile environment of the macrophage phagolysosome. As against protein antigens presented by HLA antigens, lipid antigens are presented in association with CD1 molecules, which exhibit very limited polymorphism, and hence would be able to induce immune responses in genetically diverse human population. Role of CD1-restricted T cells recognizing MA in active TB infection has been studied by Damien, et. al.

The authors used immature Dentritic cells from peripheralblood mononuclear cells (PBMCs) of active TB patients and BCG vaccinated controls pulsed with various antigens such as PPD, M.tb total Lipid 2, ESAT-6 and CFP-10, and MA for stimulating T cells ex vivo, and performed ELISPOT assays and flow cytometry analysis of the response. Longitudinal study was also performed on few patients on antitubercular treatment for correlating response to these antigens with declining mycobacterial load. MA-specific T cells were predominant in TB patients at diagnosis, but were absent in uninfected BCG vaccinated controls. These T cells were CD1b restricted, detectable in blood and disease sites, producing both IFN-γ and IL-2, and exhibited effector and central memory phenotypes. MA-specific responses contracted markedly with declining pathogen burden, and in patients followed longitudinally, exhibited recall expansion upon antigen reencounter in vitro long after successful treatment, indicative of lipid specific immunological memory.

Currently, BCG is the only vaccine that is employed to prevent TB. But recent field studies have shown that BCG induces variable protection in infants and adults demonstrating a need for new vaccine approaches. A total of 11 vaccine candidates against tuberculosis have entered clinical trials within the last several years. Most of these are based on proteins secreted by M.tb and have the disadvantage of induction of variable responses because of presentation through polymorphic HLA types.

A vaccine, to be effective at a population level, should induce equivalent responses in diverse population. Hence the conventional T cell vaccine approaches need to be further supported by unconventional T cell vaccine approaches that recognize antigens presented by less polymorphic CD1 molecules. CD1 restricted MA-specific T cells can thus be potentially important as new components of future vaccine induced responses against TB, making MA as a potential vaccine candidate.

References:

Montamat-Sicotte DJ, Millington KA, Willcox CR, Hingley-Wilson S, Hackforth S, Innes J, Kon OM, Lammas DA, Minnikin DE, Besra GS, Willcox BE, Lalvani A. A mycolic acid-specific CD1-restricted T cell population contributes to acute and memory immune responses in human tuberculosis infection. *J Clin Invest* Jun 2011; 121(6): 2493-503.

Happenings at NARI

Guest Lecture Series

This was one of the initiatives undertaken by NARI with the objective to know the advances in various fields apart from HIV. This was open for the NARI staff and there was interesting interactions in the form of question and answers with the faculty members.

The first lecture was given by Dr. Amitav Banerjee, Head of the Epidemiology Department in AFMC, Pune on 17th February on the topic 'How to develop a research proposal.'

The second lecture was by Dr. Shirish Hiremath, renowned cardiologist in Pune on 30th March. He talked on 'Cardiology: Procedures and Advances.' Dr. Hiremath presented the current trends in managing cardiac problems and their management in a very informative way.

Dr. Shirish Hiremath

Science Day Celebration

28th February is celebrated as National Science Day in India as a tribute to Sir CV Raman, who reported the discovery of Raman Effect. NARI takes the opportunity of the Science Day for increasing research awareness and for generating scientific curiosity among students by observing open day on 28th February every year. As every year, NARI laboratories were shown to the students on the occasion of Open House programme. These included laboratories performing serological tests for HIV diagnosis, CD4 testing and viral load assays. Also, immunological assays on HIV infected patients and HIV vaccine trial participants were shown to the visitors. In the microbiology laboratory, investigations done for diagnosis of opportunistic infections and sexually transmitted infections were shown. At the molecular virology laboratory, PCR, sequencing, and other molecular biology related techniques were shown.

Around 110 students from 7 colleges from and outside Pune visited NARI on this occasion. The activity helped in dissemination of knowledge regarding NARI's activities as well as updated knowledge in the research related to HIV/AIDS to the young students.

Publications

★ **In Vitro Sensitization of T Cells with DC-Associated/Delivered HIV Constructs Can Induce a Polyfunctional CTL Response, Memory T-Cell Response, and Virus Suppression.**

Swarali Kurle, Madhuri Thakar, Ashwini Shete, Ramesh Paranjape.

Viral Immunology 2012; 25 (1): 45-54

A major obstacle in developing a preventive HIV vaccine is the lack of suitable animal model. Several HIV vaccines have demonstrated failure in phase I human clinical trials even after encouraging results in the animal studies. Hence, in vitro studies that will bridge the animal studies and human clinical trials are essential. In this study, we have reported dendritic cell based- in vitro sensitization of human T cells against three HIV constructs. The in vitro sensitized T cells exhibited HIV-specific responses in various assays. This study provides evidence of an in vitro system that can be used to assess the immune response against a candidate vaccine, and may also provide the opportunity to modify vaccine constructs to achieve the goal of developing an ideal vaccine.

★ **Sexually Transmitted Infections and Risk Behaviors Among Transgender persons (Hijras) of Pune, India.**

Sahastrabuddhe S, Gupta A, Stuart E, Godbole S, Ghate M, Sahay S, Gangakhedkar R, Risbud A, Divekar A, Bollinger R, Mehendale S.

J Acquir Immune Defic Syndr 2012; 59(1):72-8.

Cross-sectional data of individuals attending 3 STI clinics in Pune, India between 1993 and 2002 were analysed with the objectives of determining the prevalence and associated risk factors of HIV and sexually transmitted infections (STI) in self-identified transgenders [TG] also known then as Hijras, and compare the prevalence with that in heterosexual men, and men having sex with men (MSM) from the same clinics.

TGs (Hijras) in the study were young with low levels of education, and the majority of them were unmarried. The study reports that the prevalence of HIV (45.2% in TG vs 20% in heterosexual men vs 18.9% in MSM, P< 0.0001) and warts (10.3% vs 4.6% vs 7.0%; P = 0.004) was higher in TGs as compared to heterosexual men and MSM. On the other hand, the prevalence of genital ulcer disease (15.3% vs 32.6% vs 21.5%; P , 0.0001) and discharge (5.4% vs 13.6% vs 9.0%; P , 0.0001) was lower.

TGs were more likely to have received money for sex and have an earlier sexual debut than the comparison groups. In multivariate analysis, receiving money for sex (adjusted odds ratio: 4.49; P < 0.04) and having genital ulcer disease (odds ratio: 3.87; P <0.08) were independently associated with high HIV prevalence in TGs.

The study findings and other recent observations among this vulnerable and neglected sexual minority in India suggest an urgent need for appropriate measures to be taken to understand and address health and sexual risk behavior vulnerabilities faced by them. The study concludes that it is important to review current prevention strategies and stress the need to engage the TG community members through appropriate targeted intervention programs.

★ **Subtle alteration of residues including N-linked glycans in V2 loop modulate HIV-1 neutralization by PG9 and PG16 monoclonal antibodies.**

Rajesh Ringe, Sanjay Phogat, Jayanta Bhattacharya.

Virology 2012; 426: 34-41

The discovery of several potent and broadly neutralizing monoclonal antibodies (MAbs) (such as PG9 and PG16) to HIV-1 provided clues on newer vaccine targets. In the present study, it was described how an env clone obtained from a slow progressor differed significantly with that of other autologous envs and what is the basis of this resistance to PG9 and PG16. By constructing chimeric envelopes and specific substitutions it was found that both loop length and spatial orientation of glycan residues in addition to the net charge of the β sheet C region that directly binds to PG9 CDRH3 within V2 loop significantly modulated HIV-1 sensitivity to PG9 and PG16 MAbs. This observation was extended to other env clones from different patients which revealed that the V2 loop length, glycan content and net positively charged residues in this region significantly modulated the Env sensitivity to PG9 and PG16 monoclonal antibodies. Our data indicated that subtle change within V2 loop alone modulates exposition of quaternary epitopes that are targets of PG9/PG16 MAbs.

★ **Antimicrobial Susceptibility Testing, Auxotyping, and Serotyping of Neisseria gonorrhoeae Strains Isolated in India.**

Kulkarni S, Bala M, Risbud A.

Sex Transm Dis 2012 ; 39 (3):188-90.

Sixty-four Neisseria gonorrhoeae strains isolated from patients attending sexually transmitted disease clinics at Pune and Delhi between January 2007 and June 2008, were subjected to antimicrobial susceptibility testing, auxotyping, and serotyping. We observed 6 antibiotic resistance patterns, 6 auxotypes, 3 serogroups, and 17 serovars. The combination of auxotyping and serotyping is a potential useful method for typing N. gonorrhoeae as a result of high discriminatory index, rapidity, ease, and relatively lower cost.

★ **Current Status of Research on HIV Epidemic, Pathogenesis, Management and Prevention in India.**

Ramesh S. Paranjape, Madhuri R. Thakar, Manisha V. Ghate and Sheela V. Godbole.

Proceedings of the National Academy of Sciences, India Section B Online First

Review article. Hence no summary given.

★ **Effects of Marathi-Hindi bilingualism on neuropsychological performance.**

Kamat R, Ghate M, Gollan TH, Meyer R, Vaida F, Heaton RK, Letendre S, Franklin D, Alexander T, Grant I, Mehendale S, Marcotte TD, HIV Neurobehavioral Research Program (HNRP) Group.

J Int Neuropsychol Soc 2012; 18(2): 305-13.

This study investigated the extent of bilingual advantages and disadvantages present in Marathi-Hindi bilinguals. The authors hypothesized that a considerable practice in exerting inhibitory control afforded by being bilingual in these two languages would impart an advantage on measures of executive function, while the verbal fluency disadvantages (especially involving nouns) associated with bilingualism may be attenuated, given the high percent of cognates

shared by Marathi and Hindi. The lower rate of cognates for verbs relative to nouns, however, was expected to limit the cognate effect, resulting in a relatively greater bilingual disadvantage on this task.

★ **Factors associated with HIV among female sex workers in high HIV prevalent state in India.**

Gajendra Kumar Medhi, Jagadish Mahanta, Ramesh S Paranjape, Rajatshruva Adhikary, Nabjyoti Laskar and P. Ngully.

AIDS Care 2011: 1-8.

While intravenous drug use is the primary driver of the HIV epidemic in the north-eastern part of India including Nagaland, the role of female sex worker is still not appreciated. Nagaland has seen an increase in the HIV prevalence in the female sex workers. They may independently drive a parallel epidemic that may reach the general population. Hence, there is need to understand the risk characteristics of the female sex worker population. This study is a part of Integrated Behavioural and Biological Assessment survey carried out as a part of a monitoring and evaluation strategy of the "Avahan" project that was implemented by the Bill and Melinda Gates Foundation. The Respondent Driven Sampling (RDS) strategy was used to recruit 400 respondents. The overall HIV and STI prevalence in this group was 13.4% and 34% respectively. Higher HIV prevalence was associated with drug use (both injecting and oral), regular clients, widowed or divorced clients, and STI infection. To contain the epidemic, sufficient attention needs to be given to the prevention of illicit drug use, early detection and treatment of STIs, and increase in consistent condom use through increased access to condoms and awareness.

★ **Increased expression of virulence attributes in oral Candida albicans isolates from human immunodeficiency virus-positive individuals.**

Arati Mane, Shraddha Gaikwad, Shilpa Bembalkar and Arun Risbud.

Journal of Medical Microbiology 2012; 61: 285-90

Oral candidiasis caused by Candida albicans is recognized as one of the most frequent opportunistic infections in human immunodeficiency virus (HIV)-infected individuals. The overall severity and chronicity of oral candidiasis has been attributed exclusively to the HIV-induced immune deficiency of the affected individuals but not to the virulence factors of the pathogen. However, genotypic and phenotypic studies have suggested that HIV infection might be associated with preferential selection of C. albicans strains with altered virulence determinants. The present work was undertaken to determine simultaneously the expression of five virulence factors in oral C. albicans isolates colonizing and infecting HIV-positive and -negative individuals. Oral swabs were collected from 335 consecutive individuals (210 HIV-positive and 125 HIV-negative). Virulence factors and genotypes were determined for all the C. albicans strains isolated. The results showed significantly increased expression of proteinase, phospholipase and haemolytic activities, as well as a greater ability to adhere, in isolates from HIV-positive compared with HIV-negative individuals. However, no significant differences in virulence factor expression in isolates colonizing or infecting HIV-positive individuals were seen. Genotype A was the predominant type; however, a relationship could not be established between the genotypes and the virulence factors, or with clinical infection. These data support the concept of preferential C. albicans strain selection with altered virulence determinants in HIV-infected individuals and emphasize the need for further molecular genetic linkage studies that could be helpful in dissecting the molecular causes of preferential strain selection, which may lead to new approaches for therapeutic intervention.

★ **Initial Virologic Response and HIV Drug Resistance Among HIV-Infected Individuals Initiating First-line Antiretroviral Therapy at 2 Clinics in Chennai and Mumbai, India.**

Nitin K. Hingankar, Smita R. Thorat, Alaka Deshpande, S. Rajasekaran, C. Chandrasekar, Suria Kumar, Padmini Srikantiah, Devidas N. Chaturbhuj, Sharda R. Datkar, Pravin S. Deshmukh, Smita S. Kulkarni, Suvarna Sane, D. C. S. Reddy, Renu Garg, Michael R. Jordan, Sandhya Kabra, Srikanth P. Tripathy, and Ramesh S. Paranjape.

Clinical Infectious Diseases 2012; 54 (S4): S348-54

The survey of acquired HIV drug resistance was performed by the National AIDS Research Institute in collaboration with the National AIDS Control Organization and WHO at the ART center, Government Hospital of thoracic Medicine, Tambaram, Chennai, and ART center, J.J. Hospital, Mumbai adapting WHO generic recommendations to identify patient and program predictors of HIVDR in order to inform national recommendations on effective management of HIVDR. A prospective survey design with sample size of 96 was utilized to estimate the clinic-level HIVDR prevention (as defined by viral load <1000 copies/mL) 12 months after initiation of ART. The survey endpoints were analyzed as HIVDR prevention, Detected HIVDR or Possible HIVDR. At Chennai site, 75% survey participants achieved HIVDR prevention, however, 64.6% achieved HIVDR prevention at the Mumbai site which is below the WHO suggested target of ≥70% viral load suppression. Only 8.33% patients at Chennai site and 9.4% at Mumbai site had detected HIVDR whereas 16.6% and 26% patients had potential HIVDR at Chennai and Mumbai site respectively.

The survey findings strengthen the past observation that high level of viral load suppression can be achieved in resource limited settings. However, it indicate the need for routine viral load testing to identify virological failure in individuals receiving first-line ART as well as underscore the need for affordable second-line ART and mechanisms to minimize loss to follow-up among patients receiving ART in India's national program.

★ **Human Immunodeficiency Virus: Biology and Natural History Infection.**

Arora SK, Paranjape R, Tripathy S, Wanchu A. In: Somesh Gupta, Bhushan Kumar,

eds. SEXUALLY TRANSMITTED INFECTIONS 2nd edn. New Delhi, India: Elsevier, 2012: Chapter 66, pages 779-794. ISBN: 978-81-312-2809-8.

Book chapter. Hence no summary given.

★ **Can we learn lessons on Ethical Issues involving women participants in clinical Trials? Is it time to act?**

Nita Mawar

In Ethics Health and Medicine: Anthropological Perspectives. Edited by U Kalpagam. 2012. Pp 109-129. (ISBN: 978-81-7831-276-7).

Book chapter. Hence no summary given.

Highlighting our Gems: NeuroAIDS team

NeuroAIDS in India

NeuroAIDS in India, an RO1 Indo-US collaborative study, funded by National Institute of Mental Health was initiated at NARI with HIV Neurobehavioral Research Centre, San Diego in 2008. The primary aims of the study are as follows:

- Determine the prevalence and nature of HIV-associated neurocognitive disorder (HAND) in HIV-1 clade C infected, treatment-naïve Indian patients in a clinic-based setting.
- Determine the impact of antiretroviral (ARV) treatment on HAND.
- Assess the viral genetics associated with HAND.
- Determine the relationship between host factors and HAND in individuals infected with clade C in India.

The secondary aims of the study are as follows:

- Establish neuropsychological (NP) and neuromedical norms in a demographically comparable Indian population
- Build upon the existing scientific and clinical expertise of the NARI investigators by transferring U.S. technology in the areas of NP assessment and interpretation, viral and host genomics, and biomarker selection and interpretation.

Patient data on age, education, and gender-corrected norms based upon 248 controls with > 4 years of education has been generated. The patients with CD4 counts between 350 and 550/mm^3 group (Non AIDS) had a median CD4 cell count of 439 (386, 515), while the AIDS group had a median CD4 cell count of 131 (82, 173).

Focusing on the groups with > 4 years of education, and using a global deficit score approach controls had a significantly lower Global Deficit Scores (median of .15 [.05, .40]) than the non-AIDS (.25 [.075, .50]) and AIDS (.35 [.15,.63]) groups; the non-AIDS/AIDS comparison approached significance (p = .052). When using a .5 GDS cutpoint, impairment rates were 16.9% in controls, 24.7% in non-AIDS, and 40.2% in the AIDS group.

The data on neurocognitive changes associated with ARV treatment initiation in patients with higher CD4 counts was presented in CROI 2012. It showed that starting the antiretroviral treatment (ART) at higher CD4 cell counts, even when NP dysfunction is mild, appears to be beneficial to the central nervous system. ART was most beneficial for those who demonstrated worse neuropsychological performance at baseline.

The first Indian data on biomarkers and neurocognition was presented in the immunology conference 2012 with findings that IL-6, RANTES, and IP-10 may predict better HIV disease prognosis in India and the pathogenesis of neurocognitive impairment may differ between people with either lower or higher CD4 counts. The findings of full study will be generated when the study is over in May 2013.

Since clade C is the more prevalent subtype in the Indian HIV 1 population, this study aimed to assess the influence of HIV 1 clade C viral (*envC2V3 & tat)* genetics on HIV associated Neurocognitive Disorders (HAND,) and also to determine the relationship between HAND and host immunogenetic factors.

Assessment of influence viral genetics on HAND:

Several HIV proteins have been shown to have neurotoxic properties *in vitro*, Env and Tat are the two important proteins which are actively involved in neuroinvasion.

The HIV-1 viral coat (*Env*) glycoproteins gp41, gp120, and their precursor gp160 have all been shown to be neurotoxic. gp120, a protein involved in regulating viral entry and in determining viral tropism, is indirectly neurotoxic.

Other than *EnvC2V3* region, Tat, a non-structural viral protein is essential for viral replication and has been previously reported to have neurotoxic properties. Mechanisms implicated in the neurotoxic actions of Tat include direct depolarization of neurons, increased levels of intracellular calcium, increased production/release of pro-inflammatory cytokines, increased macrophage infiltration, activation of excitatory amino acid receptors by, as yet, uncharacterized mechanisms, and increased incidence of programmed cell death (apoptosis).

Highlighting our Gems: NeuroAIDS team at NARI

NARI has been involved in neuroAIDS study since 2008. This is the first comprehensive study in India that will be generating the data on various aspects of neurocognition in HIV infected individuals. A pilot study was conducted in 2006 in 60 participants to standardize the neuropsychological test battery developed at San Diego. The standardization required changes to make it culture-specific for our population.

The full study was initiated in 2008. Enrollment of all 540 participants (253 HIV+ and 287 HIV-) was done at NIV and Model Colony Clinic by the neuroAIDS team and the follow ups are ongoing. HPTN 052 team supported the co-enrollment of their study participants with higher CD4 counts in this study. It was challenging to get 287 HIV- participants with age, gender, and education similar to HIV+ participants but the clinic and community teams worked together for the recruitment. The literacy test for the study was developed by Dr. Balkrishna Bokil, who has made important contributions in the National Literacy Mission.

The study involved extensive work by the clinic, laboratory, and community teams. The training of all the clinical psychologists for the administration of the neuropsychological battery was conducted by the HNRC group, who was certified for the same prior to study enrolments. The clinicians were trained and certified for conducting neurological examination of the study participants. The consultant psychiatrist was involved in managing the cases of depression.

Extensive laboratory work in the study addressed primary objectives on viral and host genetics. The investigations for clinical monitoring were also a part of extensive laboratory work.

Viral genetics: env C2V3 clade typing:

To prevent confounding by non-clade C infections in our investigations, all HIV infected subjects (n=252) were processed for HIV genotyping by standard bulk sequencing methods to determine the clade of infection. The determination of the HIV clade infection was done by the Subtype Reference Set through the web-based Los Alamos HIV sequence database (http://www.hiv.lanl.gov/content/hiv-db/SUBTYPE_REF/align.html).

Results pertaining to clade typing showed that out of 252 cases, 230 (91.3%) belonged to HIV-1 subtype 'C', followed by 14 (5.5%) from subtype 'B' and 8 (3.2%) from subtype 'A1'. Further these *env* C2V3 sequences obtained through clade typing protocol will be categorized based on the degree of impairment. Specific amino acid residues associated with degree of HNCI within HIV-1 clade C *env* will be identified using highly sensitive computational methods (Shannon entropy & Machine learning tool WEKA).

Biomarker estimation:

Nine biomarkers (IL-6, IL-8, IL- 10, IFN-γ, IP-10, MCP-1, MIP-1α, RANTES & TNF-α) were measured in serum using a multiplex array system. This evaluation involved standardization of the procedures for blood collection, processing, and storage. The laboratory already had a multiplex protein array system for estimation of secretory biomarkers in the samples. The training of the newly appointed staff, development, and execution of the robust internal quality control system gave strength to the data generated and presented at the national immunology conference.

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