

Human Brain Tissue Repository: A National Facility Fostering Neuroscience Research

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Abstract The lack of appropriate animal models to understand the biology and evolution of neurodegenerative and psychiatric disorders and the fact that studies on experimental animals cannot substitute evaluation on human tissues has led to the establishment of Human Brain Bank for tissues and tissue fluids as resource centres. These Biobanks, collect, preserve and distribute fresh and fixed human tissue samples collected following autopsy and surgical resection with informed consent of patient/close relatives to bank and use the material for research following scientific ethics guidelines of ICMR. This attempt constitutes “converting biological waste into precious human tissue resource for research”. Many significant discoveries are made in the past utilising the human brain, establishing the usefulness of Biobanking in medical research understanding pathogenesis of human diseases, their genetic basis of neurological and psychiatric illnesses. Continued functioning of such a facility needs close co-operation and commitment of clinical neuroscientists, pathologists and basic scientists. A Human Brain Tissue Repository at National Institute of Mental Health and

Neurosciences, Bangalore, South India has been functioning for the past 18 years contributing to the progress of neuroscience. With the occurrence of natural and man-made calamities like Bhopal gas tragedy, Co-60 leak in West Delhi, it is imperative for us to learn lessons by banking and analysing the tissues to understand altered biology and face the future events in preparedness. This makes the establishment of Brain Bank and Biobanks in India, a national priority as a planned strategy to face the disease and disaster in well informed and planned way.

Keywords Brain Bank · Degenerative diseases · Molecular biology · Neurobiology · Promotion of neuroscience

Introduction

Studies on the central nervous system (CNS) are fuelled by significant interest among researchers to understand the complexity of its function, to investigate various pathologies and evolve diagnostic and therapeutic strategies. With the development of molecular methods, studies on brain aging, neuro-infections, degenerative and psychiatric conditions have intensified over the past couple of decades. Though our understanding of brain function and pathology has advanced with some progress in biomarker discovery and pharmacotherapy, many endeavours have not reached their logical end since the experimental results are not validated in the human condition. This is clinically pertinent since *in vitro* and *in vivo* models cannot substitute for the human system, due to species barrier and significant differences in molecular mechanisms and genetic/epigenetic diversity. To fill this lacuna, several countries world-wide have set-up human brain and biospecimen repositories that store, characterise

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and annotate human CNS tissues and related material for clinical and fundamental neuroscience research. The Brain Banks record the clinical details of the human tissue specimens including pre and post mortem details such as demographic information, disease stage, and clinical data from the medical records of the donor. Thus, Brain Banks link the clinician, neuropathologist and basic scientist by providing well documented specimens, which prove to be precious resources for neuroscience research, aimed at developing novel diagnostics and personalized therapies.

Time magazine (May 12, 2009) identified Biobanking as one of the “ten ideas changing the world right now”. The Biobanks by nature are essentially prospective to meet the stringent needs of the scientist. Population based Biobanks are useful for the study of health status of community, the interplay of life style, environment and human response manifesting with disease. These are essential for policy planning. The project driven ones are usually small, located in the corridors of anatomic pathology division managed and controlled by the surgeon and/or pathologist. They collect specimens to pursue a research question, and may be closed later. Now the idea of ‘Biospecimen Banking’ has progressed to ‘Biospecimen Science’, addressing primarily the procedures of collection and the molecular integrity of the specimen banked for research [1, 2]. This is following hard lessons learnt in cancer research, where poor tissue integrity in the archived tissue has resulted in a serious setback to the studies.

Why Study the Human Brain When Animal Models are Available?

It is essential to realize that the newly developed transgenic animal models do not reflect all the aspects of human disease especially the neurodegenerative diseases with variable cognitive failure [3]. The human brain has evolved over centuries acquiring language, consciousness and self-awareness as we understand. This does not deny these attributes in the animals, but our understanding of cognition and learning in them does not enable us to fathom the neurodegenerative diseases like Alzheimer’s disease (AD), Parkinson’s disease (PD), various synucleinopathies and tauopathies which have no counterpart in animals. With aging, humans and primates develop neurofibrillary tangles in the brain [4], while the aged rodents manifest amyloidosis of the kidney, but not the brain [5]. Similarly, appropriate equivalents to psychiatric disorders like schizophrenia and bipolar disorders have no appropriate equivalents in animal models to understand the disease. These facts highlight the need to study the human brain and refraining from extrapolation from lower animals. Ethnic and gender differences in functional genomics of the right and left brain, various Brodmann areas of the human brain are emerging as a new avenue of research.

With the evolution of Biobanks in various countries, an International Society for Biological and Environmental Repositories has been established to monitor and assign accreditation. The Human Brain Tissue Repository at the National Institute of Mental Health and Neurosciences (NIMHANS) is not listed, though the Principal Co-ordinator has been a member of the society for the past 6 years. To meet the expanding demand for well characterised and good number of relatively homogenous group of tissues, Networks of Biobanks as consortia have emerged. They share the clinical details and specimens with the investigators. The key feature of formally linked Brain Bank network is a centralised, harmonized data base for all the specimens archived, which is a critical component for broad based research.

Global Scenario

Biobanks in the world, to be the reliable resource centres of quality biomaterial (including brain, tumors, tissue fluids etc.) need to be accredited and registered [6].

Number of Brain Banks in the world indexed (may not be registered)

USA	46	Europe	19
Australia, New Zealand	8	Canada	2
China	1	Korea	1
India	1	Russia	1

This number is increasing enrolling many centres.

The following information reflects the magnitude of the biospecimens stored and used for research in Europe [7].

<http://www.bbmri.eu/index.php/catalog> of European Biobanks (as on 15th of April 2013)

247—Biobanks (of various sizes and categories)

1.8 million Samples of DNA

80,000 samples of cDNA

330,000 cell lines

1.8 million serum/plasma samples

50,000 cryopreserved samples

8 million paraffin embedded tissues

Total >16 million samples

Diseases: Neoplasm, CNS diseases, circulatory metabolic endocrine, nutritional and rare diseases.

The gold standard protocols of modern Brain Banking include:

- (a) A well-established donor system in which an informed consent has been obtained to conduct the autopsy and retain the organs for future research and

store with investigators, protecting the confidentiality of the donor.

- (b) Developing a system of rapid autopsies with short post-mortem delay and freezing freshly dissected brain and spinal cord. This logistic planning is a prerequisite for enhancing the range of modern molecular biological genomic, proteomic and transcriptomic studies on human tissues in health and disease.
- (c) Developing uniform and well accepted clinical and neuropathological diagnostic criteria.
- (d) Ensuring quality control of samples distributed to investigators.
- (e) Ensuring distribution of brain tissue only after confirming that the material is negative for infective conditions like HIV, HBs Ag etc., for the safety of the investigators.
- (f) Honour the religious and social sentiments of the donors and maintain contact with them. Public education and dissemination of knowledge to the general public is the corner stone in enrolling them in cadaver organ donation.

The research areas benefitted from the HBTR could be broadly classified into infectious and non-infectious CNS diseases and other studies and the number of publications from each research area is indicted in Table 1. Many investigators in India though utilised the tissue, have not provided the results of their study and this remains a perpetual lacuna.

Some discoveries are possible due to Brain Banking in the World

Among the plethora of studies and knowledge emerged from analysing cryopreserved tissues, the most epoch making are those that helped determine the pathogenesis or evolve treatment modalities. They include:

- (a) Demonstration of decrease in dopamine levels in PD leading to the development of L-Dopa therapy [8].
- (b) Detection of fall in the levels of choline acyltransferase and loss of cholinergic neurons in cases of AD helped instituting cholinergic and anti-choline esterase regimens [9].
- (c) Dopaminergic over activity in nigrostriatal pathway was implicated in the pathophysiology of chorea in Huntington's disease [9]. The present treatment for chorea centres on the use of agents that diminish dopaminergic activity, including post synaptic dopamine receptor antagonists (neuroleptic phenothiazines, butyrophenones) and drugs acting in presynaptic like reserpine, tetrabenazine. All these

Table 1 Scientific publications using the tissues obtained from HBTR

Field of study	No. of publications
HIV	12
Parkinson's disease	9
Xenobiotics	8
Epilepsy	5
Brain Banking	7
Alzheimer's disease	4
Rabies	4
JEV	3
Tumor	3
CJD	3
Brain aging	2
Tuberculosis	2
TBI	2
Stroke	2
Psychiatry	1
Madras type motor neuron disease	1
Cryptococcal meningitis	1
Toxoplasma	1
Measles	1
Wilson's disease	1
OCD	1
ALS	1
Fungal infections of CNS	1
Bacterial meningitis	1
Fungal infections of CNS	1
Depression	1
Others	3
Total research publications	81

drugs ameliorate chorea and psychiatric complications of the disease.

- (d) The identification of etiological agent in SSPE (measles virus) [10, 11] and progressive multifocal leukoencephalopathy (JC virus) [12].
- (e) Central role of amyloid β protein in the evolution of AD, associated with tau protein, ubiquitin and exploring immunotherapy (though found not successful) [13].
- (f) Discovery of intrathecal immunoglobulin synthesis secondary to antigenic stimulus, without direct participation of peripheral immune system introduced the concept of immunomodulation in CNS and immune mediated neurological disorders [14].
- (g) Discovery of AIDS encephalopathy as a distinct entity in the paediatric age group [15].

Need for Brain Bank in India

In India, certain demographic and medical variables specific to the country are recognized which do not permit extrapolation of observations from the West as universal and secular. For example, Indian population reacts adversely to doses of antiepileptic drugs well tolerated in the West [16]. Indians and Japanese react differently to the ethanol levels in hard liquor because of genetic and biochemical polymorphism [17]. There is dose variation in reaching 'high' among individuals consuming addiction forming drugs. In Northern India, endemic goitre and genetically transmitted goitre are common [18]. South India is home for many inborn errors of metabolism and other inherited disorders probably resulting from consanguineous marriages and genetic inbreeding [19], manifesting with Lafora body disease with myoclonic epilepsy, hot-water epilepsy, Wilson's disease etc. A form of leukodystrophy is recognized in Agarwal families [20] and high prevalence of PD in Parsi Community [21] unlike in others. There are endemic pockets for Thalassaemia/sickle cell anaemia, specific tropical infections. HIV infection in India is essentially caused by HIV-1 clade C, unlike the West (HIV-1 clade B), with different biology and low prevalence of HIV-dementia [22]. Madras type of motor neuron disease is different from the one reported from the West. Most of these diseases primarily or secondarily affect the neuron system with clinical manifestations. In India, non-availability of human autopsy material has hampered most of the researchers, as there is lack of awareness about the clinical autopsies among basic scientists and minimal interaction between the clinicians, pathologists and research scientists.

Origin of Human Brain Tissue Repository in India

The first human brain tissue repository (HBTR) in India was established in 1995 in NIMHANS, Bangalore and is currently housed in the Neurobiology Research Centre of the same institute. This facility took birth nearly 10 years after "Brain Storming Session in Neurobiology" held at NIMHANS in 1985. It was the persistent efforts of Prof. P.N. Tandon, an eminent neurosurgeon who facilitated the establishment of this "national research facility". For the first 5 years, it was collectively funded by Department of Science and Technology (DST), Department of Biotechnology (DBT) and Indian Council of Medical Research (ICMR). From March 2000, the facility is maintained by NIMHANS, allotting the money from the Planned Budget. The HBTR has documented and stored human brain, serum and cerebrospinal fluid samples, from various neurological, psychiatric disorders over the past 18 years and has made significant contribution to neuroscience research in India.

The HBTR has actively collaborated with various national and international research groups and provided precious human material for research projects. Using such material, several novel observations related to brain pathology have been made thus establishing the importance of the HBTR in neuroscience research in the country utilising the human samples collected at autopsy with informed consent of deceased while alive or close relatives after death, from India. Many of these studies have been carried out in pathologies involving Indian population.

The Objectives of Human Brain Bank at NIMHANS

- (a) To collect, categorize and store human brain tissue from cases of psychiatric disorders, neurodegenerative diseases, developmental and metabolic diseases. To serve as controls, brains are collected from individuals who succumbed to road traffic accidents but have no prior neurological illness. The tissue is provided for biochemical, molecular biological, genetic and patho-morphological investigations.
- (b) The tissue fluid bank collects samples of plasma, serum and cerebrospinal fluid at autopsy from cases of various infectious disorders like tuberculous meningitis, viral encephalitis, AIDS, various neurodegenerative disorders like PD, AD, dementias, CJD, psychiatric diseases like schizophrenia, bipolar affective disorders, neurological diseases like stroke and epilepsy. In addition, serum and CSF are collected at autopsy from individuals with no neurological illness and unused plasma from blood bank serve as controls. The large volume of CSF (collected at autopsy and characterized) and unused plasma (to be discarded) from blood banks are used to develop diagnostic kits.
- (c) Peripheral blood leukocytes from index cases and family members of cases with suspected genetic and familial disorders like Lafora body disease, various forms of epilepsy, muscular dystrophies are stored for DNA bank for genetic studies. In house scientists and collaborators are utilising these material.

What Does Brain Bank do with the Brain Collected at Autopsy?

The Brain Bank personnel where a neuropathologist is actively involved, collects the brain at autopsy with informed consent. The brain is sliced in sagittal plane into two halves; one half is fixed in 10 % buffered formalin, and the other half is sliced coronally into 1 cm thick slabs and frozen in plastic bags at -80°C . Required anatomical areas dissected can be provided to the investigators, with diagnosis and relevant clinical data corresponding brain

tissue samples from cases of road traffic accident form the controls. Paired samples of serum and CSF are frozen from cases of infective disorders (tuberculous meningitis, viral encephalitis, parasitic diseases, AIDS, tumours, cerebrovascular accidents, some of the neurodegenerative disease, etc.) are frozen in small aliquots to provide to the investigators.

Is the human brain collected at autopsy useful for biochemical and molecular biological studies?

- (a) The tissues are best suitable for research when the interval between the time of death and freezing [post-mortem interval (PMI) after autopsy] is kept to a minimum, preferably under 6 h [23, 24].
- (b) PMI of 72 h: nucleic acids, proteins are intact while catecholamine and neuropeptide levels fall.
- (c) Terminal agonal changes do not significantly affect RNA/Protein concentration. RNA degradation occurs with repeated freeze thaw cycles. If the patient before death was in coma, the quality of RNA is not good.
- (d) No significant effect of pH on the brain (indicator of tissue integrity) with post-mortem delay of 4–18 h. Similarly premortem agonal state (Glasgow coma scale 3–18) and storage of the brain at -70°C for 1–12 years [25] do not significantly affect the tissue integrity for investigations.
- (e) With premortem agonal state, glutathione (GSH) reductase and GSH peroxidase levels are reduced [26].
- (f) In the brains from female subjects, GSH levels are high in brainstem and cerebellum.

In animal models, the investigator has the control to choose species, gender, genetic homogeneity (inbred-animals) the weight and other characteristics to suit the work plan. However when using the human material various patient related variables play a crucial part starting from administrative, legal, ethical issues, antemortem variability and post-mortem events. It is extremely difficult and impractical to control them in the human system. Yet, the material properly collected and characterized is extremely precious. Unless round the clock facility exists, the patient who dies during working hours are autopsied within 6–8 h, while at long stay clinics and psychiatric hospitals, the autopsy procedure is delayed thus partially denaturing the tissue. These are related to the factors like availability of relatives to give informed consent, the interest of the clinicians and pathologist and ready availability of facility for transportation to the mortuary and autopsy facilities at the hospital. In scientific literature, the details of post-mortem events are seldom described in publications using the human autopsy material, thus making the meta-analysis difficult.

Biobanking and Post-mortem Studies

For successful experimentation using post-mortem brains, the biochemical, molecular and structural integrity of the tissue should be maintained. It is quite plausible that pre-mortem factors (metabolic state, infections, seizures, hypoxia, exposure to toxins, drugs, and terminal agonal state before death) and post-mortem factors (post-mortem delay in extracting the brain from the deceased and the storage conditions) can critically influence post-mortem brains. While low PMI traditionally represents high tissue quality, tissue pH, tryptophan and RNA quality are also considered as quality markers [27–29]. PMI is pertinent while studying enzyme activities, protein expression, post-translational modifications in neurodegenerative pathologies [30, 31]. In several projects, the human brain tissue used from HBTR was assessed biochemically and pathologically for the post-mortem stability of different intracellular parameters in the stored human brains:

- Tissue pH in different brain samples increased with increasing PMI, while the protein solubility was relatively unaltered in different anatomical regions [25]. Among different regions, substantianigra displayed increased protein oxidation/nitration events, but the tissues did not display abnormal histology. The expression of glial fibrillary acidic protein and neurofilament were not altered by PMI in the frontal cortex.
- Mitochondrial enzyme activities including citrate synthase, malate dehydrogenase, succinate dehydrogenase and complex I were altered by gender difference, increasing age, severe agonal state and increased storage time [32].
- GSH and related enzymes were affected by agonal state, PMI and gender in cerebellum and medulla and not in hippocampus and substantianigra, while other activities such as superoxide dismutase and catalase were relatively unaffected [26]. Further, the antioxidant activities in different sub-cellular compartments were significantly affected by age and not by other pre-mortem and post-mortem factors [33]. Hence matching the age of control and test samples are essential in interpreting the studies.
- The stability of glial fibrillary acidic protein (astroglial marker), oxidized and nitrated proteins was significantly decreased with increasing storage time in the medulla and that of oxidized proteins in cerebellum, but not in the frontal cortex. However, PMI and agonal state did not affect the status of these markers. Nitrated proteins were relatively unstable among women in cerebellum compared with men [34]. Knowledge of this neuroanatomical heterogeneity in biochemical parameters is essential in planning the studies.

Xenobiotic Metabolism

Since environmental toxins are directly implicated in the pathogenesis of CNS diseases, there is a lot of interest in investigating the metabolism of xenobiotics in the brain. The major drug metabolizing enzymes, cytochromes P-450 (P450) and flavin-containing mono-oxygenase (FMO) have been extensively studied. The brain has unique P450s that metabolize drugs and endogenous compounds and these metabolic pathways are significantly different from that seen in liver [35]. Hence, analysis of the brain P450s and their mechanism of action have significant implications for drug and xenobiotic metabolism in the brain. HBTR was the source for various studies on xenobiotic metabolism in the human brain.

- Contrary to the earlier understanding that xenobiotic metabolism occurs only in the liver, P450 and FMO were detected for the first time in post-mortem brains [36, 37] and spinal cord tissues [38]. These enzymes showed regional heterogeneity in the brain and were predominantly localized to neurons [36, 38].
- A novel FMO (MW: 71 kDa) which efficiently catalyzed the metabolism of imipramine to its N-oxide was purified from the human brain [39]. This FMO was heat labile and cross-reacted with the antibody to rabbit pulmonary FMO.
- Mitochondria from different human brain regions displayed multiple isoforms of P450 belonging to the 1A, 2B, and 2E subfamilies that are involved in xenobiotic metabolism [40].
- Functionally active P4502E (the major ethanol-inducible P450 that metabolizes ethanol to acetaldehyde causing adduct-formation in cytoskeletal proteins) was detected in different anatomical areas of post-mortem human brains [41]. P4502E was constitutively expressed in neurons within the cerebral cortex, cerebellum, dentate gyrus and pyramidal neurons of Ammon's horn subfields.

The availability of quality human specimens has decreased throughout the world due to alarming decline in autopsy rate in the medical centres. The new imaging modalities have led to a sense of over confidence among the clinicians though misplaced, that they can peep into the body and offer definitive diagnosis. This is further compounded by the phobia of pathologists to handle high risk autopsies, thus rendering the autopsy service redundant in the minds of clinicians and anatomic pathologists. The general public, especially the cadaver organ donors are not informed enough regarding the philosophy and benefits of brain donation, as a 'gift of hope' promoting research in neuroscience research and possible discoveries. This explains vividly why no other Brain Bank is established in

India though there is imperative need. The new trend of 'needle autopsy'—a practise of sampling various tissues and brain with long needles and by stereotaxy, from the accessible organs does not reflect the true picture. Many of the neurological diseases are neuroanatomical site specific and routine needle biopsy does not fulfil the need. Research on rare CNS diseases has become difficult as necessary tissue sample are not available. In spite of these impediments and limitations, the Brain Banks are functioning throughout the World.

Human Brain Bank at NIMHANS Bangalore—15 years of functioning

- Established round the clock autopsy service. Brains are collected 4–20 h after death. In the West usually autopsies are conducted 24–72 h after death.
- Provides fresh brain samples and tissue fluids for research.
- Promotes neuroscience and cadaver organ donation by participating in exhibitions at colleges and schools.

The existence of Brain Bank is justified only when the clinicians, neuropathologists and basic scientists work in tandem. The clinician has an unenviable role to convince the relatives to consider donation of brain after death, to promote neuroscience research as a 'gift of hope' to future generation. The neuropathologist has the crucial role in confirming the diagnosis by collecting the tissue in time and providing tissue for research. Only after the clinician and the pathologist come to an agreement about diagnosis, can the basic scientist try to decipher the molecular changes in the diseased brain. To overcome the social and logistic problems involved in tissue banking and promoting research in neurosciences, the governmental agencies involved need to recognize the importance and declare the facility as one of the national priorities for health policy planning for the present and future.

What is the contribution of Human Brain Bank to the progress of Neuroscience in India in collaboration with various investigators? (Some salient observations)

- Age related pathological changes in the brains from India are similar to the ones noted from West [42].
- The prevalence of ApoE (ϵ 4) in Indian population is low, while association with AD is comparable with that in West.
- CJD in humans (Prion disease) is seen in India, even in vegetarians (CJD Registry at NIMHANS) [43].
- Following traumatic injury, brain damage is greater in children than in adults. Contrary to the Western

- literature, ApoE- ϵ 3 is associated with worse prognosis following traumatic closed head injury [44].
- (e) PD is related to reduced number of melanised neurons in substantianigra, correlating with decreased dopaminergic neurons and the neurochemicals. The number of melanised neurons in substantianigra are same in Indian, British and Nigerian brains though in India and Nigeria the prevalence of PD in general population is low unlike in USA and UK, suggesting alternative protective biochemical pathways [45, 46].
 - (f) DNA topology in aging and AD—A transition of super coiled β -helical brain genomic DNA acquires left handed Z helix with rigid grooves, in normal aging and AD, probably secondary to oxidative stress, intracellular ionic imbalance and cell shrinkage [47].
 - (g) Demonstration of different isoforms of cytochrome P₄₅₀ in human brain in conditions like alcoholism and treated epilepsy. The observation that the brain can metabolize a variety of lipophilic xenobiotics that enter via the blood stream, without the intermediate stage of metabolism in the liver is a seminal observation made collaborating with the HBTR at NIMHANS [37, 48]
 - (h) Decrease in 5-HT induced IP3 formation and decrease in 5H2 receptor levels have been demonstrated in psychiatric diseases in contrast to controls [49].
 - (i) In both AD and multi infarct dementia both ionisable Ca, P in CSF is significantly decreased [50].
 - (j) In India, the HIV infection is caused by HIV-1 Clade C unlike West (Clade B), with reduced macrophage fusion and giant cell formation. In India, following HIV infection, opportunistic bacterial, viral and parasitic infections are common than AIDS dementia, probably related to amino acid substitution at position 31 in viral 'tat' protein. The 'tat' protein causes neuronal damage via NMDA receptor mediated toxicity [51, 52]—this is repeated in the next section—bullet point no. 4.
 - (k) Cellular localization of Malin/Laforin in Lafora body disease with myoclonic epilepsy and their nuclear or cytoplasmic localization determining the clinical course [53, 54] has been established.
 - (l) Genomic and proteomic studies in cases of chronic meningitis encephalitis using frozen human brains [55] to identify biomarkers.

Infectious Diseases

India has the second largest burden of HIV related pathology, next only to the sub-Saharan Africa, essentially caused by HIV-1 clade C [56]. Although there is a lot of research undertaken towards the diagnostics and therapeutics of AIDS, one area of research that requires

attention is the evolution of HIV-mediated CNS pathology especially in the initial asymptomatic stage. In this regard, pathological phenomena including HIV induced encephalitis, vasculitic neuropathy and dementia require systematic study. The HBTR at NIMHANS has contributed in understanding the neuropathology of HIV in the following collaborative studies:

- A specific and sensitive PCR-based strategy to identify HIV-1 subtype C-virus (the subtype, which contributes to ~50 % of HIV-1 infections worldwide and predominantly in India) with implications for vaccine development and therapeutics in HIV has been developed [22].
- Analysis of HIV-infected cells in the inflammatory infiltrates of 15 HIV samples indicated brain pathology and suggested multiple portals of entry into the brain and homing for latency. Further, the HIV patients treated for an opportunistic infection could develop HIV associated leucoencephalitis, clinically manifesting with dementia [57].
- During HIV infection, the virus attaches and enters into target cells via a specific receptor and secondary receptor (co-receptor) and the virus could change its preference of the co-receptor. But, the pathological significance of the co-receptor switch in HIV-1 is not understood. Dash et al. [58] screened several primary viral isolates and generated novel clones to understand the unique variable pathogenic characteristics of HIV subtype C.
- The HIV-Tat protein may cause neurotoxicity via NMDA receptor-dependent excitotoxicity and Cys 30–Cys 31 motif in Tat is critical for this function. The clade C Tat has a Cys31Ser mutation which might significantly attenuate the neurotoxic response [51]. This could also contribute to lowered monocyte migration to the brain in clade C and lowered incidence of HIV-induced dementia in India compared to US and European population [52].
- Analysis of nerve biopsies that HIV is both neurotropic and lymphotropic and direct viral invasion might be involved in the pathogenesis of peripheral nerve vasculitis, even in clinically asymptomatic cases [59].
- In addition to the predominance of subtype C infection, B/C recombinant viruses were identified in primary HIV-1 isolates in south India [60].

Non-communicable Pathologies

Brain aging The free radical theory of biological aging proposes a role for altered redox balance in physiological aging of the brain, which in turn might lead to several age-associated brain pathologies. However, the role of

oxidative damage in aging and neurodegeneration is not completely understood.

- Brain aging contributes to neurodegeneration in PD and AD. Accordingly, human substantianigra, displayed extensive protein oxidation and astrocytosis and lowered mitochondrial function with concomitant loss of antioxidant function during aging compared to striatum, thus making it more vulnerable to selective degeneration during PD [61]. Similarly, enhanced protein damage, elevated GFAP expression and lowered mitochondrial complex I and antioxidant activities were observed in HC and FC (regions implicated in AD) compared to cerebellum with increasing age [62].

PD PD is a movement disorder associated with loss of pigmented neurons in the substantianigra pars compacta of the ventral midbrain. Although several research advances have delineated the pathology and different molecular pathways associated with the neurodegeneration in PD, the mechanistic basis of PD pathology has not been well understood. The HBTR been part of the following interesting projects related to PD:

- Brains from India had 40 % lower number of melanised nigral neurons than the brains from UK and this number did not decrease with age while the motor function and dopamine content were unaffected [63]. This suggests that the dopamine loss during the progression of PD may not be due to absolute numbers of melanised neurons in substantia nigra but related to critical fractional depletion from the pre-existing ones in the subject. The lower prevalence of PD among Indians compared to the British suggested an alternative neuroprotective mechanism. Further, neuronal loss noted in PD is exclusively due to the disease process itself and progressive to evolve from sub clinical stage to full blown disease.
- Oxidative stress in the mid brain could contribute to neurodegeneration in PD brains. The markers of oxidative damage and mitochondrial function were found to be relatively very low in striatum and frontal cortex, probably due to 3–5 fold increase in the total GSH secondary to decreased breakdown [64]. Further, synaptic terminals from the frontal cortex displayed higher GSH and related enzymes compared to other intracellular compartments [65], thus controlling the oxidative damage related neuronal degeneration.

Some Issues to Ponder

Whether the Brain Bank/Tissue Banks should provide the tissue collected from the donors/patients with an informed

consent, to the commercial entities is a matter to be thought of dispassionately. The specimens collected and stored are provided to the in-house scientist and other investigators and some of them funded by commercial organizations under the oversight of Institutional Review Boards. The patient donors do not expect and want their specimen to be stored indefinitely waiting for a worthy project to evolve and use. While the Biobanks need to maximize the utility of the stored specimens, the patient wants the progress of translatable research, so that cure or at least palliative therapy can be evolved based on the investigation. A national, pragmatic debate is needed to address the issue of providing the BioBank specimen to commercial organizations, instead of treading carefully with the fear of companies making profit and fatten at the cost of generous donors. The donors can be given the choice in the consent form whether their donated and bio-banked specimen can be shared with commercial organization that may have the money power to take the discovery through the final phases of trial and bring it into market. The other approach, mostly followed by many is keeping the commercial organization at arm length with a blanket statement of “not worth the risk” The HBTR at NIMHANS, at present is playing safe, looking forward to a national debate and guide lines to evolve and to be followed.

In line with the stipulation of ICMR, normally no tissue is provided outside the country from the HBTR. In case of international collaboration, the foreign collaborator is encouraged to utilize the biological material with in the country, setting up a laboratory and facilitating technology transfer. However, for the sake of validation of the result, maximum of 10 % of the material may be provided to the foreign investigator. The analysis of the data is carried out at both the national and international laboratories. This facilitates transparency in scientific interaction and fruitful sharing of technology. The international Bio Banks have mooted the idea of ‘empty tissue banks’ figuratively to indicate active sharing and utilization of the stored specimens, instead of hoarding away and waiting for a “highly productive and really worthy project” to use it.

With the advent of cancer Biobanks to discover biomarkers and evaluate response to therapy, ‘tissue micro array’ has become handy in handling large sample size. In cancer Biobanks and Brain Banks investigating neurodegenerative diseases, there is a need to monitor the pre analytical variables influencing the gene expression profile (RNA levels) than the gene structure in DNA. These are influenced by the pre-mortem agonal changes and the PMI in harvesting the brain. Luckily at the HBTR, because of the infrastructure developed, the autopsies are conducted within 6–24 h, unlike many foreign countries. Some of the autopsies were conducted within 2 h after the death. The sociological and religious urgency to cremate body soon

has contributed to early autopsy at HBTR, Bangalore. Due to shortage of trained and committed scientific manpower, it is becoming difficult to be responsive to new technical advances in tissue banking and distribution. Some of these issues can be addressed to an extent by active interdisciplinary collaboration. Proteomic, transcriptomic studies could be initiated at HBTR, in some of the infective conditions relevant to India in view of the availability of good quality neuronal tissue stored.

Problems in Developing Brain Banks

In India, clinical post-mortems are regularly performed as a policy at KEM Hospital and JJ Hospital in Mumbai, Postgraduate Institute of Medical Education and Research at Chandigarh, and NIMHANS at Bangalore, in addition to sporadic autopsies in some other medical colleges. Medico legal autopsies are carried out as mandated by Police, but the performance is delayed due to logistic and administrative reasons. The policy of conducting autopsies at NIMHANS on infective conditions like HIV, Rabies, HSV, CJD, Tuberculosis etc. has facilitated to collect and store very valuable material from brain and thus facilitate distribution and in house research. In other major Institutes like ACTREC-TMH at Mumbai, Department of Neuropathology at AIIMS, New Delhi, limited Cancer Tissue Biobanks catering to the in-house research are functioning, but have not evolved to the status of National Research Facility with networking.

Increase in awareness among the scientific fraternity regarding the need to study human brain for the progress of neuroscience, enrolling the clinicians and administrators in cadaver organ donation process and proactive education and public awareness in the society can facilitate the organ/tissue collection. But unless the pathologists and scientists join together with committed philosophy the tissue banking and Brain Banking will remain stunted in India slowing the progress of neuroscience.

Let Us Not Miss Opportunities To Learn Lessons From Those Who Succumbed Due To Ignorance And Unpreparedness.

Natural calamities and man-made accidents usually do not give time to be prepared to meet the challenge. Recent early warning system of cyclones and earth quakes has facilitated mass mobilization of the people from the epicentre of activity. Japan as a nation is a standing example of how the standard operating procedure has been systematized with professional precision. Repeated occurrence of these natural calamities including 'once in a life time, Tsunami' and nuclear power plant failure in Japan has taught lessons; and their government is quick to initiate life saving measures. However, India has witnessed many man-made calamities destroying the habitat and ecosystems

suddenly and adversely altering the response of the human body to the events. The learning continues even after the event has passed, though the 'events are made to forget' for various considerations. This applies to biological and chemical terrorism with element of surprise and variable biological effects on the human body. These events have long term effects on the human biology and nervous system, realized as late events on the health of the people exposed. With progress in science and evolving sophisticated analytical methods, the study of human nervous system provides valuable new insights into the events, effects on the biological system and techniques to combat the late effects. This is possible only by having the access to the human tissues and tissue fluids for analysis following the accident.

To state a few recent examples:

- (a) Bhopal gas tragedy-1984.
- (b) Endosulphan tragedy in Kasargod and Kerala.
- (c) BuddhaNallah—pollution in Sutlej River due to industrial effluent.
- (d) High levels of uranium and heavy metals in the ecosystem in Malwa region (Punjab).
- (e) Cobalt-60 leak in Delhi (April 2010)
- (f) H1N1 Influenza epidemic.
- (g) Assisted reproductive biology and foetal anomalies.

In India, assisted reproductive biology clinics have mushroomed utilizing various advanced and expensive in vitro technologies. Some of these foetuses are aborted spontaneously in the first trimester, because of cytogenetic abnormalities detected in the embryos [66]. Banking and study of these aborted foetuses will provide insight into the prevalence and phenotypes of anomalies and developing cell lines for advanced neurobiological studies. Pre-implantation genetic diagnosis in these cases can enhance the early diagnosis of possible anomalies and reduce foetal wastage.

In India, every year, outbreaks of various viral infections do occur with significant mortality and long term morbidity. Because of unforeseen accidents and phobias, fresh tissues are not banked except at the HBTR in Bangalore. With change in ecology, new viral infections are emerging, and we have inadequate knowledge. The only way to confront the situation and prepare for unforeseen eventualities is banking of tissues and tissue fluids for research to understand the biology and evolve vaccine and drug related strategies forming research consortia and share the material and knowledge. It is essential for the nations to learn lessons following these disasters and develop strategies for preparedness. This is feasible only by banking the human and animal biological tissues and leaving them accessible to the scientists for systematic analysis.

A Road Map

- (a) Educate the Medical Professionals about the need and benefits of ‘Brain Banking–Biobanking’ for future research and to “Convert the biological waste into precious resource for research”.
- (b) Increase public awareness about organ donation.
- (c) Clarify the legal and ethical issues keeping public interest in mind (not prejudices) regarding cadaver organ donation act 1994 and encourage the participation of public and scientists.
- (d) Ensure development of standardized diagnostic criteria and digitized medical record keeping, maintaining the essential patient confidentiality.
- (e) Recognition of the Tissue Banks/Fluid Banks as a national asset for the progress of medical/neuroscience by the National Scientific bodies (DST/DBT/ICMR)
- (f) Encourage establishment of small or big brain tissue/tissue fluid banks in major medical centres and ensure networking for sharing of material/technology/ideas.
- (g) Develop infrastructure and trained and dedicated manpower and retain the talented scientific pool.
- (h) Human tissue is not a commodity to be owned or sold. Brain Bank managers have to act as custodians of such a resource and facilitate scientific studies utilizing the material. The pathologist forms a bridge between the clinician and basic scientist for the promotion of science. All the Brain Banks have to subscribe in thought and spirit, to the overarching guidelines encoded in Helsinki declaration (World Medical Association Declaration of Helsinki Ethical Principles for Medical Research Involving Human Subjects (1964), Adopted by the 18th WMA General Assembly, Helsinki, Finland) and Ethics in Science and New Technologies in respect of Human Tissue Banking (Council of Europe Opinion of the European Group on Ethics in Science and New Technologies to the European Commission. Ethical Aspects of Human Tissue Banking, 21st July 1998) and the Nuffield Council on Bioethics (<http://www.nuffieldbioethics.org>). Brain Banks must be run on non-profit basis. A national consensus/policy need to be evolved regarding sharing of the banked material with large pharma companies for R&D activities.
- (i) Encourage networking with International Biobanks and Brain Banks. Encourage thinking, sharing, and innovation. Brain Donation is the greatest legacy one can leave as a ‘Gift of Hope’ for generations to come.

I shall pass this way but once in life. If I may do any good, let it be no; for I shall not pass this way again

...Anonymous

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