

ALZHEIMER'S DISEASE - HISTOLOGICAL ULTRASTRUCTURAL AND IMMUNOCHEMICAL STUDY OF AN AUTOPSY PROVEN CASE

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SUMMARY

We report a detailed morphological, ultrastructural and immunochemical features of neuronal pathology in a case of Alzheimer's disease. This is probably the first detailed study of an autopsy confirmed case from India. The features noted are similar to the ones described from the West.

Introduction

Indian literature on dementia is rather scanty (Bharucha et al. 1964, Mani et al. 1964; Kalyanasundaram et al. 1979, Srinivas et al. 1982). Though there are many hospitals for the psychiatrically ill, data regarding the incidence and the biological basis of this condition is meagre. Only recently some serious interest is evinced by various workers in understanding the basis and trying different therapeutic modalities in management. The two main types of pathological states in old age known to culminate in dementia are (a) arteriopathic dementia, and (b) senile/pre-senile Alzheimer's dementia. Though rapid strides are made in the West in elucidating the pathogenesis, evolution, diagnosis and treatment of Alzheimer's disease (Masters 1984, Kosik et al. 1986,

Perl et al. 1987, Young 1987, Wolozin et al. 1986, Chan-Paley 1987, Grundke-Iqbal 1986, Bahmanyar et al. 1987, Weiner 1986) surprisingly no pathologically verified case is reported in Indian literature, except for the report by Somasundaram and Sarada Menon (1975) based on a frontal lobe brain biopsy.

We report an autopsy confirmed case of Alzheimer's disease along with ultrastructural and immunochemical characterisation of the neuronal pathology. To the best of our knowledge, this is the first detailed report of the nature, from India.

Case Report

A 73 year old male scientist, was admitted at National Institute of Mental Health and Neuro Science (NIMHANS), Bangalore in November 1978 with a his-

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tory of gradual decline in personality, forgetfulness, disorientation to place and occasional bouts of violence of two months duration. The symptoms were progressive with rapid worsening 15 days prior to admission, in the form of double incontinence and confusion. There were no gait disturbances or symptoms of raised intracranial tension.

The patient was the eldest of six sibs. One of the brothers died of diabetes. The youngest sister was admitted to this hospital, about 20 years earlier for Psychosis. No other family member, had either dementia or any other degenerative neurological disease.

General physical and systemic examination were within normal limits. Mental status examination revealed a dishevelled individual with lability of affect, incoherent talk with perseveration and impaired concentration. Detailed cognitive function assessment revealed grossly impaired recent and remote memory, nominal aphasia, impaired judgement and insight. Neurological examination demonstrated primitive reflexes in the form of snout, glabellar tap and palmomenta. There were no lateralizing or localizing signs.

Laboratory investigations done indicated a serum creatinine of 1.6 mg%; blood urea of 40 mg%; the others being normal. The patient was detected to be a diabetic with random blood sugar values between 400-500 mg%. Serum protein analysis, electrocardiogram, electroencephalogram and X-rays of skull and chest were normal. Lumbar CSF analysis showed a protein level of 45 mg%, glucose 48 mg%, absent cells and globulin and a non reactive VDRL. Patient was investigated for dementia with a right carotid angiogram which showed evidence of gross ventricular dilatation. Pneumoence-

phalography confirmed a communicating hydrocephalus with moderate cortical atrophy. A clinical diagnosis of senile dementia of Alzheimer type was made. The patient was treated for diabetes with insulin and 300 mg/day of thioridazine was administered for his behaviour problems. Patient's condition gradually worsened and he died in hospital of diabetic ketoacidosis, 2 years after initial admission. Partial autopsy confined to the examination of brain only was performed.

Gross Pathology:

The dura was firmly adherent to the vault of the skull. The leptomeninges were thickened and opaque and the subarachnoid space has widened markedly. The brain was atrophic, especially the frontal lobe and middle and inferior temporal gyri on the right side. Mild, but diffuse atherosclerosis of the cerebral vessels was noted. Serial coronal slicing of the brain revealed thinning of the frontal and temporal gyri, widening of the sulci and marked ventricular dilatation. The diencephalic nuclear areas, the brain stem and cerebellum were essentially normal. Substantia nigra seemed to be normally pigmented.

Material and Methods

The brain was fixed in 10% buffered formalin. Representative sections from various parts of the brain were processed. Six micron thick, paraffin sections were stained with H.E., Kluver Berrera for myelin and Nissl, PTAH for glia, and Bodian Silver to demonstrate the neurofibrillary tangles, Alzheimer's neuritic plaques, dystrophic neurites and axons. Formalin fixed, cryocut sections from frontal lobes were also stained with Von Braunmühl's silver technique for better delineation of the neuritic plaques and Congo Red to

demonstrate the amyloid in the plaques and the neurofibrillary tangles (NFT).

Bits of formalin fixed tissue from the frontal cortex was processed for ultrastructural study and viewed under JEOL-100 CX electron microscope.

For the immunochemical study cryocut serial section (10 micron thick) of formalin fixed, 10% sucrose rinsed bits from frontal lobe and hippocampus were mounted on gelatinised slides. The sections were immunostained by Sternberger's peroxidase-anti-peroxidase technique (1982) using monoclonal antibodies against phosphorylated (SMI - 31) and non phosphorylated (SMI - 32) epitopes of 200 KDa neurofilament (NF) polypeptide isolated from rat (Sternberger - Mayer, Immunochemical Inc, Jerrettesville, Maryland, U.S.A.); human foetal microtubule associated protein 'tau' (Courtesy K.S. Kosik, Boston, USA) and paired helical filament (PHF) purified from Alzheimer's disease brain (Courtesy I.G. Iqbal, New York, USA). SMI 31 and SMI-32 were used at 1:1000, anti PHF at 1:10,000 and anti 'tau' at 1:5 dilution. The secondary antibody - HRP tagged rabbit anti-mouse serum was used at 1:40 dilution. All the antibody dilutions were done in 1% normal rabbit serum. The washing for NF staining was performed with 0.05 M Tris - HCL (PH 7.5) containing 0.2 M NaCl and for PHF and 'tau' staining, phosphate buffered saline (pH 7.2) containing 0.2 M NaCl. Sections were incubated with the primary antibody for 2 hours and secondary antibody for 30 minutes at room temperature. Colour was developed using diaminobenzidine / hydrogen peroxide. Necessary controls were incorporated in the study.

Results

Histologically numerous senile plaques (Fig. 1) were seen through out the cerebral cortex but the greatest concentration was in the frontal and temporal lobes, less frequently in the occipital lobe and other nuclear areas. Large majority of the plaques were immature to mature ones with relative paucity of central amyloid core. Argentophilic Alzheimer's neurofibrillary tangles (NFT) (Fig. 1) were present, more



Fig. 1 Bodian silver stained frontal cortex showing mature senile plaque with argenteophilic core (arrow) and many neurofibrillary tangle bearing neurons.

frequently in Sommer sector, glomerular formation of the hippocampal gyrus, amygdaloid nucleus, the adjacent temporal lobe and the orbital aspect of the frontal lobe. These were seen in different stages of evolution, some neurons containing dark, flame shaped skeins of neurofibrils displacing the nucleus to ghost tangles, totally occupying the cells, with loss of cell membrane. These tangles showed congo-

phelia and greenish birefringence under polarised light, characteristic of amyloid. An occasional NFT was noted in the substantia nigra, reticular formation of pons and medulla, but none in Purkinje cells, the dentate nucleus and inferior olivary nucleus. The other pathognomonic feature of Alzheimer's disease, the granulovacuolar

degeneration (GVD) was frequently noted in the hippocampus (Fig. 3 Inset) and occasionally in the frontal and temporal lobes. Variable degree of neuronal loss and gliosis was appreciated in the cortex. Large amount of lipofuscin was seen in most of the neurons displacing the Nissl substance. No evidence of congophilic-angiopathy was noted in any of the vessels.

Ultrastructural Features

The senile plaques were found to be made up of distended neurites containing many membrane bound dark bodies, admixed with glial cell processes and other structures in neuropil.

The central part of it occasionally contained fibrillary mass representing the amyloid. The neurons containing neurofibrillary tangles at light microscopy were found distended by stacks of straight and twisted fibrillar masses (Fig. 2A). The twisted filaments were found to be paired, helically wound with a diameter of 20nm and a periodic constriction of about 80nm (Fig. 2B). The straight ones were found to measure about 15 nm in diameter. In addition to these neurofilaments, large amount of lipofuscin was seen in the neurons, replacing the RER. Focal aggregates of mitochondria, lysosomal bodies were seen in some of the neurons. Under electron microscope granulovacuolar change revealed a membrane bound inclusion with translucent matrix and a dense core (Fig. 3).

Immunochemical Study:

The monoclonal antibody (m Ab) to phosphorylated epitope of 200 KDa NF polypeptide immuno labelled the normal axons in the white matter. Among the hippocampal neurons only the ones bearing the NFT were stained while the extracellular ghost NFT were not labelled (Fig. 4a). Only a few of the dystrophic neurites of the

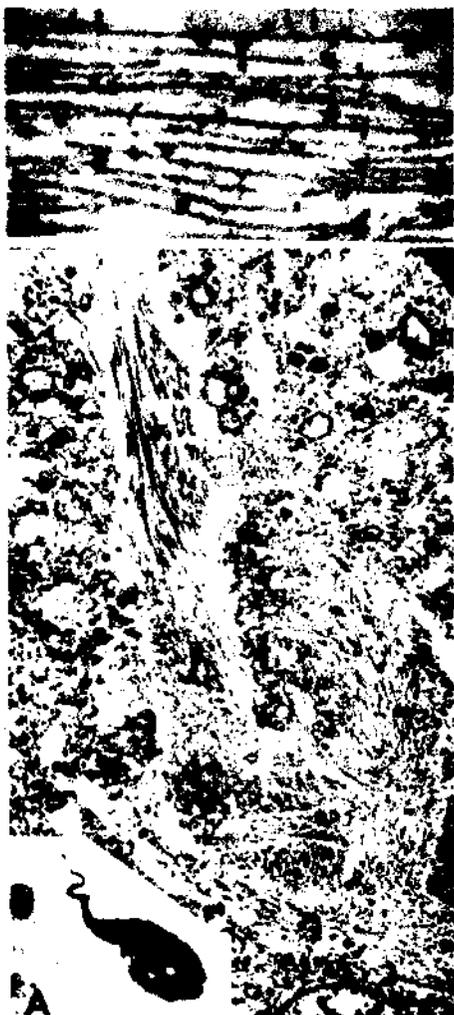


Fig. 2A Electron micrograph showing a neurofibrillary mass totally occupying the neuron. Note masses of lipofuscin in between. X 3500
Inset - A neurofibrillary tangle stained by Von Braunmühl silver.

Fig. 2B Higher magnification of the fibrills showing paired helical nature with constrictions (arrows) X 22,000



Fig. 3 Electron micrograph showing a neuron containing a membrane bound granular mass (arrow) - the granulo vascular change

A. In set showing argentophilic granulovascular change in hippocampal neuron.

senile plaques were visualised by this antibody. On the other hand, a number of NFT, including the ghost tangles were lighted up by the m Ab to PHF indicating the distribution of the PHF (Fig 4c). Similarly many of the neurites and lightly the central core of the plaques were stained indicating the presence of PHF in these areas. The m Ab to 'tau' labelled the maximum number of NFT in the hippocampus, but not the extracellular ghost tangles similar to NF antibody (Fig. 4b). The dystrophic neurites of the plaques and the ones diffusely distributed in the white matter were maximally visualised by this antibody. In addition, the granular component of the granulo vacuolar change was also stained positive by 'tau' m Ab. The m Ab to nonphosphorilated epitope of 200 KDa NF polypeptide (SMI-32) failed to react with any of the components. This variable immune reaction by different m Ab suggests that microtubule associated protein 'tau' is the major antigenic component in the various neuro pathological elements and takes part in the evolution of NFT and PHF.

Discussion

It is generally felt that in India, there is an apparent absence of diseases like Alzheimer's disease, Pick's disease and multiple sclerosis (Sarasa Bharati 1983, 1986). Similarly in a limited pathological study of dementias, Shankar et al. (1982) expressed that there may be a relative paucity of cases of Alzheimer's disease in this subcontinent. However, in an unpublished prospective study at National Institute of Mental Health and Neurosciences, it is observed that over the past 5 years nearly 30-35% of cases of dementia seen at Neurology-Psychiatry services of the Institute fall into the category of Senile/presenile dementia of Alzheimer's type satisfying the DSM III Criteria (Personal communication Nagaraja, D.N.). Probably the lack of autopsy confirmed cases and subjective impression based on limited sample lead to this view. The present case is the first autopsy confirmed case of Alzheimer's disease in India. The ultrastructural and immunochemical study further confirmed and delineated the lesions. All the features are identical to the ones reported from the West. (Hirano et al. 1962; Tomlinson 1970; Terry 1980; Wang et al. 1984; Kosik et al. 1986; Dickson et al. 1987). In spite of vast amount of literature on the morphological and immunochemical character of the lesions, the basic pathogenetic mechanisms leading to dementia eluded definition (Masters 1984). Toxic effect of elements like Aluminium, Silicon and Calcium (Garruto et al. 1984; Perl et al. 1982; Munoz Garcia et al. 1986; Kobayashi et al. 1987; Perl et al. 1987); aberration in the metabolism and utilisation of neuropeptides, neurotransmitters (Young 1987; Chan-Paley 1987) and defects in the axoplasmic transport of cytoskeletal and other formed elements of neuron (Gajdusek, 1985; Cork et al. 1986) have been incriminated as the causative factors.

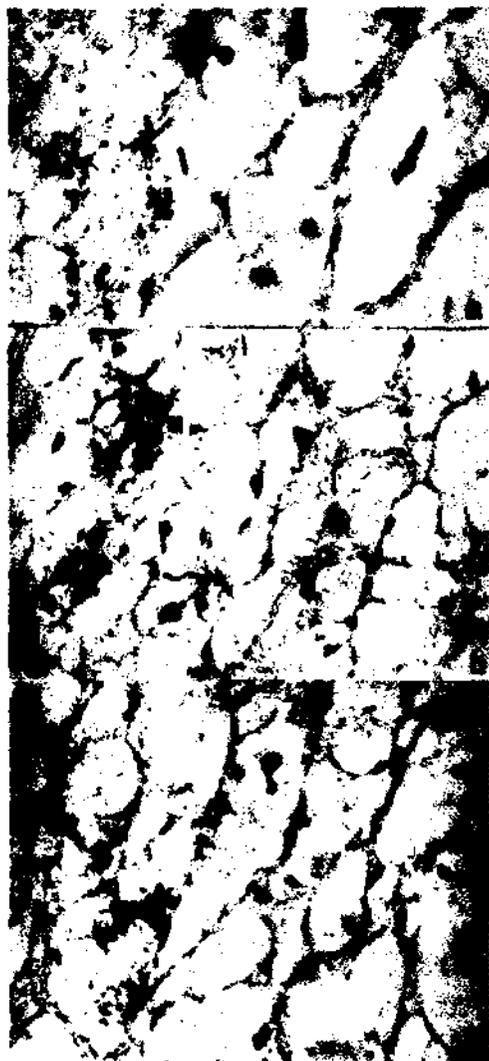


Fig. 4 Immunocytochemistry - PAP technique
 a. Monoclonal antibodies to '200 KDa NF Phosphorylated epitope' showing positive staining of a few NFT and vaguely the senile plaque.
 b. Monoclonal antibody to 'tau' protein. Note positive immunostaining of numerous NFT, robust staining of the plaque and many neurites
 c. Monoclonal antibody to 'PHF' showing dark staining of the NFT and plaque.

It is generally believed that the density distribution of NFT, GVD, and plaques in the cortex correlate well with the evolution and degree of dementia (Neary et al. 1986). However it is not still certain as to whether their presence and

accumulation within the critical areas of nerve cells is actually associated with damage to the functional integrity of the neurons. A recent quantitative morphometric ultrastructural study (Sumpster et al. 1986) has shown that a reduction in the cytoplasmic RNA, and the nuclear and nucleolar volume in the affected NFT bearing neurons are related to the degree of dementia. This suggests that the capacity of protein synthesis in tangled cells appear to decrease, whereas oxidative and lysosomal activity is preserved. The NFT formation and accumulation of the cytoskeletal elements may therefore lead to cell death and thus form the major cause of neuron loss in Alzheimer's disease.

A detailed in depth study is needed to verify the facts about the prevalence and the clinical progression of senile/pre-senile dementia in this country. It is also necessary to evaluate the impression of relative paucity of NFT and plaques in senile brains in India in contrast to the West.

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