# Review of recent publications on Alzheimer's disease

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ALZHEIMER'S disease (AD) has been recognized as the fourth leading cause of death in adults in the US. It accounts for 55% of cases with senile dementia; which in turn occurs in about 15% of the population above 65 years of age. Research on clinical and basic aspects of AD has emerged as one of the leading areas of research on ageing. A Medlar search of recent literature on Alzheimer's disease showed that in addition to a large number of individual review articles, three monographs have appeared on the subject in the last two years. One of these[1] is based on selected papers from the 2nd International Conference on Alzheimer's Disease and Related Disorders (ADRD), held in July 1990, at Toronto, Canada. This book makes interesting reading. It has been carefully edited. In spite of individual papers from 84 different groups, a coherence of theme has been maintained. In fact, individuality of each paper is one of the strengths of this book. At this juncture, when a lot is yet to be learnt about AD, access to original data is quite important, both for research and clinical care of these patients. The 3rd International Conference on ADRD was organized at Podova, Italy in July 1992. The other two monographs include, a) proceedings of a symposium on molecular and cellular mechanisms of neuronal plasticity in normal ageing and Alzheimer's disease[2] and (b) a set of articles published in *Advances in Experimental Medicine and Biology* (vol. 282, 1990). Here an attempt is made to summarize the research reported in the Proceedings of the 2nd International Conference on ADRD since it provides a single readily accessible source of current literature on the subject.

## Clinical research

Clinical research on Alzheimer's disease can be broadly categorized under four headings, viz. 1) definition of the disease which includes establishment of diagnostic criteria and tools for resolution of heterogeneity, 2) identification of biological markers for early diagnosis and prevention, 3) epidemiological studies and 4) clinical trials to identify appropriate therapeutic strategies. Progress in each of the above areas is reviewed separately.

*Definition of disease.* Definitive ante-mortem diagnosis of AD, short of brain biopsy, continues to remain elusive. For clinical care and research several clinical criteria have emerged for diagnosis of probable AD. The criteria evolved by NINCDS-ADRDA Work Group[3] appear to have found universal acceptance. Key diagnostic features include cortical type of dementia (loss of stored memory), parietal lobe symptoms and cognitive impairment. Remainder of the neurological functions largely remain intact. It is important to exclude history of strokes, cerebral infarcts on CT, Hachinski's ischaemia score of equal to or greater than 7, positive serological tests for syphilis, history of significant head trauma, alcoholism, seizures, multiple sclerosis, and premorbid psychiatric disorder for entertaining the diagnosis of AD. For detailed neuropsychological evaluation a battery of psychometric tests is required to be employed. The articles by Diaz *et al.* (p. 9), Verma *et al.* (p. 13), Blennow *et al.* (p. 21), Brun and Gustafson, (p. 26) and Clark *et al.* (p. 37) provide several references to clinical evaluation of patients with ADRD.

Simultaneously, histologic morphometric criteria are being sharpened to achieve better clinical correlation and precise postmortem diagnosis (Jellinger *et al.*, p. 75; Braak and Braak p. 91; Flood *et al.*, p. 136). This is necessary to resolve growing controversy regarding specificity of senile plaques and neurofibrillary tangles. AD-type changes have been described in brains of patients with Down syndrome (Hyman and Mann, p. 105) as well as in normal ageing individuals. A set of diagnostic pathologic criteria have been evolved by an NIH AARP workshop[4] which could be followed for correct definitive diagnosis. The use of functional markers such as cerebellar glutamate receptor subtypes (Dwear *et al.*, p. 123) and adrenergic receptors at blood brain barrier is not yet established (Grammas, Roher and Ball, p. 129).

*Heterogeneity.* In the patient originally described by Alzheimer in 1907 the symptoms started before the age of 65 years and the lesions were located in gray matter of the brain. Today a significant number of cases are diagnosed as AD on the basis of characteristic neuropathologic findings despite onset of symptoms over 65 years of age and concomitant presence of white matter lesions on computerized tomography (leuko-araiosis = LA). Blennow *et al.* have presented evidence that there are two subgroups of patients with AD; one of early-onset (EAD) who have marked parietal lobe

symptoms and the other of late-onset (LAD) who have marked congnitive impairment and confusional symptoms. Leuko-araiosis was found predominantly in the LAD subgroup. Similar observations have been made by Diaz *et al.* In their study, the mean age of patients with significant LA was $75.3 \pm 6.1$ (s.d.) years compared to $67.6 \pm 9.6$ (s.d.) years in the group without LA ($p < 0.0001$). Also, the LA was more common in females (65% v/s 17%; $p < 0.0001$). The extended scale dementia (ESD) score in the group with LA was $106.4 \pm 77$ compared to $160.3 \pm 71.7$ in the group without it ($p < 0.001$). Leuko-araiosis is located in white matter around lateral ventricles (subcortically). It is considered to arise as a result of selective infarction of white matter. On the basis of CSF : blood albumin ratio the patients with LA have been shown to have impairment of blood brain barrier (Blennow *et al.*, p. 21). The significance of age of onset and LA in the definition of AD needs to be resolved.

## *Biological markers of AD*

Search for biological markers of AD is being pursued to overcome the limitation of clinical tools in arriving at definitive diagnosis of AD. They may also help in identifying individuals at risk and in epidemiological studies.

Since the major pathological finding in patients with AD has been the loss of cholinergic neurons in neocortical areas, several investigators have attempted to measure neurotransmitters in CSF. Reinikainen *et al.* (p. 525) have reported that cholinesterase (CE), homovanillic acid (HVA), 5-hydroxyindoleacetic acid (5-HIAA) and somatostatin like immunoreactivity (SLI) are all reduced in CSF of patients with histologically confirmed AD compared to controls, but these changes are only subtle and usually present in advanced stages of the disease. Another approach that has been used for this purpose is the use of positron emission tomography (PET). Nordberg *et al.* (p. 517) using (11C) nicotine and PET have shown that there is marked decrease in both (S) (−) and (R) (+) (11C) nicotine uptake in frontal and temporal cortex of patients with AD. It may be worth further evaluation.

The second major pathological feature in AD is the deposition of amyloid in brain. This has led to several studies on amyloid precursor protein (APP) (B/A4) to see if it can serve as a biological marker. Prior *et al.* (p. 533) have measured its concentration in CSF by double sandwich ELISA test, which measures all 3 major isoforms of APP (APP 695, APP 751, APP 770). They found that the concentration of APP is slightly decreased in both AD and multiinfarct dementia cases, but the difference was not significant statistically. Longitudinal assays have been proposed to further evaluate the role of this probe. The APP levels and isoforms have been studied in circulation and platelets by Bush *et al.* (p. 547) but no definite conclusions could be drawn.

In the same direction, Shinohara *et al.* (p. 541) have evaluated the use of measuring alpha 1-antichymotrypsin (ACT), which is another important constituent of amyloid deposits in AD. While ACT values in serum were not significantly different, the ACT in CSF was elevated ($p < 0.01$). It also appears to be related to duration of the disease.

Mortilla *et al.* (p. 565) have shown that a subset of patients with familial Alzheimer's disease have a modification in the activity of hexokinase.

Hossein *et al.* (p. 569) have studied the utility of an AD-associated protein (ADAP-A68). Using Alz.−50 antibody, they have shown that high concentrations of ADAP are found in patients with AD in areas of temporal cortex, hippocampus, subiculum and amygdala; the brain areas which are known to be involved in AD. Similar observations have been made by Ravid *et al.* (p. 577). It may serve as an useful laboratory test for postmortem diagnosis of AD.

The search for useful markers will surely continue.

## *Epidemiology*

There are five articles in the proceedings on epidemiology of AD. One of them is a preliminary report on the Kungsholmen Project (Stockholm, Sweden) by Fratiglioni *et al.* (p. 359). The investigators have studied almost 90% of the 2368 subjects born in 1912 or earlier living in one parish of Stockholm. Dementia was found in 15% of the subjects (12% in age group 75–84 years compared to 34% in people aged 85 years and over), of which 54% were considered to be due to AD. The cases and controls are going to be followed up longitudinally. One limitation of this study could be that authors have used the DSM III-R criteria instead of NINCDS-ADRDA for diagnosis of dementia and AD.

## *Therapeutic strategies*

There are eight chapters in the book on therapeutic approaches for AD. These include both clinical and experimental studies which cover a wide spectrum of design protocols and assessment strategies. One major area of drug trial has been the evaluation of cholinomimetic agents. This approach is based on the observation that in patients with AD a major neuronal dysfunction is in cholinergic transmission. The effort is to develop a safe, long acting cholinesterase inhibitor which is used as the reference standard for these studies. Preliminary results indicate that THA (tetrahydroamino acridine) and its analogues could be worth pursuing

(Davis and Haroutunian p. 605; Alhainen *et al.* p. 611; Ahlin *et al.* p. 621). Heptylphysostigmine, Metrifonate (MIF) and Huperzine A are also being evaluated (Giacobini and Becker p. 627). Studies with physostigmine and other cholinesterase inhibitors have shown that all patients with AD do not respond to these agents. Alhainen and colleagues (p. 611) have reported on the use of spectral analysis of alpha/theta ratio to distinguish between responders and nonresponders.

Lamp *et al.* (p. 643) have carried out a pilot study to evaluate the efficacy of TRH in combination with lecithin in the treatment of AD. Both TRH and its analogues are known to facilitate cholinergic transmission and lecithin is a precursor of acetylcholine. Preliminary results indicate that the combination is devoid of serious side effects and may improve performance.

A new approach has been the use of growth factors (NGF, bFGF, EGF, TGF-alpha). Potter and Morrison (p. 639) have described two experimental models for the evaluation of these products: 1) cholinergic neurotoxin AF 64 A induced loss of hippocampal choline acetyl transferase activity and 2) septal-hippocampal cholinergic neuron loss following transection of the fimbriafornix pathway. FGF was found to be significantly effective in preventing decrease in cholinesterase activity and both FGF and NG protected against loss of cholinergic neurons.

## Basic research

### *Genetic studies*

In a considerable number of patients with AD, particularly with earlier ages of onset, the disease appears to be inherited as an autosomal dominant trait (Van Duijn *et al.* p. 423; Bruni *et al.* p. 451). However, most patients with AD appear to be sporadic (non-familial). The importance of genetic factors is supported by the observation that characteristic histopathological changes of plaques and tangles are also seen in patients with Down syndrome. Accordingly, major effort had been to identify the genes which could be responsible for AD.

Genetic studies on AD are complicated because of the small number of families, poor pedigree structure, diagnostic uncertainty, late age of onset of disorder, possible genetic heterogeneity and occurrence of non-genetic phenocopies. Genetic linkage studies in early onset families have demonstrated linkage of AD to the proximal long arm of chromosome 21 around marker loci D21S13/D21S16, D21S52 and D21S1/D21S11 (Van Hul *et al.*, p. 457). The structural gene for APP has also been mapped distally (telomeric) to this region.

### *Biochemistry of amyloid and paired helical filaments*

Senile plaques and neurofibrillary tangles in neocortex are characteristic of AD. The former consists of extracellular and vascular deposits of amyloid (congophilic) while the latter are formed of paired helical filaments (PHF). Considerable basic research is going on to unravel the biochemical nature of amyloid and PHF to understand the aetiology and pathophysiology of AD. Results of various studies reported in the proceedings are summarized below.

### *Amyloid*

Amyloid of AD is made up of $\beta$-protein (also known as A4 protein). It is about 40 amino acid long (4 kD amyloidogenic degradation product of a transmembrane amyloid precursor protein (APP)) the gene for which is located on chromosome 21. APP is produced in various organs of the body including brain, thymus, lung, adrenal gland, small intestine and liver. There are several isoforms of APP (770, 751, 695 and 563 amino acid long polypeptides) which appear to arise as a result of alternative splicing of exons 7 and 8. Tanaka *et al.* (p. 313) have studied the expression of APP m-RNA in brain and various non-neural tissues. In brain mRNA 695 was found to account for 67.1% of total APP mRNA. In other organs of the body mRNA 770 or 751 predominate. It has been proposed that in AD there may be qualitative/quantitative variation in expression of different isoforms of APP in different parts of the brain compared to controls, but the results are not yet conclusive. APP is a 90 130 kD transmembrane protein. It has been purified and immunochemically characterized to a large extent. Constitutively APP is cleaved into a large (>100/kD) soluble $NH_2$-terminal fragment and a small (9 kD), membrane associated, COOH-terminal fragment. Esch *et al.* (p. 269) have shown that the normal cleavage site of APP is located within the $\beta$- protein sequence of AD amyloid protein. Thus, in normal individuals APP does not usually give rise to generation of amyloidogenic fragment. It has been postulated that in patients with AD the cleavage of APP at the normal site is blocked. Gandy *et al.* (p. 155) have presented evidence that modulation of APP processing may be influenced by its phosphorylation either as a result of abnormality in protein kinases or phosphatases.

The $\beta$-protein component is postulated to lie at the junction of ectodomain and transmembrane domain of APP, 28 residues lying outside the cell and 14–15 within the membrane. The $NH_2$ terminus of the $\beta$-protein has been identified to be aspartic acid (position 597 of APP). Cleavage at this site is proposed to give rise to amyloidogenic fragments. Wisniewski *et al.* (p. 332) have shown that amyloid fibrils are formed by microglial cells. In well-formed plaques alpha1-anti-chymotrypsin and heparan sulphate proteoglycan are

also seen regularly. However, their significance in the pathogenesis of AD is unknown.

Lahiri and Robakis (p. 443) have studied the regulators of APP gene activity. They have found two *cis* acting elements in the APP gene promoter, viz. one enhancer element (−600 to 450 bp) and one negative element (−450 to 150) in the 5′ flanking region of the APP gene. The promoter appears to extend from −150 bp upstream to the transcription start site. This is a high GC region comprising of five GGGGCGC boxes, indicating that APP gene is probably a housekeeping gene.

Morgan *et al.* (p. 407) have studied the RNA from brain samples of over 300 patients with AD and age-matched controls. Their main finding was a 3–4 fold increase in glial fibrillary acidic protein (GFAP) RNA in the cerebral cortex of patients with AD. This is consistent with increased number of GFAP positive astrocytes and elevation of GFAP protein in AD patients.

### *Paired helical filaments*

Paired helical filaments (PHF) are the major constituents of neurofibrillary tangles of AD. Their composition appears to be quite complex. PHF proteins are only sparingly soluble even in presence of detergents and are relatively resistant to proteolysis. They predominantly consist of 45–62 kD polypeptides. In addition, there are varying numbers of low molecular polypeptides as well as high molecular weight aggregates. Immunochemically the 45–62 kD polypeptides cross react with the microtubule-associated polypeptides, 'tau', but the 'tau' extracted from NFT's appears to be significantly modified. 'Tau' of PHF has been shown to be abnormally phosphorylated. Calcium/calmodulin dependent protein kinase activity has been shown to be enhanced in hippocampal neurons in AD. Presence of phosphoserine, phosphothreonine and phosphotyrosine in isolated PHF suggests that more than one protein kinase may be involved. Abnormality of 'tau' interferes with assembly of microtubules, presumably leading to a defect in intracellular transport. Altered tau is proposed to accumulate as PHF. In addition to tau, ubiquitin has also been demonstrated in PHF. Disorganization of cytoskeletal system leads to dystrophy of neurons.

In *vitro* studies employing cultured cortical neurons have shown that elevation of intracellular calcium can lead to loss of microtubules, accumulation of 8–15 nm straight filaments and abnormal tau, and appearance of PHF-like structures.

### *Aetiology of AD*

While there has been considerable progress in understanding the biochemistry and molecular biology of amyloid and PHF, the basic lesion which is responsible for these alternations still remains elusive. Both genetic and environmental factors have been implicated. Realizing the limitations of studies on humans, several animal models are being explored.

1. Iqbal, K., McLachlan, D. R. C., Winblad, B. and Wisniewski, H. M. (eds.), *Alzheimer's Disease: Basic Mechanisms, Diagnosis and Therapeutic Strategies* (Selected papers from 2nd International Conference on ADRD) John Wiley and Sons, New York, 1991, pp. 1–675.
2. Coleman, P. D., Higgins, G. A. and Phelps, C. H., *Progr. Brain Res.*, 1990, **86**, 1–364.
3. McKhann, G., Drachman, D., Folstein, M., Katzman, R., Price, D. and Stadlan, E. M., *Neurology*, 1984, **39**, 939–944.
4. Khachaturian, Z., *Arch. Neurol.*, 1985, **42**, 1097–1105.