



Neuropathological hallmarks of Alzheimer's disease

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Alzheimer's disease (AD) is the most common progressive chronic neurodegenerative disorder and one of the leading causes of dementia. It is characterized by cortical amyloidogenesis, loss of neurones particularly in those regions associated with cognitive functions and cortical atrophy. Neuropathological hallmarks include neurofibrillary tangles (NFTs), neuritic plaques, neuropil threads, Hirano's bodies, granulovacuolar bodies and cerebral amyloid angiopathy. To demonstrate these changes it is necessary to perform special stains such as silver stains (von Brownmühl, Bielschowsky) immunohistochemistry and electron microscopy. NFT, the major pathological hallmark of AD, consists of abnormally phosphorylated tau protein, which is neurotoxic and can cause the neuronal death. NFTs show characteristic regional and laminar distribution affecting pyramidal neurones of hippocampus, parahippocampus, amygdala, neocortex and some subcortical neurones. NFTs are characteristic but not specific findings of AD as they can be found in some other chronic brain diseases. Because of that the diagnosis of AD must be based on correlation between clinical features (dementia) and neuropathological findings (NFTs density and distribution). As the possible causes of AD evaluated are some risk factors, neurotransmitter abnormalities, decreased cytochrome oxidase and genetic mutations. An increasing number of genetic loci are determined on different chromosomes. Their mutations account for the development of different clinical forms of AD.

KEY WORDS: *Alzheimer's disease; Neurofibrillary tangles; Brain Diseases+etiology+morphology*

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INTRODUCTION

C Alzheimer's disease (AD) is the most common chronic neurodegenerative disorder and the leading cause of dementia especially in the Western countries. The onset of disease is rarely before the age of 60 and after that period the incidence and prevalence rise with increasing age (1). AD is clinically manifested by progressive memory loss and a general decline in cognitive function. With progression of disease due to severe cortical dysfunction, patient becomes demented, aphasic, disorientated, immobile and emaciated. Pneumonia or urinary infections are the common causes of death.

AD is usually divided into the following clinical forms: sporadic form with late onset (most common, 85-90% cases), familial AD with early onset, familial AD with late onset, and AD associated with Down's syndrome (2).

MORPHOLOGICAL CHANGES

There is cortical atrophy with narrowing of gyri, widening of sulci and hydrocephalus *ex vacuo*. Most severely affected are temporal lobes (hippocampus, parahippocampus, and amygdala), than frontal and parietal lobes. Occipital lobes and motor cortex are usually spared.

The disease is characterized by deposition of beta amyloid protein in cerebral cortex and dramatic loss of neurones and synapses. A number of important neuropathological changes occur in the brains of AD.

Neurofibrillary tangles (NFTs)

NFTs are considered to be a major pathological hallmark of Alzheimer's disease. Alois Alzheimer (1907) was the first who

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described the NFT in the soma of cortical neurons in a 51-years old women who had had a 5-year history of progressive dementia. NFTs develop within the pyramidal neuronal soma as argen-tophilic filamentous inclusions, which extend into the neuronal processes (Figure 1). They are flame or globoid in shape.

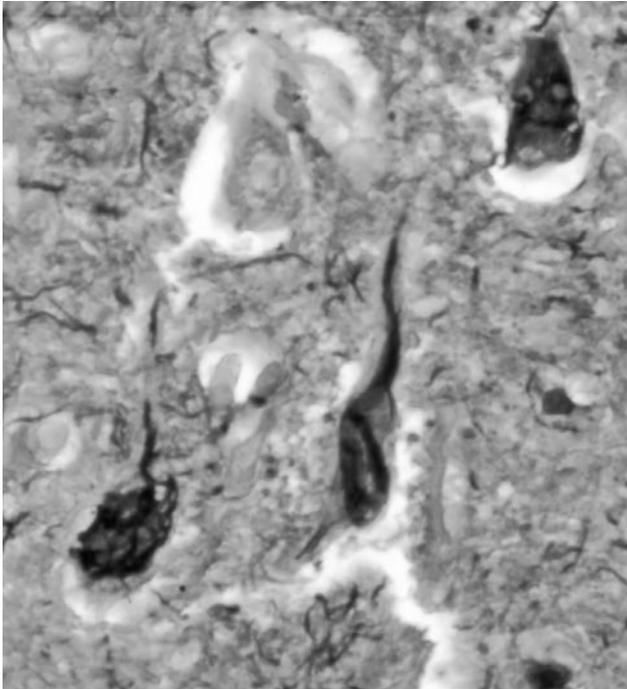


Figure 1. Alzheimer's disease; Hippocampal pyramidal neurons with darkly stained -neurofibrillary tangles; Bielschowsky silver stain modification

After deterioration of the parent cell, the NFT persists in neuropile for a long time as an extraneuronal structure.

NFT consists of highly insoluble and proteolysis-resistant paired helical filaments (PHF) in addition to 15 nm wide straight filaments and amorphous material of unknown biochemical composition. PHFs are composed of protofilaments containing proteins that are immunologically related to normal cytoskeletal proteins. PHFs appear as left handed double helices with diameter of 20-24 nm and periodicity of 160 nm. The main subunits of PHFs are altered forms of microtubule associated tau protein which undergo abnormal phosphorylation (3). Abnormally phosphorylated tau protein is believed to be neurotoxic and can be the cause of neuronal death. Apart from perikaryal NFT, PHFs are also found in dystrophic neurites associated with plaques formation and neuropil threads (4). Besides tau protein, the immunoreactivity for beta amyloid protein (beta /A4 protein) and ubiquitin can be found in NFT (5).

In general, NFTs show a rather striking predilection to affect particular areas of the AD brains. Their density is highest in the pyramidal neurones of the medial temporal lobe (amygdala, CA1 area of hippocampus, subiculum, layers II and IV of the entorhinal cortex) and moderate in the layers III and V of the association cortex

of the frontal, temporal and parietal lobe. The major subcortical neurones affected by NFT are cholinergic neurones of the basal nucleus of Meynert, noradrenergic neurones of locus coeruleus and serotonergic neurones of raphe nuclei. The characteristic and laminar distribution of NFTs supports the hypothesis that pathological process in AD may spread along a sequence of cortico-cortical connections between the association cortical areas and the hippocampal formation. The NFTs occur in neuron clusters that give rise to the feed forward and feedback cortico-cortical projections occurring between cortical and subcortical region (6). The loss of these systems leads to the disconnection between hippocampus and neocortex and between neocortical association areas resulting in the disintegration of intellectual function (7). NFTs can also be found in several other disorders (Down's syndrome, postencephalic parkinsonism, subacute sclerosing panencephalitis, amyotrophic lateral sclerosis, parkinson-dementia complex from Guam, and dementia pugilistica) as well as in normal aging brains of nondemented individuals. Because NFTs are characteristic but not specific findings of AD, the diagnosis of AD must be based on correlation between clinical features (dementia) and neuropathological findings (NFT density and characteristic distribution) (2).

Using H&E stains it is difficult to identify NFTs in tissue sections. They can be visualized by various silver stains (von Brownmühl, Bielschowsky and modifications), using histochemical methods such as thioflavine S, immunohistochemically with anti tau antibody and by electronmicroscopical investigations.

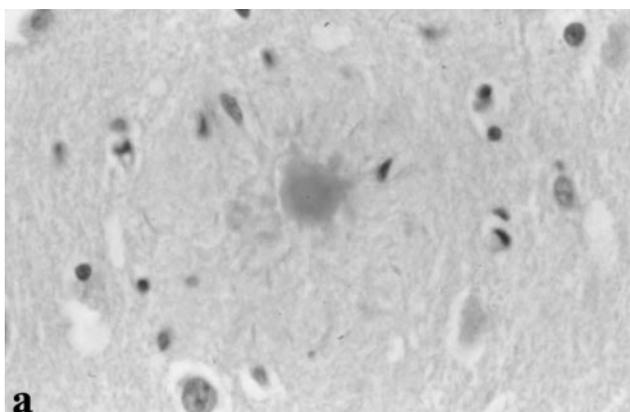
Neuritic plaques (NP)

Neuritic (amyloid, senile) plaques are foci of enlarged axons, synaptic terminals and dendrites, associated with extracellular beta/A4 amyloid. They appear as spherical areas with amyloid-positive core surrounded by argentophilic material. NPs are generally confined to the cerebral cortex (Figures 2a and 2b). The sites of predilection are amygdala, CA1 area of hippocampus, subiculum and layers II, III and V of the entorhinal cortex.

There are several plaque subtypes. The two most prominent are diffuse and classic plaques. Diffuse or immature plaque consists of beta/A4 in a non-aggregated form, free of any neuritic involvement. This form should be aggregated at some stage of the disease. It was shown that beta/A4 deposits promote neuritic interactions that result in neurite dystrophy. Classic plaque consists of fibrils of aggregated beta/A4 core surrounded by clear halo with dystrophic neurites (DN), activated microglia and reactive astrocytes at the periphery. DN within plaque consists of distended axons, dendrites and synaptic terminals. DN exhibits immunoreactivity for amyloid precursor protein, growth associated protein (GAP43), tau, ubiquitin and neurofilaments (4,8).

Histologic and immunohistochemical methods used to demon-

strate NP include Congo red, thioflavine S, silver stains and immunohistochemical stains that can demonstrate various biochemical components of the plaques. It is difficult to see NP in ordinary H&E preparations.



Hirano's bodies (HBs)

HBs are eosinophilic intraneural structures, most often found in the hippocampal pyramidal neurones. They are best seen in H&E preparations (Figure 4). Ultrastructurally HB consists of crystal-

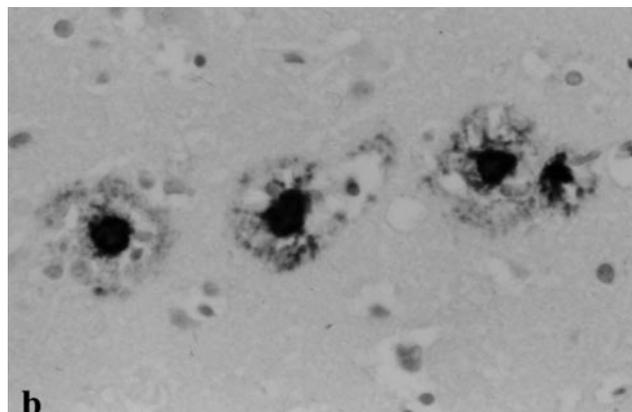


Figure 2. Alzheimer's disease; Neuritic plaques are seen in the cerebral cortex of the frontal gyrus. a) Congo red; b) Beta-amyloid immunostaining

Neuropil threads (NTs)

NTs appear as argentophilic network of fragmented and twisted fibers in the neuropil (Figure 3). They are formed within axons, dendrites and presynaptic terminals. NTs are often associated with NFTs, but are independent of NPs. Ultrastructurally, NTs are composed of PHF. Immunohistochemical techniques revealed that they contain tau and ubiquitin (4).

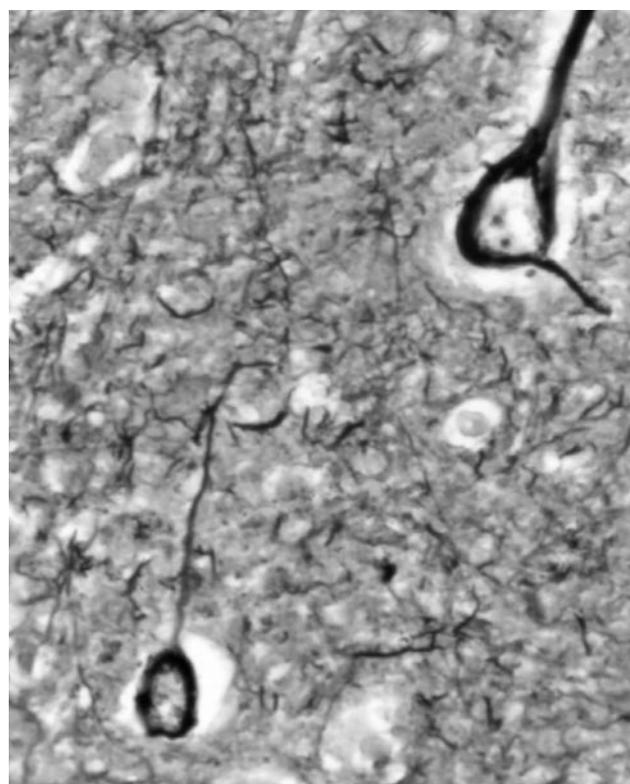


Figure 3. Alzheimer's disease. Neuropil threads. Argentophilic network of fragmented and twisted fibers in the neuropil is seen between two neurons with "corkscrew-shaped" neurofibrillary tangles. Bielschowsky silver stain modification

loid arrays of interlacing filaments displaying either a lattice-like or herringbone configuration (9).

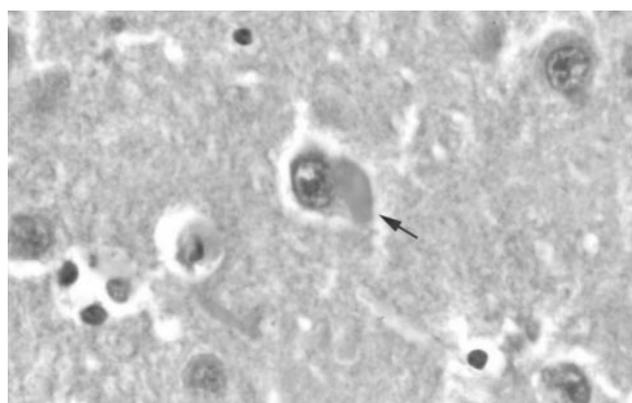


Figure 4. Alzheimer's disease; Hirano's body; Eosinophilic intraneural structure in the hippocampal pyramidal neuron (arrow); Hematoxylin-eosin stain

Granulovacuolar bodies (GVB)

GVBs appear as round vacuoles (3-4 microns) with a dense core which stains blue in H&E and are argentophilic (Figure 5). They are confined to the soma of hippocampal pyramidal neurone (9). The significance of HB and GVB is unknown.

Cerebral amyloid (conophilic) angiopathy (CAA)

CAA appears as an accumulation of beta/A4 amyloid filaments within walls of small arteries and arterioles of the leptomeninges and cerebral cortex (Figure 6). Beta/A4 can also be deposited within cerebral cortical capillaries when it usually makes a spike-like projections into the brain parenchyma (10). CAA is rarely demonstrable in the white matter and brainstem. CAA may be the cause of small cortical infarcts and hemorrhage. The methods used to demonstrate CAA are Congo red, thioflavine S, silver

stains and immunoperoxidase staining with antibody to beta/A4 protein.

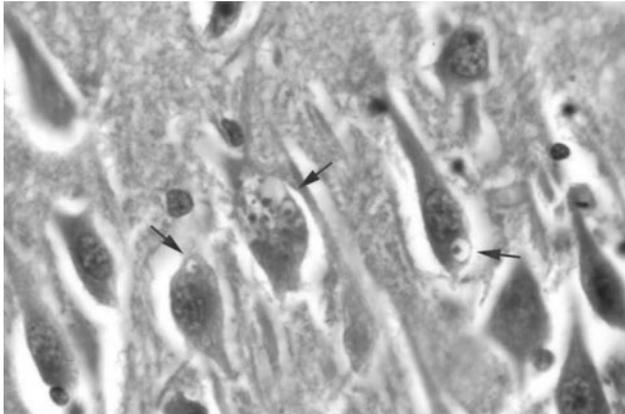


Figure 5. Alzheimer's disease; Granulovacuolar bodies; Round vacuoles with dense core in the hippocampal pyramidal neurons (arrows); Hematoxylin-eosin stain

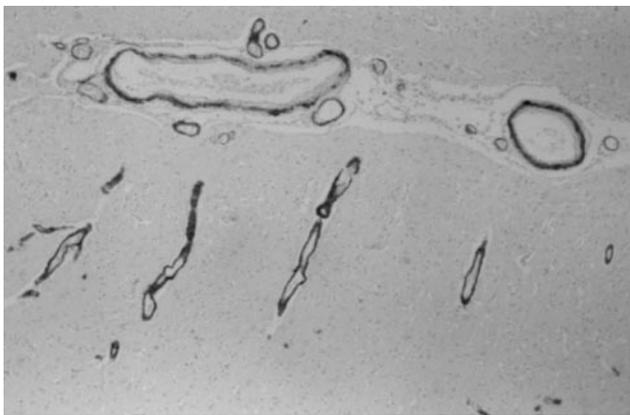


Figure 6. Alzheimer's disease; Cerebral amyloid angiopathy; Beta-amyloid is present in the leptomeningeal arteries and in arterioles penetrating into the cortex; Beta-amyloid immunostaining

PATHOGENESIS

In attempt to explain the causes of AD, only two factors stand out as having a major influence on the occurrence of AD. These are age and genetics. The association with age is not completely understood, but is likely to involve both environmental and genetic factors. Other possible risk factors are family history, maternal age, head injury, socioeconomic status (level of education and occupation), and environmental exposures (electromagnetic fields or aluminum in drinking water). Treatable diseases such as cardiac arrhythmia and diabetes have recently been reported to increase the risk for AD, while long-term use of estrogen replacement therapy in post menopausal women may act as protective factor (1).

Neurotransmitter abnormalities

There is a dramatic decline in activity of cortical choline acetyltransferase (ChAT) with depletion of cortical acetylcholine in AD

brains. The cholinergic neurones in the nucleus basalis of Meynert are markedly affected. Other neurotransmitters such as serotonin, noradrenaline, dopamine and glutamate are less affected (1).

Cytochrome oxidase abnormalities

It was postulated that defect in the key mitochondrial electron transport chain enzyme cytochrome oxidase (CO) may be the fundamental cause of AD (11). This notion is particularly attractive as in vitro data indicate that a CO defect could alter processing of the beta-amyloid precursor protein. Proteins levels of mitochondrial- and nuclear- encoded CO subunits are moderately reduced in temporal and parietal cortex, but not in relatively spared brain areas in AD. The decreased CO in brain areas having reduced neuronal activity may be the secondary event consequent to the primary neurodegenerative process (12).

Molecular pathogenesis

An increasing number of genetic loci are determined on different chromosomes.

Molecular analysis of beta amyloid of AD reveals that it is derived from a large molecule termed amyloid precursor protein (APP). APP is an integral membrane glycoprotein, which is expressed in almost all tissues and cell lines, with gene locus on chromosome 21. Under the normal APP processing there is the formation of soluble APP molecule, which appears to have a function on neuronal regulation. The normal regulation of APP processing is controlled by a positive feedback system from cholinergic neurones. As a result of abnormal APP processing arises beta-pleated sheet configuration of amyloid, also known as beta/A4 amyloid. There is some convincing evidence that beta/A4 is directly neurotoxic by disrupting the function of membrane proteins involved in the neuron's calcium homeostasis. It seems that the mutations at the APP gene are clustered within and around the amyloidogenic region and increase the amount of beta/A4 amyloid formations by various mechanisms. These mutations account for the early onset familial AD (13).

Recently it was shown that many other early onset familial ADs were linked to a locus on chromosome 14. The mutant gene encodes a protein-designated presenilin-1 (PS-1) that is predicted to be an integral membrane protein (14). In addition, a few Italian and Volga German families with early onset familial AD were linked to locus on chromosome 1. The mutant gene encodes a protein presenilin-2 (PS-2) (15). How these proteins participate in the pathogenesis of AD is unknown. Preliminary data suggest that these mutations act through the APP/betaA4 final common pathway.

Many other genetic risk factors are now being evaluated, the most important of which is the e4 allele of apolipoprotein E (ApoE) (16). It was described that ApoE family of lipoproteins with gene

locus on chromosome 19 is associated with the most common forms of AD starting after 60-years of age (late onset sporadic AD and late onset familial AD) (16,17). Apolipoproteins are lipid carrier molecules, which play a significant role in the regulation of lipid metabolism in the CNS. Apo E is the major lipoprotein expressed in the CNS. A possible role of ApoE- ϵ 4 in pathogenesis of AD consists of increasing the rate of deposition of beta /A4 and plaque formation (18). It also may facilitate hyperphosphorylation of tau protein with formation of PHF. However, possession of the ApoE ϵ 4 is not sufficient for the development of AD. Recent studies show that the increased risk for AD is associated with additional candidate genes among which are those encoding α 2-macroglobulin (19), α 1-antichymotripsin (20), butyrylcholinesterase and interleukin-1 (21).

There is no definitive treatment for AD. The most advanced treatment strategy is the use of cholinomimetic agents, which can enhance the cognition and slow down neurodegeneration by inhibiting abnormal APP processing. Other potential therapeutic manipulations include the use of estrogen, antipsychotic agents, antioxidant compounds, etc. The possibility of modifying the risk for AD by controlling some of these factors is a challenge for researchers in this field.

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Erratum

By the oversight of the guest editor serious mistakes were made in the literature references of the paper titled "Neuropathological Hallmarks of Alzheimer's Disease", which was printed in the Archive of Oncology 9(1), 2001, on the occasion of the 9th Yugoslav Congress on Pathology with International Participation. Consequently, the Editorial Board has decided to publish the paper in extenso, although its content does not belong to the thematic scope of our journal. We express our apology to all our readers and authors.

Editorial Board