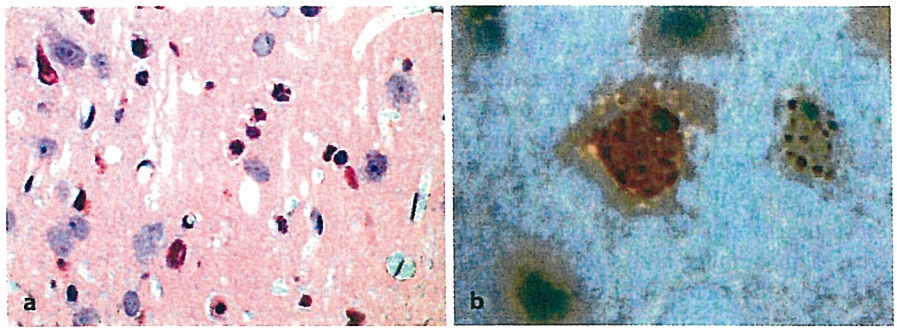

Fig. 2a,b. Light microscopy of neurons showing eosinophilic ischemic change: **a** the ischemic neurons are scattered among the normal-looking neurons in the ischemic penumbra of the cerebral cortex, and have a homogeneous eosinophilic cytosol containing a pycnotic and/or karyorrhectic nucleus (H.E. ×400); **b** TUNEL-positive nuclei showing strong positivity on the dotted chromatin condensations of the neuron are observed scattered in the ischemic penumbra of the cerebral cortex (TUNEL ×800)

Discussion

Kerr and Wyllie (1972) [15] found apoptosis after a mild ischemic insult to the liver cells; i.e., as the isolated cell died, the nucleus became condensed with large crescent-shaped chromatin condensates and fragmented into separate pieces, the condensed cytosol was also fragmented into separate apoptotic bodies containing fragmented nuclear material and normal cytosolic organelles; and these apoptotic bodies were phagocytized by macrophages. While, in necrosis, not isolated but a massive number of cells die with swollen cytosol and organelles; and the nucleus is also swollen with small chromatin condensates. Finally all cellular and nuclear membranes are ruptured. As some neurons die isolatedly with cellular condensation after a mild ischemic insult to the brain, the possibility of apoptotic neuronal death has been discussed.

The present study was aimed at elucidating time course of the ultrastructural morphological changes occurring in isolated cells undergoing neuronal death in the cerebral cortex with special regard to the behavior of the surrounding astrocytic cell processes after a mild ischemic insult, in order to discuss better the topic of apoptosis vs. necrosis for the neuronal death.

In the present model, post ischemic injuries mature slowly in the cerebral cortex. The temporal profile of histopathology revealed the development of disseminated selective neuronal necrosis (DSNN) only in the penumbra area of the cerebral cortex after the ischemic insult. However, in the infarcted focus of the frontal lobe, following temporary development of DSNN, all neurons and astrocytes except for the capillary wall itself undergo massive necrosis with marked swelling and destruction of the entire membranous system of the cells [10–12, 22].

In the penumbra, under EMS observation, the DSNN appeared from the early post ischemic stage at 15 min after the start of recirculation, as isolated dark neurons with different grades of high electron density of their cytosol and nucleus. In these dark neurons, no swelling of mitochondria and other cytosolic organelles

was observed, except in shrunken neurons with very high electron density of the entire cell. As the dark neuron showed small loosely aggregated chromatin condensates scattered in the nuclear matrix as well as along the nuclear membrane, cellular activity had probably decreased [22]. These isolated dark neurons with different grades of electron density increased in number from 5 min to 24 h, and were still newly appeared 3 weeks after the ischemic insult.

The astrocytic cell bodies and processes were remarkably swollen, especially those nearby the dendritic synapses of the dark neurons; their mitochondria increased in number and size; and they accumulated glycogen granules from 5 to 24 h after the ischemic insult. These astrocytic mitochondria showed moderately swollen cristae and increased electron densities of their matrices [12, 13]. These findings highly suggest activated astrocytic energy metabolism, generating lactate as a neuronal fuel [2, 6], scavenging potassium [25], neurotransmitters [19, 24], and other metabolites from the neuron and dendritic synapses, and also promoting survival of the astrocytes themselves [18]. As the neuronal fuel would not be transferred smoothly to neurons due to deranged neuronal energy metabolism, glycogen would accumulate in the astrocytic cell processes via gluco-neogenesis from lactate followed by glycogenesis [3, 6, 28–30].

Among the dark neurons with increased electron density, completely shrunken neurons with very high electron density of the entire cell body and axons increased in number from 5 to 24 h after the ischemic insult. Later than 12 h after the ischemic insult, some mitochondria of the isolated dark neurons showed partial swelling of their matrices, and disintegrated cristae with woolly densities. However, many of the mitochondria in the shrunken neurons showed swollen matrices with occasional woolly densities and disintegrated cristae; and such cells were considered to irreversibly damaged and unable to survive [5]. From our findings, not every dark neuron died to become a shrunken neuron, but some of them survived. Further quantitative study of the fate of these isolated dark neuron is necessary.

Four days after the ischemic insult, these condensed neuronal bodies became separated and fragmented by invasion of tiny astrocytic cell processes. Some of these fragments were phagocytized by astrocytic cell processes, and others were seen moving in the intercellular spaces. No inflammatory cells or phagocytes were seen in this study. Only the perivascular microglia showed phagocytic activities. Our present study suggests that the astrocytic cell processes around the dark neuron reacted to rescue the injured neuron by generating fuels, scavenging neuronal and synaptic metabolites [14, 16, 19, 25, 27, 30] and disposed dead neurons by shrinking, smashing and phagocitizing them.

Necrosis versus apoptosis in ischemic neuronal death has been controversial. In the present study, some of the morphological findings on the dark neurons indicated classical necrosis: i.e., on eosinophilic ghosting of cells in terms of histology; small loose aggregates of nuclear chromatin in the nuclear matrix and margin, instead of the marginates of condensed coarse granular aggregates seen in apoptosis; and a lack of apoptotic bodies. Others corresponded to apoptosis: scattered individual cells affected in terms of histology (shrinkage necrosis); lack of exudative inflammation, and condensation of the cytosol with structurally intact mitochondria and other organelles, instead of swelling of all cell component followed by

rupture of cell membrane and destruction of cytosolic organelles as seen in necrosis [21, 33]. But, sometimes dead cells are swollen and sometimes they are shrunken depending on the cellular environment [21]. In the ischemic penumbra, swollen perineuronal astrocytes seemed to cause condensation of the necrotic neuron, resulting in shrinkage. While, when astrocytes died in the infarcted area, all neurons and astrocytes swelled and all of their membrane systems were ruptured. The macrophage seemed not to be able to enter the intact neuropils like those in the penumbra, but could enter the infarct focus where neuropils have been disrupted.

Conclusion

In conclusion: 1) The ultrastructural characteristics of DSNN, in the present study, suggested necrotic neuronal death instead of apoptosis. Condensation of the isolated neuron was induced by swelling of astrocytic cell processes surrounding the dark neuron. 2) Nuclear chromatin condensations observed from the early stage in appearance of the dark neurons indicated an initiating factor of the neuronal derangement. As the TUNEL procedure resulted in staining at the nuclear chromatin condensates of the dark neuron, in the present study, some DNA derangement seemed to be involved in this process [20, 32]. Further ultrastructural TUNEL staining is necessary to determine the ultrastructural characteristics of the TUNEL-positive neurons. 3) Not all dark neurons seems to die, but some of them to survive. Mitochondrial dysfunction seemed to be a determining factor of the irreversibility.

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Amyotrophic Lateral Sclerosis-Dementia with Severe Degeneration of the Substantia Nigra and Clinical Parkinsonism: Report of an Autopsy Case

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Abstract

An autopsy case of amyotrophic lateral sclerosis-dementia (ALS-D) with clinically overt parkinsonism and severe degeneration of the substantia nigra is reported. The patient is a 78-year-old man, who died after a clinical course of two years characterized by parkinsonism which was responsive to L-DOPA treatment. Motor neuron symptoms and dementia were not apparent antemortem. Autopsy revealed severe degeneration of the substantia nigra without α-synucleinopathy-related changes. Finely granular mineralization of necrotic neurons was a unique finding in the substantia nigra. Mild loss of spinal anterior horn cells with the appearance of several Bunina bodies and degeneration of the hippocampal subiculum and temporal cortex were also noted. A small number of ubiquitinated intracytoplasmic inclusions were found in neurons of the dentate fascia of the hippocampus and the temporal and frontal cortices. Although degeneration of the substantia nigra is a common finding in ALS-D, patients seldom develop clinically overt parkinsonism. This case indicates that patients with ALS-D ~~rarely~~ present ~~with~~ predominantly parkinsonian clinical features and these symptoms and signs can be improved by L-DOPA treatment.

Key words: amyotrophic lateral sclerosis-dementia, degeneration, mineralization, parkinsonism, substantia nigra

Introduction

Degeneration of the substantia nigra is almost constantly found as a subsidiary finding in patients with amyotrophic lateral sclerosis-dementia (ALS-D)[1-6]. However, in patients with this disorder, clinical symptoms and signs of parkinsonism are usually not apparent[2,4,7]. They seem to be masked by clinical features of motor neuron disease[1,8]. Neuropathologically, degeneration of the substantia nigra found in this disorder does not exhibit α-synucleinopathy-related changes such as Lewy bodies, Lewy neurites, and pale bodies, and the pathogenetic mechanisms of nigral degeneration remain obscure.

In this report, we document an autopsy case of ALS-D, in which the clinical features simulated those of idiopathic Parkinson disease and they were moderately responsive to L-DOPA treatment. Motor neuron symptoms and dementia were very mild or almost absent, and the diagnosis of ALS-D could not be made antemortem. Autopsy revealed severe degeneration with neuronal mineralization of the substantia nigra. Pathological changes of the motor neuron system, hippocampus, and fronto-temporal lobes were typical of ALS-D but mild in degree, and they were considered to be in an early stage of the development.

Case Report

The patient is a 78-year-old man, a retired worker of a pharmaceutical company. His family history is noteworthy in that his mother died of a dementing illness after a clinical course of more than seven years and one of his sisters has been treated under

the diagnosis of Alzheimer disease. No further information is available concerning the medical history of these two persons. He was noticed to walk with short steps at the age of 76. One year later, he frequently dropped food during a meal and walked dragging the left foot. Resting tremors of both hands and swallowing difficulty also developed, and he was diagnosed idiopathic Parkinson disease by his family physician. Although magnetic resonance imaging of the brain at that time showed atrophy of the bilateral temporal lobes, impairment of memory or other intellectual abilities was not noted. He was a heavy smoker and was hospitalized for a short term because of pulmonary emphysema and pneumonia at the age of 77. During this hospitalization visual hallucinations appeared transiently. After discharge his activities of daily living deteriorated remarkably, and he could not walk nor take a meal without assistance.

He was admitted to our hospital 7 months before death. The intelligence was approximately at the normal level and no evidence of dementia was present. Neither change of the personality nor abnormal behavior was noted. Muscle atrophy, weakness, and fasciculation were not observed. Hyperreflexia and spasticity in the extremities were also absent, and Babinski reflex was not elicited. Cogwheel rigidity and resting tremors were noted in the upper extremities, and the Myerson sign and retropulsion were also observed. These parkinsonian symptoms and signs were moderately responsive to the administration of L-DOPA (100 mg/day), and his activities improved from Yahr's stage V to IV. One month prior to death, he could make a short trip of two days, but after that he complained of shortness of breath and asthma-like respiratory difficulty and died of respiratory failure after a short term hospitalization. The clinical

course from the onset of neurological symptoms to death was about two years, and the final neurological diagnosis was idiopathic Parkinson disease.

General autopsy revealed severe emphysematous changes of both lungs and bronchopneumonia of the right lung. Remarkable emaciation and atrophy of the heart and liver were noted. Atrophy of skeletal muscles was not pronounced, but the diaphragm showed mild neurogenic atrophy of muscle fibers.

Neuropathological Findings

The brain weighed 1,380 grams and showed mild atrophy of the tips of the bilateral temporal lobes. Otherwise the cerebrum retained its normal appearance, and atrophy of the motor cortex, hippocampus, and amygdala was not found. Remarkable depigmentation of the substantia nigra was the most prominent change (Fig. 1), whereas pigmentation of the locus ceruleus was preserved. The cerebellum and spinal cord appeared grossly normal.

On histopathological examination, the substantia nigra showed severe neuronal loss accompanied by astrocytosis and rarefaction of the neuropil (Fig. 2). These changes were accentuated in the ventro-medial portion. There were many free pigment granules in the neuropil, and small aggregates of pigment-laden macrophages were also observed. Deeply basophilic and finely granular or stellate-shaped mineralization in the zona compacta, probably coinciding with necrotic neurons, was a unique finding (Fig. 3). This mineralization was not stained with the von Kossa method. No Lewy bodies, Lewy neurites, and pale bodies were detected by use of

immunohistochemistry employing an anti-α-synuclein antibody (Affinity Research Products, Exeter, UK). Neurofibrillary tangles were not observed, but a few Marinesco bodies were seen. No ubiquitinated abnormal structures were identified in the substantia nigra with immunohistochemistry using an anti-ubiquitin antibody (DakoCytomation, Glostrup, Denmark). Neurons of the locus ceruleus were well preserved, but a few neurons contained tangles. The other structures in the midbrain and pons did not show any remarkable alterations. In the medulla oblongata, the hypoglossal nucleus showed mild neuronal loss, and a few neurons contained Bunina bodies. The pyramids did not show axonal loss or myelin pallor.

The spinal cord exhibited mild loss of anterior horn cells accompanied by astrocytosis at the level of the cervical cord (Fig. 4). Bunina bodies were detected in several anterior horn cells of the cervical and lumbar cords (Fig. 5), and many axonal spheroids and a few chromatolytic neurons were also noted. Neither globular nor skein-like, ubiquitin-positive inclusions were detected in anterior horn cells. The lateral and anterior cortico-spinal tracts did not show degenerative changes.

In the cerebrum, Betz cells in the motor cortex were well preserved. Circumscribed neuronal loss with astrocytosis was found in the transitional zone between the CA1 of the hippocampus and the subiculum (Fig. 6). A small number of ubiquitin-positive intracytoplasmic inclusions were found in neurons of the dentate fascia of the hippocampus and also of the temporal and frontal cortices (Fig. 7). These inclusions were of globular or semicircular shapes and showed finely fibrillary internal structures. Many ubiquitin-positive, dot-like structures were also found in the

neuropil of the affected cortices. Scattered neurofibrillary tangles and ghost tangles were found in the hippocampus, parahippocampal cortex, temporal cortex and amygdala. The superficial layers of the parahippocampal cortex and the anterior dorso-medial portion of the superior temporal gyrus showed mild microvacuolation of the neuropil and astrocytosis, but neuronal loss was not apparent. Similar changes were not observed in the frontal cortex. Many senile plaques, mainly of the diffuse type, were distributed throughout the cerebral cortex.

The striatum, globus pallidus, thalamus, subthalamic nucleus, and cerebellum did not show any significant pathological alterations.

Discussion

ALS-D or motor neuron disease with dementia is a disease entity which has well-defined clinico-pathological features, although its nosological position within the disease groups of fronto-temporal dementia or motor neuron disease is still in controversy[6,7,9,10,11]. Clinically the disease is characterized by dementia and predominantly lower motor neuron symptoms and signs[6,7]. Neuropathologically, in addition to degeneration of the motor neuron system, degeneration of the hippocampus and the anterior dorso-medial portion of the temporal cortex is a constant finding[10], and ubiquitinated intraneuronal inclusions are seen in the dentate fascia of the hippocampus and some other regions[12]. Emphasizing the pathological significance of these inclusions, the term "motor neuron disease-inclusion dementia" has been advocated recently[13].

Degeneration of the substantia nigra has been frequently described in patients with ALS-D[1,2,5,8,9], and in autopsy series studied by Yoshida[6] and by Tsuchiya et al.[3] the involvement of this nucleus was a constant finding. Rare cases of ALS-D combined with degeneration of the striato-nigral and pallido-luysian systems have also been reported[14,15]. In these cases, neither α-synucleinopathy-related alterations nor neurofibrillary tangles are seen in the nigral neurons, and the pathogenesis of nigral degeneration remains obscure, although ubiquitinated inclusions were demonstrated in nigral neurons in some cases[4,5,6,11]. The absence of degeneration of the locus ceruleus in these cases also suggests the involvement of a mechanism different from that operating in idiopathic Parkinson disease. There are presumably several different

mechanisms leading to neuronal death of the substantia nigra, and several case reports attest the presence of primary nigral degeneration not associated with α-synucleinopathy[16,17,18]. In the present case, finely granular or stellate-shaped mineralization of necrotic neurons (encrustation) in the substantia nigra was a unique finding which has not been described previously. It remains unknown whether the mineralization is essentially related to the pathogenesis of nigral degeneration or merely a coincidental finding secondary to neuronal death. Mineralization of necrotic neurons is not an uncommon finding in ischemic lesions of the cerebrum, but it is not usually seen in the substantia nigra in various degenerative conditions affecting this nucleus. Severe neuronal loss with mineralization in the substantia nigra suggests that nigral degeneration occurred in the early period of the disease progression, probably preceding degeneration of the motor neuron system and the temporal lobe. In the present case degeneration of the substantia nigra was not merely a subsidiary lesion but rather a principal lesion both clinically and neuropathologically. As suggested by Sudo et al.[14], ALS-D might be a multisystem degenerative disorder which constantly involves the dopaminergic system.

In spite of the almost constant nigral degeneration, patients with ALS-D are seldom presented with clinical features of Parkinson disease[1,2,4,7]. In the autopsy series studied by Yoshida[6], which comprises 28 cases, parkinsonism was a prominent symptom apparently in only one case. Parkinsonian clinical features seem to be usually masked by symptoms and signs of motor neuron disease in this disorder[1,8]. The present case is therefore very instructive clinically and indicates that in some

patients with ALS-D the neurological features are dominated by parkinsonism and it can be improved by L-DOPA treatment. Itoh et al.[19] reported a clinical case of ALS-D in which the concentration of homovanillic acid, a dopamine metabolite, in the cerebrospinal fluid was abnormally low and the administration of L-DOPA brought the improvement of dementia, and suggested the involvement of the dopaminergic system in this disorder.

In contrast to the symptoms and signs of parkinsonism, those of motor neuron disease were not apparent in the present case, although mild swallowing difficulty suggestive of bulbar palsy was noted. Corresponding with these clinical features, neuropathological changes of the motor neuron system were mild. Neuronal loss with astrocytosis was mild, and Bunina bodies and axonal spheroids were found in the anterior horn. These findings are in common with the early changes in the classical ALS[20] and suggest that the pathogenetic mechanisms of anterior horn cell degeneration are at least partly common in the two disorders.

The lack of clinically overt dementia is explicable by the mild degree of neuronal loss in the hippocampus and the temporal lobe, and the relative paucity of ubiquitinated inclusions. These suggest that the lesions were in the early stage of the development. The lack of overt dementia in spite of the presence of temporal lobe degeneration in patients with ALS-D has been reported by a few authors, and the possibility of the presence of the *form fruste* of ALS-D is suggested[21].

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Figure Legends

Fig. 1: Coronal sections of the midbrain show remarkable depigmentation of the substantia nigra.

Fig. 2: Severe neuronal loss accompanied by astrocytosis is seen in the substantia nigra. (Hematoxylin-Eosin stain)

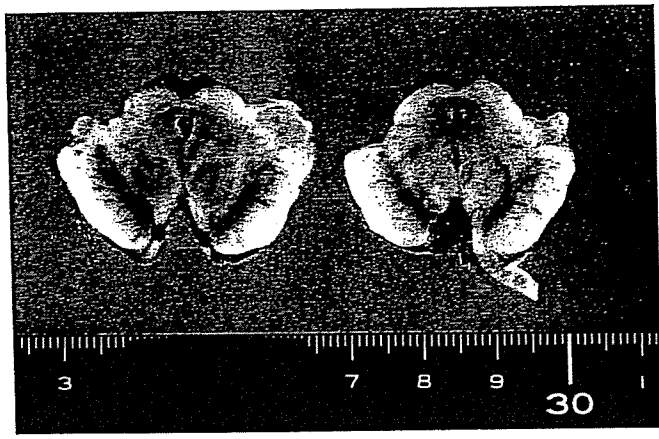
Fig. 3: Deeply basophilic, finely granular or stellate-shaped mineralization, probably coinciding with necrotic neurons, is seen in the substantia nigra. (Hematoxylin-Eosin stain)

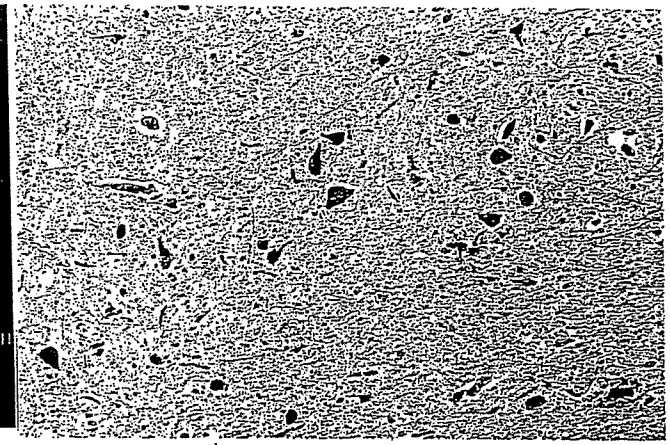
Fig. 4: The anterior horn of the cervical cord exhibits mild loss of large motor neurons and astrocytosis. (Nissl stain)

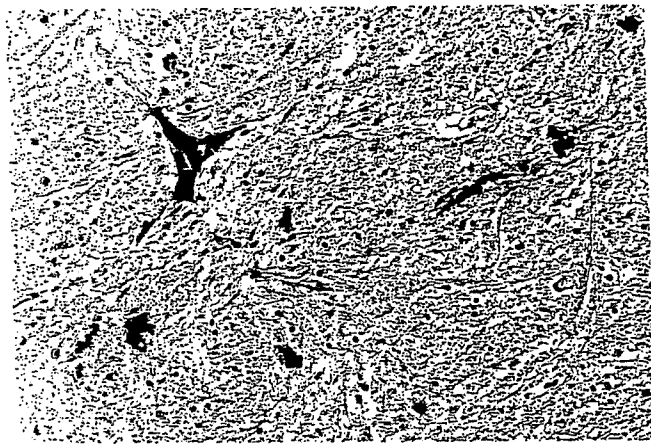
Fig. 5: Bunina bodies are found in several anterior horn cells. (Hematoxylin-Eosin stain)

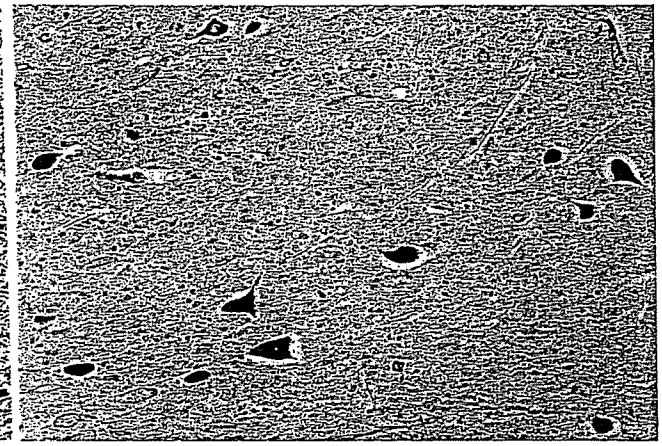
Fig. 6: Circumscribed neuronal loss with astrocytosis is found in the transitional region between CA1 of the hippocampus and the subiculum. (Hematoxylin-Eosin stain)

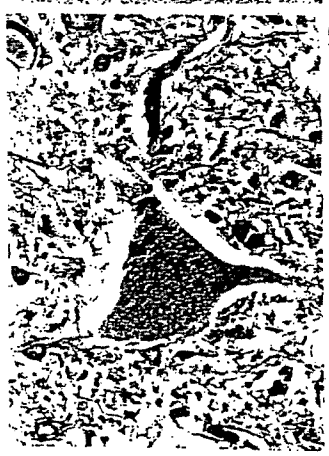
Fig. 7: A small number of ubiquitin-positive intraneuronal inclusions are found in the temporal cortex. (Immunostain for ubiquitin)

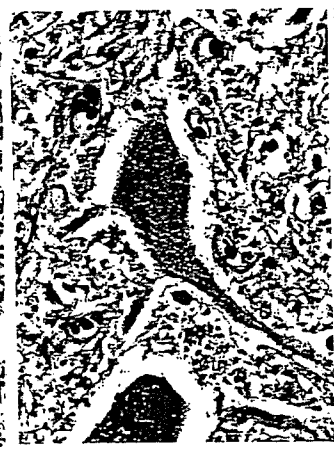


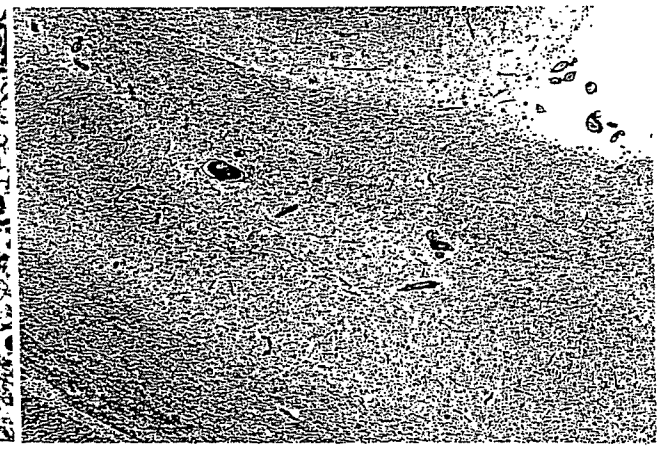


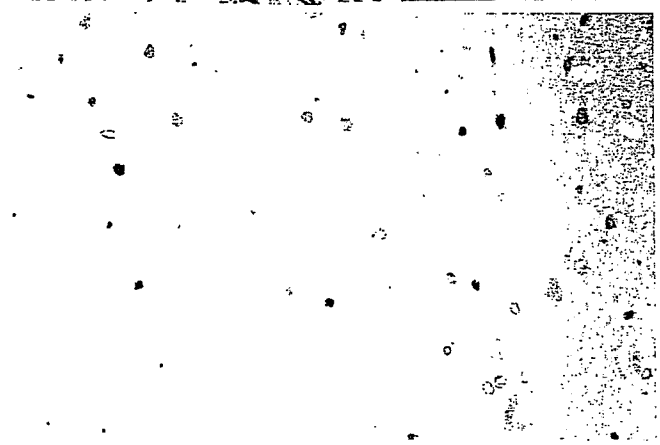












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Neuropathology, in press.

Forme fruste or incipient form of widespread-type ALS, or MND with PNLA? An autopsy case report

Running title: An ALS of widespread type or with PNLA?

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Abstract