

PAPER**PATHOLOGY AND BIOLOGY**

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Burden of Neurodegenerative Diseases in a Cohort of Medical Examiner Subjects*

ABSTRACT: Here we report studies of the burden of neurodegenerative neuropathologies in a cohort of Medical Examiner (ME) subjects from the County of Santa Clara (California) to determine if this unique population of decedents manifested evidence of neurodegeneration that might underlie causes of death seen in an ME practice. We found that 13% of the brains from ME cases showed significant tau pathology, including 55% of those 65 years old and older and 63% of those 70 years old and older. The histochemical and immunohistochemical findings were consistent with Alzheimer's disease (AD) in 7 subjects and frontotemporal lobar degeneration (FTLD) tauopathy type in six cases. There were no cases of Parkinson's disease, dementia with Lewy Bodies or other neurodegenerative conditions. Our study suggests that decedents >65 years of age in an ME practice are afflicted by common causes of dementia such as AD and FTLT which could contribute wholly or in part to their causes of death.

KEYWORDS: forensic science, ementia, Alzheimer's disease, medical examiner, neurodegenerative diseases, pathology

The United States and other developed countries are experiencing a demographic revolution such that between 2000 and 2030 the number of individuals 65 years of age and older will double (1,2). As a consequence, the prevalence of aging-related diseases will increase and pose new medical, political, social, and economic challenges to society. For example, cognitive impairment caused by neurodegenerative diseases such as Alzheimer's disease (AD) or frontotemporal lobar degeneration (FTLD) will increase proportionate to longevity.

While there are accurate statistics on aging-related increases in neurodegenerative disease in the general U.S. population, less is known about the burden of neurodegenerative diseases in individuals whose deaths fall within the jurisdiction of Medical Examiner (ME) offices. For example, according to a U.S. government report to Congress in 2008, chronic homeless individuals, who are estimated to number *c.* 124,000 people, are one segment of the U.S. population (3) whose deaths are typically investigated by ME offices. There is an undercurrent problem of demographics of homelessness at this very moment of our society. However, while the prevalence of psychiatric disturbances has been a focus of many studies of the homeless, little is known about the burden of neurodegenerative diseases in this group. Thus, a recent epidemiology study indicated that 13.9% of people 71 years old or older in the

general population of the United States is affected by dementia (4), but there are no studies on autopsy-confirmed neurodegenerative diseases in deceased members of the homeless community, although a few studies have been reported on cognitive impairment of people in homeless shelters in Australia (5,6). These studies showed a variable ratio of affected people in the group, between *c.* 10% and *c.* 40%, based upon interviews using the Mini Mental Status Examination (MMSE).

The prevalence of neurodegenerative diseases in other populations whose deaths are investigated by MEs have not been described previously. These populations include abusers of ethanol and prescription or illicit drugs and individuals with mental illness.

Obviously there are technical challenges in extracting accurate information on cognitive status, in addition to understanding causes of cognitive impairment from living homeless individuals (7), but cognitive malfunction in this group could be caused by AD, Parkinson's disease (PD), diffuse Lewy body disease (DLB), or FTLT as well as schizophrenia and substance abuse. However, since a neuropathological examination is required to make a final diagnosis of neurodegenerative diseases, we sought to determine the burden of neurodegenerative disease in a cross section of individuals whose deaths are investigated by an ME office, including homeless people.

Materials and Methods

Medical Examiners Case

With the consent and assistance of the supervisors and forensic pathologists at the Santa Clara County Medical Examiner-Coroner's Office in San Jose, CA, pathologic material was identified from 100 cases from 2005–2006. The inclusion criteria were age greater than 15 years and postmortem interval of less than 72 h. Tissue from cases determined to be confirmed or suspected homicides were excluded. The brain sections were obtained by one author

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(TH) from tissue routinely retained and preserved in 10% buffered formalin by the forensic pathologists (“stock jar”) during the usual course of autopsy examination. Sections of the hippocampus (CA—cornu ammonis) and entorhinal cortex (EC) were initially sampled from all cases. After initial screening, other areas of the brain (mid-frontal gyrus, superior temporal gyrus, cingulate gyrus, amygdala, midbrain, and medulla) were sampled as tissue was available. Minimal demographic (e.g., age) and cause of death information were also obtained in concert with one of the involved forensic pathologists. Additional details about the circumstances surrounding the death, past medical history (available in the ME’s investigative file) and toxicological and autopsy findings were obtained for a subset of subjects (13 total) with a significant degree of neurodegenerative tau pathology. Notably the brains from subjects in this cohort with significant neurodegenerative pathology were all examined in the fresh, unfixed state.

Neuropathological Assessments

Representative sections of the formalin-fixed tissue samples were embedded and 6 µm thick paraffin sections were prepared for immunohistochemical (IHC) and histochemical analysis. Previously described neuropathological diagnostic procedures conducted in the University of Pennsylvania Center for Neurodegenerative Disease Research (CNDR) were adapted to available brain sections to assess pathological changes associated with neurodegenerative diseases. The sections were stained with hematoxylin/eosin and Thioflavin-S methods as well as with antibodies to tau, α-synuclein, and TDP-43 (Tdp43; Tar DNA-binding protein-43) that detect inclusions found in many common neurodegenerative disorders including FTLN, tauopathies, and α-synucleinopathies. IHC visualized pathological protein aggregates considered to be hallmarks of these brain diseases were semi-quantitatively analyzed by following methods from CERAD criteria (8) and graded degree of pathology 0, 1 (mild), 2 (moderate), or 3 (severe). The same grading method was adapted to α-synuclein and TDP-43 pathology analysis as well. Accepted neuropathologic diagnostic criteria for AD (9,10), FTLN (11), PD (12), and DLB (13) were applied.

Results

A group of 100 ME cases aged 19–91 years (average age 50.5 ± 17.4 years; median 50 years) was examined. Fourteen cases were 19–30 years old, 15 cases 31–40 years old, 24 cases 41–50 years old, 20 cases 51–60 years old, 10 cases 61–70 years old, 12 cases 71–80 years old, and five cases 81 years old or older. The cohort consisted of both males and females. Death was attributed to natural causes (hypertensive cardiovascular disease, atherosclerotic cardiovascular disease, etc.) in 42% of cases, acute drug or ethanol intoxication in 24% of cases, suicidal means in 18% of cases (by hanging in 10 of 18 cases), transportation-related accidents in 10% of cases, and other accidental mechanisms in 4% of cases. In two cases no cause of death could be determined. In only one case was dementia listed as a contributory cause of death.

At least some degree of neurodegenerative pathology was found in 33 cases aged 44–91 years (average age 65 ± 13 years): 33 cases had tau pathology, two cases had α-synuclein pathology, and six cases had TDP-43 pathology. At least 91% of the cases 65 years of age or older (n = 20) displayed neurodegenerative disease pathology, and this age group made up 22% of the total cohort. Only 26% of the younger age group 44–65 years (n = 13/42) had some level of neurodegenerative pathology. No

neurodegenerative pathology was found in the youngest age group, less than 44 years (n = 36).

A group of 13 cases had a significant degree of neurodegenerative tau pathology, of high or moderate density in the hippocampus and EC (see Table 1). The average age of these subjects was 78 ± 8.3 years. Sections of the medulla were available for further analysis for every case in this population. Additional sections available for analysis included the midbrain (10 cases), superior temporal gyrus (three cases), amygdala (two cases), mid-frontal gyrus (one case), and cingulate gyrus (one case). A subset of these cases (n = 5) possessed additional tau pathology elsewhere, including the midbrain, medulla, and lentiform nuclei. Tau positive pathology was always present in the form of neurofibrillary tangles (NFT) or neuropil threads. Loss of neurons and activation of glia cells were noted in the areas with these tau lesions. No astrocytic or oligodendroglial tau pathologies were noted in these cases. Eight of these 13 cases showed Thioflavin-S positive staining associated with a moderate or high density of NFT; no Thioflavin-S positivity was found in the other five. The cases with Thioflavin-S positive NFTs also had co-existent moderate or high burdens of senile plaques (SP) in the hippocampus and EC. The rest of NFT tau positive cases without Thioflavin-S positive lesions were free of SPs, except for one case (#59). Due to limited availability of brain regions for histological examination, precise Braak and Braak stages could not be determined, but it can be inferred that 13 tau positive cases reported here had at least stage III–IV or higher scores.

All α-synuclein positive cases (n = 2) showed immunoreactivity in the medulla oblongata with a single case showing additional pathology in the substantia nigra (n = 1, #27). The density of α-synuclein pathology was modest in both cases and was not associated with a noticeable loss of neurons. α-Synuclein was not detected in any cortical region of any case.

A modest amount of TDP-43 pathology was found in six cases. While one case had cytoplasmic inclusions in the dentate gyrus (DG), the other cases showed reactivity in the motor neurons in the brain stem.

TABLE 1—Medical Examiner cases with significant neuropathological lesions.

ID #	Age	Phf1		NFT/ Thio-S		PL/ Thio-S		Tdp43		Possible NP DX
		CA	EC	CA	EC	CA	EC	CA	EC	
18	74	3	3	3	3	2	3	0	0	AD
29	66	2	3	3	2	3	3	0	0	AD
59	79	3	2	2	2	2	3	2	0	AD
60	91	3	3	3	3	3	3	0	0	AD
63	72	3	3	2	2	2	2	0	0	AD
64	80	2	3	2	3	2	2	0	0	AD
65	83	2	3	2	2	2	3	0	0	AD
95	89	3	3	3	3	2	3	0	0	AD
96	73	3	3	2	2	3	3	0	0	AD
12	77	3	3	0	0	0	0	0	0	Tau
24	79	3	3	2	2	0	0	0	0	Tau
62	58	3	3	2	3	0	0	0	0	Tau
77	83	3	2	0	1	0	0	0	0	Tau

0,1,2,3 represent degree of pathology. Phf1 (paired helical filaments), TDP-43: pathology visualized by immunohistochemistry with antibody against tau or TDP-43.

Thio-S, pathology visualized by Thio-S histochemistry; CA, cornu ammonis; EC, entorhinal cortex; PL/Thio-S, plaques with Thioflavin-S reactivity; AD, Alzheimer’s disease; NFT, neurofibrillary tangles; Tau, frontotemporal lobar degeneration tauopathy; Tdp43, Tar DNA-binding protein-43.

Despite limited sampling in these cases, we attempted to make a pathological diagnosis based on examination of available sections. Thus, a significant degree of Thioflavin-S positive NFTs accompanied with SPs in the hippocampus and EC suggested a diagnosis of AD, while Thioflavin-S negative and tau positive pathology devoid of SP could represent FTLD of the tauopathy type. Cases with TDP-43 pathology in the DG are consistent with FTLD-U or FTLD of the TDP-43 proteinopathy type. Finally, although we had cases with α -synuclein pathology, none had pathology that rose to a level consistent with a pathological diagnosis of PD or DLB. In summary there were seven possible AD cases and six possible FTLD cases (see Table 1).

Subsequent investigative information obtained for the 13 subjects with significant neurodegenerative pathology revealed a prior medical history of dementia (or at least significant cognitive impairment) in four (cases 18, 59, 63, and 95). A fleeting comment of a suspected diagnosis of PD (without supporting medical documentation) was noted for one subject (case 18). MMSE results were reported for two within the last year of life (case 60 [MMSE score 28/30 1 month prior to death] and case 95 [MMSE score 7/30 several months prior to death in which some degree of delirium was also suspected]). While only one subject (case 64) was considered to live a transient lifestyle, nine additional subjects were found to live in squalid surroundings and/or have evidence of significant malnutrition (cases 12, 29, 59, 60, 62, 63, 77, 95, and 96). Two of these subjects had a co-morbid condition (neoplasia) that likely contributed to their malnutrition. Squalid surroundings or poor personal hygiene was found in equal proportions irrespective of the presence or absence of cohabitating family members or caregivers. Only one subject (case 79) had a reported psychiatric history (major depression).

Subsequent pathologic information obtained for the 13 subjects with significant neurodegenerative pathology indicated the presence of cortical atrophy in two subjects, case 63 (the oldest subject with a moderate degree of atrophy) and case 96 (a slight degree of atrophy). Ventriculomegaly was identified in three subjects (cases 12, 24, and 63) and slight cerebral edema was noted in one subject (case 18, with the heaviest fresh brain weight). A single infarction (pontine) was identified in one subject (case 24); one subject (case 62) had a history of a remote cerebral infarction although none was described in the postmortem examination.

Discussion

The current study is the first neuropathological assessment of neurodegenerative disease pathology in an unselected cross-sectional cohort of ME cases which includes members of society about whom there is limited data on their cognitive status at death. Further, there are few available studies on cognitive impairment linked to neurodegenerative disease in individuals who come to autopsy at ME offices. The results from the current study indicated that 13% of the brains from ME cases in the study period showed a significant burden of tau pathology, which accounted for 55% and *c.* 63% of the population in this cohort 65 years old and older and 70 years old and older, respectively.

Due to a lack of clinical data at the time of death of these ME subjects and limited CNS regions available for study, our diagnostic assessments do not meet consensus criteria for AD, FTLD, PD, DLB, or other neurodegenerative disorders (14). However, it is widely accepted that clinical progression of AD is associated with increasing density and wider distribution of plaque and tangle pathology in the brains of AD patients. Such a notion has been applied to other disease pathologies, including TDP-43 positive

inclusions in FTLD with ubiquitin inclusions or Lewy bodies and Lewy neurites in PD or DLB. Nonetheless, some of the mild pathological changes in this ME cohort may be age-related and incidental since earlier reports indicate that AD pathologies are virtually absent until the sixth decade of life (15). Thus, while it is critical to have clinical assessment to measure cognitive function of patients, our postmortem neuropathological studies provide at least an estimate of the burden of neurodegenerative disease found in subjects investigated by ME offices. It is noteworthy that all four cases in our entire study cohort which carried a diagnosis of dementia or at least substantial cognitive impairment had significant pathologic neurodegenerative findings. Among homeless individuals who would be expected to fall within ME cohorts, there were two clinical surveys of cognitive impairment done by an Australian group in 1993 (5) and 2000 (6). The studies showed that 31% and 10%, respectively, of subjects had evidence of cognitive impairment. The Australian study in 2000 indicated that homeless individuals with an average age of 57 ± 16.2 years have cognitive impairments and our study showed abundant AD pathology in members of our cohort with an average age of 76.8 ± 9.2 years old. Thus, our study provides evidence of a significant burden of neurodegenerative disease pathology in members of an ME autopsy cohort over the age of 65 years old. Additionally in our study cohort, only one individual with significant neurodegenerative findings, in contrast to the Australian studies, was homeless. According to our analyses, the neuropathological assessment in this study is consistent with diagnoses of AD and FTLD tauopathy, a group of neurodegenerative diseases with extensive neuron loss and gliosis associated with filamentous tau inclusions. In contrast, the burden of other neurodegenerative lesions was insufficient to suggest the presence of a synucleinopathy or TDP-43 proteinopathy. In conclusion, our study suggests that a significant percentage of elderly individuals who come to autopsy in the ME office in the County of Santa Clara, encompassing a representative large metropolitan area in the United States (San Jose, estimated population of 990,000), harbor neurodegenerative disease pathology consistent with a diagnosis of AD and FTLD tauopathy. In only one subject, with a pre-existing diagnosis, was dementia considered a contributory cause of death. In five additional subjects, three with pre-existing diagnoses, could the presence of a neurodegenerative process arguably be considered contributory to the death. No death certificates were amended based upon the results of this study.

This study provides objective documentation of the true burden of neurodegenerative disease processes in a representative ME cohort, which is over three times higher than reported by the medical-legal investigators in the office from which these cases were derived. Documentation of prior (not terminal) medical treatment was available in 6 of 13 subjects with significant neurodegenerative findings. In many of the other subjects, comments of a lack of recent medical attendance were made. In only one subject, who was a passenger in a motor vehicle collision, was no apparent effort made to clarify the subject's prior medical history. In this study, a large percentage of our cohort of 13 subjects with significant neurodegenerative findings had evidence of significant malnutrition and/or lived in squalid surroundings. While the clinical correlation may not always be available to fulfill the criteria for a specific neurodegenerative disease, an age greater than 65 years coupled with malnutrition and poor hygiene/squalid surroundings suggests the possible need for more complete neuropathologic evaluation. Indeed while it is not possible in busy ME offices to preserve and carefully examine brains from all cases, the presence of these traits in concert with other circumstantial evidence may help highlight the cases likely to yield neurodegenerative pathology.

Lastly, the rate of neurodegenerative disease in this ME office may be underreported in our study as only subjects from whom tissue specimens were available were included; elderly individuals with a history of neurodegenerative disease without a full postmortem examination (i.e., only an external examination) were not included in this study. We hope this study will stimulate further research into the significance of these disorders in the community of individuals who fall within the jurisdiction of ME or coroner's offices.

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