Research Study – Infectious involvement with heavy metal toxicities in an Alzheimer's disease case study

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ABSTRACT

Introduction: Alzheimer's disease (AD) is characterized by a progressive neurodegenerative deterioration of brain cells that eventually destroys cognitive and executive functions, resulting in memory loss, mental decline, and behavioral changes. The neurocognitive decline is believed to result from cell death and the loss of neuronal plasticity associated with neuropathological hallmarks, including the accumulation of extracellular amyloid-β (Aβ) plaques and intracellular hyperphosphorylated tau (pTau) neurofibrillary tangles (NFT). Given the multifactorial nature of AD, there is growing evidence that infections, particularly with Borrelia burgdorferi and Chlamydia pneumoniae, may play a role in its pathogenesis. In this retrospective case study, we evaluated the six-year post-diagnosis journey of a man diagnosed with advanced, end-stage AD at 60-years-old, with subsequent post-mortem analysis.

Methods: Following death, a brain-only autopsy was performed with the brain being hemisected, with the left hemisphere frozen and the right hemisphere formalin-fixed for further analysis. Post-mortem analysis of his brain tissues was initiated to evaluate pathological changes that would help to explain the symptomatology. Immunohistochemistry on the fixed sections was performed for the characteristic pathology of AD, Aβ plaques, and pTau neurofibrillary tangles, as well as evidence for pathogens such as *B. burgdorferi* and *C. pneumoniae*. Analysis concentrated on specific areas of the brain that would correlate with AD symptoms. The comparable regions of the frozen hemisphere were molecularly analyzed with PCR for infectious agents.

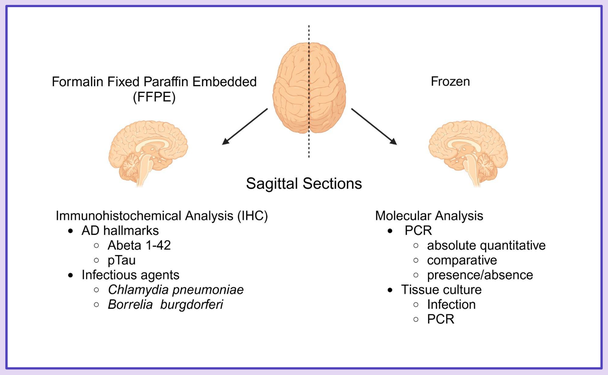
Results: Immunohistochemical analysis showed pTau and Aβ within numerous regions of the brain, including the amygdala, hippocampus, and frontal cortex. The Aβ pathology present in the hippocampus was both intraneuronal and diffuse. The brain was also positive for *B. burgdorferi* and *C. pneumoniae* immunoreactivity in regions of particular importance in AD, including the hippocampus, temporal lobe, and frontal cortex.

Conclusions: The brain did not show the typical distribution of pTau and Aβ expected in an advanced-stage AD brain. In particular, the extensive intraneuronal Aβ pathology in the hippocampus was atypical. Evidence of infection with *B. burgdorferi* and *C. pneumoniae* was detected in the brain by immunohistochemistry. Additionally, the presence of atypical pathology, exposure to heavy metals, and organisms in this case suggests that the exposome contributed to the pathogenesis of AD in this individual. Given the clinical diagnosis and post-mortem findings, variable exposures such as those of heavy metals and infectious involvement should be seriously considered during a neurological diagnostic evaluation for AD.

INTRODUCTION

- **Alzheimer's disease (AD)** is the leading cause of dementia worldwide, with nearly 7 million Americans affected by the disease.
  - A slowly progressive neurodegenerative disease
  - It affects the amygdala, entorhinal cortex, hippocampus, and frontal and parietal cortices
- Two predominant **neuropathological hallmarks:** extracellular accumulation of amyloid-beta peptides (Aβ) plaques and intracellular neurofibrillary tangles comprised of the hyperphosphorylated tau protein
  - Accumulation of Aβ oligomers disrupts neuronal function, leading to cell damage and synapse loss
  - Intracellular neurofibrillary tangles reside in the neuronal cytoplasm and disrupt microtubule structure and function
- Autosomal dominant AD accounts for ~ 5% of early-onset AD and is commonly associated with mutations in the **Aβ precursor protein (βAPP), Presenilin 1 (PSEN1), and Presenilin 2 (PSEN2**)
- Other genetic factors include the ε4 allele of **Apolipoprotein E (APOE),** a strong risk factor for late-onset AD
- The multifactorial theory of AD highlights **environmental** associations along with those of genetic predispositions in AD pathogenesis
  - infections with various microbes and heavy metal toxicity may be potential etiological agents in the disease pathogenesis
    - ***B. burgdorferi***, a spirochete, is the causative agent of Lyme disease
      - In the late stage of this disease, invasion of the brain may lead to neuroborreliosis leading to cortical atrophy and microgliosis
    - ***C. pneumoniae***, a gram-negative obligate intracellular pathogen that typically causes community-acquired pneumonia
      - May penetrate the central nervous system via the olfactory route and/or through the blood-brain barrier to infect neurons, glia, and endothelial cells
    - Toxic effects of **heavy metals** such as lead, aluminum, and lithium may be involved in neurodegeneration
      - At high levels, they can disrupt the BBB, leading to increased permeability and further increasing their toxicity

METHODS



CASE

- A previously healthy, well-educated 60-year-old-man, with a passion for the outdoors and hunting presented to his PCP with significant memory loss and marked changes in mood
- MRI was unremarkable;however, subsequent PET scan demonstrated severe hypometabolism in the parietal and temporal lobes
  - Officially diagnosed with AD, advanced end-stage
- Follow up genetic history was negative for *POEε4*, *APP*, *PSEN1*, and *PSEN2*
- Due to a history of extensive outdoor time and many tick removals over the years, his family requested a Lyme disease serology panel
  - IgG bands seen but did not meet the threshold for diagnosis
  - Subsequent urinalysis was positive for *B. burgdorferi* and *Bartonella henselae*
  - IgG antibodies to *Mycoplasma pneumoniae*, Epstein-Barr Virus (EBV), and *Babesia duncani*
- A toxic metal urinalysis showed abnormally high levels of aluminum, antimony, gadolinium, and nickel, lead, and mercury
- Given his test results, he was placed on an extensive antibiotic regimen
  - Herbal remedies, chelation therapies, and stem-cell treatments were tried
    - Some symptomatic improvement, but no evidence of slowed disease progression
- Over the next 5 years his symptoms rapidly progressed to include increasing cognitive deficits, emotional instability, paranoia, and hallucinations
- His condition progressively deteriorated, with continued behavioral decline and loss of ability to perform activities of daily living, ultimately resulting in death six years later.

RESULTS

- Aβ plaques and pTau aggregates were detected in the temporal cortex, amygdala, hippocampus, and frontal cortex
  - Greatest accumulation of both found in the amygdala and hippocampus
  - High levels of intracellular Aβ, accompanied by extracellular diffuse and dense-core Aβ plaques
- Numerous intracellular Aβ accumulations, dense-core plaques found in the temporal lobe
- The frontal cortex had diffuse extracellular Aβ plaques throughout the grey matter
  - Less involvement compared to other regions
  - No intracellular Aβ
- *C. pneumoniae* immunolabeling (CM) and *Borrelia burgdorferi* (Bbg) were observed intracellularly and in the highest concentrations in the hippocampus, amygdala, and temporal region
  - Moderate immunolabeling seen in the cerebellum
- Chlamydial antibody (CR) immunoreactivity was detected within the presumptive **endothelium** of both large and small vessels of most sections evaluated
  - Most observed in the parietal cortex and cerebellum

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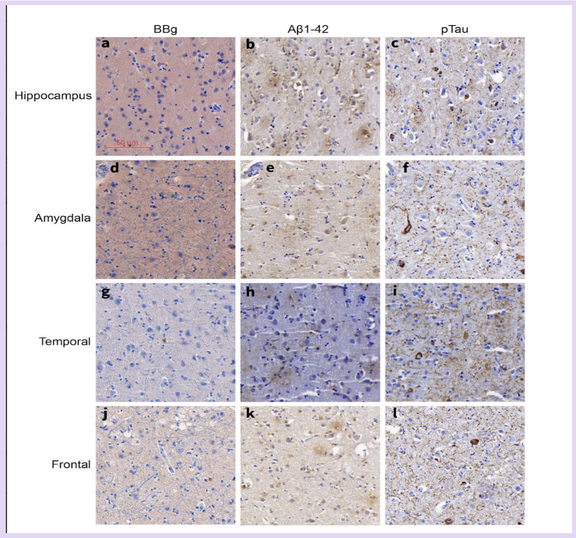

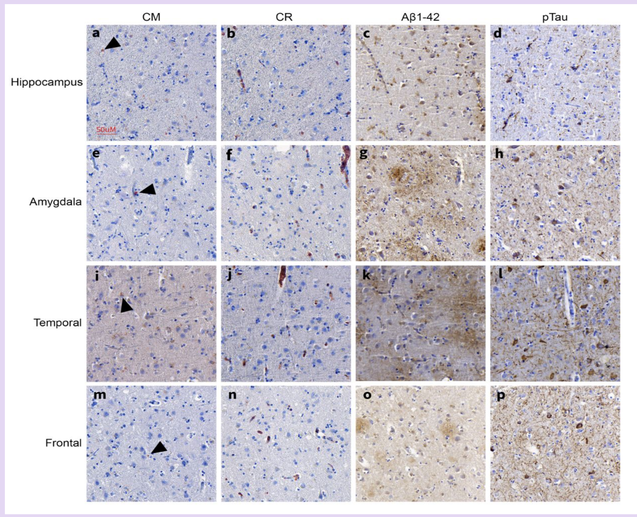

Antibody	secondary conjugate	color
Abeta 1- 42 (ab42)	HRP	brown
pTau (pTau)	HRP	brown
Borrelia burgdorferi (Bbg)	AP	red
Chlamydia pneumoniae (CM)	AP	red
Chlamydia genus (CR)	AP	red

CONCLUSIONS

- This case exemplifies the multifactorial nature of neurodegeneration and highlights how exposure to infection and metal toxicity may contibute to the abnormal accumulation of pathology and subsequent rapid decline of a 60-year-old man with no genetic hallmarks of AD
  - early-onset AD was tested ruled out by genetic testing
- Extracellular Aβ plaques typical of AD pathology were observed as well as widespread intracellular Aβ accumulations especially seen in the amygdala and cortex
  - resembles early-stage AD rather than advanced, late stage AD, suggesting ongoing Aβ production by neurons and glial cells
  - Intracellular *Chlamydia pneumoniae* and *Borrelia burgdorferi* immunolabeling patterns and concentrations were also greatest in the amygdala and hippocampus
- Exposure not only to infectious agents but also to heavy metals suggests the multifactorial development of neurological disorders
  - the premortem abnormally high levels of heavy metals may have contributed to the findings and accelerated the changes
  - may have contributed to or triggered disruption of the oxidative, metabolic, and immunological functions of brain neurons, leading to pathogenesis
  - have been reported to increase the levels of pTau and Aβ and to decrease Aβ clearance from the brain, similarly to the changes seen in AD
  - may enhance the pathogen's ability to enter the brain and create an environment where they thrive

ACKNOWLEDGEMENTS

- We would like to thank Maegan McCall, Alaha Abdul Faruq, Sanya Bhambhani, and Rachel Corwin for assistance in the dissection of the frozen hemisphere.
- We would like to thank Meejin Ahn for assistance in interpreting our results and applying them clinically.
- We would like to thank Jacquelyn Gerhart and the staff of the PCOM Bio-Imaging center for their assistance with the preparation of our FFPE specimens.
- We would like to thank Nicole Bell for all her insights and her tenacity in finding answers.
- We would like to thank the CCDA, the DOR, the AlzPI, and Sim Einstein Foundation for their support.

REFERENCES