ISSN 3060-4567

NEURODEGENERATIVE CHANGES IN THE HIPPOCAMPUS IN ALZHEIMER'S DISEASE: IMMUNOHISTOCHEMICAL AND MORPHOMETRIC ANALYSIS

Assistant of the Department of Anatomy and Clinical Anatomy,
Bukhara State Medical Institute named after Abu Ali ibn Sina
Davronov U.T.

Abstract: *Alzheimer's disease (AD) is a chronic and progressive neurodegenerative disorder that significantly impairs memory, cognition, and behavior. Central to the pathophysiology of AD is the degeneration of the hippocampus, a brain structure integral to learning and memory. This study explores the morphological and immunohistochemical alterations in the hippocampus of individuals diagnosed with Alzheimer's disease. Using post-mortem brain samples, we employed specific immunohistochemical markers to detect neuronal damage, amyloid-beta (Aβ) plaques, and hyperphosphorylated tau protein tangles. Morphometric analysis was performed to quantify neuronal density and lesion burden in various subregions of the hippocampus. The findings demonstrate significant neurodegeneration and structural abnormalities in AD-affected hippocampal tissues, supporting the clinical presentation of the disease and offering insight into potential therapeutic targets.*

1. Introduction

Alzheimer's disease (AD) is the leading cause of dementia worldwide, accounting for 60–80% of cases. The disease typically manifests as progressive memory loss, language difficulties, and impaired executive function, eventually leading to complete cognitive and functional decline. One of the earliest and most vulnerable brain regions affected in AD is the hippocampus, which plays a crucial role in forming, organizing, and storing memories. Pathologically, AD is characterized by the presence of extracellular amyloid-beta (Aβ) plaques and intracellular neurofibrillary tangles composed of hyperphosphorylated tau protein.

These pathological changes result in synaptic dysfunction, neuronal loss, and brain atrophy, particularly in the medial temporal lobe and hippocampus. The objective of this study is to investigate neurodegenerative changes in the hippocampus using both immunohistochemical and morphometric analyses, focusing on quantifying pathological hallmarks of AD and correlating them with structural and cellular damage.

2. Materials and Methods 2.1. Sample Collection

Human post-mortem hippocampal tissues were obtained from 10 patients diagnosed with Alzheimer's disease and 5 age-matched control individuals. All AD diagnoses were confirmed clinically and pathologically according to the criteria established by the National Institute on Aging and the Alzheimer’s Association.

2.2. Tissue Preparation and Immunohistochemistry

Tissues were fixed in 10% buffered formalin, embedded in paraffin, and sectioned at 5 μm thickness. Sections underwent deparaffinization, rehydration, and antigen retrieval using citrate buffer (pH 6.0). The following primary antibodies were used:

- Anti-Aβ (1:100)
- Anti-Tau (AT8, 1:200)
- Anti-NeuN (1:300) for neuronal nuclei

Secondary antibodies conjugated to horseradish peroxidase were applied, and sections were developed with DAB and counterstained with hematoxylin.

2.3. Morphometric Analysis

Quantitative assessments were performed using ImageJ software. Five random non-overlapping fields were selected per region: CA1, CA3, and dentate gyrus (DG). Neuronal counts, plaque areas, and tangle frequencies were measured and statistically analyzed.

2.4. Statistical Analysis

Statistical analysis was carried out using SPSS version 25. Data were tested for normality using the Shapiro–Wilk test. Student’s t-test and ANOVA were used to compare groups, with a p-value < 0.05 considered statistically significant.

ISSN 3060-4567

3. Results

3.1. Neuronal Loss There was a marked reduction in neuronal density in AD tissues compared to controls. The CA1 region showed a 45% reduction in neuronal count ($p < 0.001$), while the dentate gyrus showed a 30% decrease ($p < 0.05$). Neurons in AD samples appeared shrunken with condensed nuclei, consistent with neurodegeneration.

3.2. Amyloid-beta Plaque Deposition Immunohistochemistry revealed extensive Aβ plaque accumulation in all hippocampal subregions, with CA1 showing the highest burden. Plaques were extracellular, amorphous, and varied in size. Quantitatively, the plaque area occupied up to 20% of the CA1 region in AD cases ($p < 0.01$).

3.3. Tau Pathology Tau immunoreactivity was predominantly intracellular, with intense staining in neuronal soma and dendrites. The number of tau-positive cells in AD tissues was significantly higher than controls ($p < 0.001$). Neurofibrillary tangles were prominent in the subiculum and CA1.

3.4. Correlation Between Pathological Markers and Neuronal Density A strong inverse correlation ($r = -0.85$, $p < 0.001$) was observed between plaque/tangle density and neuronal counts, indicating that greater pathological burden was associated with increased neurodegeneration.

4. Discussion

This study provides compelling evidence that the hippocampus undergoes profound neurodegenerative changes in Alzheimer's disease. Our findings confirm that neuronal loss, amyloid plaques, and tau tangles are significantly more prevalent in AD patients compared to age-matched controls. The CA1 region appears to be the most susceptible to neurodegeneration, which aligns with previous studies (Braak & Braak, 1991). This region is critical for memory encoding, and damage here likely contributes to early memory impairment seen in AD. The observed correlation between pathological protein aggregates and neuronal loss emphasizes the pathogenic role of Aβ and tau in disrupting neural circuits. The findings also highlight the potential for using hippocampal biomarkers in early AD diagnosis and monitoring

ISSN 3060-4567

disease progression. Our results underscore the utility of immunohistochemical and morphometric techniques in understanding the anatomical basis of cognitive decline and provide a foundation for future therapeutic research.

5. Conclusion

In conclusion, this study demonstrates significant neurodegenerative changes in the hippocampus of patients with Alzheimer's disease. These changes include marked neuronal loss, widespread amyloid-beta plaque deposition, and tau pathology. The findings support the central role of hippocampal damage in the cognitive deficits associated with AD. Further research into the molecular mechanisms driving these changes may aid in developing targeted therapies to slow or halt disease progression.

REFERENCES

1. Braak H., Braak E. (1991). Neuropathological stageing of Alzheimer-related changes. Acta Neuropathol, 82(4), 239–259.

2. Hardy J., Selkoe D.J. (2002). The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. Science, 297(5580), 353–356.

3. Thal D.R., Rüb U., Orantes M., Braak H. (2002). Phases of Aβ-deposition in the human brain and its relevance for the development of AD. Neurology, 58(12), 1791–1800.

4. Serrano-Pozo A., Frosch M.P., Masliah E., Hyman B.T. (2011). Neuropathological alterations in Alzheimer disease. Cold Spring Harb Perspect Med, 1(1): a006189.

5. Hyman B.T., Phelps C.H., Beach T.G., Bigio E.H., Cairns N.J., Carrillo M.C., Montine T.J. (2012). National Institute on Aging–Alzheimer's Association guidelines for the neuropathologic assessment of Alzheimer's disease. Alzheimers Dement, 8(1), 1–13.

6. DeTure M.A., Dickson D.W. (2019). The neuropathological diagnosis of Alzheimer's disease. Mol Neurodegener, 14(1), 32.