

Elective Report – John Baxter

The role of β -APP and Clusterin Immunohistochemistry in Neurodegenerative Disease: A Comparison with Forensic Practice.

I spent two weeks in the Neuropathology department at Royal Victoria Hospital in Belfast as part of my medical elective. During this time I was able to assist my supervisor with a project investigating the relationship between different biomarkers, clusterin and β -APP, and neurodegenerative disease.

Part of my role was to take samples from the donor brains. All of the brains used in this project had been donated for diagnostic and research purposes. In each case, the brain had already been sectioned for diagnostic purposes this meant I had to reassemble it in order to orientate myself before I could proceed with taking further samples. This was a real test of my neuroanatomical knowledge. When I first started, it took me a long time to get all the samples required but after I had done a few more cases I became much faster. In doing this I was also able to learn more about sample collection, fixation, and staining through interacting with the other laboratory staff who would be processing the samples for staining.

Another part of my role was to create a case database. This involved reading autopsy reports and entering the correct information in to a spreadsheet. Through this I was able to see how autopsy reports are formatted and learn more about the neuropathological signs of neurodegenerative disease such as the neurofibrillary tangles and amyloid plaques in Alzheimer's disease and congophilic staining in cerebral amyloid angiopathy.

In the future we have hopes to publish the results of this project. Unfortunately, at the time of writing the samples are still in the process of staining.

Below is the outline for a poster presentation that I am preparing for this project

Introduction

Axons are nerve processes. Axonal transport is a process whereby chemicals, proteins, and organelles may be moved along the axons of nerve cells. This can be toward the soma, anterograde transport, or away from the soma toward the axon endings, retrograde transport. Axonal transport can also be classified in to fast and slow transport. Dysfunctional axonal transport is linked to neurodegenerative disease.

Immunohistochemistry involves the use of antibodies, which are linked to enzymes or fluorescent dyes, to bind to specific antigens located within tissue. When the antibody binds to its specific antigen it causes the associated enzyme or dye to activate and this allows the antigen to be visualised. β -APP and clusterin are thought to be immunohistochemical markers of disruption of axonal transport. They are also thought to be the earliest markers for stasis of transport and therefore are useful in forensic practice. Their specificity and sensitivity however is unclear and different laboratories have different interpretations of their significance. It is known that β -APP and possibly clusterin are upregulated in non-ischaemic and traumatic disease.

Methods

20 brains of patients with neurodegenerative disease were used in this project. These were made available by the Belfast Neuropathology Laboratory Brain Bank according to strict protocols for the purpose of research. In all cases the brain had already undergone dissection and sampling for diagnostic purposes.

For each brain one hemisphere was frozen and the other was preserved in formalin. Samples were taken from the frozen hemisphere. The following samples were selected; internal capsule; corpus callosum (anterior, middle, and posterior); cerebellar peduncle; parasagittal white matter. These areas were selected as they best demonstrate axonal disruption in forensic practice.

For each sample the following stains were used; standard haematoxylin & eosin (H&E), Luxol Fast Blue and H&E which stains for myelin in the brain, CD68 which stains for microglial cells, clusterin, β -APP, and controls.

The β -APP and clusterin staining will be carried out by the Belfast Neuropathology Laboratory and assessed following completion of this. They will be analysed by semi-quantitative analysis.

Results

At time of writing sample staining is still ongoing.

Discussion

Clusterin (apolipoprotein J or ApoJ) is a complement inhibitor and a stress-induced chaperone protein which can inhibit neuronal apoptosis. It is also a reported modulator of acute and chronic neuroinflammatory responses following traumatic brain injury.

Upregulation of the clusterin gene can be due to cellular stress, injury, or disease states such as neurodegeneration or atherosclerosis. This means clusterin is not a specific marker for trauma.

Beta-amyloid precursor protein (β -APP) is an integral glycoprotein ubiquitously expressed in the plasma membrane. Its normal function is unclear. Notably, β -APP is involved in the generation of β -amyloid in Alzheimer's Disease. It can be used to assess axonal cell injury in forensic practice. However, axonal cell injury can be caused by several mechanisms so, like clusterin, β -APP immunoreactivity is not a specific marker for trauma.

- Further understanding of utility of β -APP and clusterin analysis
- Clinical benefit in having additional biomarkers for neurodegenerative disease? - Familial link for screening/ early intervention purposes? Insurance?
- Further understanding of the neurodegenerative disease process - potential for targeted treatments, prognostic indications?

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β -APP and clusterin are immunohistochemical markers of disruption of axonal transport. They are also thought to be the earliest markers for stasis of transport and therefore are useful in forensic practice as they may be upregulated within 30-35 minutes of cerebral insult.

Their specificity and sensitivity however is unclear.

β -APP and possibly clusterin are upregulated in non-ischaemic and traumatic disease.

The Belfast Neuropathology Laboratory in conjunction with Dublin, operates a Brain Bank for neurodegenerative disease. Patients and relatives of patients with neurodegenerative disease, donate their brains for diagnosis and research.

Tissue has been available according to strict protocols for research projects. This tissue can be interrogated by these immunohistochemical methods for β -APP and clusterin immunohistochemistry.

Methodology

Eighteen cases of neurodegeneration were selected (with normal controls) and analysed by immunohistochemistry and histology. β -APP and clusterin staining, along with CD68 staining was performed and analysed semi-quantitatively.

Results and conclusions

1. β -APP and clusterin staining were detected in all cases
2. In most, this was in an ischaemic pattern
3. There were scattered axons staining in a non-ischaemic pattern
4. Clusterin was more sensitive but also showed more background
5. Multiple Sclerosis case showed an additional pattern of axonal staining-of significance to the causation of disability in this condition
6. Two cases of possible Chronic Traumatic Encephalopathy showed strong staining

These results have significant importance for forensic practice.

β -APP is present in control patients and non-traumatic conditions and therefore in itself, without further supporting evidence, does not signify trauma as a factor in a death