Identifying Protein Aggregation in Dementia With Lewy Bodies

Koh Zhi Wei (200820945) MBBS Stage 4, NUMed Malaysia, Z.W.Koh1@newcastle.edu.my
Supervisor: Dr Chris Morris, c.m.morris@ncl.ac.uk

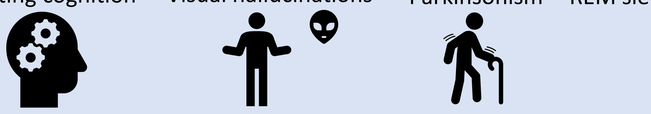
Newcastle University

INTRODUCTION

Dementia with Lewy Bodies (DLB) is a common form of memory loss in the elderly, second only to Alzheimer's disease (AD).(2)

DLB is believed to result from the accumulation of abnormally folded alpha-synuclein proteins. These proteins interfere with signal conduction between nerve cells, leading to characteristic symptoms of DLB.(1)

Core symptoms: Fluctuating cognition | Visual hallucinations | Parkinsonism | REM sleep behavioural disorder



AIMS

- To detect the presence of misfolded alpha-synuclein proteins in post-mortem brain tissue samples of patients with DLB and compare with non DLB samples.

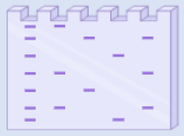
METHOD

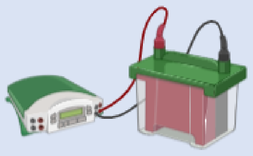
Sample preparation

Post-mortem brain tissue was homogenized, centrifuged and separated into different cellular fractions: nuclear, mitochondrial, synaptosomes and myelin.

Western blot (3)

1. **Protein separation:** Using SDS-PAGE (gel electrophoresis) followed by transfer to a nitrocellulose membrane.
2. **Antibody incubation**: Primary antibodies (NDUFS3, Complex I Sub 8, NDUFV1, Rieske Fe-S, Ubiquinol, VDAC1) were added to detect specific synapse markers, followed by secondary antibodies (anti-mouse and anti-rabbit) for visualization.
3. **Detection**: Membrane was imaged using a fluorescence imager to reveal presence of synapse markers.





Protein quantification (Bradford assay) (4)

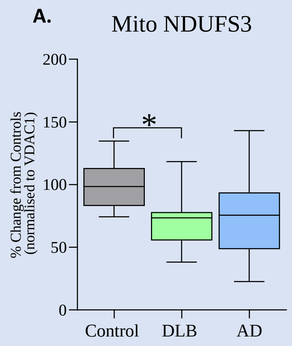
1. **Reagent preparation**: BSA standards at various concentrations.
2. **Assay procedure**: 10 µL of samples/BSA standards into a 96 well plate. Add 250 µL of Bradford reagent, incubate at room temperature for 5 minutes. Measure absorbance at 595 nm using an absorbance microplate reader.
3. **Quantification**: Protein concentrations calculated using a standard curve from known BSA concentrations

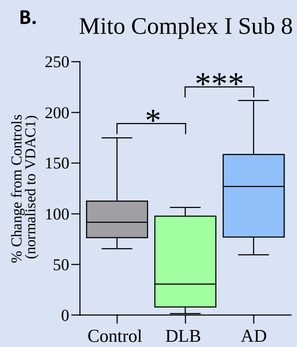
Seeded aggregation assay (5)

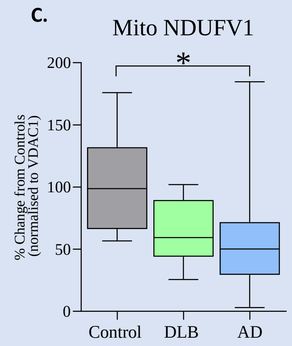
Objective: To detect pathological form of alpha synuclein using DLB, AD and non-demented controls.

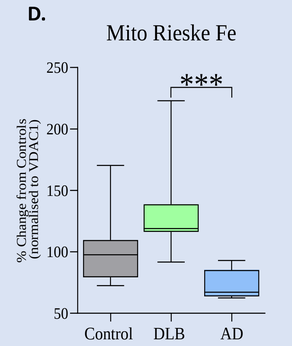
1. Suspend and dissolve brain homogenates in PBS. Perform serial dilutions to 1:5,000.
2. Dissolve Thioflavin T (ThT) in assay buffer.
3. Mix alpha synuclein monomer with brain homogenates.
4. Add the mixture, synuclein and homogenates to wells of a 96 well plate.
5. Seal and incubate the plate at 37°C
6. Measure ThT fluorescence every 10 minutes for up to 5 days
7. Analyze data and determine the lag phase, aggregation rate and final fluorescence intensity.
8. Compare results among DLB, AD and control samples.

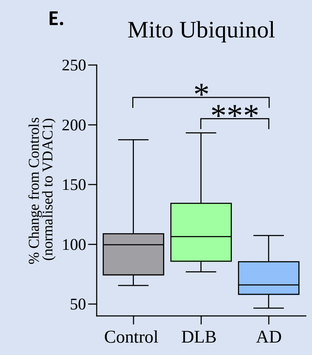
RESULTS











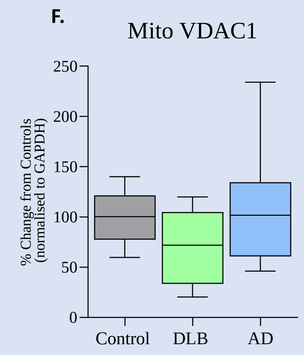


Figure 1. Mitochondrial Protein Determination in Isolated Brain Homogenates
Mitochondrial proteins were determined using specific antibodies and western blotting to demonstrate:
A. Complex I NDUFS3, **B.** Complex I Sub 8, **C.** Complex I NDUFV1, **D.** Complex III Rieske Fe-S protein, **E.** Complex III Ubiquinol, **F.** VDAC1

*= p-value < 0.05 post-hoc Kruskal-Wallis t-test
** = p-value < 0.01 post-hoc Kruskal-Wallis t-test
*** = p-value < 0.001 post-hoc Kruskal-Wallis t-test

RESULTS AND CONCLUSION

Isolation of synaptosomes from DLB and AD brain allowed us to determine if mitochondrial protein changes occurred in neurodegeneration. Several Complex I markers were seen to be reduced in DLB, while Complex III proteins appeared elevated, despite no major change in mitochondrial mass (Figure 1). No change in synaptosome SAA was observed between groups.

The differential expression of various mitochondrial subunits in DLB brain homogenates highlights the link between mitochondrial dysfunction and neurodegeneration in DLB. Previous studies have shown a change in Complex I expression in Parkinson's, and the current data demonstrates similar changes in DLB synaptosomes

The results show:

● Increased levels Complex III subunits ● Reduced levels of Complex I subunits ● Preserved levels of mitochondria

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