Journal of Alzheimer's Parkinsonism & Dementia

Research Article

Phosphorylation-Dependent TDP-43 Antibodies: Validation in a Neurodegenerative Brain Bank Cohort

This article was published in the following Scient Open Access Journal:
Journal of Alzheimer's Parkinsonism & Dementia
Received December 16, 2019; **Accepted** December 30, 2019; **Published** January 06, 2020

Keywords: TDP-43, Neurodegeneration.

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Introduction

Transactive response DNA binding protein (TDP-43) is 43 kDa protein encoded by the TARDBP gene on chromosome 1, which has in recent years been identified as the primary constituent protein in the pathological neuronal and glial inclusions characterising frontotemporal lobar degeneration (FTLD) and amyotrophic lateral sclerosis (ALS) [1]. TDP-43 aggregates have also been identified in other neurodegenerative diseases including Alzheimer's disease (AD) [1, 2], hippocampal sclerosis of aging [3], dementia with Lewy bodies [1, 2], Huntington's disease [5], chronic traumatic encephalopathy [6] and normal aged controls [7].

TDP-43 shuttles between nuclear and cytoplasmic compartments but is primarily located in the nucleus where it is involved in numerous aspects of RNA metabolism. Pathological aggregation of the protein in the diseases above occurs via a number of steps: 1) translocation to the neuronal/glial cytoplasm; 2) cleavage by caspases and other proteases to 35kDA, 25 kDa and 22kDa C-terminal fragments; 3) aberrant phosphorylation at multiple serine residues (including S409/410); 4) and ubiquitination at multiple unknown residues [8]. This aggregation process gives rise to a number of distinct inclusions such as neuronal cytoplasmic inclusions, neuronal intranuclear inclusions, glial cytoplasmic inclusions, skein-like inclusions and more diffuse granular pre-inclusions, in addition to dystrophic neurites [1, 9].

Hasegawa et al. identified the sites at which phosphorylation occurs and demonstrated that phosphorylation-dependent TDP-43 antibodies did not stain normal nuclei (unlike the commercially available phosphorylation-independent TDP-43 antibodies) [10]. This allowed TDP-43 inclusions to be identified more readily, in particular neuronal cytoplasmic inclusions and dystrophic neurites. Immunohistochemistry targeting the S409/410 phosphorylation site appeared particularly robust. However, since Hasegawa et al. first outlined the advantages of these antibodies, there has been a paucity of studies validating the use of phosphorylation-dependent TDP-43 antibodies in a practical setting.

Aim of the Study

The aim of this study was to compare the qualitative performance of a phosphorylation-dependent antibody with a phosphorylation-independent antibody in a practical, ecological setting using a cohort of patients with and without TDP-43-related diseases from the Dublin Brain Bank.

Materials and Methods

Twenty-eight cases were selected from the Dublin Brain Bank including FTLD (N=5), ALS (N=9), AD (N=7), elderly healthy controls (N=5, mean age 72.4 years) and young healthy controls (N=2, mean age 35.6 years). Formalin-fixed paraffin-embedded blocks for each case were selected corresponding to hippocampus, cerebellum and spinal cord (when sampled). Immunostaining was performed using a phosphorylation-dependent anti-TDP-43 (pS409/410, mouse monoclonal, cat. No TIP-PTD-M01, Cosmo Bio) antibody and a phosphorylation-independent anti-TDP-43 (rabbit polyclonal, cat. no. 10782-2-AP, Proteintech) antibody. Immunohistochemical staining was

performed on the Leica Bond-III (automated IHC slide stainer). Staining conditions used were Protocol F and epitope retrieval using ER2 EDTA-based buffer with surfactant (cat. no. AR9640, Leica) for 40 minutes as follows: 5 minute peroxide block; bond wash (3 quick washes); 15 minute marker; bond wash (3 quick washes); 8 minutes post-primary; bond wash (3 x 2 minutes); 8 minute polymer; bond wash (2 x 2 minutes); deionised water (1 quick wash); 10 minute mixed DAB refine; deionised water (3 quick washes); 5 minutes haematoxylin; deionised water (3 quick washes). Both antibodies were used at a dilution 1/3000. Qualitative analysis was performed by four experienced neuropathologists (AB, JC, MF, FB) and consensus agreement was reached on the presence and type of pathological inclusions for each slide. Given that the phosphorylation-independent antibody stains normal nuclei, the presence of nuclear staining was considered a negative finding.

Results

Employing the phosphorylation-dependent antibody, pathological TDP-43 inclusions were detected in 18/26 cases (excluding young healthy controls). In comparison, only 5/26 cases were felt to be positive for TDP-43 aggregates using the phosphorylation-independent stain. The phosphorylation-dependent approach was more sensitive in the detection of inclusions for each individual disease, with clear identification in all fourteen cases of ALS and FTLD. In addition, glial cytoplasmic inclusions and dystrophic neurites were identified in two cases of AD and two older healthy adult cases (not seen using phosphorylation-independent anti-TDP-43), consistent with previous reports of similar findings in normal aged controls [7]. The inclusions identified in each case are outlined in (Table 1). Apart from overall identification of TDP-43 aggregates, the clarity with which inclusions were seen was far superior utilising the phosphorylation-dependent antibody, facilitating diagnostic certainty in most cases. Examples of staining patterns using both antibodies are shown in (Figure 1 and 2).

Discussion

We have demonstrated, in a practical setting, the utility of phosphorylation-dependent anti-TDP-43 immunostaining on a small cohort of patients with and without TDP-43-related diseases. Hasegawa et al. demonstrated that phosphorylation-dependent TDP-43 antibodies did not stain normal nuclei (unlike the commercially available phosphorylation-independent TDP-43 antibodies) [10].

This approach should facilitate the detection of subtle nuclear and cytoplasmic inclusions, including pre-inclusions. In spite of the widespread use of phosphorylation-dependent antibodies, the benefit of these antibodies in facilitating easy identification of TDP-43 aggregates has not been analysed in a qualitative, ecological way. Although TDP-43 aggregates were not found in all cases with TDP-43-related diseases, the detection of inclusions was significantly improved using the phosphorylation-dependent

Table 1: Type of inclusion seen by case using phosphorylation-independent and phosphorylation-dependent antibodies. ALS = amyotrophic lateral sclerosis, FTLD = frontotemporal lobar degeneration, AD = Alzheimer's disease, DN = dystrophic neurites, GCIs = glial cytoplasmic inclusions, Skein = Skein-like inclusions.

Case	Phosphorylation-independent	Phosphorylation-dependent	Site of inclusions
ALS 1	DNs, GCIs	DNs, Skein, GCI	Spinal cord
ALS 2	Nuclear only	Negative	-
ALS 3	Nuclear only	DNs, Skein, GCI	Spinal cord
ALS 4	DNs, GCIs	DNs, GCIs	-
ALS 5	Nuclear only	DNs, GCIs	Hippocampus, spinal cord
ALS 6	Nuclear only	DNs, Skein, GCI	Spinal cord
ALS 7	Nuclear only	DNs, Skein, GCI	Spinal cord
ALS 8	Nuclear only	DNs, Skein, GCI	Hippocampus, spinal cord
ALS 9	Nuclear only	DNs, Skein, GCI	Hippocampus, spinal cord, cerebellum
FTLD 1	GCI	DNs, GCI	Hippocampus
FTLD 2	GCI	GCIs, dot like neurites	Hippocampus
FTLD 3	Skein, GCI	DNs, Skein, GCI	Hippocampus
FTLD 4	Nuclear only	Preinclusions, DNs, GCIs	Hippocampus, cerebellum
FTLD 5	Nuclear only	DNs, Preinclusions	Spinal cord
AD 1	Nuclear only	Non-specific staining	-
AD 2	Nuclear only	Pre-inclusions	Hippocampus, cerebellum
AD 3	Nuclear only	Negative	-
AD 4	Nuclear only	Negative	-
AD 5	Nuclear only	Negative	-
AD 6	Nuclear only	DNs, Preinclusions	Hippocampus
AD 7	Nuclear only	Non-specific staining	-
Aged Control 1	Nuclear only	DNs, GCI	Hippocampus
Aged Control 2	Nuclear only	Negative	-
Aged Control 3	Nuclear only	Negative	-
Aged Control 4	Nuclear only	DNs, GCIs	Hippocampus
Aged Control 5	Nuclear only	Negative	-
Young Control 1	Nuclear only	Negative	-
Young Control 2	Nuclear only	Negative	-

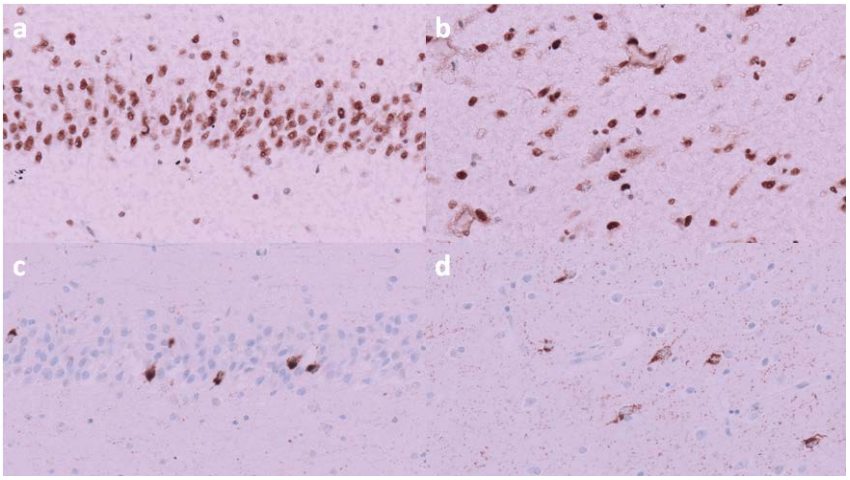

Figure1: Examples of staining patterns of two representative cases of ALS comparing the phosporylation-independent antibody (a,b) and phosphorylation-dependent antibody (c,d) of hippocampus (a,c) and frontal cortex (b,d). The phosphorylation-independent images (upper) are dominated by nuclear-staining but the lack of nuclear staining in the phosphorylation-dependent images (lower) facilitates identification of cytoplasmic inclusions and dystrophic neurites in particular.

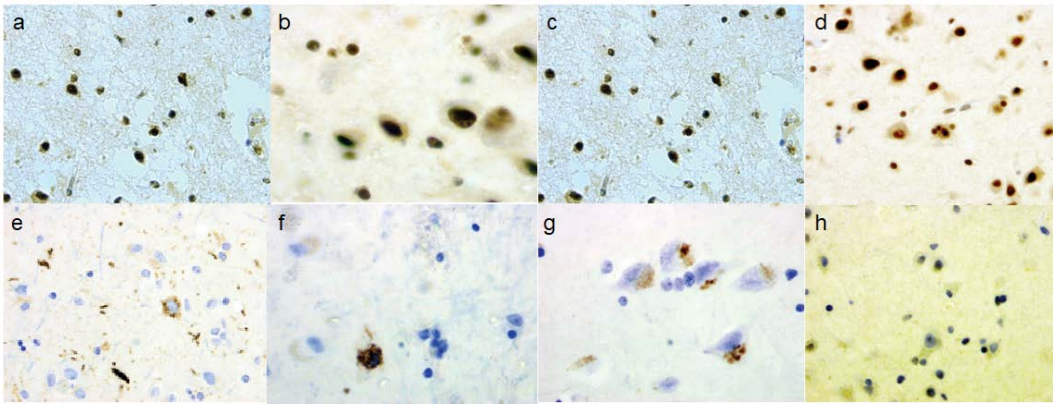

Figure 2: Examples of staining patterns of representative cases of FTLD (a,e), AD (b,f), aged healthy control (c,g) and young healthy control (d,h), comparing the phosporylation-independent antibody (a-d) and phosphorylation-dependent antibody (e-h) of hippocampus (a,c) and frontal cortex (b,d). The phosphorylation-independent images (upper) are dominated by nuclear-staining but the lack of nuclear staining in the phosphorylation-dependent images (lower) facilitates identification of cytoplasmic inclusions and dystrophic neurites in particular.

antibody compared with the phosphorylation-independent approach.

The limitations of this study include a relatively small sample size and number of raters. This could be validated on a larger cohort which would allow statistical analysis to be performed. Future studies quantifying TDP-43 aggregates using these antibodies could be undertaken to confirm our findings but this was beyond the scope of the current study.

Conclusion

This study demonstrates, in a practical setting, the utility of phosphorylation-dependent anti-TDP-43 immunostaining on a small cohort of patients with and without TDP-43-related diseases.

Funding

There are no funding sources relevant to this manuscript.

Conflict of Interest

The authors declare that they have no conflicts of interest.

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