TDP-43 is elevated in plasma neuronal-derived exosomes of patients with Alzheimer's disease

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Abstract

Background Recently, TDP-43 has been recognized as a common proteinopathy in the “oldest old” and a neuropathological comorbidity in patients with Alzheimer’s disease (AD). However, since it has a low concentration in cerebrospinal fluid, the presence of TDP-43 in AD is rarely investigated in vivo.

Methods Twenty-four patients with amyloid PET confirmed AD and 15 healthy controls (HCs) were included in this study. TDP-43 level in plasma neuronal-derived exosomes (NDEs) was measured by enzyme-linked immunosorbent assay.

Results TDP-43 level was elevated in patients with AD compared with HCs (1.20 ± 0.91 ng/ml vs 0.64 ± 0.20 ng/ml, $P < 0.039$), after controlling for age. There was no correlation between TDP-43 level and cognitive function, neuropsychiatric symptoms or APOE genotype in patients with AD.

Conclusions This study demonstrated increased TDP-43 accumulation in AD patients by examining plasma NDEs, which may provide a window into the effects of TDP-43 on AD progression.

Background

TAR DNA binding protein of 43 kDa (TDP-43), which plays a fundamental role in exon skipping, nuclear transcription, splicing and stability of RNA transcripts, micro-RNA processing, and other cellular functions (1–3), has been identified in an abnormal phosphorylated state in cellular inclusions and is associated with neurodegeneration and cognitive impairment in the majority of patients with tau-negative frontotemporal lobar degeneration (FTLD) and nearly all patients with amyotrophic lateral sclerosis (ALS) (4, 5). Moreover, TDP-43 is considered to be an independently pathogenic proteinopathy causing an amnesic dementia syndrome, which was recently named limbic-predominant age-related TDP-43 encephalopathy (LATE) (6).

In the past decade, several studies reported that TDP-43 accumulates pathologically in the brains of patients with AD. The proportion of AD patients with TDP-43 pathology has been reported to range from 19 to 73.9% (7, 8). The pathological comorbidity of TDP-43 is first observed in the medial temporal lobe and eventually spreads to occipitotemporal cortex, basal ganglia, and frontal neocortex in patients with AD (9). In a large autopsy sample, a pathological diagnosis of AD mixed with TDP-43 was the most common mixed pathology in subjects with AD (compared to pathological AD mixed with infarcts, arteriolosclerosis, Lewy bodies or hippocampal sclerosis) (10).

The burden of TDP-43 accumulation was observed to be correlated with progression of AD, such as volumetric reductions in the hippocampal and entorhinal cortex, decline in memory, naming, general cognition and global function, particularly during the early phases of neurodegeneration (7, 8, 11–15). Furthermore, TDP-43 could be a risk factor or a protective factor in terms of neuropsychiatric symptoms for patients with AD according to previously pathological studies (9, 16, 17).

Although the plasma concentration of TDP-43 has been observed to be elevated in patients with FTLD, since TDP-43 has a low concentration in cerebrospinal fluid (CSF) and may mainly originate from blood, its correlation with neurodegeneration in AD is still controversial (18–20). Exosomes are a subtype of extracellular vesicles that arise from a wide range of cells and contain molecular cargo, including a variety of proteins (21). It has been demonstrated that TDP-43 is secreted via exosomes in neuronal cells contributing to both propagation and clearance of TDP-43 in ALS brains (22), and can be detected in exosomes from CSF, which originate in the brain (23).

Neuronal-derived exosomes (NDEs) have been successfully isolated from plasma, and analyzed for the expression of AD biomarkers, such as A β , tau, cellular survival factors, lysosomal proteins, insulin receptor substrate and synaptic proteins in previous studies (24–29). In the present study, we aimed to measure expression of TDP-43 in patients with AD by isolating and analyzing plasma NDEs, and to further explore the association between TDP-43 and cognitive function, neuropsychiatric symptoms and APOE genotype.

Methods

Participants

Twenty-four patients with AD and 15 healthy controls (HCs) recruited from our longitudinal MRI study of AD and subcortical ischemic vascular dementia were included in this study. Patients with AD met the International Working Group-2 (IWG-2) diagnostic criteria for AD (30), had an amnesic symptom and an amyloid-positive ¹¹C-Pittsburgh compound B (PiB) PET scan, were aged 50-85 years, with a Mini-Mental State Examination (MMSE) score of 10-26 and a Clinical Dementia Rating (CDR) score of 0.5-2. Patients whose cognitive decline was caused by other neurological diseases, mental disorders, or medical conditions, such as dementia with Lewy bodies, Parkinson's disease, vascular dementia, multiple sclerosis, severe depression, vitamin B12 deficiency, or thyroid dysfunction, were excluded. Age- and sex-matched HCs had no complaint of cognitive decline, with an MMSE score >24 and a CDR score=0. All participants underwent comprehensive neuropsychological testing and multimodal brain MRI scans, and blood samples were collected for further investigation. This study was approved by the Ethics Committee of Tianjin Medical University General Hospital. Written informed consent was obtained from all participants.

Neuropsychological assessment

A neuropsychological battery was performed to test various cognitive domains for all subjects, including the Rey Auditory Verbal Learning Test (AVLT) (31), the Symbol Digit Modalities Test (SDMT) (32), the Trail Making Test-A (TMT-A) and B (TMT-B) (33), the Stroop test (34), the Animal Verbal Fluency Test (AFT) (35), the Controlled Oral Word Association Test (COWAT) (36), the Boston Naming Test (BNT) (37) and the Benton Judgment of Line Orientation (JLO) (38). All of the above tests have a higher score indicating better performance on the specific tasks, except for the TMT-A and TMT-B with an opposite implication.

Raw scores were converted to Z scores using the mean and SD of the HC group. Five main cognitive domains were calculated: (1) memory composite = average Z score of total learning, delayed recall and recognition on the AVLT; (2) language composite = average of the AFT, the COWAT and the BNT; (3) attention and information processing speed composite = average of the SDMT and the TMT-A; (4) executive function composite = average of the TMT-B and the Stroop color-word test; (5) visuospatial function = Z score of the JLO.

Behavioral and psychological symptoms of dementia (BPSD) were assessed using the 12-item Neuropsychiatric Inventory (NPI) (39). Apart from the total score of the NPI, the presence of motor disturbance and four symptom clusters (40), including psychosis (delusions, hallucinations), hyperactivity (agitation, disinhibition, irritability), affect (depression, anxiety) and apathy/vegetative (apathy, sleep, appetite), was also analyzed in this study.

APOE genotyping

A 500ul sample of blood was collected in ethylenediaminetetraacetic acid-containing (EDTA) vacutainer tubes from all participants. Genomic DNA was extracted using the TIANamp Blood DNA Kit (Tiangen Biotech Co., Ltd, Beijing, China) following the manufacturer's protocol. Then polymerase chain reaction amplification of the APOE gene was followed by using genomic DNA. The accuracy of genotyping was further confirmed with Sanger sequencing by using an ABI 3730xl DNA analyzer (Applied Biosystems) in Allwegene Clinical Testing Laboratory (Tianjin, China). The sequences were analyzed using the software Sequencing Analysis 5.2. The APOE status of all participants was determined by two single nucleotide polymorphisms (rs429358 and rs7412) that define the epsilon 2, 3, and 4 alleles.

Exosome isolation and identification

The isolation of NDEs was performed according to the methods previously developed by Goetzl (24). 0.5ml of plasma was incubated with 0.15ml of thromboplastin-D (Fisher Scientific, Inc., Hanover Park, IL) at room temperature for 60 minutes, followed by the addition of 0.35ml of calcium- and magnesium-free Dulbecco's balanced salt solution (DBS⁻²) with 20μl protease inhibitor cocktail (Roche Applied Sciences, Inc., Indianapolis, IN) and 5μl phosphatase inhibitor cocktail (Pierce Halt, Thermo Scientific, Inc., Rockford, IL). After centrifugation at 3000 × g for 20 minutes, supernates were mixed with 252 μl of ExoQuick exosome precipitation solution (EXOQ; System Biosciences, Inc., Mountainview, CA), and incubated for 1 hour at 4°C. Resultant exosome suspensions were centrifuged at 1500 × g for 30 minutes at 4°C and each pellet was resuspended in 350μl of DBS⁻² with inhibitor cocktails.

Each sample was mixed with 50μl of 3% bovine serum albumin (BSA) (Thermo Scientific, Inc.) and was incubated for 1 hour at 4°C with 1μg of mouse anti-human CD171 antibody (L1CAM neural adhesion protein, eBio5G3, Biotin, eBioscience, San Diego, CA), then followed by addition of 25μl streptavidin-agarose resin (Thermo Scientific, Inc.) plus 50μl of 3% BSA for 30 minutes at 4°C. After centrifugation at 400 × g for 10 minutes at 4°C and removal of the supernates, each pellet was suspended in 50μl of 0.05 M glycine-HCl (pH 3.0) by vortex-mixing for 10 seconds. Each suspension was then combined with 0.5 ml

M-PER mammalian protein extraction reagent that had been adjusted to pH 8.0 with 1 M Tris-HCl (pH 8.6) and the inhibitor cocktails followed by incubation for 10 minutes at 37°C with vortex-mixing for 15 s and was stored at -80°C before enzyme-linked immunosorbent assay (ELISA).

NDEs were identified by both transmission electron microscopy and a nanoparticle tracking system. Transmission electron microscopy measurement was conducted using a Talos F200c electron microscope (FEI, USA) at an acceleration voltage of 200 kV to characterize the size and shape of NDEs. In addition, exosomes were visualized with a NanoSight 500 instrument (NanoSight, Amesbury, UK) and characterized according to the size distribution of vesicles.

TDP-43 assay

TDP-43 protein level in plasma NDEs was assayed by ELISA kits (Signalway Antibody, CollegePark, MD). Human CD81 (Cusabio- American Research Products, Inc.) was used for normalization of TDP-43 concentration. The mean value of all CD81 levels was set at 1.00, and the relative value for each sample was used to normalize their recovery.

Statistical analysis

Statistical analyses were performed using SPSS 13.0 (SPSS Inc., USA). Demographic and clinical data of patients with AD and HCs were analyzed using Pearson chi-square test for categorical variables or independent-sample t-test for continuous variables. The difference in TDP-43 level between the two groups was analyzed using a general linear model, with age as a covariate. Partial correlation analyses were conducted to test the correlations between TDP-43 and the neuropsychological scores in patients with AD, controlling for age, sex and educational level. With respect to the NPI clusters and APOE analyses, dichotomized classification was used according to the presence or absence of the symptoms, or the epsilon 4 allele carrier status. Then chi-square tests were used to test the difference in TDP-43 level between AD patients with and without the specific neuropsychiatric symptoms, and between APOE epsilon 4 carriers vs non-carriers within the AD group. Values of $P < .05$ were regarded as significant.

Results

Demographic and clinical features

Twenty-four patients with AD (age range: 53 to 84, mean age: 67.8 ± 8.2 years, median age: 68 years, 17 females) and 15 HCs (age range: 55 to 77, mean age: 64.8 ± 6.0 years, median age: 64 years, 10 females) were included in this study. There was no significant difference in age, sex or education between the AD patients and HCs. The AD group had a significantly lower score on MMSE (16.3 ± 6.1 vs 27.7 ± 1.7) and a much higher proportion of APOE $\epsilon 4$ carriers (3 with 4/4, 10 with 3/4, 10 with 3/3, 1 with 2/3 vs 0 with 4/4, 2 with 3/4, 9 with 3/3, 4 with 2/3) compared with the HC group. Table 1 presents the demographic characteristics of the two groups.

TDP-43 level in exosomes

NDEs were identified with an electron microscope (Fig. 1A) and a nanoparticle tracking system (Fig. 1B). The size and shape of plasma NDEs from AD patients are similar to those previously reported (41). CD81, an exosome membrane marker, was measured and used to normalize the concentration of NDEs for all participants. The AD group showed a lower CD81 level (4.97 ± 1.52 ng/ml vs 6.33 ± 1.42 ng/ml, $P = 0.008$) than the HC group (Fig. 2A). Normalized plasma neuronal-derived exosomal concentration of TDP-43 (1.20 ± 0.91 ng/ml vs 0.64 ± 0.20 ng/ml, $P = 0.039$) was higher in patients with AD compared to HCs after controlling for age (Fig. 2B).

The association between TDP-43 and cognitive function, neuropsychiatric symptoms and APOE genotype in patients with AD

Z scores for all cognitive domains, including memory, language, attention and information processing speed, executive function and visuospatial function, were prominently decreased in patients with AD compared with HCs. TDP-43 level in NDEs did not correlate with the total MMSE score or the Z score of any cognitive domain, after controlling for age, sex and educational level (Table 2).

There was no difference in neuronal-derived exosomal concentration of TDP-43 between AD patients with and without symptoms of any neuropsychiatric cluster according to the NPI, including psychosis (1.36 ± 1.40 ng/ml vs 1.10 ± 0.46 ng/ml), hyperactivity (1.30 ± 1.12 ng/ml vs 1.06 ± 0.50 ng/ml), affect (1.16 ± 1.06 ng/ml vs 1.30 ± 0.35 ng/ml), apathy/vegetative (1.21 ± 1.08 ng/ml vs 1.18 ± 0.47 ng/ml) and motor disturbance (0.90 ± 0.47 ng/ml vs 1.33 ± 1.02 ng/ml) (Fig. 3A-E).

TDP-43 level did not differ between APOE $\epsilon 4$ carriers (1.41 ± 1.29 ng/ml) and non-carriers (1.03 ± 0.36 ng/ml) in patients with AD (Fig. 3F).

Discussion

TDP-43 has been increasingly recognized as an independent proteinopathy associated with an amnesic syndrome that can mimic AD, as well as a common neuropathological comorbidity in patients with AD. In the present study, we demonstrated that TDP-43 level in NDEs from plasma is elevated in patients with AD. However, we did not observe any correlation between TDP-43 level and cognitive function, neuropsychiatric symptoms or APOE genotype in AD.

It has been reported that TDP-43 pathology is strongly correlated with advanced AD and arteriosclerotic pathologies in the aged human brain according to a neuropathology data set study (42). We found that TDP-43 level was increased in patients with AD compared to cognitively healthy individuals through analysis of plasma NDEs, which may reflect neuropathological status. Our present finding supports previous studies that TDP-43 pathology could be a common comorbidity in patients with AD. Recently, it has been observed that there is a correlation between TDP-43 burden and A β deposition (9, 43), and increased hippocampal TDP-43 pathology is associated with advanced tau neurofibrillary tangle pathology (44) in patients with AD. Moreover, TDP-43 contributed to reducing plaque burden and

increasing pre-fibril oligomers of A β (45), as well as exacerbating tau aggregation (46) in an APP/PS1 mouse background.

TDP-43 has been found to be associated with cognitive decline and dementia conversion in a cohort of older persons without dementia at study entry (47). However, the correlation between TDP-43 and cognitive deficits in patients with AD has not been established. We did not find a relationship between TDP-43 level in plasma NDEs and global cognition measured with the MMSE, or any specific cognitive domain, including memory, language, attention and processing speed, executive function, and visuospatial function. The current National Institute on Aging and Alzheimer's Association (NIA-AA) research framework posits that the decline in cognitive function of AD is mainly attributable to the interaction of amyloid plaques, neurofibrillary tau deposits and neurodegeneration (48), in which TDP-43 is involved but may not be a determinant.

In the present study, TDP-43 level was not associated with any cluster of neuropsychiatric symptoms measured with the NPI, including psychosis, hyperactivity, affect, apathy/vegetative, and motor disturbance. A few previous studies focused on the contribution of TDP-43 to BPSD in patients with AD, but the findings were inconclusive. For instance, TDP-43 was observed to be a risk factor for agitation/aggression assessed by the NPI-Q in AD patients with high pathology load of neurofibrillary tangles (17). In another study using the NPI-Q to evaluate BPSD, TDP-43 was associated with increased severity of aberrant motor behavior and decreased severity of depression, and the correlations between TDP-43 and neuropsychiatric symptoms interacted with amyloid and Lewy body pathologies (9). Additionally, there was no reported correlation between global TDP-43 pathology and psychosis, which was rated with the Consortium to Establish a Registry for Alzheimer's disease Behavioral Rating Scale. However, TDP-43 in the frontal cortex may have a protective effect regarding the risk of psychosis in patients with AD (16). Therefore, the effect of TDP-43 on BPSD may interact with core AD pathology and other neuropathological changes, and may be dependent on the brain region of its accumulation.

It has been observed that APOE ϵ 4 is associated with TDP-43 burden in community-based individuals (49) and patients with AD (11, 14) according to post mortem studies. In another study, although APOE ϵ 4 was directly correlated with TDP-43, this effect was mediated by A β and tau (43). However, we did not identify a relationship between APOE genotype and TDP-43. From our point of view, the effect of APOE genotype on TDP-43 accumulation in patients with AD should be carefully interpreted, since APOE affects core AD neuropathology and cerebrovascular disease, both of which also interact with TDP-43.

In the present study, all participants were recruited from our prospective research study, and all patients with AD had a biomarker-supported diagnosis with PiB PET. However, there are some limitations to our study. L1CAM was used to extract NDEs from plasma; however, it is not exclusively expressed in the CNS, but also in other tissues including skeletal muscle and fat. In addition, the concentration of exosomes identified by CD81 was different between the AD patient group and the HC group, although we standardized TDP-43 level with CD81 for subsequent analysis. This discrepancy could be partly explained by the impact of APOE ϵ 4, which has been observed to reduce exosome expression in mouse

and human brain tissues (50). In this study, 54.2% of AD subjects were APOE ϵ 4 carriers, compared with only 13.3% of HCs. Moreover, some neuropsychological testing scales have “floor effects” and “ceiling effects”. For instance, several AD patients were unable to complete the TMT-B task within the set time of 300s; on the other hand, some items were scored 0 for the NPI in very mild patients. These effects may have affected the correlation analyses between TDP-43 and cognitive function and neuropsychiatric symptoms. Furthermore, the samples size was relatively small, especially for the HCs. Our findings need to be validated in a large population cohort.

Conclusion

TDP-43 level in plasma NDEs is increased in patients with AD. Although we did not find any correlation between TDP-43 level and cognitive function, neuropsychiatric symptoms or APOE genotype in patients with AD, the relationship between TDP-43 pathology, cognition and BPSD is complicated, and may interact with other neuropathology in the AD context. The measurement of TDP-43 in plasma NDEs is a promising non-invasive biomarker with the potential to provide insight into the role of TDP-43 in neurodegeneration and progression in AD.

Abbreviations

AD: Alzheimer’s disease; AFT: Animal Verbal Fluency Test; ALS: amyotrophic lateral sclerosis; APOE: Apolipoprotein E; AVLT: Rey Auditory Verbal Learning Test; BNT: Boston Naming Test; BPSD: behavioral and psychological symptoms of dementia; BSA: bovine serum albumin; CDR: Clinical Dementia Rating; COWAT: Controlled Oral Word Association Test; CSF: cerebrospinal fluid; DBS⁻²: Dulbecco’s balanced salt solution; ELISA: enzyme-linked immunosorbent assay; FTLD: frontotemporal lobar degeneration; HCs: healthy controls; IWG-2: International Working Group-2; JLO: Benton Judgment of Line Orientation; LATE: limbic-predominant age-related TDP-43 encephalopathy; MMSE: Mini-Mental State Examination; NDEs: neuronal-derived exosomes; NIA-AA: National Institute on Aging and Alzheimer’s Association; NPI: Neuropsychiatric Inventory; PiB: ¹¹C-Pittsburgh compound B; SDMT: Symbol Digit Modalities Test; TDP-43: TAR DNA binding protein of 43 kDa; TMT: Trail Making Test

Declarations

Acknowledgements

Not applicable

Authors’ contributions

NZ and DG were responsible for concept, study design, statistical analysis and drafting of the manuscript. NZ, DG and MM were responsible for acquisition, analysis and interpretation of data. NZ and

MG substantively revised the manuscript for important intellectual content.

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Availability of data and materials

The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

The Ethics Committee of Tianjin Medical University General Hospital approved this study. Written informed consent was obtained from all participants.

Consent for publication

Not applicable

Competing interests

Within the past 2 years, MG has received research support without direct compensation from MSD (Merck), Eisai, AbbVie, and Janssen. MG has also served on an advisory board for Eisai. The remaining authors declare that they have no competing interests.

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Tables

Table 1 Demographic and clinical features of patients with AD and HCs

	AD	HC	<i>P</i>
	N = 24	N = 15	
Age, y	67.8 ± 8.2	64.8 ± 6.0	0.228
Sex, F/M	17/7	10/5	0.784
Education, y	11.0 ± 3.6	12.6 ± 2.4	0.125
APOE ε4 carrier/non-carrier	11/13	2/13	0.036
MMSE	16.3 ± 6.1	27.7 ± 1.7	0.000

Age, education and MMSE are provided as mean ± SD.

AD: Alzheimer's disease; HC: healthy control; MMSE: Mini-Mental State Examination.

Table 2 Correlation between TDP-43 level and cognitive function in patients with AD

	Score	<i>r</i>	<i>P</i>
MMSE	16.33 ± 6.14*	0.062	0.791
Memory	-3.38 ± 1.36**	0.304	0.181
Language	-2.29 ± 1.81**	0.067	0.772
Information processing speed	-2.37 ± 1.42**	0.056	0.809
Executive function	-1.79 ± 0.90**	0.161	0.485
Visuospatial function	-3.22 ± 2.14**	0.143	0.537

AD: Alzheimer's disease; MMSE: Mini-Mental State Examination.

* raw score of the MMSE.

** Z score of the cognitive domains

Figures

Figure 1

Plasma neuronal-derived exosomes identified with TEM and NTA. (A) A representative image detected with transmission electron microscopy of exosomes extracted from an AD patient. The scale bar equals 100nm. (B) A representative plot of size/concentration determined with nanoparticle tracking analysis for plasma exosomes derived from an AD patient.

Figure 2

Protein concentrations of plasma neuronal-derived exosomes detected with ELISA. (A) CD81 level was lower in patients with AD compared to HCs. (B) Normalized TDP-43 concentration was higher in patients with AD compared to HCs. AD: Alzheimer's disease; HC: healthy control.

Figure 3

The comparison of TDP-43 concentration between patients with and without specific neuropsychiatric symptom clusters, and APOE ϵ 4 carriers and non-carriers in AD. (A-E) There were no correlations between TDP-43 and psychosis, hyperactivity, affect, apathy/vegetative, and motor disturbance measured with NPI in patients with AD ($P > 0.05$). (F) The difference in TDP-43 level between APOE ϵ 4 carriers and non-carriers of AD patients was not statistically significant ($P > 0.05$).